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**BIOCONVERSÃO DA POLPA DE BOCAIUVA VERDE POR  
*Pleurotus ostreatus* PARA OBTENÇÃO DE  
BOLO *LOW CARB***

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Dourados – MS

Fevereiro/2021

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Tese apresentada à Universidade Federal da Grande Dourados (UFGD), como parte dos requisitos exigidos para obtenção do título de DOUTOR em Biotecnologia e Biodiversidade.

Área de Concentração: Biotecnologia e Biodiversidade

Orientador: Dr. Gustavo Graciano Fonseca

Coorientadores: Dr. Marcelo Fossa da Paz e

Dr. Fernando Araripe Gonçalves Torres

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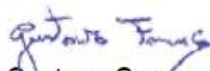
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*OSTREATUS* PARA OBTENÇÃO DE BOLO LOW CARB”

Por

**ALINE JANAINA GIUNCO**

Tese apresentada à Universidade Federal da Grande Dourados (UFGD),  
como parte dos requisitos exigidos para obtenção do título de  
DOUTORA EM BIOTECNOLOGIA E BIODIVERSIDADE  
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## RESUMO

GIUNCO, Aline Janaina. Universidade Federal da Grande Dourados, novembro de 2020. **Bioconversão da polpa de bocaiuva verde por *Pleurotus ostreatus* para obtenção de bolo *low carb***. Orientador: Prof. Dr. Gustavo Graciano Fonseca. Coorientadores: Dr. Marcelo Fossa da Paz e Dr. Fernando Araripe Gonçalves Torres.

A bocaiuva é uma palmeira do cerrado, amplamente distribuída em quase todo o Brasil, sendo abundante no Mato Grosso do Sul e Mato Grosso. A polpa do fruto maduro apresenta características nutricionais, sensoriais e funcionais importantes para a saúde. Contudo é um fruto pouco utilizado na alimentação humana, principalmente no estágio de maturação verde e existe pouca informação sobre as propriedades nutricionais e funcionais desse fruto nas suas diferentes espécies, principalmente da *Acrocomia totai*. Diante disso, o objetivo do estudo foi a bioconversão da polpa de bocaiuva verde por *Pleurotus ostreatus* para obtenção de bolo *low carb*, visando a obtenção de um produto alimentício com propriedades nutricionais, funcionais e sensorialmente aceito pelos consumidores. Na avaliação do crescimento e a composição proximal do cogumelo comestível *Pleurotus ostreatus* em substratos de polpa de bocaiuva verde com diferentes fontes de nitrogênio (8 tratamentos) mostrou que a adição de nitrato de amônio como fonte de nitrogênio no tratamento T3 potencializou a bioconversão de carboidratos da polpa de bocaiuva verde de *Pleurotus ostreatus* em proteínas, fibras e lipídios de valioso potencial nutricional, o que se revela promissor para o desenvolvimento de novos produtos alimentícios com valor nutracêutico agregado. No desenvolvimento e avaliação de bolos com baixo teor de carboidratos produzidos a partir da polpa de bocaiuva verde enriquecida com *pleurotus ostreatus*, mostrou que a farinha de bocaiuva verde enriquecida com *P. ostreatus* pode ser utilizada para o desenvolvimento de bolo *low carb* com componentes nutricionais aprimorados e características sensoriais aceitáveis. Essas informações podem subsidiar estudos sobre as propriedades nutricionais partir da polpa de bocaiuva verde enriquecida com *pleurotus ostreatus*, bem como viabilizar a aplicação biotecnológica e a produção em larga escala das espécies de *Acrocomia*.

**Palavras-chave:** *Acrocomia*, cogumelo comestível, fonte de nitrogênio, composição proximal e cultivo em estado sólido.



## ABSTRACT

GIUNCO, Aline Janaina. Federal University of Grande Dourados, November 2020.  
**Bioconversion of the green bocaiuva pulp by *Pleurotus ostreatus* to obtain low carb cake.** Advisor: Prof. Dr. Gustavo Graciano Fonseca. Co-supervisors: Dr. Marcelo Fossa da Paz and Dr. Fernando Araripe Gonçalves Torres.

The bocaiuva is a cerrado palm, widely distributed in almost all of Brazil, being abundant in Mato Grosso do Sul and Mato Grosso. The pulp of the ripe fruit has important nutritional, sensory and functional characteristics for health. However, it is a fruit little used in human food, mainly in the stage of green maturation and there is little information about the nutritional and functional properties of this fruit in its different species, mainly of *Acrocomia totai*. Therefore, the objective of the study was the bioconversion of the green bocaiuva pulp by *Pleurotus ostreatus* to obtain a low carb cake, aiming to obtain a food product with nutritional, functional and sensorially accepted by consumers. In the evaluation of the growth and the proximal composition of the edible mushroom *Pleurotus ostreatus* in green pulp substrates with different nitrogen sources (8 treatments) showed that the addition of ammonium nitrate as a nitrogen source in the T3 treatment potentiated the bioconversion of carbohydrates from green bocaiúva pulp of *Pleurotus ostreatus* in proteins, fibers and lipids of valuable nutritional potential, which is promising for the development of new food products with added nutraceutical value. In the development and evaluation of low-carbohydrate cakes produced from the pulp of green bocaiúva enriched with *pleurotus ostreatus*, it showed that the green bocaiúva flour enriched with *P. ostreatus* can be used for the development of low carb cake with enhanced nutritional components and acceptable sensory characteristics. This information can support studies on the nutritional properties of the green pulp of bocaiúva enriched with *pleurotus ostreatus*, as well as enabling the biotechnological application and large-scale production of *Acrocomia* species.

**Keywords:** *Acrocomy*, edible mushroom, nitrogen source, proximal composition and solid state cultivation.

## SUMÁRIO

CAPÍTULO 1 .....	1
1.1 APRESENTAÇÃO.....	1
1.2 INTRODUÇÃO GERAL .....	2
1.3 REFERÊNCIAS .....	4
CAPÍTULO 2 .....	8
CHARACTERISTICS, PROPERTIES AND POTENTIAL APPLICATIONS OF <i>Acrocomia</i> SPECIES.....	8
2.1 <i>Acrocomia</i> .....	9
2.2 PROPERTIES OF THE FRUITS .....	18
2.3 POTENTIAL APPLICATIONS.....	25
2.4 CONCLUSION .....	29
2.5 REFERENCES .....	29
CAPÍTULO 3 .....	39
GROWTH AND PROXIMATE COMPOSITION OF <i>Pleurotus ostreatus</i> CULTIVATED ON GREEN BOCAIUVA PULP SUBSTRATES WITH DIFFERENT NITROGEN SOURCES	39
3.1 INTRODUCTION .....	40
3.2 MATERIAL AND METHODS.....	41
3.3 RESULTS AND DISCUSSION.....	44
3.4 CONCLUSION .....	49
3.6 REFERENCES .....	49
CAPÍTULO 4 .....	58
DEVELOPMENT AND EVALUATION OF LOW-CARB CAKES PRODUCED FROM GREEN BOCAIUVA PULP ENRICHED WITH <i>Pleurotus ostreatus</i> .....	58
4.1 INTRODUCTION .....	59
4.2 MATERIAL AND METHODS.....	60
4.3 RESULTS AND DISCUSSION.....	63
4.4 CONCLUSION .....	69
4.7 REFERENCES .....	70
CAPÍTULO 5 .....	75
5.1 CONCLUSÃO GERAL .....	75
APÊNDICES .....	76

## LISTA DE FIGURAS

<b>Figure 3.1</b> Growth curves of <i>Pleurotus ostreatus</i> .....	45
<b>Figure 3.2</b> Proximate composition for different cultivation treatments with <i>Pleurotus ostreatus</i> .....	48
<b>Figure 3.3</b> Energetic value and composition variation for lipids, proteins and fibers for different cultivation treatments with <i>Pleurotus ostreatus</i> .....	49
<b>Figure 4.1</b> Averages of the values of the scores attributed in the sensory analysis for the four <i>low-carb</i> cake formulations.....	67
<b>Figure 4.2</b> Acceptance index of the four <i>low-carb</i> cake formulations.....	68
<b>Figure 4.3</b> Purchase intention of the four <i>low-carb</i> cake formulations.....	69

## LISTA DE TABELAS

<b>Table 2.1</b> Occurrence of <i>Acrocomia</i> sp. ....	11
<b>Table 2.2</b> Morphological characteristics of <i>Acrocomia</i> sp. ....	13
<b>Table 2.3</b> Biometric characteristics of <i>Acrocomia</i> sp. ....	14
<b>Table 2.4</b> Proximate composition and energetic values of fruits from <i>Acrocomia</i> sp.....	21
<b>Table 2.5</b> Proximate composition and energetic value of products elaborated with fruits of <i>Acrocomia aculeata</i> .....	22
<b>Table 2.6</b> Mineral content of fruits of <i>Acrocomia</i> sp.....	22
<b>Table 2.7</b> Physical characteristics of the fruits of <i>Acrocomia aculeata</i> .....	23
<b>Table 2.8</b> Composition of fatty acids of pulp of <i>Acrocomia</i> sp.....	23
<b>Table 2.9</b> Activity of enzymes produced by <i>Acrocomia</i> sp. ....	24
<b>Table 2.10</b> Variation of the composition of <i>Acrocomia aculeata</i> residue by bioprocess using the filamentous fungi <i>Lichtheimia ramosa</i> .....	24
<b>Table 2.11</b> Bioactive compound of <i>Acrocomia</i> sp.....	25
<b>Table 3.1</b> Treatments for growth experiments ....	42
<b>Table 4.1</b> Low-carb cake formulations.....	62
<b>Table 4.2</b> Proximate composition obtained for the low-carb cake formulations.....	65

## LISTA DE SIGLAS E ABREVIATURAS

5-LOX	arachidonate 5-lipoxygenase
AI	adiposity index
AKI	acute kidney injury
AN	ammonium nitrate
ANOVA	Analysis of variance
AS	ammonia sulphate
BP	green bocaiuva pulp
BPWB	dehydrated green bocaiuva pulp plus wheat bran
COX-2	cyclooxygenase-2
CP	cyclophosphamide
DTF	transverse diameter of the fruit
ED	endocarp mass
EP	epicarp mass
ET	endocarp thickness
F1	Low-carb cake with coconut flour
F2	Low-carb cake with green bocaiuva flour without the increment
F3	Low-carb cake with mushroom enriched green bocaiuva flour and ammonia nitrate
F4	Low-carb cake with green enriched mushroom flour without supplementation
FAO	United Nations Food and Agriculture Organization
FV	fruit volume
GBIF	Global Biodiversity Information Facility
HPD	High protein diets
HPV	papilomavírus humano
LD	oral lethal dose
LDA	longitudinal diameter of the almond
LDF	longitudinal diameter of the fruit
LDL	Low Density Lipoproteins
MA	almond mass

MF	fruit mas
MM	mesocarp mass
N	ammonium nitrate
ND	not determined
Org	Organização
PCV	proximate composition variation
PDA	potato dextrose agar
ST	strength training
T1	dehydrated green bocaiuva pulp and water
T2	dehydrated green bocaiuva pulp and Urea
T3	dehydrated green bocaiuva pulp and ammonium nitrate
T4	dehydrated green bocaiuva pulp and ammonium
T5	green bocaiuva pulp / wheat bran and water
T6	green bocaiuva pulp / wheat bran and urea
T7	green bocaiuva pulp / wheat bran and ammonium nitrate
T8	green bocaiuva pulp / wheat bran and ammonia sulphate
U	urea
WB	wheat bran
WHO	World Health Organization

# 1    **CAPÍTULO 1**

## 2    **1.1 APRESENTAÇÃO**

3            A estrutura da tese está disposta em capítulos e no formato de manuscrito científico.  
4    Assim, este Capítulo 1 traz a introdução geral, justificativa e objetivo do trabalho. No Capítulo  
5    2 é apresentada uma revisão de literatura referente as características, propriedades e potencial  
6    de aplicação das espécies de *Acrocomia*. Essa pesquisa mostra que as espécies não têm sido  
7    valorizadas quanto ao seu potencial nutricional para formulação de produtos alimentícios,  
8    potencial de compostos bioativos para elaboração de produtos funcionais ou nutracêuticos e  
9    produtos fitoterápicos. Além disso, há escassez de informações sobre o potencial de uso dos  
10   frutos no estágio de maturação verde. Com isso, sugere-se estudos do potencial nutricional,  
11   terapêutico de aplicação biotecnológica com as outras espécies e também no estágio de  
12   maturação verde.

13           Diante disso, foram elaborados os capítulos 3 e 4. No Capítulo 3 é apresentada a  
14   avaliação do crescimento e a composição proximal do cogumelo comestível *Pleurotus ostreatus*  
15   em substratos de polpa de bociúva verde com diferentes fontes de nitrogênio. A finalidade  
16   desse capítulo foi verificar as fontes de nitrogênio que contribuem para o crescimento do  
17   cogumelo comestível *Pleurotus ostreatus*, visando a obtenção de um produto com baixo teor  
18   de carboidratos e enriquecidos em proteínas e fibras.

19           O Capítulo 4 apresenta o desenvolvimento e avaliação de bolos com baixo teor de  
20   carboidratos produzidos a partir da polpa de bociúva verde enriquecida com *Pleurotus*  
21   *ostreatus*, a fim de desenvolver um produto alimentício fontes de nutrientes e substâncias  
22   benéficas à saúde, que possa ser consumido com regularidade pela população e que atenda as  
23   restrições alimentares.

24           Finalmente, no Capítulo 5 são apresentadas as conclusões gerais.

## 25 1.2 INTRODUÇÃO GERAL

26 A relação positiva entre o consumo de frutas e cereais ricos em fibras na prevenção e  
27 tratamento das doenças crônicas, estimula a prospecção de espécies vegetais que apresentam  
28 quantidades significativas de fibra alimentar para o desenvolvimento de novos produtos  
29 alimentícios (Bernaud & Rodrigues, 2013; Fayet-Moore et al., 2017).

30 A bociuva é uma palmeira do cerrado, amplamente distribuída em quase todo o Brasil,  
31 sendo abundante no Mato Grosso do Sul e Mato Grosso (Hiane et al., 2006, Lorenzi et al.,  
32 2010). A polpa do fruto maduro apresenta características nutricionais (Bora & Rocha, 2004;  
33 Ramos et al., 2008; Orsi et al., 2015), sensoriais (Silva et al., 2008; Ramos et al., 2008; Kinupp  
34 & Lorenzi, 2014) e funcionais importantes para a saúde (Ramos, Ramos Filho, Hiane, Braga  
35 Neto, & Siqueira, 2008; Ramiro, 2010; Silva, 2012; Orsi et al., 2015; Coimbra & Jorge, 2011;  
36 Lescano, et al., 2015; Costa et al., 2020). Contudo é um fruto pouco utilizado na alimentação  
37 humana, principalmente no estágio de maturação verde e existe pouca informação sobre as  
38 propriedades nutricionais e funcionais desse fruto nas suas diferentes espécies.

39 A farinha da polpa de bociuva verde pode apresentar ação terapêutica no controle de  
40 peso, glicose e dislipidemia, devido os seus componentes nutricionais (Ramiro, 2010; Silva,  
41 2012; Giunco, 2018). Dessa forma, produtos alimentícios formulados com adição da farinha  
42 poderão ser uma opção alimentar contribuindo para a prevenção de doenças crônicas. Além  
43 disso, os frutos de bociuva, atualmente obtidos pelo extrativismo, podem ganhar mercado e  
44 serem mais valorizados e contribuindo para preservação das palmeiras, pois muitas vezes são  
45 desmatadas para fins de exploração agrícola da área.

46 O consumo de bociuva verde pode ser favorecido a partir da bioconversão da polpa de  
47 bociuva verde por *Pleurotus ostreatus* para obtenção de produtos alimentícios com baixo teor  
48 de carboidratos e enriquecidos em proteínas e fibras, e aceitos pela maioria dos consumidores  
49 (Ritota & Manzi, 2019).

50 O *P. ostreatus* é um cogumelo comestível conhecido como cogumelo ostra, que possui  
51 coloração clara (branco, cinza ou castanho) e seu basidiocarpo possui formato de folha carnuda  
52 (Jonathan & Esho, 2010). Os *Pleurotus* são fonte de nutrientes, principalmente proteínas,  
53 minerais e vitaminas B, C e D (Panjikaran & Mathew, 2013). Esses cogumelos contêm 20-35  
54 % de proteína (peso seco), possuem baixos teores de lipídeos e aminoácidos essenciais (Li et  
55 al., 2017; Lavelli et al., 2018). Os cogumelos podem contribuir para prevenção de algumas  
56 doenças, como hipertensão (Vaz et al., 2011), colesterol (Wei et al., 2018; Liu et al., 2019),  
57 diabetes (Asrafuzzaman et al., 2018; Liu et al., 2019; Khatun et al., 2020; Balaji et al., 2020),



58 estresse (Akata et al., 2012), obesidade (Khatun et al., 2020) e atuar como um inibidor de apetite  
59 (Sheng et al., 2019). O *Pleurotus* por apresentar baixo valor calórico pode ser incluído em dietas  
60 com ingestão controladas de calorias (Jaworska & Bernás, 2009).

61 Os substratos pectocelulósicos, como cascas, polpas e bagaços de frutas, apresentam  
62 condições favorável para o cultivo de cogumelos comestíveis, pois possui menor relação  
63 carbono: nitrogênio e altas concentrações de açúcares simples, o que facilita a sua  
64 disponibilização ao fungo (Rivas et al., 2010; Cardoso et al., 2013; Silva et al., 2014; Silva et  
65 al., 2020). A adaptação das espécies de *Pleurotus* a novos substratos representa, um dos  
66 principais processo de bioconversão em produtos alimentícios com valor agregado e de  
67 qualidade nutricional (Mbassi et al., 2018; Ritota & Manzi, 2019). Dessa forma, a utilização de  
68 substratos de frutas para o cultivo de cogumelos comestível torna uma alternativa viável para o  
69 enriquecimento nutricional desses produtos e valorização das espécies dentro de uma cadeia  
70 produtiva, com produção permanente e sustentável (Carrasco-González et al., 2017; Ritota &  
71 Manzi, 2019).

72 Diante disso, o objetivo do estudo foi a bioconversão da polpa de bocaiuva verde por  
73 *Pleurotus ostreatus* para obtenção de bolo *low carb*, visando a obtenção de um produto  
74 alimentício com propriedades nutricionais, funcionais e sensorialmente aceito pelos  
75 consumidores.

