

Carla Cristina Bauermann Brasil  
(Organizadora)



# ALIMENTOS: TOXICOLOGIA E MICROBIOLOGIA & QUÍMICA E BIOQUÍMICA

Carla Cristina Bauermann Brasil  
(Organizadora)



# ALIMENTOS: TOXICOLOGIA E MICROBIOLOGIA & QUÍMICA E BIOQUÍMICA

**Editora chefe**

Profª Drª Antonella Carvalho de Oliveira

**Editora executiva**

Natalia Oliveira

**Assistente editorial**

Flávia Roberta Barão

**Bibliotecária**

Janaina Ramos

**Projeto gráfico**

Camila Alves de Cremo

Daphynny Pamplona

Gabriel Motomu Teshima

Luiza Alves Batista

Natália Sandrini de Azevedo

**Imagens da capa**

iStock

**Edição de arte**

Luiza Alves Batista

2022 by Atena Editora

Copyright © Atena Editora

Copyright do texto © 2022 Os autores

Copyright da edição © 2022 Atena Editora

Direitos para esta edição cedidos à Atena Editora pelos autores.

Open access publication by Atena Editora



Todo o conteúdo deste livro está licenciado sob uma Licença de Atribuição *Creative Commons*. Atribuição-Não-Comercial-NãoDerivativos 4.0 Internacional (CC BY-NC-ND 4.0).

O conteúdo dos artigos e seus dados em sua forma, correção e confiabilidade são de responsabilidade exclusiva dos autores, inclusive não representam necessariamente a posição oficial da Atena Editora. Permitido o *download* da obra e o compartilhamento desde que sejam atribuídos créditos aos autores, mas sem a possibilidade de alterá-la de nenhuma forma ou utilizá-la para fins comerciais.

Todos os manuscritos foram previamente submetidos à avaliação cega pelos pares, membros do Conselho Editorial desta Editora, tendo sido aprovados para a publicação com base em critérios de neutralidade e imparcialidade acadêmica.

A Atena Editora é comprometida em garantir a integridade editorial em todas as etapas do processo de publicação, evitando plágio, dados ou resultados fraudulentos e impedindo que interesses financeiros comprometam os padrões éticos da publicação. Situações suspeitas de má conduta científica serão investigadas sob o mais alto padrão de rigor acadêmico e ético.

**Conselho Editorial****Ciências Agrárias e Multidisciplinar**

Prof. Dr. Alexandre Igor Azevedo Pereira – Instituto Federal Goiano

Profª Drª Amanda Vasconcelos Guimarães – Universidade Federal de Lavras

Profª Drª Andrezza Miguel da Silva – Universidade do Estado de Mato Grosso

Prof. Dr. Arinaldo Pereira da Silva – Universidade Federal do Sul e Sudeste do Pará

