Universidade Estadual de Londrina

Centro de Ciências Agrárias
Depto. De Ciência e Tecnologia de Alimentos
Programa de Pós-Graduação em Ciência de Alimentos

CAROLINA SAORI ISHII MAURO

FARINHAS DE FRUTOS DO CERRADO E PANTANAL BRASILEIRO: BIOACESSIBILIDADE DE COMPOSTOS FENÓLICOS, CAPACIDADE ANTIOXIDANTE E EFEITO NA MICROBIOTA COLÔNICA HUMANA

Londrina

2023

CAROLINA SAORI ISHII MAURO

FARINHAS DE FRUTOS DO CERRADO E PANTANAL BRASILEIRO: BIOACESSIBILIDADE DE COMPOSTOS FENÓLICOS, CAPACIDADE ANTIOXIDANTE E EFEITO NA MICROBIOTA COLÔNICA HUMANA

Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos, nível Doutorado, da Universidade Estadual de Londrina, como requisito parcial à obtenção do Título de Doutor em Ciência de Alimentos

Orientadora: Profa. Dra. Sandra Garcia

Londrina

2023

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática do Sistema de Bibliotecas da UEL

M457f Mauro, Carolina Saori Ishii .
FARINHAS DE FRUTOS DO CERRADO E PANTANAL BRASILEIRO: BIOACESSIBILIDADE DE COMPOSTOS FENÓLICOS, CAPACIDADE ANTIOXIDANTE E EFEITO NA MICROBIOTA COLÔNICA HUMANA / Carolina Saori Ishii Mauro. - Londrina, 2023.
166 f. : il.

Orientador: Sandra Garcia.
Tese (Doutorado em Ciência de Alimentos) - Universidade Estadual de Londrina, Centro de Ciências Agrárias, Programa de Pós-Graduação em Ciência de Alimentos, 2023.
Inclui bibliografia.

1. Alimentos funcionais - Tese. 2. Compostos bioativos - Tese. 3. Frutos brasileiros - Tese. 4. Microbiologia - Tese. I. Garcia, Sandra. II. Universidade Estadual de Londrina. Centro de Ciências Agrárias. Programa de Pós-Graduação em Ciência de Alimentos. III. Título.

CDU 641.1

CAROLINA SAORI ISHII MAURO

FARINHAS DE FRUTOS DO CERRADO E PANTANAL BRASILEIRO: BIOACESSIBILIDADE DE COMPOSTOS FENÓLICOS, CAPACIDADE ANTIOXIDANTE E EFEITO NA MICROBIOTA COLÔNICA HUMANA

Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos, nível Doutorado, da Universidade Estadual de Londrina, como requisito parcial à obtenção do Título de Doutor em Ciência de Alimentos

BANCA EXAMINADORA

Orientadora: Profa. Dra. Sandra Garcia
Universidade Estadual de Londrina

Profa. Dra. Adele Costabile
University of Roehampton

Profa. Dra. Karla Bigetti Guergoletto
Universidade Estadual de Londrina

Profa. Dra. Ieda Maria Bortolotto
Universidade Federal de Mato Grosso do Sul

Prof. Dra. Thais de Souza Rocha
Universidade Estadual de Londrina

Londrina, 09 de março de 2023

To my fiance Carlos Henrique,
for all the love and partnership.
To my family for supporting me
in all my decisions.

AGRADECIMENTOS

À minha mãe, professora Dra. Iria Hiromi Ishii, pelos ensinamentos sobre árvores e frutos nativos do Mato Grosso do Sul, pelo exemplo de superação e ética na profissão de bióloga e por me transferir este amor pela ciência e pelos frutos do Brasil.

À minha orientadora professora Dra. Sandra Garcia pela paciência e amizade ao longo destes quase nove anos de orientação, entre iniciação científica, mestrado e doutorado. Obrigada por estar sempre preocupada com nosso bem estar, estar disponível para sanar dúvidas e pelo apoio em todas as horas.

À professora Dra. Adele Costabile por ter me acolhido muito bem University of Roehampton em Londres e ter me dado todo o suporte necessário para que eu completasse minhas análises do doutorado sanduíche. Por ser um exemplo de profissional e uma mulher na ciência de excelência.

À CAPES, CNPq e Fundação Araucária, pelas bolsas de estudos concedidas.

Agradeço aos professores da University of Roehampton Maria Teresa Esposito, Yolanda Calle e Volker Behrends pelos ensinamentos neste período, à Ms. Maryame Khadiri que ajudou na parte experimental do trabalho e aos funcionários da universidade pela ajuda e apoio ao longo dos 5 meses que passei lá. Gostaria também de agradecer às professoras da Monica Barone, Patrizia Brigidi and Silvia Turroni da Universidade de Bollogna pelo auxílio nas análises de microbiota e todo o apoio fornecido para a publicação do nosso artigo científico.

Ao Professor César Ricardo Teixeira Tarley, à doutoranda Milena do Prado Ferreira e à mestranda Ingrid do Departamento de Química da Universidade Estadual de Londrina por cederem o material necessário às análises de digestão *in vitro* pelo método unificado BARGE UBM e auxílio na realização das análises.

À professora Dra. Suzana Lucy Nixdorf e ao Dr. Lycio Shinji Watanabe pelas análises cromatográficas realizadas no laboratório de Desenvolvimento de Instrumentação e Automação (DIA) da Universidade Estadual de Londrina.

À Profa Dra. Renata Kobayashi do Departamento de Microbiologia da Universidade Estadual de Londrina e aos seus doutorandos pela doação da cultura microbiana de *Escherichia coli.*

Aos docentes e funcionários do departamento de Ciência e Tecnologia de Alimentos, em especial às professoras Dra Karla Bigetti Guergoletto e Dra. Thais de Souza Rocha pelo apoio e auxílio para que eu pudesse realizar o doutorado sanduíche

no exterior.

À amigas queridas e colegas de pesquisa Fernanda Silva Farinazzo e Maria Thereza Carlos Fernandes pela parceria e cumplicidade em todos estes anos, pela parceria tanto em publicações como em idas a congressos juntas.

Aos companheiros de laboratório José Renato, Rebeca, Ana Clara, Fabíola, José Augusto, Viviane, Vinícius e Jaquellyne por deixarem o ambiente de trabalho mais leve e divertido, pelas conversas e apoio nos dias bons e nos dias mais difíceis.

Aos meus gatos, companheiros de escrita da tese e de todos os dias.

A todos que colaboraram para a realização deste trabalho e que estavam ao meu lado para que eu pudesse concluir esta importante etapa da minha vida.

ACKNOWLEDGEMENTS

To my mother Dr. Iria Hiromi Ishii for teaching me about native trees and fruits of Mato Grosso do Sul, for the example of resilience and ethics in the profession of biologist and for transferring to me this love for science and for the fruits of Brazil.

To my advisor Dr. Sandra Garcia for her patience and friendship throughout these almost nine years of supervision, including scientific initiation, master's and PhD. Thank you for always being concerned about our well-being, being available to answer every questions and for your support at all times.

To my advisor abroad Dr. Adele Costabile for accepting me so well at the University of Roehampton in London and for giving me all the support I needed to complete my doctorate analysis. For being an example of a professional and a woman in science of excellence.

To CAPES, CNPq and Fundação Araucária, for the study grants.

I would like to thank the University of Roehampton Lecturers Dr. Maria Teresa Esposito, Dr. Yolanda Calle and Dr. Volker Behrends for teaching me and for the experimental analysis performed. To Ms. Maryame Khadiri who helped with most of the pratical analysis of the work and the university staff for their help and support throughout the 5 months I spent there. I would also like to thank Dr. Monica Barone, Dr. Patrizia Brigidi and Dr. Silvia Turroni from the University of Bollogna for their help in the microbiota analyzes and all the support provided for the publication of our scientific article.

To Dr. César Ricardo Teixeira Tarley, PhD student Milena do Prado Ferreira and master's student Ingrid from the Department of Chemistry at the State University of Londrina for providing the necessary material for the *in vitro* digestion using the BARGE UBM method and assistance in carrying out the analyses.

To Dr. Suzana Lucy Nixdorf and Dr. Lycio Shinji Watanabe for the chromatographic analyzes performed at the Instrumentation and Automation Development Laboratory (DIA) at the State University of Londrina.

To Prof Dr. Renata Kobayashi from the Department of Microbiology at the State University of Londrina and her doctoral students for donating a strain of *Escherichia coli*.

To all the lecturers, professors and staff of the Department of Food Science and Technology, especially to Dr. Karla Bigetti Guergoletto and Dr. Thais de Souza Rocha

for the support and assistance so that I could carry out part of my PhD abroad.

To my dear friends and research colleagues Fernanda Silva Farinazzo and Maria Thereza Carlos Fernandes for their support and complicity over all these years, for their partnership both in publications and in going to congresses together.

To my lab mates José Renato, Rebeca, Ana Clara, Fabíola, José Augusto, Viviane, Vinícius and Jaquellyne for making the work environment lighter and more fun, for the conversations and support on the good days and the hardest days.

To my cats, my writing and everyday companions.

To all those who collaborated for the accomplishment of this work and who were by my side so that I could conclude this important stage of my life.

"Life is not easy for any of us. But what of that?
We must have perseverance and above all confidence in ourselves.
We must believe that we are gifted for something
and that this thing must be attained."

"I am among those who think that science has great beauty.
A scientist in his laboratory is not only a technician:
he is also a child placed before natural phenomena
which impress him like a fairy tale."

Marie Curie

MAURO, Carolina Saori Ishii. **Farinhas de Frutos Do Cerrado e Pantanal Brasileiro: Bioacessibilidade de Compostos Fenólicos, Capacidade Antioxidante e Efeito na Microbiota Colônica Humana**. 2023. 166 f. Tese (Doutorado em Ciência de Alimentos) – Universidade Estadual de Londrina, Londrina, 2023.