76

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196

197 **CAPÍTULO 2**

198 **CHARACTERISTICS, PROPERTIES AND POTENTIAL APPLICATIONS OF**  
199 ***Acrocomia* SPECIES.**

200 **ABSTRACT:** The *Acrocomia* genus is found in several countries and is explored natively and  
201 commercially in different ways. There is no consensus on the exact number of species of this  
202 genus, in addition to the lack of information on properties: nutritional, therapeutic, biometric,  
203 antioxidant and application potential of existing species. Therefore, this study aimed to analyze  
204 species of *Acrocomia* genus to confirm their properties and potential nutraceutical and  
205 nutritional. Seven species were found that represent this group, *A. aculeata*, *A. intumescens*  
206 *Drude*, *A. totai* Mart., *A. hassleri*, *A. glaucescens* Lorenzi e *A. emensis* (Toledo) Lorenzi e *A.*  
207 *crispa* (Kunth) C.F. Baker ex Becc. Of those that stands out the most, for being widely  
208 distributed worldwide, is *A. aculeata*. Biometric, morphological and nutritional characteristics  
209 vary according to the species and region where it is found, which can influence the exploration  
210 potential. Despite the economic diversity of *Acrocomia* exploitation and its therapeutic and  
211 application potential, they are generally used for subsistence of native peoples throughout  
212 tropical America. In addition to commercial exploitation the species is destined for oil and oil  
213 production. The species of greatest commercial interest in Brazil and with high potential for  
214 exploration in the countries where they occur are *A. aculeata*, *A. totai* and *A. intumescens*. This  
215 shows that the species have not been valued in terms of their nutritional potential for the  
216 formulation of food products, the potential of bioactive compounds for the elaboration of  
217 functional or nutraceutical products and herbal products. In addition, there is a scarcity of  
218 information on the potential use of fruits in the green ripening stage. Thus, it is suggested studies  
219 of the nutritional, therapeutic potential of biotechnological application with the other species  
220 and also in the green maturation stage.

221

222 **Keywords:** Acrocomy, fatty acids, antioxidants, co-products, nutritional properties.

## 223 **2.1 *Acrocomia***

### 224 **2.1.1 General aspects**

225 *Acrocomia* is a genus of palm trees of arboreal size belonging to the Palmae family  
226 (Arecaceae). It is native from South America and is widely distributed in Argentina, Bolivia,  
227 Brazil, and Venezuela, and Central American countries, including Mexico. It is commercially  
228 exploited in the countries where it occurs. Due to its wide geographical distribution, the  
229 phenology varies according to the region (Vianna et al., 2015, 2017).

230 There is no consensus regarding the number of species of this genus (Vianna et al., 2015,  
231 2017), but the *Acrocomia aculeata* species is the one that has the most extensive occurrence  
232 and, therefore, is named with different colloquial terms in the different regions where it occurs  
233 (Almeida et al., 1998; Lorenzi et al., 2010; Galeano & Bernal, 2010; Smith, 2015; Vianna et  
234 al., 2015; Lieb et al., 2019).

235 All *Acrocomia* species have bunches with several round shaped fruits, consisting of  
236 epicarp (peel), mucilaginous and fibrous mesocarp (pulp), hard and dense endocarp  
237 (integument), and seed (almond) covered by the endocarp (Lorenzi et al., 2006; Vianna et al.,  
238 2017). These fruit species are considered native to the Savannah region and have nutritional,  
239 therapeutic potential (Reis & Schmiele, 2019), potential for the production of biofuels and  
240 vegetable oil (César et al., 2015; Del Río et al., 2016, Nunes et al., 2018), in addition to being  
241 commonly used for subsistence of native peoples across tropical America (Vianna et al., 2017).

242 The mature *Acrocomia* pulp is rich in lipids, carbohydrates, fibers (Bora and Rocha,  
243 2004; Ramos et al., 2008; Orsi et al., 2015), copper, potassium and zinc (Ramos et al., 2008)  
244 and can supply the nutritional needs of human beings through its consumption *in natura* or  
245 processed (Ramos et al., 2008). In addition, the pulp is rich in  $\beta$ -carotene (Ramos et al., 2008;  
246 Orsi et al., 2015; Coimbra & Jorge, 2011) and  $\alpha$ -tocopherol (Coimbra & Jorge, 2011), which  
247 are important carotenoids with antioxidant action and anti-inflammatory effect (Lescano et al.,  
248 2015a; Costa et al., 2020).

249 Several studies have analyzed the morphology (Sanjinez-Argandoña and Chuba, 2011;  
250 Brandão et al., 2014; Lescano et al., 2015b), the nutritional constituents (Hiane et al., 1990;  
251 Coimbra & Jorge, 2011; Machado et al., 2015; Lescano et al., 2015b), fatty acids (Hiane et al.,  
252 1990; Lescano et al., 2015a; Lescano et al., 2015b), carotenoids (Ramos et al., 2008; Coimbra  
253 & Jorge, 2011; Orsi et al., 2015), and minerals (Ramos et al., 2008; Lescano et al., 2015b) from  
254 the fruits.

255 Despite the exploration potential of the *Acrocomia* genus, most of the information reported  
256 in the literature is related mostly to the biometric characteristics, nutritional properties, and

257 therapeutics of the *A. aculeata* species. There is still a lack of studies that present information  
258 about other species of this genus. In this sense, there is a need for studies with different species  
259 to underline their properties to justify their permanent and sustainable production within a  
260 productive chain.

261

### 262 **2.1.2 Species and occurrence**

263 The *Acrocomia* genus is represented by seven species, six occurring in regions of Brazil  
264 (Pará, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Federal District, Goiás, Mato  
265 Grosso do Sul, Mato Grosso, Minas Gerais, Rio de Janeiro, São Paulo, and Paraná states. They  
266 are *A. aculeata*, *A. intumescens* Drude, *A. totai* Mart., *A. hassleri*, *A. glaucescens* Lorenzi, and  
267 *A. emensis* (Toledo) Lorenzi. The species *A. crispa* (Kunth) C.F. Baker ex Becc. is more  
268 common in Cuba (Lorenzi et al. 2010; Vianna et al., 2016; Vianna & Campos-Rocha, 2020).  
269 The species known as bocaiuva, macauba or macaiba are all *Acrocomia* sp. (Lorenzi et al.,  
270 2006; Vianna et al., 2017).

271 The species *A. aculeata* is named with different common terms (Table 2.1), depending  
272 on the occurrence region (Almeida et al., 1998; Galeano & Bernal 2010; Lorenzi et al., 2010;  
273 Sanjinez-Argandoña & Chuba, 2011; Smith, 2015; Vianna et al., 2015; Hernández-Zardón,  
274 2016; Lieb et al., 2019). In Belize and Panama were found reports of the use of *Acrocomia*  
275 species receiving the common name of corozo or cohune, but with the scientific name of *Attalea*  
276 *cohune* in Belize (Hernández-Zardón, 2016).

277 Among the species found in Brazil, *A. aculeata*, *A. intumescens*, and *A. totai* are  
278 exploited by extraction for fruits' consumption. *A. intumescens* is named locally by macaiba.  
279 This species is endemic to the Brazilian Northeast region, occurring in areas of the called Wood  
280 Zone and in highland forests. *A. totai* is known as bocaiuva, distributed in most of the state of  
281 Mato Grosso do Sul, associated with the regions of the Brazilian Savannah and Pantanal  
282 (Lorenzi et al., 2010; Vianna et al., 2017).

283 *A. hassleri* occurs on the border between Brazil (at Mato Grosso do Sul state) and  
284 Paraguay, and it frequently mistaken with *A. emensis* (Toledo) Lorenzi. *A. glaucescens* Lorenzi  
285 occurs in the Brazilian state of Goiás but is also mentioned in the literature as of occurrence in  
286 the Mato Grosso do Sul state (Lorenzi et al., 2010). *A. emensis* (Toledo) Lorenzi, in its turn, is  
287 popularly known as crawling tucum. It occurs in the Brazilian states of Goiás, Mato Grosso do  
288 Sul, Minas Gerais, São Paulo and Paraná (Neiva et al., 2016). This palm is considered a species  
289 at risk of extinction due to the destruction of its natural habitat and the fact that it is not being  
290 reforested (Lorenzi et al., 2010).



291 **Table 2.1** Occurrence of *Acrocomia* sp.

Species	Common names	Occurrences*	Occurrences by country*	References
<i>A. aculeata</i>	Butter tree, bacaiuveira, bocaiuva, chonta, corozo, Bahian gum, catarrh coconut, viscous coconut, thorned coconut, slobber coconut, cherished coconut, coyol, palm oil, grou grou, imbocaia , jabara, korondia, macauba, macauva, macaiba, macacauba, macajuba, macaibeira, macaja, marcova, mucaja, mucaia, macaia, Paraguayan nut, mascara oil, macaw palm, cormorant palm, Paraguayan palm, uba	666	Mexico (208); Brazil (124); Colombia (113); Costa Rica (57); Bolivia (24); Argentina (20); Paraguay (20); Nicaragua (15); Puerto Rico (11); United States (11); Honduras (10); Belize (7); Panama (7); Dominican Republic (6); El Salvador (4); Jamaica (4); Guatemala (3); Suriname (3); Trinidad and Tobago (3); and other countries***	Almeida et al,1998; Galeano & Bernal, 2010; Sanjinez-Argandoña & Chuba, 2011; Lorenzi et al. 2010; Smith 2015; Vianna et al., 2015; Hernandez-Zardón, 2016; Lieb et al., 2019
<i>A. intumescens</i>	Macauba, macaiba, bocaiuva, slobber coconut, bellid palm, bellid macauba, thorned coconut	27	Brazil	Lorenzi et al., 2010
<i>A. totai</i>	Bocaiuva, totai, bocaiuveira, macabira, mocajuba, como mbokaja (Guarani language)	44	Bolivia (14); Brazil (12); Paraguay (11); Argentina (4); United States (3)	Lorenzi et al. 2010; Moraes, 2015
<i>A. hassleri</i>	Field's small coconut, dwarf tucum	40	Brazil (9); Paraguay (31)	Lorenzi et al., 2010
<i>A. glaucescens</i>	Small macauba	19	Brazil	Lorenzi et al., 2010
<i>A. emensis</i>	Crawling tucum, field's small coconut, tucum	7	Brazil	Lorenzi et al., 2010; Neiva et al., 2016
<i>A. crispa</i>	Corojo palm	13	Cuba	Pérez et al., 2015
<i>A. corumbaensis</i>		2	Brazil	

292 \*Data obtained from GBIF.org (2020). \*Data that contained the geographic coordinates. \*\*Occurrence of species in different countries ranging from 1 to 2 (adding up to a total  
 293 of 16 occurrences).

294 **2.1.3 Morphological characteristics**

295 The morphological characteristics refers to the size of the trees, which can be  
296 distinguished mainly by their stem characteristics, in addition to other characteristics (Table  
297 2.2).

298 *A. aculeata* presents rounded fruit, the epicarp has a green to matte green color, quite  
299 hard when the fruits are green, but brittle when ripe, the mesocarp 3 to 7 mm thick, green,  
300 cream, yellow or orange. It usually blooms at the end of the dry season. Variations in the fruiting  
301 period can occur and may be related to the geographic region (Hernández-Zardón, 2016).

302 *A. hassleri* is a species characterized by the underground stem (acaulescent) and short  
303 tomentuous peduncular bract, with sparse thorns in the median and / or apical region. It can be  
304 confused with *A. emensis*, which has a larger size, peduncular bract greater than 15 cm in length,  
305 and is all covered with thorns (Lorenzi et al., 2010). *A. emensis* is an underbrush terrestrial palm  
306 tree with many thorns on the leaves and petioles. The leaves are pinnate and arched. The fruits  
307 are globose, and their ripening occurs at the beginning of southern hemisphere summer, with  
308 fruiting occurring once a year (Lorenzi et al., 2010; Neiva et al., 2016).

309

310 **2.1.4 Biometric characteristics**

311 The fruits of *Acrocomia* sp. are arranged in clusters, being considered a drupa globosa  
312 constituted by cardiac epicarp (peel), thin, mucilaginous, fibrous and oily mesocarp (pulp) and  
313 hard and dense endocarp (integument), containing seed (almond) adhered to the lipid-rich  
314 endocarp (Almeida et al., 1998; Lorenzi et al., 2006; Vianna et al., 2017; Lieb et al., 2019).  
315 Depending on the species ecotype, the mature pulp has a yellow or orange color (da Silva et al.,  
316 2018), suggesting the presence of bioactive compounds, such as carotenoids.

317 The palm fruits ripen unevenly inside the bunch and are considered ripe when they start  
318 to come off the bunches and fall to the ground, which occurs from September to January in the  
319 South hemisphere (Lorenzi, 2010; Lieb et al., 2019). On the ground, the fruits are exposed to  
320 attack by insects, rodents or microbial contamination, which causes rapid degradation with  
321 quantitative and qualitative losses of their constituents, especially in the pulp.

322 Table 2.3 show the biometric characteristics of some *Acrocomia* sp. The values of the  
323 mass of the whole fruit and of the parts that compose show a great variation between the same  
324 species and among others, depending of their origin, especially the *A. aculeata* collected in  
325 Costa Rica. The main information found in the literature is related to the species *A. aculeata*  
326 found in Brazil.

327

**Table 2.2** Morphological characteristics of *Acrocomia* sp.

Species	Stipe	Plant height (m)	Leaf length (m)	Fruit diameter (cm)	Thorns	Fruit color	Habitat	Reference
<i>A. aculeata</i>	Cylindrical, straight, with base remains of fallen leaves	14-20	1.9-3	3.0-6.0, rarely up to 9	Stipe and leaves	Varied (opaque green and brown)	In general, dry and open area	Viana et al., 2017; Hernandez-Zardón, 2016
<i>A. intumescens</i>	Cylindrical, swollen near the middle	-	2.4-3	3.1-4.5	Stipe of young plants	Greenish, light yellow	Atlantic Forest	Vianna et al., 2017
<i>A. totai</i>	Cylindrical, straight, with base remains of fallen leaves	-	2.0-2.6	2.3-4.3	It may occur in the stipe and leaves	Varied	Dry and open area	Vianna et al., 2017
<i>A. hassleri</i>	Espinescent with underground stem	0.4-0.8	0.31-0.38	Unseen fruits	Along the rachis, more abundant at the base	Not seen	Savanna, well-drained soils	Caxambú et al., 2015
<i>A. glaucescens</i>	Visible scars on the leaf base	0.8-6.5	1.5-2.0	1.5-2.6	Without	Yellowish green	Savanna, sandy soils	Lorenzi et al., 2010
<i>A. emensis</i>	Creeper	0.40-0.60	-	1.8-2.2	Sparse in the median and / or apical region	-	-	Lorenzi et al., 2010
<i>A. crispa</i>	Slightly swollen stems	-	-	1.0-3.0	Spiny stems	Soft orange	-	Pérez et al., 2015; Lorenzi et al., 2010

330 **Table 2.3** Biometric characteristics of *Acrocomia* sp.

Species	MF (g)	EP (g)	MM (g)	ED (g)	MA (g)	LDF (cm)	DTF (cm)	LDA (cm)	ET (cm)	FV (mL)	Reference
<i>A. aculeata</i> *	19.64±3.57	4.13±0.52	9.20±1.18	6.89±1.21	1.14±0.23	3.85±0.80	3.35±0.40	ND	ND	ND	Ramos et al., 2008; Sanjinez-Argandoña & Chuba, 2011; Lescano et al., 2015b; Vianna et al., 2017; Oliveira et al., 2020
<i>A. aculeata</i>	39.2	10.13	18.23	8.03	2.93	4.2	4.27	ND	ND	ND	Alfaro-Solís et al., 2020
<i>A. aculeata</i>	11.55	2.16	5.07	3.15	1.17	ND	ND	ND	ND	ND	Bonet et al., 2020
<i>A. intumescens</i>	29.08	ND	ND	ND	ND	3.88	3.69	ND	ND	ND	Vianna et al., 2017
<i>A. totai</i> *	18.03±1.16	3.76	10.49	4.05	1.37	3.13±0.21	2.72	1.53	0.38	19.00	Machado et al., 2015; Vianna et al., 2017

331 MF: fruit mass; EP: epicarp mass; MM: mesocarp mass; ED: endocarp mass; MA: almond mass; LDF: longitudinal diameter of the fruit; DTF: transverse diameter of  
 332 the fruit; LDA: longitudinal diameter of the almond; ET: endocarp thickness; FV: fruit volume. \*Data calculated by means of the results obtained by the authors. ND: not  
 333 determined.

334

335

336 Considering the average of the values obtained in the biometric characteristics of *A.*  
337 *aculeata* fruits collected in two municipalities in Brazil, the pulp represented approximately  
338 42% of the whole fruit, the peel 20%, the mass of the endocarp (seed with almond) 38%, the  
339 endocarp 31% and the almond 7% in relation to the whole fruit. In addition, each raceme  
340 produced 6.32 kg of pulp and 1.36 kg of endosperm (almond) (Sanjinez-Argandoña & Chuba,  
341 2011). In another two localities (Brazilian Savannah and Pantanal), the fruits of this same  
342 species presented, on average, 19.6% and 17% of epicarp, 46.9% and 51% mesocarp and 5.28%  
343 and 6.75% almonds, respectively. In the Savannah, the trees presented, on average, 3.8 racemes,  
344 with an average length of 72.5 cm and an average number of 168.8 fruits per raceme, with a  
345 mass of 5.8 kg per raceme, and 645 fruits per palm, totaling 19.6 kg. In the Pantanal region,  
346 they presented, on average, 3.7 racemes, with an average length of 66.6 cm, and an average  
347 number of 258 fruits per raceme, with a mass of 4.4 kg per raceme, and 923 fruits per palm,  
348 totaling 16.1 kg (Ciconini et al., 2013). These data show the high productivity of this plant,  
349 while the high percentage of the edible part indicates the technological potential of the fruit as  
350 a raw material for the food, cosmetic, biofuel, among other products (Conceição et al., 2015).

351

### 352 **2.1.5 Regional and economic importance**

353 *Acrocomia* sp. are important oil palm trees, due to their role in local ecosystems and  
354 economies and their potential to produce vegetable oil and biofuels (Nunes et al., 2018). They  
355 are utilized for subsistence by native peoples throughout tropical America. The commercial  
356 exploitation occurs mainly for oil production in Brazil and Paraguay. They are also used for  
357 various other purposes: the palms in ornamentation, the roots as medicine, the sap in the  
358 preparation of alcoholic drinks, and the leaves to obtain fibers for the production of threads,  
359 ropes, nets, and forage, while the ripe fruits are consumed fresh or processed (Ramos et al.,  
360 2008; Vieira et al., 2017).

361 However, despite the economic diversity of exploitation of the species, the greatest  
362 interest in exploration is in the fruits, as they have a high potential to produce oil. It is estimated  
363 that more than 4,000-5,000 Kg / ha / year of oil can be obtained for the food, cosmetic and  
364 pharmaceutical industries, and alternatively to produce biodiesel (Del Río et al., 2016; Vianna  
365 et al., 2017). The average oil extraction yield is 19.62% from the pulp and 3.43% from the  
366 almond (Del Río et al., 2016).