Prof. Dr. Antonio Pasqualetto – Pontifícia Universidade Católica de Goiás

Profª Drª Carla Cristina Bauermann Brasil – Universidade Federal de Santa Maria



Prof. Dr. Cleberton Correia Santos – Universidade Federal da Grande Dourados  
Prof<sup>o</sup> Dr<sup>a</sup> Diocléa Almeida Seabra Silva – Universidade Federal Rural da Amazônia  
Prof. Dr. Écio Souza Diniz – Universidade Federal de Viçosa  
Prof. Dr. Edevaldo de Castro Monteiro – Universidade Federal Rural do Rio de Janeiro  
Prof. Dr. Fábio Steiner – Universidade Estadual de Mato Grosso do Sul  
Prof. Dr. Fágner Cavalcante Patrocínio dos Santos – Universidade Federal do Ceará  
Prof<sup>o</sup> Dr<sup>a</sup> Girlene Santos de Souza – Universidade Federal do Recôncavo da Bahia  
Prof. Dr. Guilherme Renato Gomes – Universidade Norte do Paraná  
Prof. Dr. Jael Soares Batista – Universidade Federal Rural do Semi-Árido  
Prof. Dr. Jayme Augusto Peres – Universidade Estadual do Centro-Oeste  
Prof. Dr. Júlio César Ribeiro – Universidade Federal Rural do Rio de Janeiro  
Prof<sup>o</sup> Dr<sup>a</sup> Lina Raquel Santos Araújo – Universidade Estadual do Ceará  
Prof. Dr. Pedro Manuel Villa – Universidade Federal de Viçosa  
Prof<sup>o</sup> Dr<sup>a</sup> Raissa Rachel Salustriano da Silva Matos – Universidade Federal do Maranhão  
Prof. Dr. Renato Jaqueto Goes – Universidade Federal de Goiás  
Prof. Dr. Ronilson Freitas de Souza – Universidade do Estado do Pará  
Prof<sup>o</sup> Dr<sup>a</sup> Talita de Santos Matos – Universidade Federal Rural do Rio de Janeiro  
Prof. Dr. Tiago da Silva Teófilo – Universidade Federal Rural do Semi-Árido  
Prof. Dr. Valdemar Antonio Paffaro Junior – Universidade Federal de Alfenas



## Alimentos: toxicologia e microbiologia & química e bioquímica

**Diagramação:** Gabriel Motomu Teshima  
**Correção:** Mariane Aparecida Freitas  
**Indexação:** Amanda Kelly da Costa Veiga  
**Revisão:** Os autores  
**Organizadora:** Carla Cristina Bauermann Brasil

### 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  
[www.atenaeditora.com.br](http://www.atenaeditora.com.br)  
contato@atenaeditora.com.br



**Atena**  
Editora  
Ano 2022

## DECLARAÇÃO DOS AUTORES

Os autores desta obra: 1. Atestam não possuir qualquer interesse comercial que constitua um conflito de interesses em relação ao artigo científico publicado; 2. Declaram que participaram ativamente da construção dos respectivos manuscritos, preferencialmente na: a) Concepção do estudo, e/ou aquisição de dados, e/ou análise e interpretação de dados; b) Elaboração do artigo ou revisão com vistas a tornar o material intelectualmente relevante; c) Aprovação final do manuscrito para submissão; 3. Certificam que os artigos científicos publicados estão completamente isentos de dados e/ou resultados fraudulentos; 4. Confirmam a citação e a referência correta de todos os dados e de interpretações de dados de outras pesquisas; 5. Reconhecem terem informado todas as fontes de financiamento recebidas para a consecução da pesquisa; 6. Autorizam a edição da obra, que incluem os registros de ficha catalográfica, ISBN, DOI e demais indexadores, projeto visual e criação de capa, diagramação de miolo, assim como lançamento e divulgação da mesma conforme critérios da Atena Editora.



## DECLARAÇÃO DA EDITORA

A Atena Editora declara, para os devidos fins de direito, que: 1. A presente publicação constitui apenas transferência temporária dos direitos autorais, direito sobre a publicação, inclusive não constitui responsabilidade solidária na criação dos manuscritos publicados, nos termos previstos na Lei sobre direitos autorais (Lei 9610/98), no art. 184 do Código Penal e no art. 927 do Código Civil; 2. Autoriza e incentiva os autores a assinarem contratos com repositórios institucionais, com fins exclusivos de divulgação da obra, desde que com o devido reconhecimento de autoria e edição e sem qualquer finalidade comercial; 3. Todos os e-book são *open access*, *desta forma* não os comercializa em seu site, sites parceiros, plataformas de *e-commerce*, ou qualquer outro meio virtual ou físico, portanto, está isenta de repasses de direitos autorais aos autores; 4. Todos os membros do conselho editorial são doutores e vinculados a instituições de ensino superior públicas, conforme recomendação da CAPES para obtenção do Qualis livro; 5. Não cede, comercializa ou autoriza a utilização dos nomes e e-mails dos autores, bem como nenhum outro dado dos mesmos, para qualquer finalidade que não o escopo da divulgação desta obra.