RESUMO

O Cerrado é o segundo maior bioma da América do Sul e a savana mais rica do mundo, abrigando 11.627 espécies de plantas nativas já catalogadas. O bioma Pantanal é considerado uma das maiores extensões úmidas contínuas do planeta. Segundo a Embrapa Pantanal, quase duas mil espécies de plantas já foram identificadas neste bioma e classificadas de acordo com seu potencial. Alguns estudos vêm sendo realizados para valorizar a cultura e biodiversidade local como, por exemplo, a geração de conhecimento através da pesquisa sobre as características dos frutos nativos. Diversos frutos da região do Cerrado e Pantanal são consumidos *in natura* ou processados pela população para venda no comércio local, entre eles as farinhas de frutos (polpa e amêndoa). As farinhas de polpa de jatobá (JA), amêndoa de cumbaru (CU), polpa de bocaiuva (PB), e amêndoa de bocaiuva (AB) são exemplos de farinhas comercializadas na região. Entretanto, existem poucos trabalhos científicos a respeito de suas características químicas e do valor nutritivo. Os prebióticos são ingredientes fermentáveis capazes de estimular seletivamente o crescimento de microrganismos intestinais que conferem benefícios à saúde do hospedeiro. Os frutos do Cerrado e Pantanal possuem propriedades ainda não estudadas quanto a quantidade de fibras funcionais e compostos bioativos benéficos à saúde. Com isso, o objetivo da pesquisa foi investigar a composição de farinhas de frutos do Cerrado e Pantanal, quantificar o teor de compostos bioativos e a bioacessibilidade de compostos antioxidantes, avaliar efeito na microbiota colônica *in vitro* e a fermentação de farinha de jatobá por linhagens probióticas. Os compostos fenólicos totais ao final do ensaio de bioacessibilidade foram maiores no método colorimétrico em relação à análise por HPLC. A bioacessibilidade da capacidade antioxidante pelo método DPPH reduziu após a simulação gastrointestinal e pelo método FRAP variou em relação ao valor inicial. Não foram detectados compostos pelo método ABTS•+ mostrando que houve degradação de compostos. Após a avaliação das quatro farinhas de frutas nativas brasileiras na microbiota intestinal humana em indivíduos saudáveis (HD) e pós-COVID-19 (PC), foram observadas habilidades promissoras de modulação da microbiota, com destaque para a farinha de amêndoa de bocaiuva, que favoreceu o crescimento de *Lactobacillus* e *Bifidobacterium* nos indivíduos PC. O óleo de JA (JAO) apresentou efeitos quimiopreventivos na proteção celular contra a citotoxicidade induzida pela daunorrubicina, estresse oxidativo e dano ao DNA. JA apresentou-se um substrato adequado para fermentação dos probióticos *Lacticaseibacillus casei* (LC-01), *Limosilactobacillus reuteri* (LR 92) e *Lactobacillus acidophillus* (LA-5), promovendo, após 24 h, contagens semelhantes à glicose para *L. casei* (9,01 ±0,15 log CFU/mL) e maior que a de frutooligossacarídeos (FOS) para *L. reuteri* (8,65 ±0,13 log CFU/mL). Na presença de JA, *L. acidophilus* apresentou contagens de 8,64 ±0,05 log CFU/mL, um resultado superior à glicose e que não diferiu de FOS. O crescimento de *Escherichia coli* na presença de JA foi menor que o observado no meio contendo glicose. Estes resultados fornecem novas informações sobre as propriedades bioativas das farinhas de frutas do Cerrado e do Pantanal.

Palavras-chave: *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart., alimentos funcionais, compostos bioativos, *Dipteryx alata* Vogel, *Hymenaea courbaril* L, microbiologia.

MAURO, Carolina Saori Ishii. **Brazilian Cerrado and Pantanal Fruit Flours: Bioacessibility of Phenolic Compounds, Antioxidant Capacity and Effect on Human Colonic Microbiota.** 2023. 166 p. Thesis (Doctor Degree in Food Science) – State University of Londrina, Londrina, 2023.

ABSTRACT

The Cerrado is the second largest biome in South America and it is the richest savannah in the world, harboring 11,627 species of native plants already catalogued. The Pantanal biome is considered one of the largest continuous wetlands on the planet. According to Embrapa Pantanal, almost two thousand species of plants have already been identified in this biome and classified according to their potential. Some studies have been carried out to value the local culture and biodiversity, such as, for example, the generation of knowledge through research on the characteristics of native fruits. Several fruits from the Cerrado and Pantanal region are consumed in natura or processed by the population for sale in local shops, including fruit flours (pulp and almond). The flours made from jatobá pulp (JA), cumbaru almond (CU), bocaiuva pulp (PB), and bocaiuva almond (AB) are examples of flours commercialized in the region, which are easily obtainable by consumers. However, there are few scientific works regarding its chemical characteristics and nutritional value. Prebiotics are fermentable ingredients capable of selectively stimulating the growth of intestinal microorganisms that confer health benefits to the host. The fruits of the Cerrado and Pantanal have properties that have not yet been studied regarding the functional fibers and bioactive compounds beneficial to health. Therefore, the objective of the research was to investigate the composition of flours from Cerrado and Pantanal fruits, quantify the content of bioactive compounds and bioacessibility of antioxidants, evaluate the effect on the colonic microbiota *in vitro* and the fermentation of jatobá pulp flour by probiotic strains. The total phenolic compounds at the end of the bioaccessibility test were higher in the colorimetric method compared to the HPLC analysis. The bioaccessibility of the antioxidant capacity by the DPPH method reduced after the gastrointestinal simulation and by the FRAP method it varied in relation to the initial value. No compounds were detected by the ABTS•+ method, showing that there was a degradation of compounds. After evaluating the four native Brazilian fruit flours on the human intestinal microbiota in healthy (HD) and post-COVID-19 (PC) individuals, promising microbiota modulation abilities were observed, with emphasis on bocaiuva almond flour, which favored the growth of *Lactobacillus* and *Bifidobacterium* in PC. Oil extracted from JA (JAO) showed chemopreventive effects in cellular protection against daunorubicin-induced cytotoxicity, oxidative stress and DNA damage. JA presented a suitable substrate for fermentation of the probiotics *Lacticaseibacillus casei* (LC-01), *Limosilactobacillus reuteri* (LR 92) and *Lactobacillus acidophillus (*LA-5), promoting, after 24 h, counts similar to glucose for *L. casei* (9.01 ±0.15 log CFU/mL) and greater than that of fructooligosaccharides (FOS) for *L. reuteri* (8.65 ±0.13 log CFU/mL). In the presence of JA, *L. acidophilus* showed counts of 8.64 ±0.05 log CFU/mL, a result superior to glucose and not different from FOS. The growth of *Escherichia coli* in the presence of JA was lower than that observed in the medium containing glucose. These results provide new information on the bioactive properties of Cerrado and Pantanal fruit flours.

Keywords: *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart., functional foods, bioactive compounds*, Dipteryx alata* Vogel, *Hymenaea courbaril* L, microbiology.

LIST OF FIGURES

CHAPTER I

CHAPTER II

CHAPTER III

TABLE LIST

CHAPTER I

CHAPTER II

CHAPTER III

CHAPTER IV

LIST OF ABBREVIATIONS AND ACRONYMS

a*	Red/Green Value
AABD-SH	4-Acetoamido-7-mercapto-2,1,3-benzoxadiazole
AAE	Ascorbic Acid Equivalent
AB	Bocaiuva Almond Flour
$ABTS^{\cdot+}$	2,2', azinobis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of Variance
ANVISA	Brazilian Health Regulatory Agency
AOAC	Official Methods of Analysis
ASVs	Amplicon Sequence Variants
b*	Blue/Yellow Value
BARGE	Bioaccessibility Research Group of Europe
BHI	Brain Heart Infusion
BSA	Bovine Serum Albumin
CFU	Colony Forming Unit
CIELAB	Commission Internationale d'Eclaire L* a* b*
COVID-19	Coronavirus Disease 2019
CU	Cumbaru Almond Flour
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DPDS	2,2'-dipyridyl disulfide
$DPPH^{\cdot}$	2,2-diphenyl-1-picrylhydrazy
FAO	Food and Agriculture Organization of the United Nations
FBS	Fetal Bovine Serum
FSC/SSC	Forward and side scatter signals profile
FRAP	Ferric Reducing Antioxidant Power
GABA	Gamma-aminobutyric acid
GAE	Gallic Acid Equivalent
GC-DIC	Gas chromatograph coupled to a flame ionization detector
GFP	Green fluorescent protein
HD	Healthy Donors
HPLC	High Performance Liquid Chromatography
ISO	International Organization for Standardization

IU	International Units
JA	Jatobá Pulp Flour
JAO	Jatobá Pulp Flour Oil
L*	Lightness
MCFA	Medium Chain Fatty Acids
MFI	Median fluorescence intensity
MRS	De Man, Rogosa and Sharpe
MODIS	Moderate Resolution Imaging Spectroradiometer
MUFA	Monounsaturated Fatty Acids
ND	Not Detected
P	p-value or probability value
PB	Bocaiuva Pulp Flour
PBS	Phosphate-buffered saline solution
PC	Post-COVID-19 Donors
PCoA	Principal Coordinates Analysis
PFA	Paraformaldehyde
QC	Quality Control
RDC	Collegiate Board Resolution
SCFA	Short-Chain Fatty Acid
SD	Standard Deviation
SFA	Saturated Fatty Acids
TE	Trolox Equivalent
TPC	Total Phenolic Content
TPP	Triphenylphosphine
HPLC	High Performance Liquid Chromatography
UBM	Unified BARGE Method
UNU	United Nations University
WHO	World Health Organization

SUMÁRIO/SUMMARY

1. INTRODUÇÃO GERAL

Os biomas Cerrado e Pantanal apresentam uma rica biodiversidade de fauna, flora e funga. O Cerrado é o segundo maior bioma da América do Sul, com uma área natural de 1.032.486 km^2, (IBGE, 2020), enquanto o bioma Pantanal é considerado uma das maiores áreas úmidas contínuas do planeta, com uma área natural de 132.096 km^2 (IBGE, 2020). Contudo, os biomas estão perdendo território de abrangência devido à ação humana. Imagens de satélite mostram que vastas áreas de vegetação nativa foram transformadas em áreas destinadas à agricultura e pecuária. Este fato é preocupante porque pode gerar desequilíbrios na fauna e flora das regiões (BONANOMI et al., 2019; PARANHOS FILHO et al., 2014). O Cerrado é um dos biomas mais ameaçado do Brasil atualmente porque diversas espécies animais e vegetais dessa região se encontram ameaçadas de extinção (STRASSBURG et al., 2015; MACHADO; AGUIAR; SILVA, 2023).

As frutas do Cerrado e Pantanal possuem um grande potencial para o crescimento econômico devido à sua grande diversidade e teor nutricional. Já há uma iniciativa de incentivo ao aproveitamento de frutos com retorno econômico para comunidades (BORTOLOTTO et al., 2017b; GUTIÉRREZ et al., 2023). O melhor aproveitamento dos frutos, de forma ambientalmente correta, permite o aumento da renda de muitas famílias, incentiva o desenvolvimento local sustentável das comunidades e consequentemente, promove a conservação dessas regiões e suas riquezas naturais (DAMASCENO-JUNIOR et al., 2010; BORTOLOTTO; DAMASCENO-JUNIOR; POTT, 2017, BORTOLOTTO et al., 2017b). Nesse sentido, é de extrema importância o estudo de frutos nativos da fauna brasileira para valorizar a cultura e biodiversidade local através do conhecimento gerado.

No mundo existem mais de 30 mil espécies de plantas que possuem partes comestíveis, porém apenas 20 destas plantas constituem 90% dos alimentos mais consumidos, mundialmente. Parte deste problema está associado à monotonia alimentar, em que um número limitado de alimentos é consumido com frequência, comprometendo a variedade da dieta. Assim, este hábito pode resultar em carências nutricionais, anemia, fome oculta e desenvolvimento de doenças crônicas, como diabetes (KINUPP; LORENZI, 2014).

Ainda há poucos trabalhos científicos sobre as características químicas e valor nutritivo dos frutos do Cerrado e Pantanal. Por isso, estudos são necessários para

determinar a composição em macronutrientes, vitaminas e minerais, a bioacessibilidade destes nutrientes e a utilização dos frutos no processamento e desenvolvimento de alimentos com elevado valor agregado.

As fibras alimentares correspondem aos carboidratos não digeríveis que passam para o intestino grosso (cólon) para serem fermentados. Alguns destes ingredientes alimentares podem ser classificados como prebióticos, que são substratos fermentados seletivamente por microrganismos hospedeiros, conferindo um benefício à saúde (GIBSON et al., 2017).

Dentre os prebióticos se destacam os frutooligossacarídeos (FOS), que podem estar presentes em diversas fontes vegetais. Os FOS constituem a maior classe de oligossacarídeos bifidogênicos, ou seja, são capazes de estimular seletivamente o crescimento de bifidobactérias no intestino, contribuindo assim para a proliferação de microrganismos benéficos e redução de microrganismos patogênicos (GIBSON; ROBERFROID, 1995). Os frutos do Cerrado e Pantanal podem ser estudados quanto à presença e quantidade de fibras funcionais prebióticas e compostos bioativos benéficos à saúde, de modo a contribuir com o conhecimento sobre estes alimentos.