367 *A. aculeata*, *A. totai* and *A. intumescens* are the three tree species of *Acrocomia* with  
368 greatest commercial interest in Brazil, with also high potential for use in other countries where  
369 they also occur. Due to the diversification of their use, they are considered an alternative to

370 generate income for several communities. In Brazil, there are reports of the use and  
371 commercialization of these three species for several applications, *e.g.*: civil construction (rods),  
372 bovine and equine nutrition (leaves), manufacture of fishing lines (fibers), gravel substitute  
373 (endocarp), oil extraction for food, cosmetic, or biofuel (endosperm), and fresh or processed  
374 consumption (pulp) (Vianna et al., 2017).

375 *A. aculeata* is currently the most utilized *Acromonia* species in the production of  
376 biofuels, with the oil present in the mesocarp ranging from 37 to 78%. However, the other  
377 species also have potential for oil production. *A. intumescens* has between 34 and 41% while  
378 *A. totai* between 14 and 31% of oil in the mesocarp (Vianna et al., 2017). *A. aculeata* is  
379 considered as the second species of palm in the world in oil production (estimated 4,200 L /  
380 ha). The high quality of the oil is comparable to that of olive, which can be used in food, as a  
381 raw material in the production of soap and cosmetics, or for the strategic production of biofuels  
382 (Hernández-Zardón, 2016).

383 Edible oils can be extracted from the mesocarp and the nucleus of *Acrocomia* sp.,  
384 differing mainly in their chemical composition and technological properties. The oil extracted  
385 from the almonds is transparent and colorless, being an alternative in cooking to replace the  
386 olive oil (Lieb et al., 2019).

387 The pulp of *A. intumescens* fruits in the Brazilian Northeast region and *A. totai* in the  
388 Mato Grosso do Sul state are exploited by local communities for fresh consumption or  
389 processed foods, such as meals, ice cream, cakes and others. Some of the elaborated products  
390 are sold by these communities to supplement the family income. On the other hand, the *A.*  
391 *aculeata* species is the most explored in the Brazilian Midwest region and in Pará, São Paulo,  
392 and Minas Gerais states, for the extraction of pulp oil for the production of biofuel, cosmetics  
393 and soap (Ramos et al., 2008; Coimbra et al., 2011; Sanjinez-Argandoña & Chuba, 2011;  
394 Vianna et al., 2017; Nunes et al., 2018).

395 The pulp of the *A. aculeata* fruit is traditionally consumed fresh but is also utilized as a  
396 source of oil by natives and people living in rural areas. Although the fruit of this species is an  
397 important component in the diet of some tribes, it is not attractive for those who are not  
398 accustomed to consuming it, due to its mucilaginous texture and oleaginous pulp (Hernández-  
399 Zardón, 2016). The pulp is also consumed in the cooked form, in the form of refreshment, as  
400 flour, pancakes, jellies, mousses, sweets and ice cream (Ramos et al., 2008; Coimbra et al., 2011;  
401 Hernández-Zardón, 2016; Nunes et al., 2018). The almonds of the *A. aculeata* fruit are  
402 consumed raw by the local population or in food products, such as sweets, or it can also be used  
403 as a source of raw material for oil extraction (Ramos et al., 2008; Coimbra et al., 2011).

404           Some communities take advantage of the pulp of the ripe fruit of *A. aculeata* in the  
405 production of flour for e.g. breads and biscuits. The pulp, in the form of flour, favors the  
406 concentration of most nutrients and increases its shelf-life, in addition to facilitating transport  
407 and storage due to the reduction in mass and volume. The flour can be incorporated into bakery  
408 products, diet products and baby foods, improving the sensory and nutritional quality of food  
409 products (Mota, 2005; Guimarães & Silva, 2008).

410           The use of *A. aculeata* fruits in the Mato Grosso do Sul state has a strong socioeconomic  
411 and environmental appeal. The pulp is rich in nutrients and has antioxidant action, and its use  
412 in the formulation of jams minimizes post-harvest losses, providing to consumers a product  
413 with high nutritional value all year round (da Silva et al., 2018).

414           *A. aculeata* leaves have been explored for feeding high-performance horses due to their  
415 high nutritional and energetic values. The press cake, by-product of the endosperm after  
416 extraction of oil by pressing, is used in the preparation of animal feed, due to its high nutritional  
417 content and is commonly found in the Brazilian semiarid region (Azevedo et al., 2014;  
418 Hernández-Zardón, 2016). The exocarp is used in the production of energy, such as charcoal,  
419 and leaf fibers in the manufacture of handicrafts and work utensils, such as ropes, fishing  
420 equipment; production of starch like sago (Hernández-Zardón, 2016).

421           It was reported that the use of *A. aculeata* has increased due to its potential for disease  
422 prevention. According to the popular knowledge, this medicinal plant has been used in the  
423 treatment of respiratory diseases and has laxative, analgesic and restorative properties (Lorenzi,  
424 2006). In popular medicine, the roots of *A. aculeata* have been used for a long time by  
425 indigenous peoples in the states of Yucatán and Tamaulipas, in Mexico, for the treatment of  
426 diabetes (Sosnowska & Balslev, 2008). The decoction of *A. aculeata* leaves is used to treat  
427 hypertension (Agra et al., 2007).

428           In Brazil, the production of *A. totai* almond is carried out through family farming. The  
429 plant has gaining attention due the high oil productivity (about six tons per hectare), which  
430 represents an alternative to the growth in energy demand. In addition, the activity enables the  
431 creation of direct and indirect jobs through its production chain (Souza et al., 2017).

432           In the Brazilian Northeastern region, the *Acromonia* palm is represented mainly by the  
433 species *A. intumescens*. However, there is still little information in the literature related to this  
434 species. The leaves are normally used for animal nutrition and the pulps for human  
435 consumption. The oil extracted from the pulp and almond is generally used as a tonic in popular  
436 medicine due to its anti-inflammatory and antioxidant activities (Nascimento et al., 2016).

437

## 438 2.2 PROPERTIES OF THE FRUITS

### 439 2.2.1 Proximate composition and physical characteristics

440 The information on the nutritional composition of foods allows to assess the availability  
441 of nutrients and the development of research for the agricultural planning and the food industry  
442 (Coimbra et al., 2011). The proximate composition and energetic value of fruits from the  
443 species *A. aculeata*, *A. intumescens* and *A. totai* has been reported elsewhere (Table 2.4).  
444 However, information on the processing, and on the physical and chemical characteristics of  
445 the trees and the composition of the fruits from the other species of *Acrocomia* were not found  
446 (Pérez et al., 2015).

447 *A. aculeata* collected in Paraguay showed higher levels of protein (6.1 g / 100 g), ash (5  
448 g / 100 g), carbohydrates (35.2 g / 100 g) and energetic value (382.1 Kcal / 100 g) compared  
449 with fruits obtained in other countries and with fruits from the other *Acromonia* species.  
450 However, *A. aculeata* harvested in Costa Rica had a higher lipid content (33.23 g / 100 g)  
451 compared to others. Regarding the almond, data were obtained only from *A. aculeata* found in  
452 Brazil and Paraguay. These almonds presented little variation for proteins, ash, fibers and  
453 energetic value. On the other hand, almonds from *A. aculeata* found in Paraguay showed higher  
454 lipid (56.75 g / 100 g) and lower carbohydrate (4.75 g / 100 g) contents compared to the almonds  
455 from fruits collected in Brazil.

456 Some food products prepared from *A. aculeata* fruits, including pulp flour, pulp flour  
457 cupcake, pulp flour alfajor, degreased flour and almond cereal bars were reported elsewhere  
458 (Table 2.5).

459 The bocaiuva almonds of *A. aculeata* fruits can be considered a valuable source of high  
460 digestible protein, as they do not contain protease inhibitors. The protein fractions of the  
461 bocaiuva almond are also potentially good sources of some essential amino acids, as they are  
462 rich in methionine + cysteine, valine and leucine. Nevertheless, they are limiting in some  
463 essential amino acids, such as threonine and lysine (Hiane, 2006).

464 Mineral content of pulp and seed from *A. aculeata* and *A. totai* fruits collected in Brazil  
465 were reported in the literature (Table 2.6). Both species had a high content of iron and zinc in  
466 the pulp, and high iron content in seed. Zinc was found higher for *A. aculeata* pulp and seed.  
467 Minerals are important nutrients for the body, as they participate in numerous functions, *e.g.*  
468 iron and zinc are essential to combat infections, increase immunity, and decreased the risk of  
469 depression (Li et al., 2017; Biasebetti et al., 2018; Nairz & Weiss, 2020).

470 The knowledge on the physical characteristics of the fruits is also relevant for the  
471 preparation of food products, as well as for the conservation of the processed food (Silva et al.,



472 2019). The pulp and almonds of *A. aculeata* fruits have low acidity and high-water activity  
473 (Table 2.7), indicating high perishability of the fresh fruits and the importance of the  
474 technological process for the obtaining and preservation of processed food products.

475

### 476 **2.2.2 Fatty acid content**

477 Literature reports the fatty acids profile of the pulp and macauba seed oils for *Acromonia*  
478 sp. (Table 2.8). The oil obtained from the pulp is mostly composed of unsaturated fatty acids  
479 (74-81%), consisting mainly of oleic acid (63-65%), and the oil obtained from the seed has a  
480 high content of lauric acid (38-45%) (Bora & Rocha, 2004; Coimbra & Jorge, 2011; Ferrari &  
481 Azevedo Filho, 2012). The composition of the extracted oils is important in order to identify  
482 the most appropriate potential use for each raw material.

483 *A. aculeata* seed oil has high oxidative stability and contains bioactive compounds. This  
484 oil is solid at low temperatures and has a low solid fat content at room temperature (Magalhães  
485 et al., 2020).

486 The *A. aculeata* pulp oil consists mainly of triglycerides (about 78.5%), followed by  
487 small amounts of diglycerides (about 13.2%), free fatty acids (about 5.6%) and sterols (1.5%).  
488 The seed oil has a higher amount of triglycerides (about 98.6%), with only traces of free fatty  
489 acids (0.9%), sterols (0.2%) and monoglycerides (0.3%), allowing several possibilities of  
490 industrial (Del Río et al., 2016).

491

### 492 **2.2.3 Enzymatic activity and Nutritional enrichment of foods**

493 Enzymes are important products used in several applications in the textile, chemical,  
494 pharmaceutical, and food industries. Among the most important industrial enzymes are the  
495 amylases (25% of the enzyme market) (Silva et al., 2016; Lopes et al., 2020).

496 In Brazil, about 14.5 tons of fruit residue are produced per year due to the extraction of  
497 oil from *A. aculeata*. This generated by-product is rich in cellulose, hemicellulose, lignin and  
498 lipids. Thus, it can be used to produce fungal enzymes and edible mushrooms that degrade these  
499 components of plant biomass (Lopes et al., 2020).

500 Studies using residues of *A. aculeata* in fermentation by microorganisms show the  
501 production of the enzymes amylase,  $\beta$ -glucosidase, endoglucanase (CMCase), lipase, protease,  
502 and xylanase, with greater production of amylases and proteases (Table 2.9).

503 It was reported elsewhere the utilization of *Acrocomia aculeata* cake and residual oil  
504 from the biodiesel industry as substrate to produce lipase by *Moniliella spathulata* R25L270,

505 reducing lipase production costs and, simultaneously, adding value to the waste from the  
506 biodiesel industry (Souza et al., 2015).

507 *A. aculeata* residues were reported to have potential for bioconversion by using the  
508 filamentous fungi *Lichtheimia ramosa*. The nutritional value of the fungus increased after  
509 cultivation (Table 2.10). A greatest decrease in carbohydrates and increase in fibers and proteins  
510 contents was pointed out. The protein enrichment reached 67.88% (Silva et al., 2014).

511

#### 512 **2.2.4 Bioactive compounds**

513 Bioactive compounds, including carotenoids, tocopherols, ascorbic acid, and phenolic  
514 compounds, may be related to antioxidant capacity and other important health benefits (Verruck  
515 et al., 2018). Bioactive compounds were found in the pulp, seed, and bark of *A. aculeata* (Table  
516 2.11). The pulp oil presents high levels of carotenoids (490 µg / g), tocopherols (212.95 mg /  
517 kg), and antioxidant capacity (63 g / g DPPH). The seeds have high content of ascorbic acid  
518 (59.34 mg / 100 g) and phenolic compounds (112.8 mg EAG / 100g).

519 The pulp and the peel of *A. aculeata* fruits were reported to have in their composition  
520 the carotenoids violaxanthin, luteoxanthin, lutein, zeoxanthin and β-carotene (Schex et al.,  
521 2018). Pro-vitamin A and non-pro-vitamin A carotenoids can act as antioxidants against  
522 cardiovascular disease, certain types of cancer, neurological disorders, strengthen the immune  
523 system, age-related macular degeneration and cataracts, in the activation of genes and  
524 inflammatory processes, modulating lipoygenase. Some carotenoids, such as β-carotene, play  
525 an important nutritional role as they are capable of being converted into vitamin A (Coimbra et  
526 al., 2011). In another study, it was revealed that the β-carotene from *A. aculeata* pulp is more  
527 bioavailable than the pure β-carotene (Ramos et al., 2007), which shows the potential of the  
528 pulp as a natural source of β-carotene and vitamin A (Ramos et al., 2008).

529

530 **Table 2.4** Proximate composition and energetic values of fruits from *Acrocomia* sp.

Species	Analyzed part	Compound (g/100 g)						Energetic value (kcal/100 g)	Reference
		Moisture	Protein	Lipids	Ash	Carbohydrates	Fibers		
<i>A. aculeata</i> *	Pulp	47.86±7.73	3.19±1.94	15.88±10.95	1.72±0.30	21.38±14.09	12.93±1.55	226.66±83.42	Ramos et al, 2008; Silva et al., 2008; Sanjinez-Argandoña & Chuba, 2011; Lescano et al., 2015b;
<i>A. aculeata</i> *	Almond	7.66±3.80	15.97±7.28	45.81±8.81	1.94±0.23	22.10±22.28	25.62±22.34	515±50.58	Hiane e al., 2006; Dessimoni-Pinto et al., 2010; Coimbra et al., 2011; Lescano et al., 2015b; Machado et al., 2015; Alves et al., 2016
<i>A. aculeata</i> *	Pulp	57.03±9.61	ND	33.23±6.50	ND	ND	ND	ND	Alfaro-Solis et al., 2020
<i>A. aculeata</i>	Pulp	56.6	6.1	24.1	5	35.2	29.6	382.1	Bonet et al., 2020
<i>A. aculeata</i> *	Almond	9.20±4.24	15.95±0.64	56.75±0.49	2.10±0.14	4.75±0.07	20.45±1.06	588.85±13.93	Bonet et al., 2020
<i>A. intumescens</i>	Pulp	62.2	2.5	29.6	2	3.5	ND	286,80	Silva et al., 2015
<i>A. totai</i>	Pulp	45.42	1.15	32.05	0.66	18.1	51.7	ND	Machado et al., 2015

531 \*Data calculated by means of the results obtained by the authors. ND: not determined.

532

533 **Table 2.5** Proximate composition and energetic value of products elaborated with fruits of *Acrocomia aculeata*.

Product	Compound (g/100 g)						Energetic value (kcal/100 g)	Reference
	Moisture	Protein	Lipids	Ash	Carbohydrates	Fibers		
Pulp flour*	9.42±1.20	5.01±2.02	20.88±0.81	4.21±1.57	66.77±6.80	13.40±0.00	434.32±24.50	Vieira et al., 2017; Rodrigues et al., 2017; Silva et al., 2020
Cupcake of pulp flour	18.35	3.63	11.93	1.56	64.53	3.91	379.12	Vieira et al., 2017
Alfajor of pulp flour	14.66	8.21	11.83	1.42	63.89	2.54	393.34	Rodrigues et al., 2017
Degreased flour	7.69	5.23	8.44	5.83	ND	ND	ND	Silva et al., 2020
Cereal bars of almond	5.79	6	7.84	1.53	75.43	3.54	396.28	Dessimoni-Pinto et al., 2010

534 \*Data calculated by means of the results obtained by the authors.

535

536

537 **Table 2.6** Mineral content of fruits of *Acrocomia* sp.

Species	Analyzed part	Mineral (mg/Kg)										Reference
		N	Ca	P	K	Mg	Na	Fe	Mn	Zn	Cu	
<i>A. aculeata</i> *	Pulp	ND	1,270±570	370	7,700	1,080±220	37.4	56.56±39.56	8.51±7.49	32.90±23.75	10.22±12.33	Ramos et al., 2008; Silva et al., 2008; Marin et al., 2009; Lescano et al., 2015b
<i>A. aculeata</i> *	Seed	ND	930±10	5,730	3,770	1,900±250	21.41	29.50±4.83	21.56±3.92	38.04±10.05	13.87±2.74	Hiane et al., 2006; Lescano et al., 2015b
<i>A. totai</i>	Pulp	730	450	3,580	3,950	2,210	ND	40.7	38.16	27.33	5.05	Machado et al., 2015
<i>A. totai</i>	Seed	3,610±1,060	810±100	640±10	9,250±20	1,420±60	ND	54.97±9.77	5.76±0.86	8.06±0.40	2.50±0.10	Machado et al., 2015

538 \*Data calculated by means of the results obtained by the authors. ND: not determined.

539  
540

**Table 2.7** Physical characteristics of the fruits of *Acrocomia aculeata*.

Species	Analyzed part	Titrateable acidity (%)	pH	Water activity	Reference
<i>A. aculeata</i>	Pulp	0.51±0.24	5.92±0.22	0.95±0.04	Sanjinez-Argandoña & Chuba, 2011; Lescano et al., 2015b; Silva et al., 2018; Souza et al., 2019
<i>A. aculeata</i>	Almond (kernel)	0.07±0.00	6.00±0.00	0.677±0.00	Lescano et al., 2015b

541 \*Data calculated by means of the results obtained by the authors.

542

543

544 **Table 2.8** Composition of fatty acids of pulp of *Acrocomia* sp.