## 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; análise 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 1..... 1**

ASPECTOS DA FERMENTAÇÃO MALOLÁTICA NO PROCESSO DE VINIFICAÇÃO DE VINHOS ARGENTINOS E BRASILEIROS

Maria Mariana Oliveira Souza

Thamyres Fernanda Moura Pedrosa Souza

 <https://doi.org/10.22533/at.ed.3702217011>

### **CAPÍTULO 2..... 11**


AVALIAÇÃO DA ATIVIDADE ANTIOXIDANTE EM MALTE FERMENTADO COM *AGARICUS BRASILIENSIS*

Mariane Daniella da Silva

Herta Stutz

Fernanda Maria Pagane Guerreschi Ernandes

Crispin Humberto Garcia-Cruz

 <https://doi.org/10.22533/at.ed.3702217012>

### **CAPÍTULO 3..... 18**

AVALIAÇÃO DA VIABILIDADE CELULAR DE *Lactobacillus plantarum* APÓS INCORPORAÇÃO EM CHOCOLATES ARTESANAIS COM ALTO TEOR DE CACAU

Kassiany Pedroso Dalmora

Thabata Maria Alvarez


 <https://doi.org/10.22533/at.ed.3702217013>

### **CAPÍTULO 4..... 29**

PROSPECÇÃO TECNOLÓGICA: USO DO MESOCARPO DE BABAÇU NAS ÁREAS DE ALIMENTOS, FÁRMACOS E COSMÉTICOS

Itaceni de Araújo Sousa

Tonicley Alexandre da Silva

 <https://doi.org/10.22533/at.ed.3702217014>

### **CAPÍTULO 5..... 39**

AVALIAÇÃO DA QUALIDADE MICROBIOLÓGICA DE FARINHA DE MANDIOCA COMERCIALIZADA EM MACEIÓ – AL

Genildo Cavalcante Ferreira Júnior

Heitor Barbosa Gomes de Messias


Eduarda Mendes de Almeida

Lucas Pedrosa Souto Maior

Eliane Costa Souza

Thiago José Matos Rocha

Jammily de Oliveira Vieira Moreira


 <https://doi.org/10.22533/at.ed.3702217015>

### **CAPÍTULO 6..... 47**

DIFERENTES POTENCIALIDADES E USOS DO ÓLEO DE MACAÚBA : UMA BREVE

## REVISÃO


Thaynara Cavalcanti Lima  
Cristhiane Maria Bazílio de Omena Messias  
Marianne Louise Marinho Mendes

 <https://doi.org/10.22533/at.ed.3702217016>

## **CAPÍTULO 7.....53**

ANÁLISE NUTRICIONAL, QUÍMICA E ANATÔMICA DE MARUPAZINHO (*Eleutherine bulbosa* (Mill.) Urb – IRIDACEAE) DE BELÉM DO PARÁ, BRASIL


Ana Paula Ribeiro de Carvalho Ferreira  
Mariana Aparecida de Almeida Souza  
João Paulo Guedes Novais  
Dayane Praxedes da Silva  
Mirian Ribeiro Leite Moura  
Ana Cláudia de Macêdo Vieira

 <https://doi.org/10.22533/at.ed.3702217017>

## **CAPÍTULO 8.....73**

DESENVOLVIMENTO E CARACTERIZAÇÃO DE DOCE DE CUMBARU (*Dipteryx alata* Vog.) ACRESCIDO DE FARINHA DE BAGAÇO DE MALTE


Drielle Suely de Souza Oliveira  
Márcia Helena Scabora  
Daiane Alves Cardoso  
Dayane Sandri Stellato

 <https://doi.org/10.22533/at.ed.3702217018>

## **CAPÍTULO 9.....87**

EXTRAÇÃO DE ÓLEO ESSENCIAL DE CAPIM-LIMÃO (*Cymbopogon citratus* (D. C.) Stapf) POR HIDRODESTILAÇÃO