Portanto, o presente trabalho visou abordar o potencial funcional de farinhas de frutos do Cerrado e Pantanal feitas com polpa de jatobá, polpa de bocaiuva, amêndoa de cumbaru e amêndoa de bocaiuva, através de análises de fermentação colônica *in vitro* e caracterização dos compostos bioativos.

1. GENERAL INTRODUCTION

The Cerrado and Pantanal biomes have rich fauna, flora and funga biodiversity. The Cerrado is the second largest biome in South America, with an natural area of 1,032,486 km^2, while the Pantanal biome is considered one of the largest continuous wetlands on the planet, with a natural area of 132,096 km^2 (IBGE, 2020). However, biomes are losing territory due to human action. Satellite images show that vast areas of native vegetation have been transformed into areas for agriculture and livestock. That is a worrysome fact because it generates imbalances in the fauna and flora of the regions (BONANOMI et al., 2019; PARANHOS FILHO et al., 2014). The Cerrado is one of the most threatened biome in Brazil because several animal and plant species in this region are threatened with extinction (STRASSBURG et al., 2015; MACHADO; AGUIAR; SILVA, 2023).

Cerrado and Pantanal fruits have great potential for economic growth due to their great diversity and nutritional content. There is already an initiative to encourage the use of fruits with economic return for communities (BORTOLOTTO et al., 2017b; GUTIÉRREZ et al., 2023). The best use of the fruits, in an environmentally correct way, allows an increase in the income of many families, encourages the sustainable local development of communities and, consequently, promotes the conservation of these regions (DAMASCENO-JUNIOR et al., 2010; BORTOLOTTO; DAMASCENO-JUNIOR; POTT, 2017; BORTOLOTTO et al., 2017b). Therefore, it is extremely important to study native fruits of the Brazilian flora to value local culture and biodiversity through the knowledge generated.

Worldwide there are more than 30 thousand species of plants that have edible parts, but only 20 of these plants constitute 90% of the most consumed foods. Part of this problem is associated with food monotony, in which a limited number of foods are frequently consumed, compromising the variety of the diet. Therefore, this habit can result in nutritional deficiencies, anemia, hidden hunger and the development of chronic diseases, such as diabetes (KINUPP; LORENZI, 2014).

There are still few scientific works on the chemical characteristics and nutritional value of fruits from the Cerrado and Pantanal. Therefore, studies are necessary to determine the composition of macronutrients, vitamins and minerals, the bioaccessibility of these nutrients and the use of fruits in the processing and development of foods with high added value.

Dietary fibers correspond to non-digestible carbohydrates that pass to the large intestine (colon) to be fermented. Some of these food ingredients can be classified as prebiotics, which are substrates selectively fermented by host microorganisms, conferring a health benefit (GIBSON et al., 2017).

Among the prebiotics, fructooligosaccharides (FOS) stand out, and can be found in several vegetable sources. FOS constitute the largest class of bifidogenic oligosaccharides, that is, they are able to selectively stimulate the growth of bifidobacteria in the intestine, thus contributing to the proliferation of beneficial microorganisms and reduction of pathogenic microorganisms (GIBSON; ROBERFROID, 1995). The fruits of the Cerrado and Pantanal can be studied for the presence and amount of functional prebiotic fibers and bioactive compounds beneficial to health, in order to contribute to the knowledge about these foods.

Therefore, the present work aimed to address the functional potential of flours from Cerrado and Pantanal fruits made with jatobá pulp, bocaiuva pulp, cumbaru almond and bocaiuva almond, through *in vitro* colonic fermentation analysis and characterization of bioactive compounds.

2. OBJETIVO

2.1 OBJETIVO GERAL

Investigar a composição de farinhas de frutos do Cerrado e Pantanal, quantificar o teor de compostos bioativos e a bioacessibilidade de compostos antioxidantes, avaliar efeito na microbiota colônica *in vitro* e a fermentação de farinha de jatobá por linhagens probióticas.

2.2 OBJETIVOS ESPECÍFICOS

- Determinar a composição centesimal (umidade, cinzas, lipídios, proteínas e carboidratos e teor de fibras solúveis e insolúveis) de farinha de polpa de jatobá, farinha de amêndoa de cumbaru, farinha de polpa de bocaiuva e farinha de amêndoa de bocaiuva (safras de 2019 e 2021);
- Determinar o melhor solvente extrator para análise de compostos fenólicos totais e capacidade antioxidante;
- Analisar a bioacessibilidade de Compostos Fenólicos Totais e Capacidade antioxidante pelos métodos $ABTS^{\cdot+}$, $DPPH^{\cdot}$ e FRAP após condições simuladas de passagem pelo trato gastrointestinal;
- Determinar o teor de carotenoides das amostras de farinha de polpa;
- Determinar *in vitro* o efeito das farinhas de frutos na fermentação colônica humana de doadores saudáveis e pós-COVID-19;
- Analisar o efeito do óleo de farinha de polpa de jatobá na proteção ao estresse oxidativo induzido por daunorrubicina e dano ao DNA;
- Analisar a fermentação das farinha de polpa de jatobá por cepas probióticas (*Lacticaseibacillus casei* LC-01, *Limosilactobacillus reuteri* LR 92 and *Lactobacillus acidophillus* LA-5) e *Escherichia coli* ATCC 25922.

2. OBJECTIVE

2.1 GENERAL OBJECTIVE

To investigate the composition of Cerrado and Pantanal fruit flours, quantify the content of bioactive compounds and bioaccessibility of antioxidants, and evaluate the effect on the colonic microbiota *in vitro* and fermentation of jatobá flour by probiotic strains.

2.2 SPECIFIC OBJECTIVES

- To determine the chemical composition (moisture, ash, lipids, proteins, carbohydrates and soluble and insoluble fiber content) of jatobá pulp flour, cumbaru almond flour, bocaiuva pulp flour and bocaiuva almond flour (2019 and 2021 harvests);
- To determine the best extractor solvent for analysis of total phenolic compounds and antioxidant capacity;
- To analyze the bioaccessibility of Total Phenolic Compounds and Antioxidant Capacity by $ABTS^{\cdot+}$, $DPPH^{\cdot}$ and FRAP methods after simulated conditions of passage through the gastrointestinal tract;
- To determine the carotenoid content of pulp flour samples;
- To determine *in vitro* the effect of fruit flours on human colonic fermentation of healthy and pos-COVID-19 donors;
- To analyze the effect of jatoba pulp flour oil on protection against daunorubicin-induced oxidative stress and DNA damage;
- Analyze the fermentation of jatobá pulp flour by probiotic strains (*Lacticaseibacillus casei* LC-01, *Limosilactobacillus reuteri* LR 92 and *Lactobacillus acidophillus* LA-5) and *Escherichia coli* ATCC 25922.

3. REFERENCES

1. BONANOMI, J., TORTATO, F. R., GOMES, R. S. R., PENHA, J. M., BUENO, A. S. & PERES, C. A. Protecting forests at the expense of native grasslands: Land-use policy encourages open-habitat loss in the Brazilian cerrado biome. **Perspectives in Ecology and Conservation**, v. 17, n. 1, p. 26-31, 2019.

2. BORTOLOTTO, I. M., DAMASCENO-JUNIOR, G.A. & POTT, A. Lista preliminar das plantas alimentícias nativas de Mato Grosso do Sul, Brasil. **Iheringia Série Botânica**, Porto Alegre, v. 73(supl.), p.101-116, 2017.

3. BORTOLOTTO, I. M.; HIANE, P. A.; ISHII, I. H.; DE SOUZA, P. R.; CAMPOS, R. P.; GOMES, R. J. B.; FARIAS, C. S.; LEME, F. M.; ARRUDA, R. C. O.; COSTA, L. B. L. C.; DAMASCENO-JUNIOR, G. A. A knowledge network to promote the use and valorization of wild food plants in the Pantanal and Cerrado, Brazil. **Regional Environmental Change**, V. 17, p. 1329–1341, 2017.

4. DAMASCENO-JUNIOR, G. A.; SOUZA, P. R.; BORTOLOTTO, I. M.; RAMOS, M. I. L.; HIANE, P. A.; BRAGA NETO, J. A.; ISHII, I. H.; COSTA, D. C.; RAMOS FILHO, M. M.; GOMES, R. J. B.; BARBOSA, M. M.; RODRIGUES, R. B. **Sabores do Cerrado e Pantanal: Receitas e boas práticas de aproveitamento**. 1. ed. Campo Grande: Editora UFMS. 2010. V. 1. 141p.

5. GIBSON, G.R. AND ROBERFROID, M.B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. **Journal of Nutrition**, v. 125, p. 1401 – 1412, 1995.

6. GIBSON, G. R.; HUTKINS, R.; SANDERS, M. E.; PRESCOTT, S. L.; REIMER, R. A.; SALMINEN, S. J.; SCOTT, K.; STANTON, C.; SWANSON, K. S.; CANI, P. D.; VERBEKE, K.; REID, G. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. **Nature Review Gastroenterology and Hepatology**, v. 14, n. 8, p. 491-502, 2017.

7. GUTIÉRREZ, L. A. L.; OLIVEIRA JR., C. J. F.; GOMES, R. J. B.; CAMPOS, R. P.; BORTOLOTTO, I. M.; FEHLAUER, T. J. Bioeconomy and sociobiodiversity in the agroecological perspective for the wellbeing. **Revista Brasileira de Agroecologia**, v. 18, n. 1, p. 129-150, 2023.

8. IBGE – INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. **Contas de ecossistemas: o uso da terra nos biomas brasileiros: 2000-2018/IBGE**, Coordenação de Recursos Naturais e Estudos Ambientais, Coordenação de Contas Nacionais. Rio de Janeiro, RJ, Brazil: IBGE; 2020.

9. KINUPP, V.F.; LORENZI, H. **Plantas Alimentícias Não-Convencionais (PANCs) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas**. Nova Odessa: Ed. Plantarum, 768p. 2014.

10. MACHADO, R. B.; AGUIAR, L. M. S.; SILVA JMC. Brazil: plan for zero vegetation loss in the Cerrado. **Nature**, v. 615(7951), p. 216, 2023.

11. PARANHOS FILHO, A. C.; MOREIRA, E. S.; OLIVEIRA, A. K. M.; PAGOTTO, T. C. S. & MIOTO, C.L. Análise da variação da cobertura do solo no Pantanal de 2003 a 2010 através de sensoriamento remoto. **Engenharia Sanitária e Ambiental***,* v.19, p. 69-76, 2014.

12. STRASSBURG, B. B. N.; BROOKS, T.; FELTRAN-BARBIERI, R.; IRIBARREM, A.; CROUZEILLES, R.; LOYOLA, R.; LATAWIEC, A. E.; OLIVEIRA FILHO, F. J. B.; SCARAMUZZA, C. A. M.; SCARANO, F. R.; SOARES-FILHO, B. & BALMFORD, A. Moment of truth for the Cerrado hotspot. **Nature Ecology and Evolution**, v. 1, p. 1-3, 2017.

Esta tese será apresentada na forma de quatro capítulos distintos, descritos abaixo, seguidos de uma conclusão geral e anexos.