Species	Oil	C4	C6	C8	C10	C12	C14	C15	C16	C16:1 (n7)	C17	C17:1	C18	C18:1 (n7)	C18:1 (n9)	C18:2 (n6)	C18:3	C20	C20:1	C22	C23	C24	Reference
<i>A. aculeata</i>	Pulp	ND	0.24±0.20	0.184±0.14	0.14±0.10	0.66±0.36	0.47±0.16	0.03	20.14±7.08	2.37±1.79	0.08	0.11	2.71±0.37	ND	66.6±9.23	5.72±2.96	1.06±0.06	0.43±0.32	0.14±0.03	0.06	ND	0.08	Hiane et al., 1990; Lescano et al., 2014; Del Río et al., 2016; Oliveira et al., 2017; Dario et al., 2018
<i>A. aculeata</i>	Pulp	ND	ND	ND	ND	0.1	0.1	ND	19.1	4.8	ND	ND	1.7	4.4	64.9	2.9	1.4	ND	ND	ND	ND	ND	Lieb et al., 2019
<i>A. aculeata</i>	Pulp	0.0	ND	0.21	0.16±0.06	1.055±0.21	0.565±0.09	ND	18.48±2.86	2.90	ND	ND	3.56±0.06	63.48±3.37	ND	4.57±0.80	0.70	0.20	ND	ND	ND	ND	Nunes et al., 2015; Bonet et al., 2020
<i>A. intumescens</i>	Pulp	ND	ND	ND	Tr	Tr	Tr	ND	14.08	ND	ND	ND	Tr	ND	74.14	11.78	ND	ND	ND	ND	ND	ND	Silva et al., 2015
<i>A. aculeata</i>	Almond	ND	0.40±0.28	3.87±2.18	3.93±0.93	42.78±4.20	9.09±1.70	ND	7.64±2.13	0.78±1.30	ND	3	3.55±1.34	0.21	26.61±5.30	10.10±20.0	0.12±0.09	0.20±0.09	0.18±0.07	0.07±0.04	ND	0.09±0.08	Lescan et al., 2015b; Del Río et al., 2016; Nunes et al., 2018; Magalhães et al., 2020
<i>A. aculeata</i>	Almond	ND	ND	4.9	3.1	41.6	13.4	ND	9.2	0.1	ND	ND	3.2	0.3	20.2	3.1	ND	ND	ND	ND	ND	ND	Lieb et al., 2019
<i>A. aculeata</i>	Almond	ND	ND	5.8	4.46±0.86	38.50±0.69	7.84±1.41	ND	6.66±0.79	0.06	ND	ND	2.62±0.55	27.56	ND	3.97	ND	ND	ND	ND	ND	ND	Lescano et al., 2015b; Bonet et al., 2020
<i>A. intumescens</i>	Almond	ND	ND	ND	5.03	45.44	12.61	ND	9.53	ND	ND	ND	4.31	ND	23.07	Tr	ND	ND	ND	ND	ND	ND	Silva et al., 2015

545 ND: not determined. Tr: traces (<1,0 g/Kg).

546

547

548 **Table 2.9** Activity of enzymes produced by *Acrocomia* sp.

Species	Microorganism	Enzyme (U/mL)						Reference
		Protease	Amylase	$\beta$ -glucosidase	CMCase	Lipase	Xylanase	
<i>A. aculeata</i>	<i>L. ramosa</i>	ND	1.80	0.01	0.58	0.02**	0.92	Silva et al., 2013, 2014
<i>A. aculeata</i>	<i>B. amyloliquefaciens*</i>	30.38±0.2	151.16±1.17	ND	ND	ND	ND	Silva et al., 2016
<i>A. aculeata</i>	<i>Moniliella spathulata</i>	ND	ND	ND	ND	2.47***	ND	Souza et al., 2015

549 \*Calculated data from the authors' experimental design (average cultivation of 36h). \*\*Data converted from U/g to U/mL. \*\*\*Maximum activity value. ND: not determined.

550

551

552 **Table 2.10** Variation of the composition of *Acrocomia aculeata* residue by bioprocess using the filamentous fungi *Lichtheimia ramosa*.

Substrate	Compound (g/100g)						Reference
	Moisture	Protein	Lipids	Ash	Carbohydrates	Fibers	
<i>A. aculeata</i> residue*	-	3.55±0.33	8.14±0.16	5.23 ± 0.41	20.90*	62.18±0.60	Silva et al., 2014
<i>A. aculeata</i> enriched residue*	-	5.07±2.28	7.56 ±3.78	8.70±2.29	10.13	69.56±6.39	

553 \*Data calculated by the author (in dry basis). \*Residue composed of pericarp, mesocarp fiber (without pulp) and endocarp. \*\*Calculated from the average of 5 to 40 days of  
554 cultivation.

555

556 **2.3 POTENTIAL APPLICATIONS**

557 **2.3.1 Activity against chronic diseases**

558 The oil extracted from the pulp of *A. aculeata* has antidiabetic and antioxidant activities,  
 559 without causing cytotoxicity in vitro in LLC-PK1 cells. The doses of oil (3, 30 or 300 mg / kg)  
 560 administered orally were able to significantly decrease the high glucose levels in fructose-  
 561 induced diabetic rats, and significantly reduce the glucose levels in normoglycemic rats. It was  
 562 also observed that treatment with doses of oil for 24 days reduced the high plasmatic glucose  
 563 induced by the administered drug streptozotocin. These findings suggested the functional  
 564 potential of *A. aculeata* oil for the consumption by diabetic patients (da Silva et al., 2018).

565

566 **Table 2.11** Bioactive compound of *Acrocomia* sp.

Species	Analyzed part	Total carotenoids (µg/g)	Total tocopherols (mg/kg)	Ascorbic acid (mg/100g)	Phenolic compounds (mg EAG/100g)	Antioxidant capacity (g/g DPPH)	Reference
<i>A. aculeata</i>	Pulp oil	490	212.95	ND	3.9	63	Oliveira et al., 2017; Coimbra et al., 2011
<i>A. aculeata</i>	Seed oil	1.82	23,10	ND	4.38	ND	Coimbra et al., 2011, 2012
<i>A. aculeata</i>	Pulp	49.0	ND	59.34	112.8	5.05	Ramos et al., 2008; da Silva et al., 2018
<i>A. aculeata</i>	Seed	1.9	ND	11,8	ND	ND	Lescano et al., 2015b; Silva et al., 2015
<i>A. aculeata</i>	Jelly	ND	ND	12.34	93	22.53	da Silva et al., 2018
<i>A. aculeata</i>	Exocarp	30.75	ND	ND	ND	ND	Schex et al., 2018

567 ND: not determined.

568

569 A study showed that the addition of *A. aculeata* seed oil to the diet of type 2 diabetic  
 570 rats had a hypoglycemic effect, reduced weight gain, and a low deposition of medium chain  
 571 fatty acids in the epididymal adipose tissue. These findings suggested that the oil should be  
 572 considered as a promising source of energy to partially replace carbohydrates in diets to control  
 573 the side effects of type 2 diabetes on blood glucose levels (Nunes et al., 2018).

574 High protein diets (HPD) are widely used for health and strength training performance.  
575 A study evaluated the activity of supplementary HPD with 6% lyophilized *Acrocomia* sp. fruit  
576 pulp (205.6 g / kg of protein) in metabolic parameters and body composition of rats submitted  
577 to strength training (ST). The pulp combined with an HPD reduced visceral fat and adiposity  
578 index (AI), in addition to improving glucose tolerance in rats submitted to ST. It was observed  
579 that after 12 weeks of ST, the maximum strength increased significantly in trained groups.  
580 However, the one who received the pulp presented significantly lower AI values (3.8-0.7% vs.  
581 6.8-1.3%) and visceral fat (0.038-0.004 g / g vs. 0.067-0.012 g / g) compared to the sedentary  
582 receiving pulp (Almeida et al., 2020).

583 Pharmacological studies reported that the oil of *Acrocomia* sp. fruit pulp reduced  
584 cholesterol and glucose in rats (Ramiro, 2010; Silva, 2012). In another study, it was shown that  
585 the flour of the green *A. aculeata* fruit pulp is rich in soluble fiber and has functional and  
586 nutraceutical properties in the reduction of body weight, hypolipidemic activity in the total  
587 cholesterol fractions, LDL-cholesterol, triglycerides and fasting glucose in male mice (Giunco,  
588 2018).

589

### 590 **2.3.2 Anti-hypovitaminosis activity**

591 Vitamin A deficient rats that received 13,475 mcg of  $\beta$ -carotene and 275 g of *A. aculeata*  
592 ripe fruit pulp per 1 kg of diet had a higher retinol accumulation compared to the animals that  
593 received a diet with pure  $\beta$ -carotene, improving the stocks of hepatic retinol, which allowed to  
594 conclude that the  $\beta$ -carotene present in the pulp had an anti-hypovitaminosis effect (Ramos et  
595 al., 2007).

596

### 597 **2.3.3 Antioxidant and anti-inflammatory activities**

598 Oxidative stress is a metabolic disorder associated with several chronic diseases that can  
599 be minimized by the action of antioxidants. The *A. aculeata* fruit pulp is widely used in the  
600 treatment of various diseases (Silva et al., 2018; Verruck et al., 2018). These properties are also  
601 present in other structures of the plant. A study recently conducted with *A. aculeata* leaf extracts  
602 obtained with water, ethanol, and methanol solvents showed highest amounts of phenolic  
603 compounds and free radical scavenging activity on the alcohol solvents. However, the extract  
604 obtained with the water solvent was more effective in protecting human erythrocytes against  
605 hemolysis and lipid peroxidation in all experimental models, which supports its use in the  
606 prevention or treatment of diseases related to oxidative stress (Monteiro-Alfredo et al., 2020).



607 The oil extracted from the pulp of *A. aculeata* fruits has effects in the prevention or in  
608 the mitigation of the reproductive toxicity induced by cyclophosphamide (CP) in male rats. The  
609 oil administered improved the changes induced by CP on the weight of reproductive organs,  
610 hormonal levels, sperm count and testicular histology, increased the expression of the Ckit gene,  
611 and normalized the levels of antioxidant enzymes that were altered by CP. Thus, the oil  
612 extracted from the pulp of *A. aculeata* can protect the male reproductive system against the  
613 adverse effects of CP. The high content of  $\beta$ -carotene in the oil was related with the antioxidant  
614 activity observed in the rats, which reinforces the need of the evaluation of the oil as a  
615 chemoprotective agent in humans (Arena et al., 2018).

616 The oil extracted from *A. aculeata* and microencapsulated showed anti-inflammatory,  
617 antiedematogenic, and diuretic effects in rats with paw edema and pleurisy. This was related to  
618 the fatty acids (bioactive compounds) that interfere in the inflammatory parameters. The  
619 microencapsulation by complex coacervation favored the bioavailability and the preservation  
620 of the bioactive components of the oil. A significant marked inhibition in the carrageenan-  
621 induced paw edema (67%) was observed. The oral administration of the oil at doses of 300 and  
622 700 mg / kg significantly inhibited (91% and 81%, respectively) the leukocyte migration  
623 induced by carrageenan to the cavity pleural. These properties strengthen the development of  
624 new products based on this natural product. However, studies are needed to identify the active  
625 components responsible for the therapeutic effects (Lescano et al., 2015a).

626 It was reported a lipid extract (D-005) obtained from the oil of ripe fruits of *A. crispa*,  
627 with 92-96% of total fatty acids and 1-3% of free acids, ranging from of 8 to 18 carbon atoms  
628 (Pérez et al., 2015). Its anti-inflammatory activity was demonstrated later in *in vitro* and *in vivo*  
629 studies (Pérez et al., 2015; Pérez et al., 2017; Oyarzábal-Yera et al., 2019). Firstly, it was  
630 observed that increasing doses from 0.9 to 1000  $\mu$ g / ml significantly inhibited the activities of  
631 the COX-2 (cyclooxygenase-2) enzyme in the microsomal fraction of the rat seminal vesicles  
632 and 5-LOX (arachidonate 5-lipoxygenase) enzyme in the cytosolic fraction of  
633 polymorphonuclear leukocytes, without modifying the activity of COX-1 in the mice  
634 microsomal platelets. The reduction of both enzymes suggested the anti-inflammatory potential  
635 of D-005 (Pérez et al., 2017).

636 In another study, for *in vivo* experimental models, all doses of D-005 (25 - 400 mg / kg)  
637 administered had a protective effect against acute kidney injury (AKI) induced by ischemia or  
638 renal reperfusion, significantly decreasing serum concentrations of creatinine, urea and uric  
639 acid), histopathological changes of AKI, and tubular damage. D-005 was also able to reverse  
640 oxidation disorder markers, decreasing the concentrations of the malondialdehyde and

641   sulfhydryl groups in plasma and renal homogenates, and increasing renal catalase activity  
642   (Oyarzábal-Yera et al., 2019). This group also found that D-005 administered in doses of 5 –  
643   200 mg / kg, in an *in vivo* experimental model of acute lung injury, moderately reduced ( $\approx$  28%  
644   inhibition) pulmonary edema, with the dose of 200 mg / kg, causing a significant reduction in  
645   the edemas and improvement of the lung injuries, except for the lowest dose (5 mg / kg), which  
646   achieved a slight improvement in the injuries (Mena et al., 2019).

647

#### 648   **2.3.4 Antimutagenic, antigenotoxic, antiproliferative, and other activities**

649         The oil extracted from the pulp of the *A. aculeata* fruit has no cytotoxic, genotoxic or  
650   mutagenic effects in rats, nor does it present acute and subacute toxicity after oral treatment  
651   with oil from this plant, suggesting that the LD50 (oral lethal dose) is greater than 2000 mg /  
652   kg. These results indicate that the exploration of the species great medical importance.  
653   However, other studies, such as chronic toxicity, reproductive toxicity and others, must be  
654   carried out to assess the total safety for use in humans (Traesel et al., 2014).

655         It was reported elsewhere several biological assays using methanolic extract of thorns  
656   from *A. totai* that have been performed against bacteria, fungi, viruses, neglected neotropical  
657   parasites, and human cancer cell strains. The extract obtained showed activities against breast,  
658   colon and cervical cancer tumor cell strains, in addition to bacteriostatic activity against  
659   *Staphylococcus aureus* and moderate activity against *Trypanosoma cruzi* forms in  
660   trypomastigote. Piceatannol (stilbenoid chemically analogous to resveratrol) was isolated from  
661   the extract and showed bacteriostatic and bactericidal effect against *S. aureus*, bacteriostatic  
662   effect against *Bacillus subtilis* and the inhibitory-evolutionary forms of *Leishmania*  
663   *amazonensis*. In addition, the stilbenoid was tested against the Zika virus, but showed no  
664   activity (Souza et al., 2017). *A. totai* leaves were described as a valuable source of active  
665   terpenoids that act against a human cancer cell modified by HPV (Souza et al., 2019).

666         The oils extracted from the pulp and the almond oil from *A. aculeata* fruit, at doses of  
667   3, 15 and 30 mg / kg, showed chemoprotective effects and immunomodulatory activity,  
668   reducing the ability of cyclophosphamide to trigger apoptosis in the liver, spleen, and kidney  
669   cells. These results suggested that these oils can be classified as a functional food and can also  
670   be used to enrich other foods or be used as nutraceuticals with chemopreventive characteristics.  
671   However, they are not suitable as sources of adjuvants to chemotherapeutic agents, particularly  
672   the cyclophosphamide, because they inhibit the genotoxic and mutagenic actions of this agent  
673   (Magosso et al., 2016). In another study, it was observed from an *in vivo* model that the oil  
674   extracted from the pulp of *A. aculeata* fruit has, in addition to anti-inflammatory properties,

675 antimutagenic properties, which were related to the the fatty acids content, *e.g.* acid oleic, and  
676 antioxidant compounds, *e.g.* carotenoids (Costa et al., 2020).

677

### 678 **2.3.5 Other applications**

679 By-products of *A. aculeata* (rachis, leaves and fruit pulp) can be used to obtain fibers  
680 and from these, cellulose nanocrystals (Corrêa et al., 2019). It is also reported the obtaining of  
681 films from *A. aculeata* pulp flour, with relevant characteristics for application as an edible  
682 coating (Silva et al., 2020).

683

684

## 685 **2.4 CONCLUSION**

686 Despite the economic importance and the diversification of use of the *Acrocomia* sp.,  
687 they are marginally explored so far. The species that has the most outstanding applications both  
688 in subsistence and commercial exploitation is the *A. aculeata*. However, its greatest emphasis  
689 is on oil production, which has neglected its potential for formulating enriched food products,  
690 obtaining bioactive compounds, and the elaboration of functional, nutraceutical or  
691 phytotherapeutical products. In addition, there is a scarcity of information on the potential use  
692 of fruits at the green ripening stage.

693

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984 **CAPÍTULO 3**

985 **GROWTH AND PROXIMATE COMPOSITION OF *Pleurotus ostreatus* CULTIVATED**  
986 **ON GREEN BOCAIUVA PULP SUBSTRATES WITH DIFFERENT NITROGEN**  
987 **SOURCES**

988

989 **ABSTRACT:** The aim of this work was to evaluate the growth and the proximate composition  
990 of the mycelium-based bocaiuva pulp with the edible mushroom *P. ostreatus* on green bocaiuva  
991 flour added with different sources of nitrogen (urea, ammonium nitrate and sulfate ammonia).  
992 Growth was monitored by kinetics. At the end, the proximate composition of the best three  
993 treatments (dehydrated green bocaiuva pulp and water, T1; dehydrated green bocaiuva pulp and  
994 ammonium nitrate, T3; and green bocaiuva pulp/wheat bran and ammonium nitrate, T7) was  
995 determined. Ammonium nitrate was the nitrogen source that showed the greatest growth in both  
996 substrates (T3:8.33 cm and T7:7.67 cm), in relation to the other treatments (4.67 to 7.17 cm),  
997 with emphasis on the green bocaiuva pulp. The substrate with green bocaiuva pulp and water  
998 was the one that showed the highest growth (7.50 cm), which was close to the treatment with  
999 mixed substrate and ammonium nitrate (7.67 cm). The treatment with the green bocaiuva pulp  
1000 and ammonium nitrate (T3) was highlighted due to its significant increase in proteins (9.42 g  
1001 100 g<sup>-1</sup>) and fibers (5.21 g 100 g<sup>-1</sup>) and decrease in carbohydrates (9.52 g 100 g<sup>-1</sup>), in comparison  
1002 to the other treatments T7 (8.94, 2.16, and 5.99 g 100 g<sup>-1</sup>, respectively) and T1 (2.78, 4.33, and  
1003 2.28 g 100 g<sup>-1</sup>, respectively). The product obtained from the growth of *P. ostreatus* in the pulp  
1004 of bocaiúva verde has promising prospects to be used as raw material in the development of  
1005 new food products with added nutraceutical value.

1006

1007 **Key words:** *Acrocomia*, Edible mushroom, Nitrogen source, Proximate composition and solid-  
1008 state cultivation.

1009

### 1010 3.1 INTRODUCTION

1011 The consumption of fruits and vegetables has been estimated in many countries  
1012 because it is associated with a lower incidence of mortality from chronic non-communicable  
1013 diseases (NCDs) (Miller et al. 2017; Rosário et al., 2018; Uddin et al., 2020). Among them,  
1014 cardiovascular diseases, diabetes, cancer and chronic respiratory disease are prevalent. They  
1015 are accentuated by smoking, physical inactivity, inadequate diet and alcohol use (Duncan et al.,  
1016 2012; Pengpid & Peltzer, 2017). On the other hand, some of these diseases can be minimized  
1017 or even prevented by changing eating habits, *e.g.* incorporating the daily consumption of fruits  
1018 and vegetables (Raigond et al., 2018; Yuan et al., 2018; Jezewska-Zychowicz et al., 2019) with  
1019 high content of vitamins, minerals, dietary fiber (Fayet-Moore et al., 2017; Arun et al., 2017;  
1020 Volpe, 2019), and low glycemic indexes (Oboh et al., 2015; Raigond et al., 2018).