Marília Assunta Sfredo  
Carina Tasso  
Daniele Bergmeier  
Cristiane Reinaldo Lisboa  
José Roberto Delalibera Finzer

 <https://doi.org/10.22533/at.ed.3702217019>

## **CAPÍTULO 10.....102**

AVALIAÇÃO DOS PARÂMETROS FÍSICOQUÍMICOS DE SALSICHA RESFRIADA TIPO HOT DOG COMERCIALIZADA EM UBERABA, MINAS GERAIS

Priscila Renata da Costa  
Claudia Maria Tomás Melo

 <https://doi.org/10.22533/at.ed.37022170110>

## **CAPÍTULO 11.....108**

RENDIMENTO DE CARÇAÇA E CORTES EM FRANGOS DE CORTE - HÍBRIDOS COMERCIAIS (*Gallus gallus domesticus*)

Carlos Eduardo da Silva Soares


Fabiano Dahlke  
Lucélia Haupti  
Priscila de Oliveira Moraes  
Priscila Arrigucci Bernardes  
André Luís Ferreira Lima - Bernardes  
Diego Peres Neto  
Juliano de Dea Lindner

 <https://doi.org/10.22533/at.ed.37022170111>

**CAPÍTULO 12..... 123**

ÓLEOS VEGETAIS EM PRODUTOS CÁRNEOS: PERSPECTIVAS FUTURAS PARA SUBSTITUIÇÃO DA GORDURA ANIMAL


Juliana de Andrade Mesquita  
Erika Cristina Rodrigues  
Katiuchia Pereira Takeuchi  
Edgar Nascimento  
Rozilaine Aparecida Pelegrine Gomes de Faria

 <https://doi.org/10.22533/at.ed.37022170112>

**CAPÍTULO 13..... 146**

EVALUATION OF TWO TOXIN BINDERS EFFECTIVNESS IN REDUCING ZEARALENONE TOXIC EFFECTS ON GILTS

José Antonio Fierro  
Juan Carlos Medina  
Luis Miguel Dong  
Elizabeth Rodríguez

 <https://doi.org/10.22533/at.ed.37022170113>

**CAPÍTULO 14..... 152**

LIPASE B FROM *Candida antarctica*: ACTIVITY AND STABILITY studies in DIFFERENT PH AND TEMPERATURES


Mirian Cristina Feiten

 <https://doi.org/10.22533/at.ed.37022170114>

**CAPÍTULO 15..... 163**

MICROSCOPIA DE ALIMENTOS: DIFICULDADES E LEGISLAÇÃO VIGENTE NA IDENTIFICAÇÃO E ANÁLISE DE CONTAMINANTES BIOLÓGICOS

Gustavo Paim de Carvalho  
André Luis de Alcantara Guimarães




 <https://doi.org/10.22533/at.ed.37022170115>

**CAPÍTULO 16..... 173**

IDENTIFICAÇÃO MICROSCÓPICA DE ADULTERANTES E MATÉRIAS ESTRANHAS NA COMPOSIÇÃO DOS ALIMENTOS E OS IMPACTOS NA SAÚDE PÚBLICA