Capítulo I – Artigo de Revisão: Frutos de Jatobá, Cumbaru e Bocaiuva: uma revisão da importância econômica, potenciais aplicações alimentícias e estudos clínicos em humanos

Capítulo II – Artigo científico: Bioacessibilidade de compostos fenólicos totais e capacidade antioxidante de farinhas de frutos do Cerrado e Pantanal

Capítulo III – Artigo científico: Farinhas de frutos do Cerrado e Pantanal afetam a composição da microbiota intestinal em indivíduos saudáveis e pós-COVID-19: um estudo piloto de fermentação *in vitro*

Capítulo IV – Artigo científico "Short Communication": Análise de Composição Química de farinha de polpa de jatobá e efeito no crescimento de bactérias probióticas

Os capítulos estão de acordo com as normas das revistas científicas que vão ser submetidos, por isso diferenças na formatação do texto podem ocorrer.

This thesis will be presented in the form of four distinct chapters, described below, followed by a general conclusion and annexes.

Chapter I – Revision Article: Jatobá, Cumbaru and Bocaiuva fruits: A review on the economic importance, potential food applications and human clinical trials

Chapter II – Original Research Article: Bioaccessibility of total phenolic compounds and antioxidant capacity of fruit flours from the Brazilian Cerrado and Pantanal

Chapter III – Original Research Article: Cerrado and Pantanal fruit flours affect gut microbiota composition in healthy and post-COVID-19 individuals: An *in vitro* pilot fermentation study

Chapter IV – Short Communication Article: Analysis of the chemical composition of jatobá pulp flour and its effect on the growth of probiotic bacteria

The chapters are in accordance with the guide for authors of the scientific journals to which they will be submitted, so differences in text formatting may occur.

Chapter I

Review Article to be submitted to the **Journal of Culinary Science & Technology**

Authors: Carolina Saori Ishii Mauro[1]* Iria Hiromi Ishii[2], Fernanda Silva Farinazzo[3], Maria Thereza Carlos Fernandes[1] and Sandra Garcia[1].

[11]Department of Food Science and Technology, State University of Londrina (UEL), Londrina 86057-970, Brazil.
[2] Department of Ambiental Sciences, Federal University of Mato Grosso do Sul (UFMS), Corumbá 79304-902, Brazil.
[3]Departament of Food Engineering, State University of Mato Grosso do Sul (UEMS), Naviraí 79950-000, Brazil.
*Corresponding author. E-mail: carol.saori@gmail.com

Jatobá, Cumbaru and Bocaiuva fruits: A review on the economic importance, potential food applications and human clinical trials

ABSTRACT

The Brazilian Cerrado and Pantanal biomes present a rich biodiversity of flora, fauna and funga. Several native fruits are nutritive options to local consumers and have a great potential for economic growth, allowing an increase in the income of many families and encouraging the sustainable local development of communities. The purpose of this study is to review literature findings on the Brazilian fruits jatobá, cumbaru and bocaiuva, its economic importance, potential food applications and health benefits based on human clinical trials. Several food preparations with sensory tests were found, with generally good acceptance of the products. Studies showed that cumbaru almond supplementations improved serum concentrations of cholesterol markers while jatobá pulp flour breads reduced the glycemic index of volunteers after consumption. Although there are studies on food formulations containing bocaiuva fruit, clinical trials involving humans were not found. This review draws the attention of culinary professionals, food scientists, chefs, nutritionists and researchers on the uses of Brazilian fruits, in order to benefit the consumers and promote conservation of these regions. The study of native fruits to diversify the local cuisine and popularize its use is a new trend in culinary science and technology.

Key words: *Acrocomia aculeata (Jacq.) Lodd. Ex Mart., Dipteryx alata* Vogel, functional foods, *Hymenaea* spp.

Título: Frutos de Jatobá, Cumbaru e Bocaiuva: uma revisão da importância econômica, potenciais aplicações alimentícias e estudos clínicos em humanos

INTRODUCTION

The Brazilian Cerrado and Pantanal biomes present a rich biodiversity of fauna, flora and funga (Figure 1). The Cerrado is the second largest biome in South America, with an area occupying 24% of the Brazilian territory (IBGE, 2020). The Brazilian Cerrado is recognized as the richest savannah in the world and a global "hotspot" of biodiversity, as it contains many endemic species, with over 4,800 plant and vertebrate species found nowhere else (Strassburg et al., 2015).

The Pantanal is considered one of the largest continuous wetlands of the planet, occupying 1.8% of the Brazilian national territory (IBGE, 2020). The Pantanal vegetation gathers botanical specimens found in the Cerrado region, estate forests, Chaco, Amazon and Atlantic Forest, with about 2,000 species of plants identified and 294 species of food plants (Pott et al., 2011; Bortolotto et al., 2017).

Despite their importance, biomes are losing territory due to human action, mainly due to agricultural activity, since vast areas have been converted into agricultural land and cattle pastures (Paranhos Filho et al., 2014, Strassburg et al., 2015; Françoso et al., 2017; Bonanomi et al., 2019, Rodrigues et al., 2022). Fauna and flora species are at risk due to destruction of the ecosystem, which makes conservation a priority to prevent the Cerrado and Pantanal environmental collapse (Strassburg et al., 2015; Bortolotto et al., 2017; Françoso et al., 2017).

Conservation and development policies must work in an integrated manner so that the natural resources of the Cerrado and Pantanal are preserved and eventually exploited economically, in a sustainable way (Strassburg et al., 2015; Bortolotto et al., 2017). This practice would also aim at the survival of species and the maintenance of environments (Paranhos Filho et al., 2014; Strassburg et al., 2015; Bortolotto et al., 2017).

Some studies have been carried out to value the culture and biodiversity of these regions, such as the generation of knowledge through research on the characteristics of native fruits (Damasceno-Junior et al., 2010; Gutiérrez et al., 2023). In the state of Mato Grosso do Sul, where the Cerrado and Pantanal predominate, several fruits of the region are consumed *in natura* or processed by the population for sale in the local market, in the form of jams, ice cream, mousses, juices, flours and cakes (Damasceno-Junior et al., 2010).

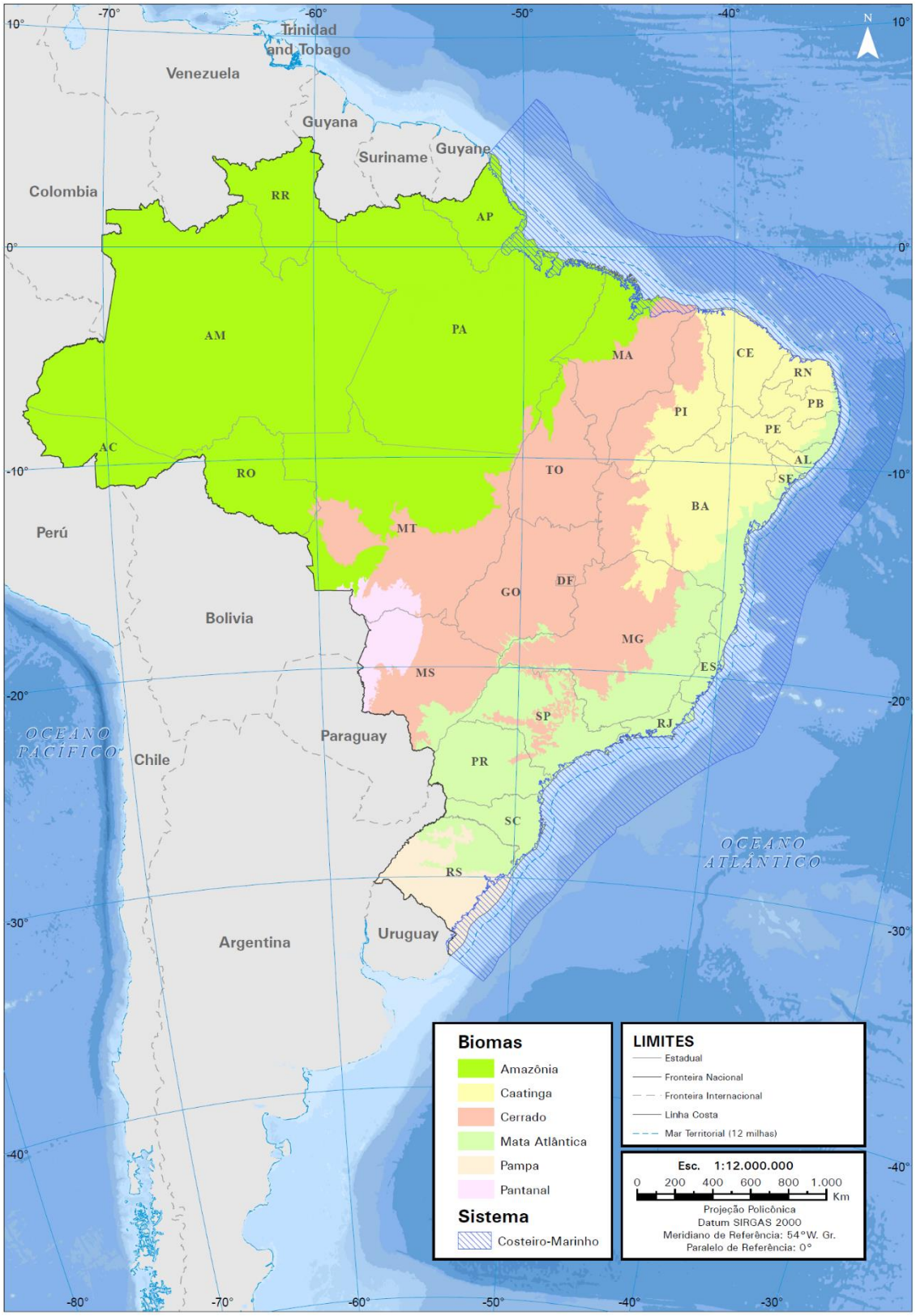


Figure 1. Map showing the six Brazilian biomes: Amazon (Amazônia), Caatinga, Cerrado, Atlantic Forest (Mata Atlântica), Pampa and Pantanal. Source: Biomas e sistema costeiro-marinho do Brasil: compatible with scale 1:12,000,000 - IBGE, 2019.

Cerrado and Pantanal fruits have a great potential for economic growth due to their great diversity. Better use of native fruits, in an environmentally correct way by commercial and extractive producers, would allow an increase in the income of many families, encourage the sustainable local development of communities and consequently promoting the conservation of these regions and its natural resources. Moreover, native fruits can provide health benefits for the consumers (Damasceno-Junior et al., 2010; Mendes et al., 2014, Melo et al., 2017).

Therefore, this work intends to provide a review of the importance of Cerrado and Pantanal fruits jatobá, cumbaru and bocaiuva, based on the current available scientific data of economic potential, food applications and human health benefits.

Search Strategy and Studies Selection

A comprehensive search of literature was carried out using the scientific eletronic databases Google Scholar, PubMed, Scopus, Science Direct and Web of Science to identify relevant original reseach papers regarding jatobá, cumbaru and bocaiuva fruits (pulp, almonds and fruit flours) from 2008 until present (15 years). Criteria for inclusion were the keywords: *Acrocomia aculeata*, bocaiuva, macaúba, *Dipteryx alata*, cumbaru, baru, *Hymenaea* spp. and jatobá. Papers were omitted if the focus of the research was not food applications or if it did not present sensory evaluation tests. Regarding the intervention studies, only clinical human trials were considered.

Cerrado and Pantanal Native Fruits

Jatobá - *Hymenaea* spp.