1021 In the Brazilian savannah biome are found species of native or adapted fruit plants with  
1022 nutritional and therapeutic properties, depending on the primary and secondary compounds  
1023 derived from the plant's metabolism, as well as, by the peculiar aroma and flavor that make  
1024 them attractive for consumption (Reis & Schmiele, 2019). Among these species, stands out  
1025 *Acrocomia* sp., which fruits are commonly known as bocaiuva, macauba or macaiba (Vianna  
1026 et al., 2017). These fruits provide mature pulp rich in lipids, carbohydrates, fibers (Bora &  
1027 Rocha 2004; Ramos et al., 2008; Orsi et al., 2015), copper, iron, manganese, potassium and  
1028 zinc (Ramos et al. 2008; Gonçalves et al., 2020) being able to supply the nutritional needs of  
1029 human beings through ingestion natural or processed (Ramos et al. 2008; de Oliveira et al.,  
1030 2020). In addition, the pulp is rich in  $\beta$ -carotene (Ramos et al. 2008; Coimbra & Jorge, 2011;  
1031 Orsi et al. 2015) and  $\alpha$ -tocopherol (Coimbra & Jorge, 2011), which are important carotenoids  
1032 due to their antioxidant action and anti-inflammatory effect (Costa et al., 2020; Lescano et al.,  
1033 2015). Most scientific studies on the nutritional and functional properties of the genus  
1034 *Acrocomia* refer to the species *A. aculeata*. Studies with other species, *e.g.* *A. totai* are scarce  
1035 and indispensable to confirm their properties, increase their value and contribute to the  
1036 preservation of palm trees, as they are often deforested for agricultural purposes in the region.

1037 The bocaiuva pulp has important nutritional, sensory and functional characteristics for  
1038 health, thus being considered a promising source for the food industry (Orsi et al., 2015; Valério  
1039 et al., 2019). However, it is little used in human food, mainly in the stage of green maturation.  
1040 Thus, its consumption can be favored from the bioconversion of the green bocaiuva pulp by  
1041 edible mushrooms in order to obtain food products with *low* carbohydrate content and enriched  
1042 in proteins and fibers, that are accepted by most of the consumers (Ritota & Manzi, 2019).

1043 Mushrooms can contribute to the prevention of some diseases, such as hypertension  
1044 (Vaz et al., 2011), cholesterol (Wei et al., 2018; Liu et al., 2019), diabetes (Asrafuzzaman et  
1045 al., 2018; Liu et al., 2019; Khatun et al., 2020; Balaji et al., 2020), stress (Akata et al., 2012),  
1046 obesity (Khatun et al., 2020) and acting as an appetite suppressant (Sheng et al., 2019).  
1047 *Pleurotus ostreatus* is an edible oyster mushroom that has a light color (white, gray or brown)  
1048 and a basidiocarp with a fleshy leaf shape (Jonathan & Esho, 2010). *Pleurotus* are a source of  
1049 nutrients, mainly proteins, minerals and vitamins B, C and D (Panjikkaran & Mathew, 2013).  
1050 This mushroom contains 20-35% protein (dry weight) and low levels of essential lipids and  
1051 amino acids (Li et al., 2017; Lavelli et al., 2018). Due to its low caloric value can be included  
1052 in diets with controlled calorie intake (Jaworska & Bernás, 2009).

1053 Pectocellulosic substrates, such as peels, pulps and fruit pomace, present favorable  
1054 conditions for the cultivation of edible mushrooms, as they have a lower carbon: nitrogen ratio  
1055 and high concentrations of simple sugars, which facilitates their availability to the fungus (Rivas  
1056 et al., 2010; Cardoso et al., 2013; Silva et al., 2014; Silva et al., 2020). The adaptation of  
1057 *Pleurotus* species to new substrates represents one of the main bioconversion processes in food  
1058 products with added value and nutritional quality (Mbassi et al., 2018; Ritota & Manzi, 2019).  
1059 Therefore, the use of fruit substrates for the cultivation of edible mushrooms makes it a viable  
1060 alternative for the nutritional enrichment of these products and the enhancement of species  
1061 within a productive chain, with permanent and sustainable production (Carrasco-González et  
1062 al., 2017; Ritota & Manzi, 2019).

1063 Thus, the objective of this work was to evaluate the growth and the proximal  
1064 composition of *P. ostreatus* grown on green bocaiuva pulp substrates with different nitrogen  
1065 sources, in order to obtain a product with a low carbohydrate content and rich in proteins and  
1066 fibers.

1067

1068

## 1069 **3.2 MATERIAL AND METHODS**

### 1070 **3.2.1 Microorganism and substrate**

1071 *Pleurotus ostreatus* URM 4072 was obtained from the Fungal Culture Collection of the  
1072 Federal University of Pernambuco. The cells were received lyophilized and, after reactivation,  
1073 kept in a medium inclined potato dextrose agar (PDA) immersed in mineral oil and stored at 4  
1074 °C (Fonseca et al., 2009; Silva et al., 2013). The strain was grown in Petri dishes containing  
1075 Sabouraud 4% glucose agar medium at 30 °C for 10 days to reactivate the mycelial growth.

1076 The substrates utilized were the dehydrated green bocaiuva pulp and the mixture of  
 1077 dehydrated green bocaiuva pulp (50%) and wheat bran (50%). The bocaiuva fruits (*Acrocomia*  
 1078 *totai* Mart) were collected in the municipality of Bela Vista-MS (56 ° 24'12.6'' West longitude,  
 1079 21° 56'15.9'' South latitude, and 153 m altitude), Brazil.

1080 After harvested, they were washed and sanitized with sodium dichloroisocyanurate  
 1081 0.66% (w / v) solution (Sumaveg de Diversery Lever) with 200 ppm of active chlorine for 15  
 1082 min. Then, the fruits were manually peeled and mechanically pulped. The pulp obtained was  
 1083 dehydrated in a circulation oven, with drying air speed of 1m / s at 50 °C for 24 h. Wheat bran  
 1084 was purchased from a local market in the city of Dourados-MS.

1085

### 1086 **3.2.2 Inoculation, cultivation and mycelial growth**

1087 The experiments were carried out with 2 pulp combinations: dehydrated green bocaiuva  
 1088 pulp (BP) or dehydrated green bocaiuva pulp plus wheat bran (BPWB) in a 1: 1 w / w  
 1089 proportion. For each combination of pulps, experiments were carried out with the addition of  
 1090 2% of external nitrogen sources: urea (U), ammonium nitrate (AN), and ammonium sulfate  
 1091 (AS), resulting in eight treatments (Table 3.1). Each treatment was placed into 50 mL test tubes,  
 1092 sterilized in an autoclave at 121 °C for 20 min and inoculated with 5 cm discs of myceliated  
 1093 agar. The tubes were incubated at 30 °C and the axemic growth of the fungus was measured  
 1094 every 2 days with a calibrated ruler (Ilyas & Avin, 2018).

1095

1096 **Table 3.1** Treatments for growth experiments.

Treatment	Pulp mixture	External N source
T1	BP	-
T2	BP	U
T3	BP	AN
T4	BP	AS
T5	BP/WB	-
T6	BP/WB	U
T7	BP/WB	AN
T8	BP/WB	AS

1097 BP: green bocaiuva pulp; BP/WB: green bocaiuva pulp / wheat bran; U: urea; AN: ammonium  
 1098 nitrate; AS: ammonium sulfate.

1099



### 1100 **3.2.3 Proximate composition**

1101 The proximate composition was determined for the dehydrated green bocaiuva pulp  
1102 (BP), the wheat bran (WB), the mixture of green bocaiuva pulp and wheat bran (BP / WB) and  
1103 the three best experiments with mycelial growth. The moisture content was determined by using  
1104 an oven with air circulation at 70 °C (Method n° 44-15.02, AACC 2010), the mineral residue  
1105 by weighing the residues from muffle incineration at 550 °C (Method n° 08-01.01, AACC,  
1106 2010), the proteins by the Kjeldahl method (Method n° 2001.11, AOAC, 2005), the total lipids  
1107 by using the Soxhlet extractor (Method n° 30-25.01, AACC, 2010), and the crude fiber by the  
1108 gravimetric method using a fiber determiner (Method n° 978.10, AOAC, 2005). The  
1109 determination of the carbohydrates was performed by difference (= total - moisture – minerals  
1110 – lipids – proteins – fibers).

1111

### 1112 **3.2.4 Energetic value and proximate composition variation (PCV)**

1113 The energy value was calculated using the Atwater coefficients that consider 4 kcal / g  
1114 of sample for proteins and carbohydrates and 9 kcal / g of sample for lipids (Merril & Watt,  
1115 1973). The proximate composition variation of the cultivated media for proteins, lipids and  
1116 fibers was calculated by the percentage difference between the concentration presented in the  
1117 cultivated medium (greater accumulation) and treated medium (inoculated) (Equation 1),  
1118 according to Fonseca et al. (2009).

1119

$$1120 \text{ PCV (\%)} = \left( \frac{\text{Final content (\%)}}{\text{Initial content (\%)}} \times 100 \right) - 100 \quad (1)$$

1121

### 1122 **3.2.5 Statistical analysis**

1123 The experiments were carried out in triplicate and the results expressed as mean and  
1124 standard deviation. Analysis of variance (ANOVA) and Tukey's multiple comparison test ( $P <$   
1125 0.05) were calculated using Statistica version 8.0 software (StatSoft, Inc, Tulsa, USA).

1126

## 1127 **3.3 RESULTS AND DISCUSSION**

### 1128 **3.3.1 Mycelial growth**

1129 Fig. 3.1 shows the growth curves of *P. ostreatus* on dehydrated green bocaiuva pulp and  
1130 the mixture of dehydrated green bocaiuva pulp and wheat bran based substrates with different  
1131 nitrogen sources (urea, ammonium nitrate and sulfate ammonia) for 40 days. In all treatments,  
1132 colonization was observed after two days of inoculation, as reported elsewhere (Patel et al.,  
1133 2009). The growth was evident up to the 30<sup>th</sup> day. After that, a stationary phase was observed.

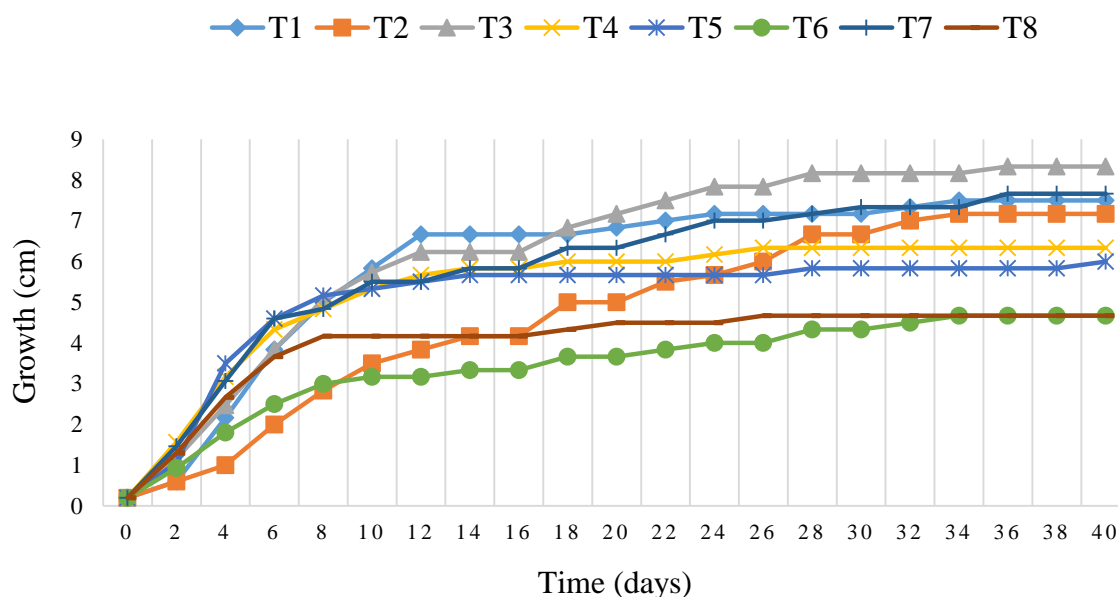
1134 Ammonium nitrate was the best nitrogen source evaluated. It allowed the highest growth  
1135 in both substrates compared to the other treatments, mainly on the green bocaiuva pulp.  
1136 Regarding the control treatments, it can be observed that the substrate with green bocaiuva pulp  
1137 and water was the one that showed the highest growth, which was close to the treatment with  
1138 ammonium nitrate and the mixture of green bocaiuva pulp and wheat bran (T7).

1139 The type of substrate utilized has an effect on the chemical, functional and sensory  
1140 characteristics of the mushrooms (Mbassi et al., 2018; Lavelli et al., 2018; Pazza et al., 2019;  
1141 Valenzuela-Cobos et al., 2019). *Pleurotus* sp. extract the nutrients from the substrate through  
1142 the mycelium, obtaining substances necessary for its development, such as carbon, nitrogen,  
1143 vitamins and minerals (Lavelli et al., 2018; Bellettini et al., 2019). The nutritional content of  
1144 the substrates can be improved by supplementing with nitrogen (Nunes et al., 2012).

1145 The nitrogen source is an important factor in the synthesis of proteins, nucleic acids,  
1146 purines, pyrimidines and polysaccharides (Drozdowski et al., 2010; Abdullah et al., 2015).  
1147 Some studies have pointed out that nitrogen supplementation can increase productivity yield,  
1148 but up to a certain level, as high nitrogen values can inhibit the fructification of *Pleurotus* sp.  
1149 (Silva et al., 2007). Thus, supplementation with 2% ammonium nitrate in the green bocaiuva  
1150 pulp-based substrate was efficient for the mycelial growth of *P. ostreatus*.

1151

1152



1153

1154 **Figure 3.1** Growth curves of *Pleurotus ostreatus*.

1155 T1: dehydrated green bocaiuva pulp (BP) and water; T2: dehydrated green bocaiuva pulp (BP)  
 1156 and Urea (U); T3: dehydrated green bocaiuva pulp (BP) and ammonium nitrate (AN); T4:  
 1157 dehydrated green bocaiuva pulp (BP) and ammonium sulfate (AS); T5: green bocaiuva pulp /  
 1158 wheat bran (BP/WB) and water; T6: green bocaiuva pulp / wheat bran (BP/WB) and urea (U);  
 1159 T7: green bocaiuva pulp / wheat bran (BP/WB) and ammonium nitrate (AN); T8: green  
 1160 bocaiuva pulp / wheat bran (BP/WB) and ammonia sulphate (AS).

1161

### 1162 3.3.2 Proximate composition

1163 The results obtained for the proximate composition of wheat bran, dehydrated green  
 1164 bocaiuva pulp and the three best treatments with dry mycelial growth are shown in Fig. 3.2. It  
 1165 observes that the wheat bran, the green bocaiuva pulp and the treatments presented moistures  
 1166 of  $1.22 \pm 0.17$  to  $3.88 \pm 0.49$  g 100 g<sup>-1</sup> (Fig. 3.2A). According to Brazilian legislation it should  
 1167 not exceed 15 g 100 g<sup>-1</sup> (ANVISA, 2005a).

1168 The type of substrate and the nitrogen source utilized for the cultivation of *Pleurotus* sp.  
 1169 influenced directly in the final proximate composition of the products. The main proximate  
 1170 components that had a significant increase ( $p < 0.05$ ) were proteins and fibers, for all treatments,  
 1171 in relation to the initial composition, without miceliation. It also underlines the treatment with  
 1172 green bocaiuva pulp and ammonium nitrate (T3) due to its significant increase in proteins ( $9.42$   
 1173 g 100 g<sup>-1</sup>) (Fig. 3.2B) and fibers ( $5.21$  g 100 g<sup>-1</sup>) (Fig. 3.2C), in comparison to the other T7

1174 treatments (8.94 and 2.16 g 100 g<sup>-1</sup>, respectively) and T1 (2.78 to 4.33 g 100 g<sup>-1</sup>, respectively)  
1175 (Fig. 3.2B and 3.2C). The variation in protein content can be explained by the addition of a  
1176 nitrogen source (ammonium nitrate) in the substrate. In accordance, it was reported elsewhere  
1177 that the protein enrichment of pineapple peels by using *Trichoderma viride* was higher, when  
1178 there was a better aeration and the addition of ammonium sulfate as nitrogen source (Aruna,  
1179 2019).

1180         The increase in fiber content during the solid state bioprocess may be associated with  
1181 the fungal cell wall, as it is composed of chitin fibers, which is a polysaccharide consisting of  
1182 a long chain of N-acetylglycosamine, insoluble in water (Garcia-Rubio et al., 2020). The cell  
1183 wall of filamentous fungi contains chitin in the proportion of 10 to 20%. These structures  
1184 withstand great pressure and, thus, become responsible for the integrity of the cell wall. When  
1185 chitin synthesis is interrupted, the cell wall becomes disorganized and the fungal cell undergoes  
1186 deformations and osmotic instability (Bowman & Free, 2006).

1187         It was demonstrated that the soluble dietary fiber in nejayote increased 45% after solid  
1188 state cultivation with *P. ostreatus* Perla, improving the bioavailability of the fiber source as a  
1189 functional ingredient (Acosta-Estrada et al., 2019).

1190         The use of *P. ostreatus* in the bioconversion of green pulp is an alternative to produce  
1191 improved foods with added value for disease prevention due to the enrichment of the nutrient  
1192 content by the fungus.

1193         According to technical regulations, T1 can be considered as a protein source and T3 and  
1194 T7 as high protein content sources, all with high fiber content (ANVISA, 2012). It is  
1195 recommended the daily consumption of 50 g of protein by adults and 34 g by children up to 10  
1196 years old (ANVISA, 2005b). In this sense, the intake of 100 g of T3 provides about 27 to 40%  
1197 of the daily protein requirement. It also provides about 93% of the daily recommendation of  
1198 fiber for adults by FAO (United Nations Food and Agriculture Organization) and WHO (World  
1199 Health Organization), being that at least 25 g of fibers per day is considered enough to prevent  
1200 chronic diseases (WHO/FAO, 2003).