Ludilaine Fiuza Barreto de Oliveira  
André Luis de Alcantara Guimarães

 <https://doi.org/10.22533/at.ed.37022170116>

<b>CAPÍTULO 17.....</b>	<b>185</b>
<b>ATIVIDADE IMUNOMODULADORA DO ÓLEO E DA NANOEMULSÃO DE MAURITIA FLEXUOSA NA INTERAÇÃO ENTRE FAGÓCITOS E ENTAMOEBIA HISTOLYTICA</b>	
Marianny Carolina Custódio da Silva Brito Núbia Andrade Silva Victor Pena Ribeiro Adenilda Cristina Honório-França Eduardo Luzia França Kellen Menezes de Oliveira Silvana de Oliveira Castro Juliana Francycle Martins de Camargo Guilherme Alves Sena Valmir André Peccini Mateus Abreu Milani Ana Beatriz dos Santos Matsubara Matheus Leal Lira Alves Lucélia Campelo de Albuquerque Moraes	
 <a href="https://doi.org/10.22533/at.ed.37022170117">https://doi.org/10.22533/at.ed.37022170117</a>	
<b>CAPÍTULO 18.....</b>	<b>204</b>
<b>DETERMINAÇÃO DE HERBICIDAS EM ÁGUA DE ABASTECIMENTO DE ESCOLAS DA REGIÃO RURAL DO MUNICÍPIO DE SANTA MARIA/RS</b>	
Rosselei Caiel da Silva Jonatan Vinicius Dias Jefferson Soares de Jesus Ionara Regina Pizzutti Rochele Cassanta Rossi	
 <a href="https://doi.org/10.22533/at.ed.37022170118">https://doi.org/10.22533/at.ed.37022170118</a>	
<b>CAPÍTULO 19.....</b>	<b>215</b>
<b>SUCO DE LIMÃO: PRODUÇÃO, COMPOSIÇÃO E PROCESSAMENTO</b>	
Lucia Maria Jaeger de Carvalho Antonio Gomes Soares Marcos José de Oliveira Fonseca José Luiz Viana de Carvalho	
 <a href="https://doi.org/10.22533/at.ed.37022170119">https://doi.org/10.22533/at.ed.37022170119</a>	
<b>SOBRE A ORGANIZADORA.....</b>	<b>246</b>
<b>ÍNDICE REMISSIVO.....</b>	<b>247</b>

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

Data de aceite: 01/11/2021

**Mirian Cristina Feiten**

Departamento de Engenharia de Alimentos e  
Tecnologia de Alimentos

Universidade Estadual de Maringá (UEM),  
Umuarama, PR, Brasil

**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 °C. 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 65°C 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 °C e 60 °C. 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 °C 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 | 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  $\alpha/\beta$  hydrolase, composed by 317 amino acids, arranged in 10  $\alpha$ -helices and 9  $\beta$ -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; Ferrario 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  $\mu\text{m}$ , maximum flow rate of 2.6 mL/min (maximum pressure of 0.3 MPa), molar mass range between  $3 \times 10^3$  and  $7 \times 10^5$  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 °C 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 (Ultraspec™ 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 °C, 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 $\times$ SYPRO orange (1:500 dilution of the stock) in a total volume of 10  $\mu\text{L}$  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
<b>A</b>	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>B</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
<b>C</b>	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
<b>D</b>	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
<b>E</b>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM MgCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM MgCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM MgCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM CaCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM CaCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM CaCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM MnCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM MnCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM MnCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM ZlCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM ZlCl <sub>2</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM ZlCl <sub>2</sub>
<b>F</b>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM FeCl <sub>3</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM FeCl <sub>3</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM FeCl <sub>3</sub>	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 <sub>4</sub> Cl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM NH <sub>4</sub> Cl	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM NH <sub>4</sub> Cl
<b>G</b>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 1 mM MgSO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 5 mM MgSO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 10 mM MgSO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM Li <sub>2</sub> SO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM Li <sub>2</sub> SO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM Li <sub>2</sub> SO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 50 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 100 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	20 mM NaHEPES pH 7.5, 100 mM NaCl, 200 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	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
<b>H</b>	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 <sub>2</sub> O	Protein in its buffer + Sypro orange (Reference)	H <sub>2</sub> O