The species *Hymenaea* spp. corresponds to trees belonging to the family *Fabaceae* (or *Leguminosae*). The representatives of this species are: *Hymenaea courbaril* L. (jatobá-mirim), *Hymenaea stigonocarpa* Mart. (jatobá-do-cerrado) *and Hymenaea stilbocarpa* Mart. (jatobá-da-mata) (Lorenzi, 1998).

Jatobá is a typical Cerrado tree that reaches from 15 to 40 meters high, with variations according to the species. It adapts to sandy, clayey, continental and high/lowland soils, and can be found in several regions of the country, from Piauí to northern Paraná (Lorenzi, 1998; Shanley; Medina, 2005). *H. courbaril* L. occurs in the forests of the Pantanal and in areas of Cerrado in Mato Grosso do Sul, with a ripening

period from July to August, while *H. stignocarpa* Mart. occurs in the Cerrado, with ripening period from September to November (Damasceno-Junior et al., 2010). Figure 2 shows the jatobá tree in the Cerrado and Pantanal region of Mato Grosso do Sul (MS).

Figure 2. Jatobá tree (*Hymenaea* spp.) in the Cerrado and Pantanal region.
Original image from Iria Hiromi Ishii

Figure 3 shows fruits and seeds of jatobá-mirim (*H. courbaril*). The fruits are dry and hard, with a bright peel of reddish-brown color. The edible part of jatobá corresponds to 5-10 % of the fruit, with slightly greenish cream-colored pulp. The pulp can be consumed *in natura,* in juices or used to obtain flour, which is used in cakes, breads, biscuits, fried dumplings, porridges and other food preparations (Damasceno-Junior et al., 2010; Schwartz, 2018). An adult tree can produce 1000–2000 fruits (50–100 kg per year) (Schwartz,2018). Therefore, the great commercial and industrial potential of the fruit, still little explored, are acknowledge.

Besides having high nutritional potential, the jatobá pulp has farinaceous quality, which facilitates its integral or partial replacement of traditional flours, in the composition of cakes, pies and other foods, making it a viable alternative to reduce cases of malnutrition. Jatobá pulp has a high caloric value and is rich in magnesium and copper for adults and children (Damasceno-Junior et al., 2010). The nutritional value of jatobá pulp and seed was investigated by Dias et al. (2013). The pulp and seed presented low lipid content (< 6 %) and there was a significant presence of the minerals sodium, potassium and phosphorus. The main component of pulp and seed was crude fiber, with 50.02% and 72.14%, respectively, in addition to considerable vitamin C content, with 51.87 and 121.45 mg/100 g, respectively. The chemical properties highlighted good quality of the oils, presenting oxidative stability index influenced by fatty acid composition. Pulp oil was more stable due to the lower amount of polyunsaturated fatty acids (12.23%), which corresponds to half of the content of these fatty acids found.

Jatobá flour contains high soluble and insoluble fiber content. Silva et al. (2014) found that jatobá flour (*H. stigonocarpa* Mart.) presented 47.8 g/100 g of insoluble fibers and 12.8 g/100 g of soluble fibers, showing a potential that can be used in the development of products containing diet fibers. Jatobá flour also presented carotenoids such as beta-carotene and lutein and minerals such as calcium (145 mg/100g, magnesium (125 mg/100 g) and potassium (1352 mg/100 g).

Figure 3. Jatobá (*Hymenaea courbaril*) fruits, its pulp and seeds. Source: Schwartz, 2018.

Economic importance of jatobá

The practice of using jatobá by the population of the region in which it occurs is increasing. In Mato Grosso do Sul, residents of the Andalúcia settlement (Nioaque, MS) collect jatobá fruits to obtain flour from the pulp, with the bark extracting natural dyes for weaving and generating energy in place of the firewood of native trees. In the Amolar region (Corumbá, MS), tree sap is used by community residents as a fortifying drink (Damasceno-Junior et al., 2010).

In addition to being consumed by humans, animals such as deers, pacas, agoutis and monkeys usually consume jatobá fruits. Rodents paca and tapir, after eating the fruit, spread the seeds in the forest and help in the dispersal of the species. There is a practice of jatobá wood extraction due to its hard, heavy and resistant wood and with acceptance in the foreign market. However, when trees are cut down, many animals lose their food source and when there is deforestation, these animals lose their natural habitat (Shanley; Medina, 2005).

Jatobá has importance in the restoration of native forest and vegetation cover. This species is used for the recovery of degraded areas due to its characteristic of fixing

nitrogen in the soil through *Rhizobium* bacteria and the ability to develop in soils poor in nutrients and water (Schwartz, 2018).

Food applications of jatobá

According to the company Frutos do Mato, a small producer of local products in Campo Grande (MS), for the preparation of jatobá flour, the fruit is harvested, peeled and grated with a common grater for separation of pulp and seed. The pulp is processed in the domestic blender until a uniform flour is obtained. After obtaining the flour, this material is exposed to the sun for drying. Table 1 shows the food applications of jatobá fruit parts in the literature.

Abed El Kader et al. (2011) produced an integral bread containing a clear and soluble gum extracted from jatobá (*H. courbaril*) seeds. The dietetic fiber content (60.7%) of the gum was used for the formulation of integral bread to be included in a diet for dyslipidemia patients. The bread formula contained 17.60% protein, 1.19% fat and 0.97% crude fiber and the product acceptability evaluation was done by a comparison test and by a hedonic scale test. The bread prepared with a gum concentration of 7% and low fat content was selected by the panelists. In the acceptance tests, the bread with 7% fiber had acceptability of 67% among the panelists.

Silva et al. (2018) analyzed the effect of the addition of jatobá-do-cerrado flour (*H. stignocarpa* Mart.) on the glycemic response of breads, replacing wheat flour with jatobá flour in the proportions of 10, 20 and 30%. The addition of jatobá flour provided products with reduced glycemic index (low to moderate), rich in fibers, and were well accepted in the sensorial test. The treatments 10 and 20% did not differ according to the attributes: aroma, texture, flavor and overall evaluation, obtaining scores between 6 (liked slightly) and 7 (liked moderately). The bread with 30% jatobá flour had lower scores, around 5 (neither liked nor disliked). The level of acceptance by the judges decreased with the increase in the proportion of jatobá flours added to the products, due to the higher residual flavor characteristic of the fruit. Despite this, jatobá flour is a nutritional ingredient for formulations that use wheat flour, making it an option for people seeking a healthier diet (SILVA et al., 2018).

Borges et al. (2022) evaluated the use of jatoba flour to replace wheat flour in plant-based cookies with 25, 50, 75 and 100% (w/w) of jatoba flour. The cooking factor showed no significant difference between the samples, only the cookies' diameters were smaller in the samples with jatobá flour. The cookies with jatoba flour were higher in

fiber, calcium, magnesium, iron, zinc content than the control sample (100% based on wheat flour).

Health benefits

Jatobá pulp flour was investigated regarding its health benefits (Table 2), since the high fiber content of jatobá-do-cerrado indicates that it may have a low glycemic index (GI). Breads with jatobá-do-cerrado pulp flour provided reduced GI (low to moderate) in a human intervention study and can be included in the diet of individuals with chronic diseases, such as diabetes, as a way to assist in glycemic control (Silva et al., 2018).

An experimental design was carried out with 30 male volunteers who presented dyslipidemia and ingested a bread containing fibers extracted from *H. courbaril* seeds. After 6 weeks, there was a significant decrease in the concentration of triacylglycerides and VLDL-cholesterol, suggesting a possible use of *H. courbaril* gum, as a diet modification for patients with dyslipidemia (Abed El Kader et al., 2011).

Jatobá pulp can also have positive effects on the human gut microbiota, impacting on host physiology and health. Most fibers cause specific shifts in microbiota composition due to competitive interactions, and which of these compositional shifts contribute to health benefits (Makki et al., 2018). After an *in vitro* pilot fermentation study, a reduction of *Veillonellaceae* (a potential opportunistic pathogens) with jatobá pulp flour was observed in post-COVID 19 individuals (Mauro et al., 2022).

Plants parts of the genus *Hymenaea* (*Fabaceae*) are used in South American and Asian traditional medicines to treat multi-factorial diseases, such as intestinal and respiratory disorders, kidney problems, viral related disorders, inflammation conditions, among others. The bioactive properties from crude extracts and pure compounds may be involved in these uses (Boniface et al., 2017).

Cumbaru - *Dipteryx alata* Vogel

The species *Dipteryx alata* Vogel is a tree of the *family Fabaceae* (or *Leguminosae*) whose popular names are: cumbaru, cumaru, baru, barujo, coco-feijão, cumarurana, emburena-brava, coco bean or pau-cumaru (Lorenzi, 2002). It is a native Brazilian species and a characteristic plant of Cerrado, occurring mainly in areas with more fertile land. The cumbaru tree reaches heights of 15 to 25 meters and the fruits are

oval, slightly long and with only one almond inside (Figure 4) (Damasceno-Junior et al., 2010).

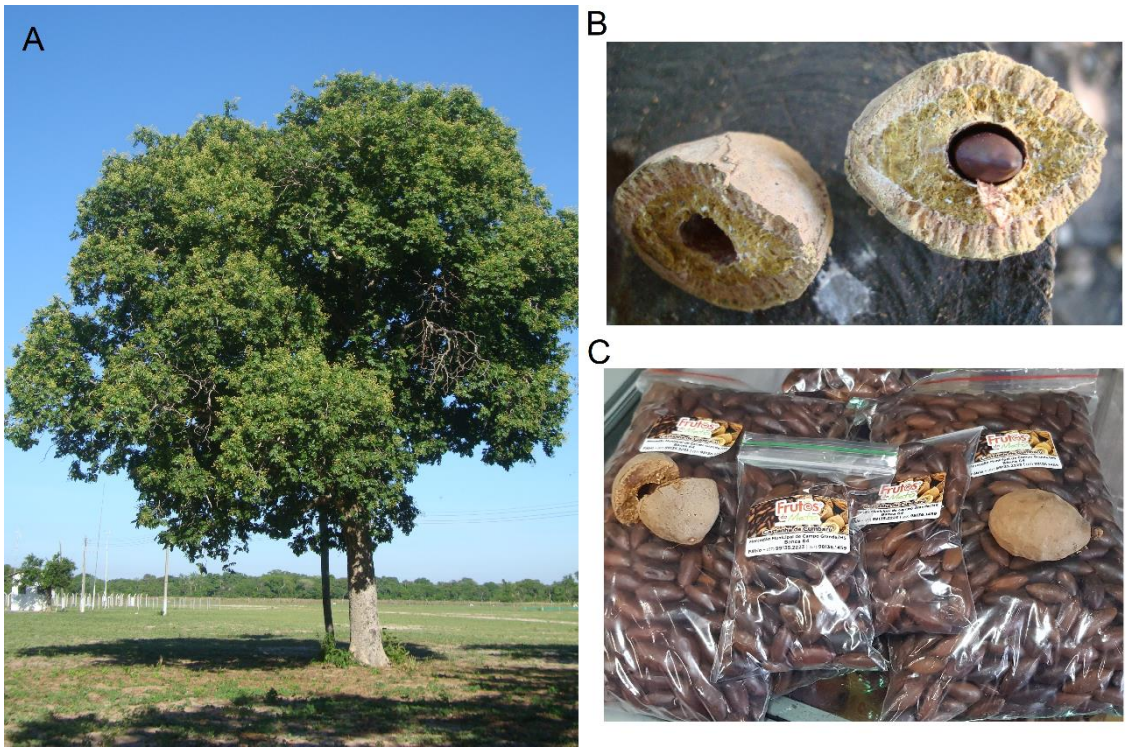


Figure 4. Cumbaru (*Dipteryx alata* Vogel) tree, dry fruit and almonds. A) cumbaru tree in the Cerrado region, B) cumbaru dry and open fruit showing an almond inside and C) almond packets found in the local market of Campo Grande – Mato Grosso do Sul. Original images from Iria Hiromi Ishii

Cumbaru has great ecological importance in the Cerrado region because its fruits ripen in the dry season and the mature pulp can feed several animals from this region, including cattle from the pastures (Sano; Brook; Brito, 2004). Therefore, the valorization of this fruit can contribute to the biodiversity of the Cerrado biome and nature conservation.