1201         Regarding the carbohydrate content, there was a significant reduction for all treatments  
1202 (Fig. 3.2D) being greater in T3 (9.52 g 100 g<sup>-1</sup>), followed by T7 (5.99 g 100 g<sup>-1</sup>) and T1 (2.28  
1203 g 100 g<sup>-1</sup>). The carbohydrates consumed were used as sources of carbon and energy for growth  
1204 and synthesis of other compounds (Fonseca et al. 2009; Silva et al. 2013).

1205         The minerals showed a decrease in the contents for all treatments compared to the initial  
1206 characterization (2.75, 2.89, and 2.81 g 100 g<sup>-1</sup> for T1, T3, and T7, respectively) (Fig. 2.2E).  
1207 This decrease may be due to leaching, as a result of microbial activities that some minerals are

1208 lost or used during solid state fermentation by *Pleurotus ostreatus* (Adebayo et al., 2019). The  
1209 range of values found in the present study (1.62 to 2.00 g 100 g<sup>-1</sup>) is below to that reported by  
1210 Silva et al. (2014), with minerals reaching up to 10.91 g 100 g<sup>-1</sup>. These contents can vary  
1211 depending on the fungal species and the substrate utilized.

1212 The treatments had an increase of 0.06 to 0.53 g 100 g<sup>-1</sup> of lipids in relation to the initial  
1213 characterization (Fig. 3.2F). The increase in lipids may have occurred due to the production of  
1214 enzymes during mycelial growth, as these lipids are intended for the construction of the cell  
1215 wall of fungi (Fonseca et al., 2009; Athenaki et al., 2017) and it is also associated that some  
1216 fungi species are able to accumulate lipids during the bioprocess (Dulf et al., 2016; Dulf et al.,  
1217 2017; Araújo et al., 2020).

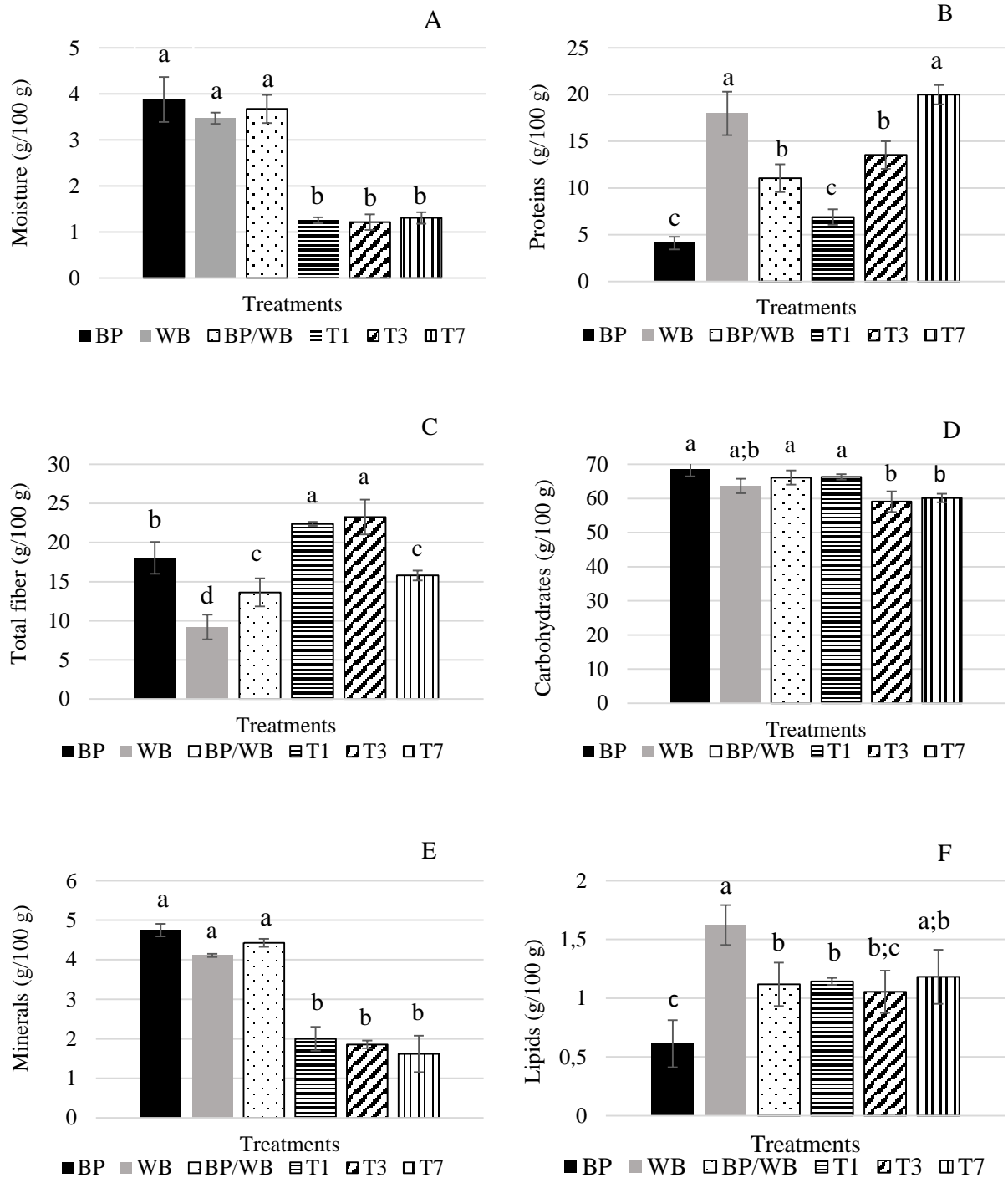
1218

### 1219 **3.3.3 Energetic value and proximate composition variation**

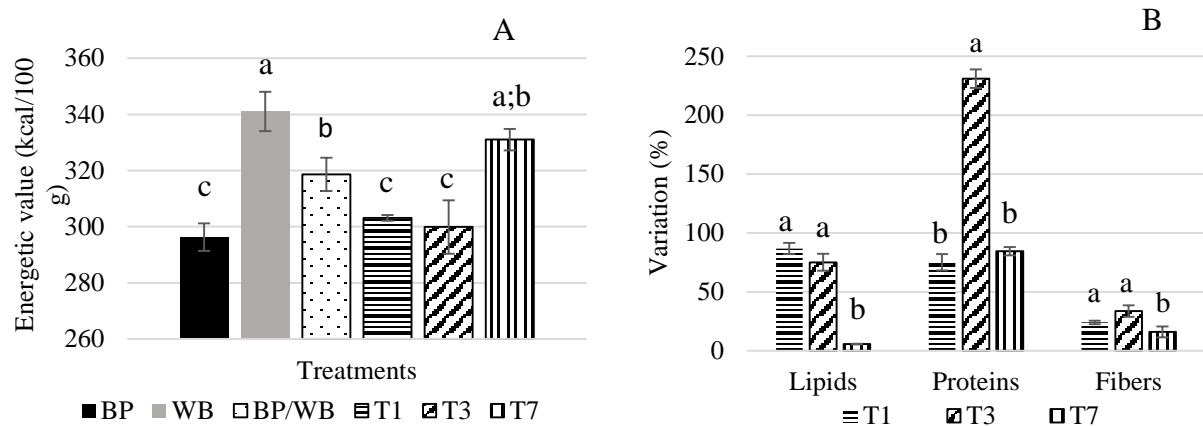
1220 It can be observed that the treatments with green bocaiuva pulp (T1 and T3) had lower  
1221 energy value in relation to the experiment with the mixture of bocaiuva pulp and wheat bran  
1222 (T7) (Fig. 3.3A). This behavior may be related to the increase in the protein content, indicating  
1223 that the microorganism utilized efficiently the source of exogenous nitrogen to the  
1224 bioconversion of carbohydrates and some fibers into protein.

1225 An expressive positive protein variation (enrichment) was observed for all treatments,  
1226 mainly for T3 with 231.12%, followed by T7 (84.44%) and T1 (75.17%) (Fig. 3.3B). These  
1227 results demonstrate that T3 was superior in terms of protein enrichment than *e.g.* the the fruit  
1228 residues of *Caryocar brasiliense* (160.04%), *Annona crassiflora* (143.31%), *Campomanesia*  
1229 *pubescens* (102.42%), and the T1 (75.17%) presented itself higher to the substrate *Acrocomia*  
1230 *aculeata* (67.88%) with *Lichtheimia ramosa* (Silva et al. 2014). Regarding the lipid variation,  
1231 it can be observed that T1 presented a higher percentage (86.76%) than that observed for T3  
1232 (75.08%) and T7 (5.57%). This enrichment was greater than that reported by Fonseca et al.  
1233 (2009) who obtained 66.70% of variation using *P. ostreatus* in cultivations containing a mixture  
1234 of rice bran, rice straw and sof rush. The fibers had a variation of 15.88, 24.00, and 33.51%, for  
1235 T7, T1 and T3, respectively. In the study by Zusman et al. (1997) it can be observed increases  
1236 up to 78 % in the fiber content in *P. ostreatus* fungi grown on corn cobs.

1237



1238 **Figure 3.2** Proximate composition for different cultivation treatments with *Pleurotus ostreatus*.  
 1239 BP: dehydrated green bocaiuva pulp without increment; WB: wheat bran without increment;  
 1240 BP/WB: green bocaiuva pulp / wheat bran without increment; T1: dehydrated green bocaiuva  
 1241 pulp (BP) and water; T3: dehydrated green bocaiuva pulp (BP) and ammonium nitrate (AN);  
 1242 T7: green bocaiuva pulp / wheat bran (BP/WB) and ammonium nitrate (AN).  
 1243



1244 **Figure 3.3** Energetic value and composition variation for lipids, proteins and fibers for different  
 1245 cultivation treatments with *Pleurotus ostreatus*.

1246 BP: dehydrated green bocaiuva pulp without increment; WB: wheat bran without increment;  
 1247 BP/WB: green bocaiuva pulp / wheat bran without incremente; T1: dehydrated green bocaiuva  
 1248 pulp (BP) and water; T3: dehydrated green bocaiuva pulp (BP) and ammonium nitrate (AN);  
 1249 T7: green bocaiuva pulp / wheat bran (BP/WB) and ammonium nitrate (AN).

1250

### 1251 3.4 CONCLUSION

1252 The addition of ammonium nitrate as nitrogen source in the T3 treatment contributed to  
 1253 better mycelial growth and potentiated the bioconversion of carbohydrates from the green pulp  
 1254 of *Pleurotus ostreatus* into proteins, fibers and lipids, which is promising for the development  
 1255 of new ones food products with nutraceutical added value.

1256

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1262

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1511 **CAPÍTULO 4**

1512 **DEVELOPMENT AND EVALUATION OF LOW-CARB CAKES PRODUCED FROM**  
1513 **GREEN BOCAIUVA PULP ENRICHED WITH *Pleurotus ostreatus***

1514 **ABSTRACT:** *Pleurotus ostreatus* is an edible fungus with interesting nutritional properties that can  
1515 be used as an ingredient to develop sustainable healthy food products. Thus, the aim of this work was  
1516 to develop and evaluate low-carb cakes by replacing wheat flour with green bocaiuva pulp enriched with  
1517 *P. ostreatus*. Four formulations were tested: F1: coconut flour, F2: green bocaiuva flour, F3: green  
1518 bocaiuva flour enriched with mushroom and ammonium nitrate and F4: green bocaiuva flour enriched  
1519 with *P. ostreatus* and water. Results indicated that all formulations can be considered as protein source  
1520 foods (7.33 to 10.47 g 100 g<sup>-1</sup>), with high fiber contents (7.16 to 9.43 g 100 g<sup>-1</sup>), and low-carbohydrate  
1521 contents (13.92 to 21.87 g 100 g<sup>-1</sup>), without sugar addition. F4 presented the highest scores for all  
1522 parameters of sensory analysis (color: 7.77; aroma: 7.55; texture: 7.32, flavor: 7.18 and overall rating:  
1523 7.73) and purchase intention (68% for the sum of probably and certainly would purchase). It was  
1524 concluded that the green bocaiuva flour enriched with *P. ostreatus* can be used for the development of  
1525 low-carb cake with improved nutritional components and acceptable sensory characteristics.

1526

1527 **Keywords:** Low-carb cake; Acrocomy; Edible fungus; Proximate composition; Sensory  
1528 analysis.



## 1529 **4.1 INTRODUCTION**

1530 Bakery products present a high content of simple sugars of rapid absorption, high fat  
1531 content and low amount of dietary fiber, which makes them highly caloric food products,  
1532 contributing negatively to chronic diseases associated with the metabolic syndrome *e.g.*  
1533 diabetes, obesity, dyslipidemia and some types of cancer (Duncan et al., 2012; Peris et al.,  
1534 2019). In 2016, more than 1.9 billion adults aged 18 and over were overweight (39%) and 650  
1535 million were obese (13%), while 340 million children and teenagers aged 5 to 19 years were  
1536 overweight or obese (WHO, 2016). Thus, there is an urgency to replace certain ingredients in  
1537 order to increase the nutritional added value of processed food products (Dimou et al., 2019).

1538 The cake is one of the most consumed food products, second only to bread (Gohara et al.,  
1539 2014). This confectionery product is the result of mixing, homogenizing and baking the dough  
1540 prepared by combining flours or starch, eggs, milk, butter or vegetable fat, sugars and leavening  
1541 agent (Soares et al., 2018; Carvalho et al., 2019; Stavale et al., 2019). Some of the ingredients  
1542 can be replaced by adding ingredients with functional or nutritional properties (Carvalho et al.,  
1543 2019; Dalmolin et al., 2019; Montagner & Storck, 2019; Stavale et al., 2019) and low-  
1544 carbohydrate content, which is beneficial for health, improving *e.g.* anthropometric and blood  
1545 markers in healthy adults (Harvey et al., 2019). The great challenge is to improve the nutritional  
1546 quality of cakes without impairing the sensory properties.

1547 Studies have shown that sugar can be substituted for sweeteners (Dalmolin et al., 2013;  
1548 Farzi et al., 2015; Pineli et al., 2016; Soares et al., 2018) and animal milk for soy milk, corn  
1549 milk, chestnut milk, coconut milk, soy oil, sunflower oil or coconut oil (Drunkler et al., 2010;  
1550 Correia et al., 2014; Pereira et al., 2017). Wheat is the main ingredient used in cake production  
1551 and can be replaced by fruit pulp flour, contributing to the nutritional and sensory characteristics  
1552 (Carvalho et al., 2019).

1553 A promising source for the food industry in the development of new products replacing  
1554 wheat flour is the pulp of the fruit of the bocaiuva (*Acrocomia sp.*), Mainly in the stage of green  
1555 ripening, which its consumption is little used in human food, and can be favored to from the  
1556 processing of fresh pulp into flour, with functional and nutraceutical properties, since the pulp  
1557 flour from the fruit of *A. aculeata* green is rich in soluble fibers and has shown an effect in  
1558 reducing body weight, a hypolipemic activity in the total cholesterol fractions, LDL-  
1559 cholesterol, triglycerides and fasting glucose in male mice (Giunco, 2018).

1560 Another promising nutrient food that can be used to replace whole or partial wheat flour  
1561 is the pulp of fruits and vegetables enriched with microorganisms. According to Kolawole,  
1562 Akinwande and Ade-Omowaye (2020), products developed from orange-fleshed sweet

1563 potatoes enriched with sclerotia from *Pleurotus tuberregium* showed higher levels of protein,  
1564 ash, crude fiber, water-soluble vitamins and mineral composition, compared to wheat flour, and  
1565 with acceptable sensory characteristics. It was also reported in the literature several nutritional  
1566 properties of *Pleurotus* sp. in the prevention of insulin resistance, dyslipidemia, hypertension  
1567 and obesity (Khan & Tania, 2012; Balaji et al., 2020; Dicks & Ellinger, 2020) by providing  
1568 various bioactive compounds like  $\beta$ -glucans. Although the consumption of edible mushrooms  
1569 is ancient (Ilavenil et al., 2017), its use in food applications, such as in the manufacture of cakes,  
1570 has been marginally studied so far.

1571 Thus, the aim of this work was to develop low-carb cakes by replacing wheat flour with  
1572 green bocaiuva pulp enriched with the edible fungus *Pleurotus ostreatus*, and evaluate their  
1573 proximate composition and sensory analysis in order to have food products rich in nutrients,  
1574 with substances beneficial to health, and sensory characteristics acceptable to consumers.

1575

## 1576 **4.2 MATERIAL AND METHODS**

### 1577 **4.2.1 Microorganism and substrate**

1578 *Pleurotus ostreatus* URM 4072 was utilized in this study. It was obtained from the Fungi  
1579 Culture Collection at the Federal University of Pernambuco. The cells were received  
1580 lyophilized and, after reactivation, kept in an inclined potato dextrose agar (PDA) medium  
1581 immersed in mineral oil and stored at 4 °C (Fonseca et al., 2009; Silva et al., 2013). This strain  
1582 was grown in Petri dishes containing 4% glucose Sabouraud medium and incubated at 30 °C  
1583 for 10 days, until obtaining adequate mycelial growth, before recovering.

1584 The substrate utilized was dehydrated green bocaiuva pulp. The bocaiuva (*Acrocomia*  
1585 *totai* Mart) fruits were collected in the municipality of Bela Vista, MS, Brazil (56°24'12.6''  
1586 West longitude, 21°56'15.9'' South latitude, and 153 m altitude). Fruits were then washed and  
1587 sanitized with 0.66% (w v<sup>-1</sup>) sodium dichloroisocyanurate solution (Sumaveg, Diversey Lever)  
1588 with 200 ppm of active chlorine for 15 min, manually peeled and mechanically pulped. The  
1589 pulp obtained was dehydrated in an air circulation oven at 50 °C for 24 h.

1590

### 1591 **4.2.2 Inoculation, cultivation and mycelial growth**

1592 The experiments were carried out by mixing 100 g of substrate (dehydrated green  
1593 bocaiuva pulp) with 200 mL of N source stock solution (2% ammonium nitrate solution  
1594 prepared in distilled water) in transparent polypropylene autoclavable bags measuring 20 x 30

1595 cm. The bags were closed with cotton plugs to allow gas exchange during the process, and  
1596 sterilized in an autoclave at 121 °C for 20 min.

1597 Inoculation of the fungus in the substrate was carried by transferring 1.3 cm diameter  
1598 mycelium discs from the plates, with the aid of a 5 mL test tube and a platinum loop, previously  
1599 sterilized. The bags containing the inoculated samples were stored in a bacteriological oven (30  
1600 °C) for 30 days. After this period, the mycelium was dehydrated in an air circulation oven at 70  
1601 °C for 24 h, crushed and sieved, obtaining a flour.

1602

#### 1603 **4.2.3 Preparation of low-carb cakes**

1604 After preliminary tests, a standard formulation was defined, using as raw materials: pure  
1605 cocoa powder, brown coconut flour, culinary sweetener, coconut milk, butter, egg, chocolate  
1606 flavoring and baking powder (F1). Three other formulations were prepared by replacing 100%  
1607 of the brown coconut flour with raw green bocaiuva flour (F2), enriched green bocaiuva flour  
1608 and ammonium nitrate (F3), or enriched green bocaiuva flour and water (F4) (Table 4.1). The  
1609 experiments were conducted in accordance with safety, hygiene and good food handling  
1610 practices established for foods (ANVISA, 2004).