## 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 °C (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.

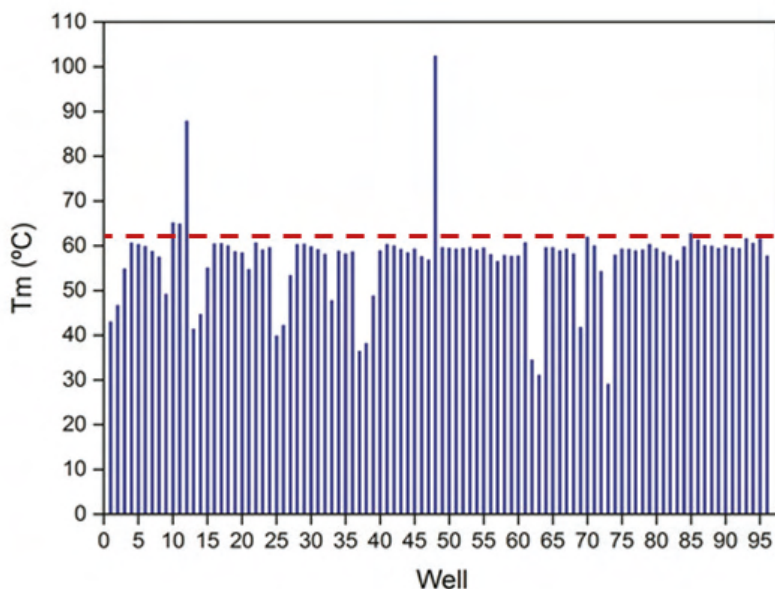


Figure 1.  $T_m$  (°C) for CalB in 96 different buffer conditions.

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 °C (Table 2). Well 48 is not reliable because a melting temperature of 102.3 °C is not realistic for any protein.

Well	Condition	$T_m$ (°C)
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.  $T_m$  (°C) 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 °C, indicating that just a few hydrophobic regions are exposed in the native structure. When temperature was increased above 65 °C, 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 °C for 1 hour.

Assay	Incubation	Temperature of measurement (°C)	pH	Hydrolysis activity (U/mL) (%) ± standard error
1	Stored at 4 °C 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 °C 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 °C, although their thermal stability changes substantially depending on their source – microbial lipases may resist to higher temperatures (65-70 °C) (Macrae and Hammond, 1985; Jaeger and Eggert, 2002). CalB activity increased 4-fold in all cases when measurements were carried out at 60 °C compared to 30 °C 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 °C compared to 30 °C. The highest hydrolysis activity was observed at 65 °C for 1 hour and measured in pH 9.2 at 60 °C.

## 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 65°C 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**. 2ª edição. Editora Acribia, Zaragoza, 1997.

BLANK, K., MORFILL, J., GUMPP, H., GAUB, H. E. **Functional expression of *Candida Antarctica* lipase B in *Escherichia 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 Universitat 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.

## ÍNDICE REMISSIVO

### A

Ácidos graxos insaturados 51, 125, 130

Acrocomia aculeata (jacq.) Lodd 49

Agaricus blazei 12, 13, 17

Agrotóxicos 205, 206, 207, 209, 210, 211, 212, 214

Água 8, 14, 21, 22, 23, 26, 27, 43, 59, 67, 80, 81, 84, 88, 91, 93, 94, 95, 96, 97, 98, 107, 112, 113, 116, 117, 133, 134, 135, 165, 168, 171, 176, 181, 188, 205, 206, 207, 208, 209, 210, 212, 214, 219, 220, 221, 224, 226, 227, 230, 232, 233, 234, 235, 236, 237, 239, 241

Alimentos funcionais 18, 19, 86

Alimentos ready-to-eat 125

Análise de Alimentos 108

Análise química, 55, 64

Análises físico-químicas 76, 103, 104, 107, 178

Artrópodes 164, 168, 169, 172

Avicultura 109, 110, 121, 122, 123

### B

Babaçu 5, 29, 30, 31, 32, 33, 34, 35, 37, 38, 39

Bacillus cereus 40, 41, 42, 43, 44, 45, 46, 47, 182

Bactérias do ácido láctico 1, 2, 8

### C

Caracterização anatômica 55

Chocolate intenso 18

Citral 88, 89, 90, 91, 101, 220, 240, 242

Citrus latifolia 216, 218, 244, 245

Coliformes 40, 42, 43, 44, 45, 46, 74, 80, 84, 86, 182

Composição centesimal 54, 55, 58, 59, 66, 67, 68, 69, 103, 108

Consumo 2, 8, 13, 27, 41, 50, 51, 57, 64, 75, 85, 110, 111, 112, 115, 116, 119, 125, 131, 144, 167, 169, 171, 172, 177, 180, 181, 205, 207, 214, 224, 231, 234, 237, 243