Cumbaru pulp can be consumed *in natura* or in the form of jam and liquor. Cumbaru almond is removed when breaking the pit, which is very hard. Cumbaru almond, consumed raw or toasted, resembles the flavor of peanuts and can be an ingredient of Brazilian traditional sweets such as pé-de-moleque and paçoca. In addition, cumbaru almonds are used to enrich breads, cakes, ice cream and as appetizers (Santos et al., 2012; Ministério da Saúde, 2015). However, the raw cumbaru almond contains a high trypsin inhibitor content, an anti-nutritional factor that makes it difficult

for the human body to absorb nutrients. Therefore, it is recommended to roast the almond to inactivate this antinutritional factor (Carazza; Ávila, 2010).

The almond has high levels of calcium, potassium, phosphorus and magnesium (Takemoto et al., 2001; Carazza; Ávila, 2010), it is considered rich in protein, manganese, zinc, copper and iron and source of potassium and calories (Damasceno-Junior et al., 2010). The protein content found is 23.9 g/100 g of food, higher than that found in other nuts and seeds, such as cashew nuts (18.5 g/100g), Brazil nuts (14.5 g/100 g) and walnut (14 g/100 g) (TACO, 2011). The cumbaru nut has lipid content lower than the mentioned almonds, with 38.2% of lipids in its composition (WHO, 2015; TACO, 2011).

Economic importance of cumbaru

Cumbaru fruits are collected mostly to obtain the almond, since it has more economic potential. The structure of the cumbaru production chain in the state of Mato Grosso (Poconé) is composed of agents producing descendants of indigenous peoples, quilombolas, families who develop activities in agriculture and families working on farms as collaborators (Melo et al., 2017). Similarly, women in the southwestern region of Mato Grosso obtain their remuneration through the sustainable extractivism of Cerrado's native fruits, which contributes to their family income. Therefore, this practice plays an important role in recognizing the women's workforce and gaining citizenship (Mendes et al., 2014).

In Mato Grosso do Sul, the extraction of the almond is important for the valorization of natural resources by the residents of settlements, increased income and self-esteem, especially of women in the region (Bortolotto et al., 2021). The generation of income through the commercialization of almonds has led residents to conserve cumbaru trees and protect young plants (Damasceno-Junior et al., 2010). According to the company Frutos do Mato (Figure 3-C), for yield of 1 kg of cumbaru nuts are used 25 to 30 kg of the fruit. There are many collection losses due to the natural infestation of insects that eat the fruit and can reach its interior, consuming the almond.

This extractive activity mentioned has great importance in the Cerrado and Pantanal regions, as it contributes to the preservation of the species, income generation and introduction of foods with high nutritional value, because cumbaru almonds are rich in macro and micronutrients. (Takemoto et al., 2001; Carazza; Ávila, 2010).

Food applications of cumbaru

According to the company Frutos do Mato (Campo Grade, MS), to produce cumbaru almond flour the dry fruits are opened with a specific machine that removes the almond, made locally for this purpose. After this process, the almond is roasted at 270 °C in a conventional oven, in which 1 kg of almonds are toasted for 20 minutes. The flour is obtained by processing the toasted almond with the peel in a domestic blender, until it reaches the appearance of a uniform flour.

Some food preparations were developed using cumbaru almonds (Table 1). Pagliarini et al. (2018) added cumbaru almond flour (BF) to produce reduced-fat cumbaru cupcakes. Four different cupcake formulations (70% wheat flour + 30% BF) were produced with reductions of 50, 75 and 100% margarine, compared to a control with 100% of wheat flour and 100% margarine. The cupcakes developed are considered "*light*", with the reduction of more than 30% margarine and *trans* fatty acids. In the sensory analysis, formulation with 75% margarine reduction had good acceptance.

The acceptability of paçocas prepared with cumbaru almonds was investigated in different proportions of peanuts and cumbaru almonds of 75:25, 50:50 and 25:75, respectively. The paçocas with 25% cumbaru almond had the best performance, in relation to the overall acceptance, indicating that this is the proportion indicated not to promote sensory alterations and the consequent acceptance of the product. This paçoca presented the lowest energy density and the highest concentration of total dietary fiber, compared to the paçoca traditionally made with peanuts (Santos et al., 2012).

An ice cream based on cumbaru almonds was developed and results showed that most consumers evaluated the attributes appearance, texture and flavor around the score 8 ("I liked it very much") and 92% declared purchase intention. The addition of cumbaru almonds increased lipid contents by 53.8% and protein contents by 45.5%, compared to the standard formulation. The zinc content was six times higher in cumbaru ice cream and the caloric value was 21.4% higher than the standard formulation. The study indicated the advantage of using cumbaru almond in a nutritious food product that pleased many consumers (Pinho et al., 2015).

Health benefits

Cumbaru almonds have considerable contents of monounsaturated fatty acids (MUFA), dietary fiber, vitamin E and zinc, which could exert positive effects on human health (Table 2). A clinical human trial showed that cumbaru almond improved lipid

profile (serum lipids and markers of oxidation) in mildly hypercholesterolemic subjects (Bento et al., 2014). In randomized, placebo-controlled clinical trials, the consumption of 20 g of cumbaru almonds promoted increases in high-density lipoprotein concentrations, and a reduction of abdominal adiposity and serum cholesteryl ester transfer protein after 8 weeks in overweight and obese women. An increase in the enzyme glutathione peroxidase activity were also observed, indicating an important role in reducing the oxidative stress and inhibition of inflammation associated with obesity, since this enzyme is involved in the cellular antioxidant defense system (Souza et al., 2018; Souza et al., 2019).

Regarding the cumbaru oil consumption, its supplementation was investigated on inflammation, oxidative stress, body composition, lipid profile, and plasma fatty acids of hemodialysis patients. The supplementation of 5 g cumbaru almond oil per day decreased ultra-sensitive C-reactive protein (us-CRP) concentration, an inflammatory marker. However, the supplementation was not effective in improving body composition, lipid profile, and oxidative stress (Schincaglia et al., 2020).

Bocaiuva - *Acrocomia aculeata* (Jacq) Lodd. Ex Mart.

The species *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. is a palm tree belonging to the family *Arecaceae*, popularly known as: macaúba, macaúva, bocaiuva, coco-de-catarro, coco-de-espinho, coco-baboso, macaíba, macacaúba, macajuba, macaibeira, mucajá, mucaia, mucajuba or chiclete-de-baiano (LORENZI et al., 2000). The bocaiuva occurs in almost all states of Brazil (from Pará to São Paulo and Mato Grosso do Sul) and in nearby countries such as Bolivia, Paraguay and Argentina, mainly in areas of open vegetation such as cerrado, semideciduous forest and troubled forests (Lorenzi et al., 2000).

The palm tree has a thorn-coated stem and can reach heights from 10 to 15 meters. The fruits of the bocaiuva are round, with peels green to orange and brown when ripe, hard-shelled, measuring 2 to 4 cm in length by 3 to 5 cm in diameter. The mature pulp is yellow and have a viscous and fibrous texture. The fruit contains a round, hard, white and oily almond, measuring from 1 to 2 cm (Damasceno-Junior et al., 2010). Figure 5 shows the bocaiuva palm tree and its fruits.

Figure 5. Bocaiuva palm (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.) and its intact and open fruits, showing its yellow pulp. Original images from Iria Hiromi Ishii

Bocaiuva is easily found in the Cerrado and Pantanal regions and is widely used by the local population (Lescano et al., 2021). The fruit can be consumed in fresh form or in the processed form of flour and incorporated into preparations such as cakes, biscuits, sweets, porridges, ice cream, etc. (Damasceno-Junior et al., 2010). Pulp flour can be a substitute for wheat flour, resulting in a fiber source food (Kopper et al., 2009).

The pulp has a high energy value and can be a natural source of minerals such as copper, potassium and zinc and β-carotene, an antioxidant carotenoid pigment. Ramos et al. (2008) evaluated the nutritional quality of bocaiuva pulp and concluded that among the evaluated minerals, the highest concentration was potassium (766.37±18.36 mg/100g), followed by calcium (61.96±2.30 mg/100g) and phosphorus (36.70 mg/100 g). When relating the results of minerals to the Recommended Daily Intake (RDI), bocaiuva pulp can be classified as rich in copper for children, a source of zinc and potassium for children and source of copper and potassium for adults. The potassium content was twice that found in bananas (333.4 mg/100 g) and passion fruit (380.0 mg/100 g), fruits that are considered important sources of this mineral. The whole pulp was also rich in β-carotene (49.0±2.0 μg/g). These nutrients found in bocaiuva pulp may contribute to the enrichment of the regional diet in food supplementation programs (Ramos et al., 2008).

Bocaiuva almonds have a high content of lipids, proteins and fibers and are rich in calcium, phosphorus and manganese, compared to other almonds such as cashew and coconut (Hiane et al., 2006). The oil extracted from the bocaiuva almond is colorless, aromatic and resembles coconut oil, with potential use in culinary preparations. The bocaiuva almond was analyzed for the presence of anti-nutritional factors to determine its safe consumption. No trypsin and chymotrypsin inhibitors were found, indicating that this almond can be consumed *in natura*, without the need for roasting (Munhoz et al., 2018).

Table 1. Food Applications and sensory evaluation of Cerrado and Pantanal fruit pulp, almonds or flours.