1611 The recipes were individually prepared using the standard technique according with the  
1612 following steps: preheating of the domestic oven at 180 °C; separation of the ingredients and  
1613 weighing on a digital scale balance with an accuracy of 0.01 g; mixing of the ingredients in an  
1614 electric mixer (Britânia, model Pérola Maxx) until obtaining a smooth and homogeneous  
1615 dough; packing the dough in silicone forms (5 x 2 cm) that were placed on the top of an  
1616 aluminum form (25 x 2 cm); and then baking at 180 °C for 30 min in a domestic gas oven  
1617 (Brastemp, model BFS6NCBUNA).

1618 To guarantee a homogeneous baking and the standardization of the formulations, a total  
1619 of 15 units of silicone forms containing the same dough formulation were disposed in each  
1620 aluminum form. After 15 min, they had their positions switched in the oven to allow each cake  
1621 to be baked at the same way. After 30 min, they were taken and tested with a toothpick. If they  
1622 did not pass the toothpick test, they would bake for another 3 min until the toothpick came out  
1623 clean.

1624 After baking, the cakes were cooled to room temperature (25 °C) and added in  
1625 polyethylene containers with a lid for a maximum of 24 h before analysis.

1626

1627 **4.2.4 Proximate composition and energy value**

1628 The moisture content was determined by using an oven with air circulation at 70 °C  
 1629 (Method n° 44-15.02, AACC, 2010), the mineral residue by weighing the residues from muffle  
 1630 incineration at 550 °C (Method n° 08-01.01, AACC, 2010), the proteins by the Kjeldahl  
 1631 procedure (Method n° 2001.11, AOAC, 2005), the total lipids by using the Soxhlet apparatus  
 1632 (Method n° 30-25.01, AACC, 2010), and the crude fiber by the gravimetric method using a  
 1633 fiber determiner (Method n° 978.10, AOAC, 2005). The determination of the carbohydrates  
 1634 was performed by difference (total – moisture – minerals – lipids – proteins – fibers). The  
 1635 energy value was calculated using the Atwater coefficients that consider 4 kcal g<sup>-1</sup> of sample  
 1636 for proteins and carbohydrates and 9 kcal g<sup>-1</sup> of sample for lipids (Merril & Watt, 1973).

1637

1638 **Table 4.1** Low-carb cake formulations.

Ingredient	Formulation (g 100 g <sup>-1</sup> )			
	F1	F2	F3	F4
Brown coconut flour	13.82	-	-	-
Green bocaiuva flour without supplementation	-	13.82	-	-
Enriched green bocaiuva flour and NH <sub>4</sub> NO <sub>3</sub>	-	-	13.82	-
Enriched green bocaiuva flour and water	-	-	-	13.82
Cocoa powder	0.31	0.31	0.31	0.31
Culinary sweetener	3.45	3.45	3.45	3.45
Coconut milk	41.48	41.48	41.48	41.48
Butter	6.91	6.91	6.91	6.91
Egg	33.18	33.18	33.18	33.18
Chocolate flavoring	0.16	0.16	0.16	0.16
Chemical yeast	0.69	0.69	0.69	0.69

1639 F1: Low-carb cake with coconut flour. F2: Low-carb cake with green bocaiuva flour without  
 1640 the increment. F3: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour and  
 1641 ammonia nitrate. F4: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour  
 1642 and water.

1643

#### 1644 **4.2.5 Sensory analysis and purchase intention**

1645 The attributes color, smell, texture, taste, and overall impression were evaluated by 11  
1646 trained judges, aged from 21 to 44 years old, all of them students or technicians from the Federal  
1647 University of Grande Dourados, through the combined acceptance-preference test, using a 9-  
1648 point hedonic scale anchored at extremes 1 (I did not like it) and 9 (I liked it). The purchase  
1649 intention test was also applied with a 5-point scale (1 = certainly would not purchase, and 5 =  
1650 certainly would purchase). The tests were carried out in individual cabins, with white lighting,  
1651 according to the recommendations of the environment and the distribution of the samples  
1652 described elsewhere (Meilgaard et al., 2007).

1653 The acceptability index (AI) was calculated according to Equation 1, where A is the  
1654 average score of the attribute and B is the highest score observed for the evaluated attribute.  
1655 The product was considered acceptable if the AI was at least 70% (Reis et al., 2020).

1656

$$1657 \quad AI(\%) = \frac{(A \times 100)}{B} \quad (1)$$

1658

#### 1659 **4.2.6 Statistical analysis**

1660 The results obtained were expressed by the average of the repetitions and standard  
1661 deviation. The results were submitted to the analysis of variance (ANOVA) and compared using  
1662 the Tukey test at a significance level of 5% using the software Statistica (Statsoft version 8.0).

1663

### 1664 **4.3 RESULTS AND DISCUSSION**

#### 1665 **4.3.1 Proximate composition and energy value**

1666 Table 2 shows the proximate composition of the *low carb* cakes made. The enrichment  
1667 of bocaiuva flour with edible mushroom (F3 and F4) contributed to a product with lower  
1668 moisture content and higher content of minerals, lipids, proteins and fibers in relation to the  
1669 formulation with green bocaiuva flour without the increment (F2). Low carb cakes enriched  
1670 with edible mushroom (F3 and F4), bocaiuva flour without the increment (F2) had a lower lipid  
1671 content, and higher minerals compared to the standard formulation with coconut flour (F1).  
1672 Carbohydrates showed no significant difference ( $p > 0.05$ ) between the four formulations. Low  
1673 carb cakes enriched with edible mushrooms (F3 and F4) showed an energy value similar to that

1674 of formulations without mycelia, which demonstrates that there is no direct relationship  
1675 between mycelia and energy value.

1676 All studied cake formulations can be considered low-carb according to the Technical  
1677 Regulation on Complementary Nutritional Information (ANVISA, 2012), as they present  
1678 protein and fibers contents above 7% for all formulations. Moreover, formulations were  
1679 prepared without sugars addition when compared to traditional cakes, which represents a  
1680 reduction of at least 58 to 60% in the carbohydrates content when compared to cakes with wheat  
1681 or coconut flour (TACO, 2011).

1682 The lower carbohydrate content and higher protein and fiber contents indicate that the  
1683 low-carb cake formulations were nutritionally better when compared to cake added to passion  
1684 fruit peel flour (42.20 g 100 g<sup>-1</sup>, 6.56 g 100 g<sup>-1</sup> and 9.12 g 100 g<sup>-1</sup> of carbohydrates, proteins and  
1685 fibers, respectively) (Reis et al., 2020) and banana and diet oat cake (26.81 g 100 g<sup>-1</sup>, 4.77 g  
1686 100 g<sup>-1</sup> and 2.04 g 100 g<sup>-1</sup> of carbohydrates, proteins and fibers, respectively) (Souza et al.,  
1687 2020). It was reported elsewhere that oyster mushroom flour can be used for the partial  
1688 replacement of wheat flour in dough formulation, due to its ability to increase nutritional values  
1689 and improve sensory attributes such as taste and texture the research by (Nordiana et al., 2019).

1690 Moreover, as the obtained low-carb cakes did not present addition of sugar, whole milk,  
1691 and wheat flour, they can be considered suitable for consumption by those who have restrictions  
1692 on the consumption of sucrose, lactose and gluten, or who only restrict these ingredients by  
1693 choice. It is worth mentioning that among all the parameters studied, the increase in the protein  
1694 and in the mineral content, and the reduction in the carbohydrate content in the F3 formulation  
1695 stood out with the supplementation of the N source due to the increased fungal myceliation on  
1696 the flour. Thus, it is expected that the incorporation of fungal biomass should have incorporated  
1697 nutritional and medical functions reported in the literature (Khan & Tania, 2012; Ilavenil et al.,  
1698 2017; Vargas-Sánchez et al., 2018; Bindh & Arunava, 2019; Balaji et al., 2020; Ellinger, 2020;  
1699 Nweze et al., 2020).

1700 Based on the recommended daily consumption of 50 g of protein by adults and 34 g by  
1701 children up to 10 years old (ANVISA, 2005), it can be considered that the intake of 100 g of  
1702 low-carb cakes prepared with enriched flour, according to the F3 and F4 formulations, provide  
1703 about 21 and 15% of the daily requirement for adults, and 31 and 22% for children, respectively.  
1704 In addition, it provides about 31 and 38% of the minimum of 25 g of fiber recommended to be  
1705 consumed daily to prevent chronic diseases, considering the F3 and F4 formulations,  
1706 respectively (WHO/FAO, 2003).

1707

1708 **Table 4.2** Proximate composition and energy value obtained for the low-carb cake  
 1709 formulations.

Component	Formulation (g 100 g <sup>-1</sup> )			
	F1	F2	F3	F4
Moisture	43.34±2.15 <sup>a,b</sup>	46.18±1.91 <sup>a</sup>	43.71±2.78 <sup>a,b</sup>	39.79±1.29 <sup>b</sup>
Minerals	2.05±0.05 <sup>d</sup>	2.42±0.20 <sup>c</sup>	3.44±0.11 <sup>a</sup>	3.00±0.03 <sup>b</sup>
Lipids	22.87±0.76 <sup>a</sup>	15.09±1.47 <sup>c</sup>	16.07±0.49 <sup>b,c</sup>	18.34±0.73 <sup>b</sup>
Proteins	8.86±0.73 <sup>b</sup>	7.33±0.75 <sup>c</sup>	10.47±0.38 <sup>a</sup>	7.57±0.06 <sup>b,c</sup>
Total fiber	8.96±0.45 <sup>a,b</sup>	7.16±0.60 <sup>b</sup>	7.70±0.49 <sup>a,b</sup>	9.43±1.05 <sup>a</sup>
Carbohydrates	13.92±3.47 <sup>a</sup>	21.82±4.00 <sup>a</sup>	18.61±2.53 <sup>a</sup>	21.87±2.40 <sup>a</sup>
Energetic value*	296.98±5.43 <sup>a</sup>	252.38±4.94 <sup>b</sup>	260.94±10.12 <sup>b</sup>	282.78±9.14 <sup>a</sup>

1710 \*In Kcal/100g<sup>-1</sup>. Values are expressed as means and standard deviation (n = 3), with equal  
 1711 letters on the same line do not differ significantly at the level of 5% (p> 0.05) by the Tukey test.  
 1712 F1: Low-carb cake with coconut flour. F2: Low-carb cake with green bocaiuva flour without  
 1713 the increment. F3: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour and  
 1714 ammonia nitrate. F4: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour  
 1715 without supplementation with nitrogen.

1716

### 1717 4.3.2 Sensory analysis

1718 The sensory characteristics of the cake are of great importance for its acceptance by  
 1719 consumers and, consequently, for its commercialization, being the flavor of the product decisive  
 1720 in this process. Figure 4.1 shows the averages of the scores attributed in the sensory analysis  
 1721 for the four *low-carb* cake formulations. The formulation with the green bocaiuva flour enriched  
 1722 with *P. ostreatus* without nitrogen supplementation (F4) was the one that presented the highest  
 1723 score for all parameters. The color and the aroma attributes showed no significant difference  
 1724 (p<0.05) in relation to the formulations. The flavor was the main parameter that negatively  
 1725 influenced in the consumers' choice, followed by the overall impression, for the F3. It may be  
 1726 related to the bitter taste caused by supplementation with ammonium nitrate (nitrogen source)  
 1727 during the mycelial growth of the fungus in the pulp. Despite this, F3 was better in all other  
 1728 attributes than the F1 (control), indicating the need for improvement in these two  
 1729 characteristics, as an example, the decrease in the amount of ammonium nitrate in the growth  
 1730 of the fungus in F3.

1731 The formulations F2 and F4 showed acceptance indexes above 70% and 80% for all  
1732 attributes (Figure 4.2). On the other hand, acceptance indexes lower than 70% were obtained  
1733 for the flavor parameter in the F3, and for texture, flavor and overall evaluation in the F1, which  
1734 indicate that products would not be accepted by consumers, if commercialized (Reis et al.,  
1735 2020). However, these results indicate that F2 and F4 present great potential for  
1736 commercialization. The low-carb cakes prepared with green bocaiuva flour, enriched or not,  
1737 without nitrogen supplementation, presented acceptance rates above 70% for all attributes and  
1738 nutritional value.

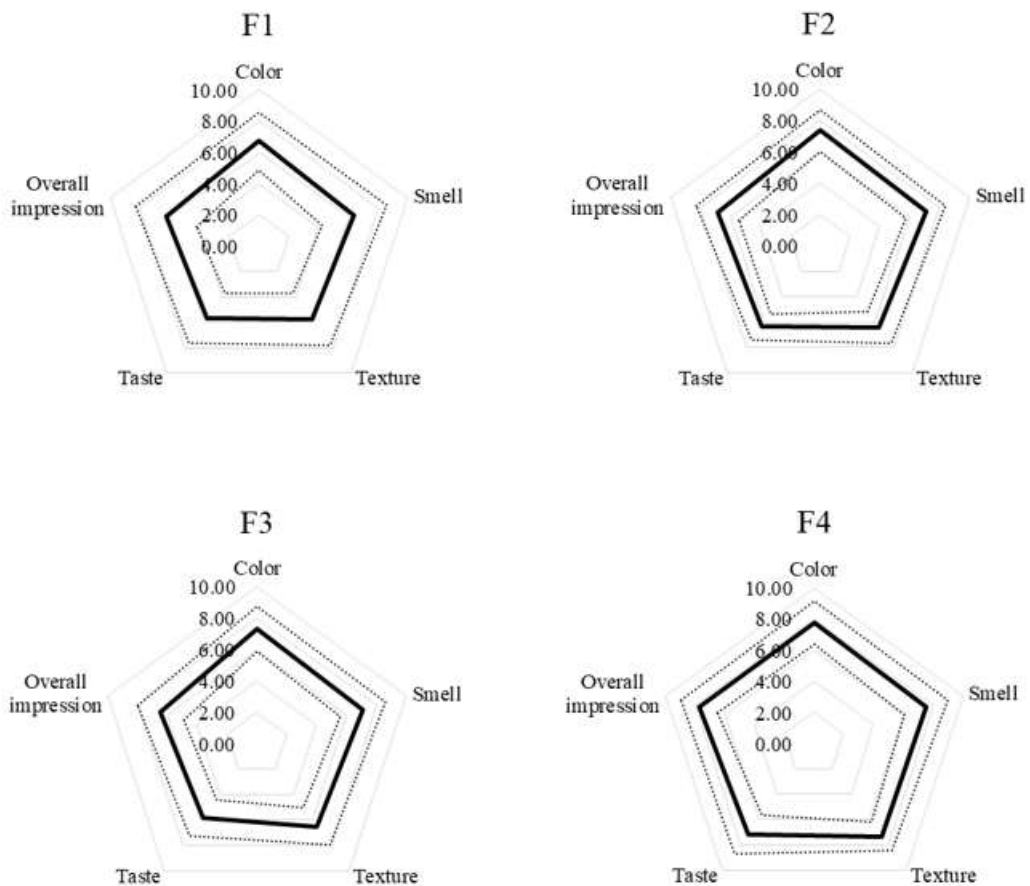
1739 Another important attribute evaluated was the purchase intention to of the four low-carb  
1740 cake's formulations (Figure 4.3). The results demonstrate that 68% of the judges certainly  
1741 would and probably would purchase the cake F4. Most of the judges perhaps would purchase  
1742 or perhaps would not purchase the cake F2 (59%). In comparison, 36% of the judges probably  
1743 or certainly would not purchase the cake F1.

1744 The average purchase intention for F4 ( $3.91 \pm 0.97$ ) was similar to that reported elsewhere  
1745 for banana cupcakes with the addition of up to 7.0% of banana peel flour ( $3.42 \pm 1.05$  to  $3.98$   
1746  $\pm 1.03$ ) (Carvalho et al., 2012). The purchase intention test revealed great market potential and  
1747 positive purchase intention for the F4, if it were actually available on the market. Therefore,  
1748 green bocaiuva flour enriched with edible mushroom *P. ostreatus* has the potential to be used  
1749 in the production of cakes and possibly other bakery products with functional properties for the  
1750 prevention of chronic diseases and contributing to inspire the food industry to produce food  
1751 products added value.

1752

1753



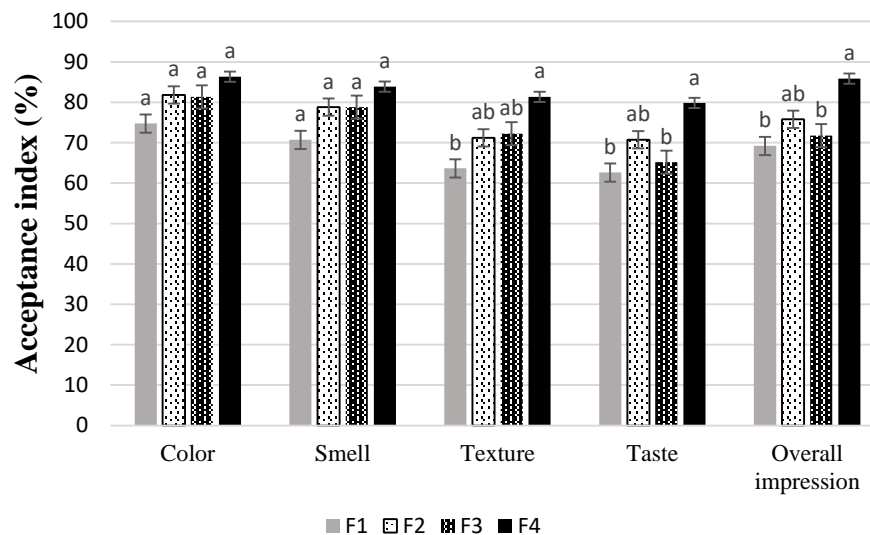


1754

1755 **Figure 4.1** Averages of the values of the scores attributed in the sensory analysis for the four  
 1756 low-carb cake formulations.

1757 F1: Low-carb cake with coconut flour. F2: Low-carb cake with green bocaiuva flour without  
 1758 the increment. F3: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour and  
 1759 ammonia nitrate. F4: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour  
 1760 without supplementation. Solid lines = Average value. Dashed lines = Standard deviation.