Cor do vinho 1, 3, 7, 8

Coxa 109, 110, 114, 115, 117, 118, 119, 120

Cultivo submerso 11, 12, 13, 14, 15

Cumbaru 6, 74, 75, 76, 77, 78, 80, 83, 85



## D

Destilação 89, 90, 91, 93, 190, 235, 241, 242

Dpph• 11, 12, 14, 16

## E

Eleutherine bulbosa 6, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 66, 68, 69, 71

Embutidos cárneos 103, 104, 108

Enologia 1, 3

Essência 89, 90, 99

## F

Farinha de bagaço de malte 6, 74, 75, 76, 77, 78, 82, 83, 84, 85

Fermentação 5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 19, 20, 75

Fermentação malolática 5, 1, 2, 3, 4, 6, 7, 8, 9, 10

## G

Gilts 7, 147, 148, 149

## H

Híbridos comerciais 6, 109, 110, 111, 117, 118, 119, 120

Hyperestrogenism 147

## I

Inovação 5, 29, 38, 39, 52, 70, 166

## L

Lima ácida 216, 217, 218, 219, 220, 221, 223, 224, 244, 245

Literatura científica 48, 183

## M

Manteiga de cacau 18, 20, 21, 23, 24, 25, 26, 27

Mesocarpo 5, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38

Monitoramento 45, 206

## O

Organoaluminosilicate 147, 149, 150, 151

## P

Peito 109, 110, 112, 115, 117, 118, 119, 120, 122

Ph 7, 153, 155

Potencial mercadológico 48

probióticos 18, 19, 20, 23, 25, 26, 27, 134

PROBIÓTICOS 23

Processamento 8, 5, 30, 40, 42, 45, 51, 76, 77, 79, 80, 122, 133, 145, 165, 166, 167, 179, 216, 222, 224, 225, 231, 232, 233, 234, 235

Prospecção 5, 20, 29, 30, 39, 59

## R

Reproduction 147

Roedores 164, 167, 168, 169, 172, 176

## S

Salmonela sp 40

Salsichas 103, 104, 106, 107, 108, 124, 133, 135, 136

Saudabilidade 50, 125, 133

Stability 7, 28, 139, 140, 142, 143, 144, 146, 153, 154, 160, 162, 163

Suco de limão 8, 216, 217, 218, 219, 220, 221, 223, 224, 225, 227, 231, 232, 233, 234, 235, 236, 237, 239, 245

## T

Taninos 1, 2, 3, 5, 7, 8, 55, 58, 63, 64, 65, 68, 69, 72

Temperature 47, 153, 154, 155, 156, 158, 159, 160

Toxin binders 7, 147, 149

Tricologia 164, 168

## V

Validação de método 206

Vigilância sanitária 40, 42, 44, 46, 69, 100, 164, 165, 166, 169, 171, 172, 174, 175, 182, 184, 185, 243, 246, 247

Vulvovaginitis 147, 148

## Y

Yeast cell walls 147, 149, 152

## Z

Zearalenone 7, 147, 148, 150, 152

www.atenaeditora.com.br  
contato@atenaeditora.com.br  
@atenaeditora  
www.facebook.com/atenaeditora.com.br



# ALIMENTOS: TOXICOLOGIA E MICROBIOLOGIA & QUÍMICA E BIOQUÍMICA

🌐 [www.atenaeditora.com.br](http://www.atenaeditora.com.br)  
✉ [contato@atenaeditora.com.br](mailto:contato@atenaeditora.com.br)  
📷 @atenaeditora  
📘 [www.facebook.com/atenaeditora.com.br](https://www.facebook.com/atenaeditora.com.br)



# ALIMENTOS: TOXICOLOGIA E MICROBIOLOGIA & QUÍMICA E BIOQUÍMICA