Species	Edible part	Product	Formulations	Participants	Sensory evaluation	Results	Authors
Dipteryx alata Vogel	Almond	Paçoca (typical Brazilian sweet)	Proportions of peanut and baru nuts of 75:25, 50:50, and 25:75, respectively	40 untrained adults	9-point hedonic scale test (flavor, aroma, texture and overall acceptance)	Paçocas made with 25% of baru nuts showed the best results for global acceptance compared to the control	Santos et al., 2012
	Almond partially deffated flour (PBDF)	Gluten free cake	Cakes were prepared with 100% wheat flour and with 100% PDBF and four different levels of xanthan gum (0%-PDBF cake, 0.1%-X1, 0.2%-X2 and 0.3%-X3 cakes)	No information	hedonic scale test (appearance, flavor, texture and overall acceptance)	No difference was found among the treatments for texture and appearance. Flavor of X2 and X3 cakes were more accepted than wheat flour cake	Pineli et al., 2015
	Almond	Ice cream	One standard formulation of ice cream and other containing cumbaru almonds (roasted baru almonds partially crushed were added in the proportion of 260 g for 10L of ice cream)	91 volunteers	9-point hedonic scale (appearance, texture and taste) and purchase intention test	More than 85% of the testers showed good acceptance of the appearance, texture and flavor and 92% stated purchase intent	Pinho et al., 2015
Acrocomia aculeata	Pulp flour	Cookie	Six cookie formulations where bocaiuva pulp flour replaced wheat flour at the 10% and 15% (w/w)	50 untrained adults, both genders	9-point hedonic scale test	All cookie formulations were well accepted sensorially, with overall acceptance scores around 6 (I liked it slightly)	Kopper, 2009
	Almond	Cereal bar	Formulation containing 15% (w/w) of bocaiuva almond and a control	45 untrained adults	5-point hedonic scale test, preference analysis and descriptive quantitative analysis	The product acceptance was 88.90% and the preference test showed 71.11% approval. The test bar obtained better accepted attributes compared to the control	Dessimoni-Pinto et al., 2010

	Pulp flour	Cupcake	Five formulations containing 0%, 5%, 10%, 15% and 20% (w/w) of bocaiuva pulp flour	43 untrained children of both genders, aged between 7 and 10 years	7-point hedonic facial scale test (appearance, aroma, taste, texture, color and overall acceptance) and a 5-point purchase intent	The products up to 20% bocaiuva flour addition were well accepted by children, with similar scores to the standard product	Vieira et al., 2017
	Pulp flour	Alfajor	Six formulations containing 0%, 6%, 12%, 18% and 24% (w/w) of bocaiuva pulp flour	60 untrained children of both genders, aged between 7 and 11 years	7-point hedonic facial scale test (appearance, aroma, taste, texture, color and overall acceptance) and a 5-point purchase intent scale	An addition of up to 12% bocaiuva flour in alfajores (reduction of 40% wheat flour) was well accepted by children	Rodrigues et al., 2017
	Pulp	Sauce	Three formulations containing 22.7% bocaiuva pulp and 0.1% xanthan gum, 0.1% gum arabic or no gum addition (control)	60 untrained adults of both genders, aged from 17 to 60 years	9-point hedonic scale test (color, acidity, flavor, aroma, viscosity and global acceptance)	There were no differences for all evaluated sensory attributes, with scores close to 7 ("I liked it moderately"). The use of pulp and gums as an emulsifier did not negatively affect the product	Fonseca et al., 2021
Hymenaea courbaril	Soluble gum extracted from seed	Bread	Bread formulations with soluble gum extract at 3.57%; 5.89%; 7.0% and 8.20% of dietary fiber	100 untrained adults (paired preference test) and 30 panelists (hedonic scale test)	Paired preference test and 4-point hedonic scale test	59% of the panelists selected the bread with 7% fiber, which was chosen to perform acceptance tests. The acceptability was 67%among the panelists	Abed El Kader et al., 2011

Hymenaea stignocarpa Mart.	Pulp flour	Bread	Bread formulations with jatobá-do-cerrado flour at 10, 20 and 30% (w/w) to replace wheat flour	99 untrained adults, of both genders	9-point hedonic scale test (color, odor, taste and overall acceptability)	Breads with 10% and 20% of flour had overall acceptance scores ranging from 6 (liked it slightly) to 7 (liked it moderately). The bread with 30% showed the lowest mean score of approximately 6.	Silva et al., 2018

Table 2. Human clinical trials with the Cerrado and Pantanal fruits cumbaru and jatobá.

Species	Edible Part	Subjects	Dosage	Study Design	Study Duration	Dependent Variables	Results	Authors
Dipteryx alata Vogel	Almond	20 mildly hypercholesterolemic subjects, total cholesterol (TC) 5.8 ± 0.2 mmol/L	20 g/day	Randomized, crossover, placebo-controlled clinical trial	2 periods of 6 weeks each and a 4-week washout period between treatments	TC, low-density lipoprotein cholesterol (LDL-c), non-high-density lipoprotein cholesterol (non-HDL-c), oxidation biomarkers	Baru almond supplementation reduced serum concentrations of TC, non-HDL-c and LDL-c. • The oxidation biomarkers did not change.	Bento et al., 2014
	Almond	46 overweight and obese women (age: 40 ± 11 years; body mass index: 33.3 ± 4.3)	20 g/day	Randomized, placebo-controlled clinical trial	8 weeks	Waist circumference, cholesteryl ester transfer protein expression, high-density lipoprotein concentrations	Reduction of abdominal adiposity and serum cholesteryl ester transfer protein. Increase in high-density lipoprotein concentrations	Souza et al., 2018
	Almond	46 overweight and obese women (age: 40 ± 11 years; body mass index: 33.3 ± 4.3)	20 g/day	Randomized, placebo-controlled clinical trial	8 weeks	Malondialdehyde, adiponectin, tumor necrosis factor-α, interleukin-6, interleukin-10, antioxidant enzymes activities (catalase; glutathione peroxidase; superoxide dismutase), and minerals in plasma samples.	Baru almond supplementation increased the glutathione peroxidase activity	Souza et al., 2019
	Almond oil	29 Hemodialysis patients (50.5 ± 2.2 years)	5 g almond oil (n=17) or placebo, (n=12)	Randomized, double-blind, placebo-controlled clinical study	12-weeks	Body composition, renal function, ultra-sensitive C-reactive protein (us-CRP), oxidative stress, plasma fatty acids, and lipid profile	Baru almond oil supplementation decreased us-CRP concentration	Schincaglia et al., 2020
Hymenaea courbaril	Soluble gum extracted	30 male volunteers with dyslipidemia (age: 40-50 years,	Bread containing *H. courbaril*	Single blind study	6 weeks	Lipid profile (triacylglycerides, total cholesterol, VLDL-	Significant decreases in the concentrations of triacylglycerol and	Abed El Kader et al., 2011

	from seed	plasma cholesterol >200 mg/dL and triacylglycerides >150 mg/dL)	gum (7.0%) compared to control bread			cholesterol, LDL-cholesterol, and HDL-cholesterol)	VLDL-cholesterol were observed. These findings suggest a possible use of *H. courbaril* gum, as a diet modifier for dyslipidemic patients.	
Hymenaea stignocarpa. Mart.	Pulp flour	11 healthy female volunteers (age: 27.4 ± 2.7, body-mass index: f 22.8 ± 3.6 $kg.m^{-2}$)	Bread with jatobá flour at 10, 20 and 30% (w/w)	Single blind study	130 min	Plasma glucose, glycemic index (GI)	Breads with 20% jatobá-do-cerrado flour addition promoted significant reduction (22%) in GI from 70 (control) to 54.3, while 30% addition presented GI of 57.4 and 10% addition a GI of 62.5.	Silva et al., 2018

Economic importance of bocaiuva

Bocaiuva flour is obtained by removing the pulp, dehydrating it in the sun and grinding until smaller particles are formed (Damasceno-Junior et al., 2010). The pulp flour is an important source of lipids, carbohydrates, fibers and vitamin A precursors and, therefore, can be used in the elaboration of new foods as a natural source of these nutrients (Kopper et al., 2009).

The oil extracted from bocaiuva pulp has the potential economic viability of biodiesel production in Brazil if the domestication of the species occurs and improvement in processing to ensure its availability for long-term use (Lopes et al., 2013). However, the extractivism destined to produce fuel can go against the principle of food generation, due to the potential use of bocaiuva as a palatable and nutritious food. In this sense, the production of biodiesel from edible vegetable oils has been criticized by non-governmental organizations (NGOs) for the conversion of food into fuel, while millions of people suffer from hunger and malnutrition (Tan et al., 2010).

Food applications of bocaiuva

The food preparations with bocaiuva fruit parts are shown in Table 1. Due to the nutrient content of bocaiuva pulp and almond, its use in culinary preparations can bring benefits to the health of consumers, besides promoting the valorization of a Brazilian fruit. Bocaiuva pulp flour was added in the formulation of a cookie type and its sensory characteristics were analyzed. Six biscuit formulations were elaborated in which bocaiuva pulp flour (FB) replaced wheat flour in the proportion of 10 to 15 %. The formulations containing 15% FB resulted in biscuits classified as food fiber sources, containing 3.46 to 3.88 g fibers in 100 g of product. The scores obtained in the sensory evaluation showed that all formulations, both sweet and salty, obtained acceptance scores around 6, which corresponds to "slightly liked" (Kopper et al., 2009).

The sensory acceptance of cupcakes containing FB by children aged 7 to 10 years old was analyzed through formulations without addition (standard) up to 20% of FB addition. Results showed that the addition of up to 20% FB in cupcakes was well accepted by the participants, with scores similar to the standard product (Vieira et al., 2017).

The addition of FB in alfajores showed that the overall acceptance by children aged 7 to 11 years was higher for the product containing up to 12% FB compared to products

containing 18 to 24% FB. Scores for all attributes (aroma, color, flavor, texture and appearance) were concentrated above 5 ("good") on a scale from 1 to 7 and note 4 ("I liked") for global acceptance, with a scale from 1 to 5 (Rodrigues et al., 2017). The studies cited aimed at reducing refined wheat flour in products intended for children's consumption, showing that bocaiuva flour can be a nutritional substitute for these foods.

The bocaiuva almond was analyzed for its application in a cereal bar. The elaborated product is a food source of lipids and energy, with a high potential for acceptance and preference compared to the control. The acceptance rate of the cereal bar formulated was 88.90% and the preference test showed 71.11% approval by the participants of the research. The bocaiuva almond conferred a higher average score of the attribute "flavor", evidencing its acceptability (Dessimoni-Pinto et al., 2010).

Health benefits

Acrocomia aculeata plant parts have bioactive compounds which can bring benefits to human health. Although there are studies on food formulations containing bocaiuva fruit, clinical trials involving humans were not found. The presence of the fatty acids linolenic acid (omega 3) content found in the pulp, flour and bocaiuva almond showed high values when compared to values of other oils such as olive, sunflower and corn (Hiane et al., 2005). There are documented scientific research involving phytochemicals found in pulp, kernel and leaves, such as rutin, quercetin and tocopherols, that are responsible for anti-inflammatory, antioxidant and antimicrobial, hypolipidemic and diuretic effects (Lescano et al., 2021). However, these studies involve cell culture or animal models.

Bocaiuva pulps and kernel cakes presents high levels of dietary fiber, high antioxidant activity and prebiotic activity *in vitro* (Andrade et al., 2020). An *in vitro* pilot fermentation study concluded that bocaiuva almond flour led to an enrichment of *Lactobacillus* and *Bifidobacterium*, showing a relevant prebiotic potential that should be further investigated (Mauro et al., 2022).

For a health claim to be considered scientifically relevant, clinical human intervention studies must be conducted to confirm the potential according to the content of bioactive compounds present in the fruit. This is a gap of knowledge since there are no human intervention studies available in the scientific literature and new research data would contribute to the knowledge and the application of this species.

Conclusions

Incentives to carry out studies and research for the development of technologies, methods and processes for more efficient handling, processing and conservation of foods are extremely important to fulfill their social function. There are increasing research papers on the use of the Cerrado and Pantanal fruits in food and for human health promotion. Bocaiuva presented more uses of the plant parts among the fruits studied, with food products as cookie, cereal bar, cupcake, alfajor and sauce. Cumbaru almond and partially deffated flour from the almond were used to prepare paçoca, ice cream and gluten free cake. Jatobá pulp flour and gum extract from seeds were used in bread formulations. However, there are still little scientific data on clinical human trials to collaborate with research on jatobá and cumbaru potential health benefits. Despite its popularization in food preparations, bocaiuva fruits do not present scientific studies involving humans.

Author contributions

Conceptualization: C.S.I.M, S.G. and I.H.I; Writing - Original Draft: C.S.I.M and M.T.C.F; Writing - Review & Editing: S.G., I.H.I., F.S.F. and M.T.C.F. Visualization: I.H.I. provided the pictures. All authors have read and agreed to publish the manuscript.