1761

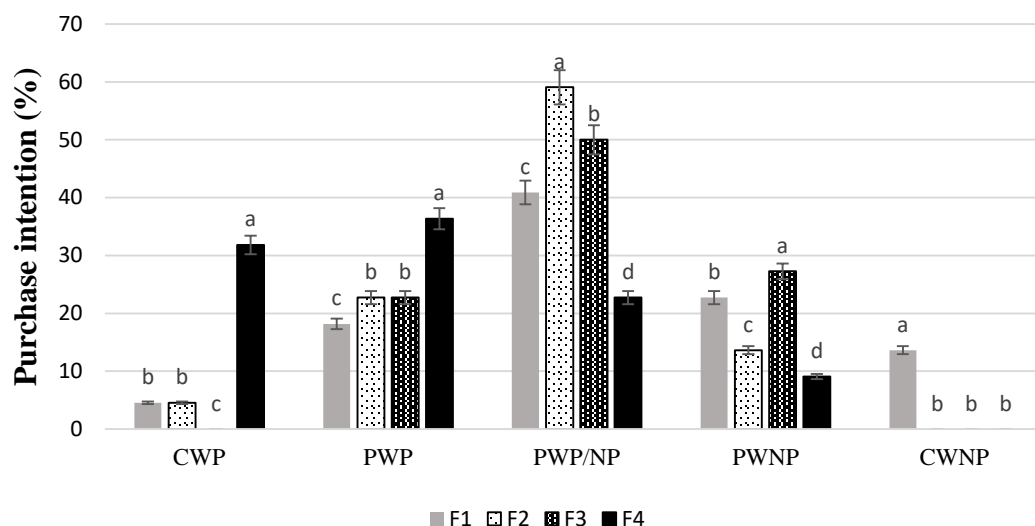


1762

1763 **Figure 4.2** Acceptance index of the four low-carb cake formulations.

1764 F1: Low-carb cake with coconut flour. F2: Low-carb cake with green bocaiuva flour without  
 1765 the increment. F3: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour and  
 1766 ammonia nitrate. F4: Low-carb cake with *Pleurotus ostreatus* enriched green bocaiuva flour  
 1767 without supplementation. Values are expressed as means and standard deviation (n = 22), with  
 1768 equal letters on the same line do not differ significantly at the level of 5% ( $p > 0.05$ ) by the  
 1769 Tukey test.

1770



1771 **Figure 4.3** Purchase intention of the four low-carb cake formulations.

1772 CWP: Certainly would purchase; PWP: Probably would purchase; PWP/NP: Perhaps would  
 1773 purchase/ not purchase; PWNP: Probably would not purchase; CWNP: Certainly would not  
 1774 purchase. F1: Low-carb cake with coconut flour. F2: Low-carb cake with *Pleurotus ostreatus*  
 1775 enriched green bocaiuva flour without the increment. F3: Low-carb cake with *Pleurotus*  
 1776 *ostreatus* enriched green bocaiuva flour and ammonia nitrate. F4: Low-carb cake with  
 1777 *Pleurotus ostreatus* enriched green bocaiuva flour without supplementation. Values are  
 1778 expressed as means and standard deviation (n = 22), with equal letters on the same line do not  
 1779 differ significantly at the level of 5% ( $p > 0.05$ ) by the Tukey test.

1780

#### 1781 4.4 CONCLUSION

1782 The green bocaiuva flour enriched with *Pleurotus ostreatus* demonstrated great  
 1783 potential for the preparation of low-carb cakes with improved nutritional composition (F3 and  
 1784 F4), acceptable sensory characteristics by trained judges (F4), and great potential for  
 1785 commercialization (F4). However, the dosage of ammonium nitrate as the nitrogen source for  
 1786 the development of the fungus negatively interfered in the taste of the elaborated product (F3).

1787

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1793

#### 1794 **4.6 DISCLOSURES STATEMENT**

1795           None of the authors have conflicts of interest to disclose.

1796

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1932  
1933



1934 **CAPÍTULO 5**

1935 **5.1 CONCLUSÃO GERAL**

1936

1937           Apesar da importância econômica do gênero *Acrocomia* e a diversificação de seu uso,  
1938 observa-se ainda subutilização das espécies. A espécie que apresenta maior destaque de  
1939 exploração tanto na subsistência quanto na exploração comercial a nível nacional e mundial é  
1940 a *A. aculeata*. Mas, seu maior destaque está voltado para exploração do óleo, sendo  
1941 negligenciado o potencial nutritivo para formulação de produtos alimentícios enriquecidos,  
1942 potencial de compostos bioativos para elaboração de produtos funcionais ou nutracêuticos, e  
1943 produtos fitoterápicos. Além disso, há escassez de informações sobre o potencial de uso dos  
1944 frutos no estágio de maturação verde. A adição de nitrato de amônio como fonte de nitrogênio  
1945 no tratamento T3 contribuiu para o melhor crescimento micelial e potencializou a bioconversão  
1946 de carboidratos da polpa de bocaiúva verde de *Pleurotus ostreatus* em proteínas, fibras e  
1947 lipídios, o que se revela promissor para o desenvolvimento de novos produtos alimentícios com  
1948 valor agregado nutracêutico. A farinha de bocaiúva verde enriquecida com *Pleurotus ostreatus*  
1949 demonstrou grande potencial para a preparação de bolos com baixo teor de carboidratos com  
1950 melhor composição nutricional (F3 e F4), características sensoriais aceitáveis por juízes  
1951 treinados (F4) e grande potencial de comercialização (F4). Porém, a dosagem de nitrato de  
1952 amônio como fonte de nitrogênio para o desenvolvimento do fungo interferiu negativamente  
1953 no sabor do produto elaborado (F3).

1954

1955

## APÊNDICES

1956 **Análise Descritiva Quantitativa (ADQ)**1957 Quadro 1- Termos descritores, definições e descrição do material de referência para extremos  
1958 da escala.

<b>Atributo</b>	<b>Termo Descrito</b>	<b>Definição</b>	<b>Referência para os extremos da escala</b>
Aparência	1. Cor da casca	Coloração característica de bolo que podem variar de bege claro a marrom escuro, dependendo dos ingredientes presentes.	Clara (fraco): Bolo de sabor coco, marca Italac. Escura (forte): Bolo de chocolate, marca: Pullman.
	2. Cor da massa	Coloração da massa dos bolos que podem variar de bege claro a marrom escuro, dependendo dos ingredientes presentes.	Clara: Bolo sabor baunilha, marca Vitarella. Escura: Bolo de chocolate, marca: Pullman.
	3. Brilho da casca	Capacidade reflexão a luz, com aparência brilhante ou opaco da casca do bolo.	Opaco: Biscoito de maisena, marca: Vitarella. Brilhante: Bolo de chocolate, marca: Migra.
	4. Uniformidade da casca	Homogeneidade da casca em relação à presença de bolhas de ar	Pouca: Queijo suíço, marca: Muito: Bolo de sabor chocolate, marca: Migra
	5. Tamanho das bolhas na massa	Tamanho das cavidades formadas pelas bolhas de ar	Pequena: Bolo de chocolate, marca: Pullman. Grande: Queijo suíço, marca: Raclette.
	6. Estrutura do massa	Atributo de aparência em relação à estrutura do centro do bolo	Leve: Bolo sabor baunilha, marca Vitarella. Pesado: Bolo embatumado.
Aroma	7. Chocolate	Aroma de chocolate percebido por aspiração, antes do produto ser colocado na boca.	Nenhum: Água mineral. Muito: Bolo de chocolate, marca: Baducco.
	8. Coco	Aroma volátil de coco percebido por aspiração, antes do produto ser colocado na boca.	Nenhum: Água mineral. Muito: Bolo de coco, marca: Italac.

	9. Bocaiuva enriquecida com cogumelo comestível	Aroma volátil de bocaiuva enriquecida com cogumelo comestível por aspiração, antes do produto ser colocado na boca.	Nenhum: Água mineral. Muito: Bocaiuva enriquecida com cogumelo comestível
	10. Farinha de bocaiuva sem cogumelo comestível	Aroma volátil de bocaiuva por aspiração, antes do produto ser colocado na boca.	Nenhum: Água mineral. Muito: Farinha de Bocaiuva sem cogumelo comestível.
Textura	11. Maciez	Força mínima necessária para comprimir a amostra entre os dentes	Pouca: Torrada, marca: Bauducco. Muita: Marshmallow, marca Haribo.
	12. Umidade	Sensação provocada pela quantidade de água presente no bolo	Pouca: Biscoito de maisena Muita: 1 fatia de bolo comercial sabor chocolate umedecida com 10 mL de leite/ Bolo de chocolate, marca: Pullman.
	13. Fraturabilidade	Intensidade de força pela qual o material quebra ou fratura.	Pouca: Bolo de chocolate, marca: Bauducco. Muita: Pé de moleque, marca DaColônia.
	14. Adesividade	Capacidade do produto em se aderir ao dente durante a mastigação.	Pouca: Biscoito de água e sal, marca: vitarella. Muita: Bala de caramelo de leite, marca: Butter Toffees.
	15. Gomosidade	Força necessária para desintegrar a massa do alimento, obtida durante a mastigação, até que atinja o ponto de engolir.	Pouca: Biscoito de maisena, marca Vitarella. Muita: Farinha de bocaiuva sem cogumelo.
Sabor	16. Doce	Sabor adocicado	Pouco: Miolo de pão de forma, marca: Pullman. Muito: Doce de coco, marca DaColônia.
	17. Chocolate	Intensidade do sabor de chocolate, percebido quando é colocado na boca e sentido pela língua por meio das papilas gustativas.	Nenhum: Água mineral. Muito: Bolo de chocolate, marca: Bauducco.

	18. Coco	Característica do sabor de coco, percebido dentro da boca, durante a mastigação.	Nenhum: Água mineral. Muito: Bolo de coco, marca: Italac.
	19. Farinha de Bociuva enriquecida com cogumelo comestível	Intensidade do sabor Bociuva enriquecida com cogumelo comestível percebido dentro da boca durante a mastigação.	Nenhum: Água mineral. Muito: Farinha de bociuva com cogumelo comestível.
	20. Farinha de bociuva sem cogumelo comestível.	Intensidade do sabor característico associado à presença de sabor de farinha de bociuva sem cogumelo comestível na boca durante a mastigação.	Nenhum: Água mineral. Muito: Farinha de bociuva.
	21. Amargo	Intensidade do sabor característico de amargo após a deglutição.	Nenhum: solução 0,06% de glutamato monossódico. Muito: solução de 0,02% de cafeína.

1959 Tabela 1 – Médias dos termos descritores para o atributo aparência das formulações de bolo  
1960 *low carb*.

Formulações	Cor da casca	Cor da massa	Brilho da casca	Uniformidade da casca	Tamanho das bolhas na massa	Estrutura da massa
F1	4,18±1,37 <sup>c</sup>	3,51±1,05 <sup>c</sup>	5,05±1,84 <sup>a</sup>	5,49±1,93 <sup>a</sup>	2,40±1,24 <sup>b</sup>	3,42±1,91 <sup>a</sup>
F2	4,95±1,68 <sup>c</sup>	4,20±1,50 <sup>c</sup>	4,54±1,71 <sup>a</sup>	6,10±1,56 <sup>a</sup>	4,23±1,89 <sup>a</sup>	3,96±1,70 <sup>a</sup>
F3	6,21±1,61 <sup>b</sup>	5,93±1,17 <sup>b</sup>	4,26±2,05 <sup>a</sup>	5,85±2,00 <sup>a</sup>	2,80±1,65 <sup>b</sup>	3,55±2,02 <sup>a</sup>
F4	7,67±0,78 <sup>a</sup>	7,50±0,79 <sup>a</sup>	4,40±1,70 <sup>a</sup>	6,75±1,75 <sup>a</sup>	2,42±0,98 <sup>b</sup>	3,26±2,05 <sup>a</sup>

1961 Valores são expressos com médias e desvio padrão, com letras iguais na mesma coluna não  
1962 diferem significativamente entre si ao nível de 5% ( $p > 0,05$ ) pelo teste de Tukey.

1963 Legenda: F1: Bolo *low carb* com farinha de coco. F2: Bolo *low carb* com farinha de bociuva  
1964 verde sem o incremento. F3: Bolo *low carb* com farinha de bociuva verde enriquecida  
1965 cogumelo e nitrato de amônia. F4: Bolo *low carb* com farinha de bociuva verde enriquecida  
1966 cogumelo sem suplementação.

1967 Tabela 2 – Médias dos termos descritores para o atributo aroma das formulações de bolo *low*  
 1968 *carb*.

Formulações	Chocolate	Coco	Bocaiuva enriquecida com cogumelo comestível	Farinha de bocaiuva sem o cogumelo comestível
F1	2,58±1,45 <sup>b</sup>	2,60±1,72 <sup>a</sup>	4,30±2,03 <sup>a</sup>	3,55±2,13 <sup>a</sup>
F2	3,30±2,06 <sup>b</sup>	2,95±1,60 <sup>a</sup>	3,43±1,93 <sup>a,b</sup>	3,81±2,17 <sup>a</sup>
F3	5,01±2,17 <sup>a</sup>	2,37±1,43 <sup>a</sup>	3,28±1,74 <sup>a,b</sup>	3,69±2,18 <sup>a</sup>
F4	6,19±2,33 <sup>a</sup>	2,73±2,04 <sup>a</sup>	2,75±1,59 <sup>b</sup>	2,95±1,53 <sup>a</sup>

1969 Valores são expressos com médias e desvio padrão, com letras iguais na mesma coluna não  
 1970 diferem significativamente entre si ao nível de 5% (p>0,05) pelo teste de Tukey.

1971 Legenda: F1: Bolo *low carb* com farinha de coco. F2: Bolo *low carb* com farinha de bocaiuva  
 1972 verde sem o incremento. F3: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 1973 cogumelo e nitrato de amônia. F4: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 1974 cogumelo sem suplementação.

1975

1976 Tabela 3 – Médias dos termos descritores para o atributo textura das formulações de bolo *low*  
 1977 *carb*.

Formulações	Maciez	Umidade	Fraturabilidade	Adesividade	Gomosidade
F1	5,72±1,93 <sup>a</sup>	6,08±2,09 <sup>a</sup>	3,65±2,17 <sup>a</sup>	3,55±1,91 <sup>a</sup>	4,00±2,16 <sup>b</sup>
F2	5,49±1,70 <sup>a</sup>	4,32±1,30 <sup>b</sup>	3,98±1,92 <sup>a</sup>	4,69±1,56 <sup>a</sup>	5,75±1,56 <sup>a</sup>
F3	6,15±2,02 <sup>a</sup>	4,53±1,58 <sup>b</sup>	3,99±2,30 <sup>a</sup>	3,54±1,75 <sup>a</sup>	3,71±2,04 <sup>b</sup>
F4	6,79±1,79 <sup>a</sup>	6,55±1,09 <sup>a</sup>	3,72±2,29 <sup>a</sup>	3,46±2,13 <sup>a</sup>	3,70±1,91 <sup>b</sup>

1978 Valores são expressos com médias e desvio padrão, com letras iguais na mesma coluna não  
 1979 diferem significativamente entre si ao nível de 5% (p>0,05) pelo teste de Tukey.

1980 Legenda: F1: Bolo *low carb* com farinha de coco. F2: Bolo *low carb* com farinha de bocaiuva  
 1981 verde sem o incremento. F3: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 1982 cogumelo e nitrato de amônia. F4: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 1983 cogumelo sem suplementação.

1984

1985 Tabela 4 – Médias dos termos descritores para o atributo sabor das formulações de bolo *low*  
 1986 *carb*.

Formulações	Doce	Chocolata	Coco	Bocaiuva enriquecida com cogumelo comestível	Farinha de bocaiuva	Amargo
F1	4,25±1,84 <sup>a</sup>	2,79±1,66 <sup>b</sup>	2,92±2,08 <sup>a</sup>	4,37±2,11 <sup>a</sup>	4,08±2,39 <sup>a</sup>	3,26±2,31 <sup>b</sup>
F2	3,70±1,47 <sup>a</sup>	2,42±1,48 <sup>b</sup>	2,61±1,61 <sup>a</sup>	3,23±1,98 <sup>a</sup>	4,98±2,35 <sup>a</sup>	2,01±0,96 <sup>b</sup>
F3	3,62±1,46 <sup>a</sup>	2,90±1,72 <sup>b</sup>	2,44±1,41 <sup>a</sup>	4,39±2,08 <sup>a</sup>	4,63±2,13 <sup>a</sup>	3,82±2,42 <sup>a</sup>
F4	4,84±2,05 <sup>a</sup>	4,37±2,24 <sup>a</sup>	2,66±1,68 <sup>a</sup>	3,46±2,15 <sup>a</sup>	3,73±1,66 <sup>a</sup>	2,51±1,55 <sup>a,b</sup>

1987 Valores são expressos com médias e desvio padrão, com letras iguais na mesma coluna não  
 1988 diferem significativamente entre si ao nível de 5% (p>0,05) pelo teste de Tukey.

1989 Legenda: F1: Bolo *low carb* com farinha de coco. F2: Bolo *low carb* com farinha de bocaiuva  
 1990 verde sem o incremento. F3: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 1991 cogumelo e nitrato de amônia. F4: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 1992 cogumelo sem suplementação.

### 1993 Escala Hedônica

1994 Tabela 5 – Médias dos termos descritores para o atributo aparência das formulações de bolo  
 1995 *low carb*.

Formulações	Cor	Aroma	Textura	Sabor	Avaliação Global	Intenção de compra
F1	6,73±1,83 <sup>a</sup>	6,36±2,15 <sup>a</sup>	5,73±2,03 <sup>b</sup>	5,64±1,94 <sup>b</sup>	6,23±2,05 <sup>b</sup>	2,77±1,07 <sup>b</sup>
F2	7,36±1,33 <sup>a</sup>	7,09±1,31 <sup>a</sup>	6,41±1,26 <sup>a,b</sup>	6,36±1,05 <sup>a,b</sup>	6,82±1,44 <sup>a,b</sup>	3,18±0,73 <sup>b</sup>
F3	7,32±1,43 <sup>a</sup>	7,09±1,48 <sup>a</sup>	6,50±1,44 <sup>a,b</sup>	5,86±1,42 <sup>b</sup>	6,45±1,53 <sup>b</sup>	2,95±0,72 <sup>b</sup>
F4	7,77±1,41 <sup>a</sup>	7,55±1,47 <sup>a</sup>	7,32±1,13 <sup>a</sup>	7,18±1,53 <sup>a</sup>	7,73±1,24 <sup>a</sup>	3,91±0,97 <sup>a</sup>

1996 Valores são expressos com médias e desvio padrão, com letras iguais na mesma coluna não  
 1997 diferem significativamente entre si ao nível de 5% (p>0,05) pelo teste de Tukey.

1998 Legenda: F1: Bolo *low carb* com farinha de coco. F2: Bolo *low carb* com farinha de bocaiuva  
 1999 verde sem o incremento. F3: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 2000 cogumelo e nitrato de amônia. F4: Bolo *low carb* com farinha de bocaiuva verde enriquecida  
 2001 cogumelo sem suplementação.

2002