Acknowledgments

We would like to thank the National Council for Scientific and Technological Development (CNPq) and Fundação Araucária for the study grants, Frutos do Mato for the information about fruit flours provided and the State University of Londrina.

Declaration of interest statement

The authors declare no conflict of interests.

References

1. Abed El Kader, D., Pinto G. L., Martínez M., Cano-Ponce C., Reyna, N. & Larrazaval, M. (2011). Effect of the intake of an integral bread formulated with gum from *Hymenaea courbaril* on the concentrations of blood lipids of patients with mild-moderate dyslipidemia. *Investigación Clínica*, 52(2),140-9. Spanish. Available in: http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S05355133201100020004&lng=es&nrm=iso

2. Andrade, A. C., Marinho, J. F. U., de Souza, A. C., de Sousa, T. T., Dias, D. R., Schwan, R. F., Nunes, C. A. & Bastos, S. C. (2020). Prebiotic potential of pulp and kernel cake from Jerivá (*Syagrus romanzoffiana*) and Macaúba palm fruits (*Acrocomia aculeata*). *Food Research International*, 136, 109595. doi: http://10.1016/j.foodres.2020.109595

3. Bento, A. P., Cominetti, C., Simões Filho, A. & Naves, M. M. (2014). Baru almond improves lipid profile in mildly hypercholesterolemic subjects: a randomized, controlled, crossover study. *Nutrition, Metabolism & Cardiovascular Diseases,* 24(12), 1330-6. doi:https://doi.org/10.1016/j.numecd.2014.07.002

4. Bonanomi, J., Tortato, F. R., Gomes, R. S. R., Penha, J. M., Bueno, A. S. & Peres, C. A. (2019). Protecting forests at the expense of native grasslands: Land-use policy encourages open-habitat loss in the Brazilian cerrado biome. *Perspectives in Ecology and Conservation*, 17 (1), 26-31. doi: https://doi.org/10.1016/j.pecon.2018.12.002

5. Boniface, P. K., Ferreira, S. B. & Kaiser, C. R. (2017). Current state of knowledge on the traditional uses, phytochemistry, and pharmacology of the genus *Hymenaea. Journal of Ethnopharmacology*, 206, 193-223. doi: https://doi.org/10.1016/j.jep.2017.05.024

6. Borges, A. V. C., Romão, B.; Zandonadi, R. P. (2022). Jatobá (*Hymenaea* sp.) as a wheat substitute in plant-based cookies to improve its nutritional quality. *Research, Society and Development*, 17, e190111739085. doi: https://doi.org/10.33448/rsd-v11i17.39085

7. Bortolotto, I. M., Damasceno-Junior, G.A. & Pott, A. (2017). Lista preliminar das plantas alimentícias nativas de Mato Grosso do Sul, Brasil. *Iheringia Série Botânica*, Porto Alegre, 73(supl.):101-116. doi: https://doi.org/10.21826/2446-8231201873s101

8. Bortolotto I. M., Hiane, P. A., Ishii, I. H., De Souza, P. R., Campos, R. P., Gomes, R. J. B., Farias, C. S., Leme, F. M., Arruda, R. C. O., Costa, L. B. L. C. & Damasceno-Junior, G. A. A. (2017). A knowledge network to promote the use and valorization of wild food plants in the Pantanal and Cerrado, Brazil. *Regional Environmental Change*, 17, 1329–1341. https://doi.org/10.1007/s10113-016-1088-y

9. Bortolotto, I. M., Ziolkowski, N. E., Gomes, R. J. B., Almeida, F. S., Campos, R. P. & Aoki, C. (2021). Mulheres em Rede: Conectando Saberes Sobre Plantas Alimentícias do Cerrado e Pantanal. *Ethnoscientia*, 6(2), 198-232. http://dx.doi.org/10.18542/ethnoscientia.v6i2.10374

10. Carazza, L. & Ávila, J. C. C. (2010). *Manual tecnológico de aproveitamento integral do fruto do baru*. Brasília: ISPN.

11. Damasceno-Junior, G. A., Souza, P. R. Bortolotto, I. M., Ramos, M. I. L., Hiane, P. A., Braga Neto, J. A., Ishii, I. H., Costa, D.C., Ramos Filho, M.M., Gomes, R. J. B., Barbosa & M. M. Rodrigues, R. B. (2010). *Sabores do Cerrado e Pantanal: conhecer para valorizar os frutos nativos; receitas e boas práticas de aproveitamento*. Editora da Universidade Federal de Mato Grosso do Sul, Campo Grande, 142 p.

12. Dessimoni-Pinto, N. A. V., Silva, V. M., Batista, A. G., Vieira, G.; Souza, C. R., Dumont, P. V. & Santos, G. K. M. (2010). Características físico-químicas da amêndoa de macaúba e seu aproveitamento na elaboração de barras de cereais. *Alimentos e Nutrição Araraquara,* 21(1), 77-84.

13. Dias, L. S., Luzia, D. M. M. & Jorge, N. (2013). Physicochemical and bioactive properties of *Hymenaea courbaril* L. pulp and seed lipid fraction. *Industrial Crops and Products*, 49, 610-618. doi: https://doi.org/10.1016/j.indcrop.2013.06.005

14. Françoso, R. D.; Brandão, R.; Nogueira, C. C.; Salmona, Y. B. Machado, R. B. & Colli, G. R. (2015). Habitat loss and the effectiveness of protected areas in the Cerrado Biodiversity Hotspot**.** *Natureza & Conservação*, 13(1), 35-40. doi: https://doi.org/10.1016/j.ncon.2015.04.001

15. Fonseca, J. V. S., Batista, J. D. F., Oliveira, M. C., Diniz, N. C. M., Lima, M. S., Madruga, M. S., Magnani, M. & Borges, G. S. C. (2021). Low-fat and rich-fibers macauba (*Acrocomia* spp.) sauces: Physical and oxidative stability, nutritional quality and sensory characteristics. *Food Bioscience*, 43, 101272. doi: https://doi.org/10.1016/j.fbio.2021.101272

16. Gutiérrez, L. A. L.; Oliveira Jr., C. J. F.; Gomes, R. J. B.; Campos, R. P.; Bortolotto, I. M.; Fehlauer, T. J. (2023). Bioeconomy and sociobiodiversity in the agroecological perspective for the wellbeing. *Revista Brasileira de Agroecologia*, 18(1), 129-150. https://doi.org/10.33240/rba.v18i1.23741

17. Hiane, P. A.; Ramos Filho, M. M.; Ramos, M. I. L. & Macedo, M. L. R. (2005). Bocaiuva, *Acrocomia aculeata* (Jacq.) Lodd., Pulp and Kernel Oils: Characterization and Fatty Acid Composition. *Brazilian Journal of Food Technology*, 8, 256-259.

18. Hiane, P. A.; Baldasso, P. A.; Marangoni, S. & Macedo, M. L. R. (2006). Chemical and nutritional evaluation of kernels of bocaiuva, *Acrocomia aculeata* (JACq.) LODD. *Food Science and Technology*, 26 (3), 683-689. doi: https://doi.org/10.1590/S0101-20612006000300031

19. IBGE - Instituto Brasileiro de Geografia e Estatística. (2019). *Biomas e sistema costeiro-marinho do Brasil: compatível com a escala 1:250 000.* Rio de Janeiro: IBGE. 161 p. (Série relatórios metodológicos, v. 45). Available at: <https://biblioteca.ibge.gov.br/biblioteca-catalogo.html?id=2101676&view=detalhes> Access 10 Jan 2021.

20. Kopper, A. C.; Saravia, A. P. K.; Ribani, R. H. & Lorenzi, G. M. A. C. (2009). Utilização tecnológica da farinha de bocaiuva na elaboração de biscoitos tipo cookie. *Alimentos e Nutrição Araraquara*, 20(3), 463-469.

21. Lescano, C.H., Pires de Oliveira, I., Freitas de Lima, F. (2021). *Acrocomia aculeata*. In: Freitas de Lima, F., Lescano, C.H., Pires de Oliveira, I. (eds) *Fruits of the Brazilian Cerrado*. Springer, Cham. doi: https://doi.org/10.1007/978-3-030-62949-6_1

22. Lopes, D.C.; Steidle Neto, A. J.; Mendes, A. A. & Pereira, D. T. V. (2013). Economic feasibility of biodiesel production from Macauba in Brazil. *Energy Economics*, 40, 819-824.

23. Lorenzi, H. (1998). *Árvores brasileiras: Manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. 2.ed. Nova Odessa, SP: Instituto Plantarum, v.1. 352p, 1998.

24. Lorenzi, H.; Souza, H.M.; Costa, J.T.M.; Cerqueira, L.S.C. & Ferreira, E. (2000). *Palmeiras Brasileiras e Exóticas Cultivadas.* Nova Odessa, SP: Editora Plantarum.

25. Lorenzi, H. (2002). *Árvores brasileiras.* Nova Odessa, SP: Instituto Plantarum, v. 1.

26. Makki, K.; Deehan, E. C.; Walter, J.; Bäckhed, F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. (2018). *Cell Host & Microbe*, 23(6), 705-715. doi: https://doi.org/10.1016/j.chom.2018.05.012

27. Melo, S. A. B. X, Silva, F. S., Melo, A. X. & Bento, T. S. (2017). Cadeia Produtiva do Cumbaru (*Dipteryx alata* Vogel) Em Poconé, Mato Grosso. *Cadernos De Ciência & Tecnologia*, 34(1), 37-58.

28. Mendes, F. M.; Neves, S. M. A. S.; Neves, R. J.; Silva, T. P. (2014). Organização de mulheres extrativistas na região sudoeste Mato-Grossense, Brasil. *Estudos feministas*, 22(1), 71-89. doi: https://doi.org/10.1590/S0104-026X2014000100005

29. Ministério da Saúde, Secretaria de Atenção à Saúde, Departamento de Atenção Básica. (2015) *Alimentos regionais brasileiros*. 2. ed. – Brasília: Ministério da Saúde, 484 p.

30. Munhoz, C. L.; Guimarães, R. C. A.; Nozaki, V. T.; Sanjinez-Argandoña, E. J. & Macedo, M. L. R. (2018). Composição química e de fatores antinutricionais de frutos de bocaiuva. *Ambiência*, Guarapuava (PR), 14(1), 212-224. http://doi.org/10.5935/ambiencia.2018.15.01

31. Paglarini, C. S., Queirós, M. S., Tuyama, S. S., Moreira, A. C. V., Chang, Y. K. & Steel, C. J. (2018). Characterization of baru nut (*Dipteryx alata* Vog) flour and its application in reduced-fat cupcakes. *Journal of Food Science and Technology*. 55(1), 164-172. http://doi.org/10.1007/s13197-017-2876-1

32. Paranhos Filho, A. C.; Moreira, E. S.; Oliveira, A. K. M.; Pagotto, T. C. S. & Mioto, C.L. (2014). Análise da variação da cobertura do solo no Pantanal de 2003 a 2010 através de sensoriamento remoto. *Engenharia Sanitária e Ambiental,* 19,69-76. Doi: https://doi.org/10.1590/S1413-41522014019010000305

33. Pineli, L. L, Aguiar, L. A., Oliveira, G. T., Botelho, R. B., Ibiapina, M. D., Lima, H. C. & Costa, A. M. (2015). Use of baru (Brazilian almond) waste from physical extraction of oil to produce gluten free cakes. *Plant Foods for Human Nutrition*, 70(1), 50-5. doi: http://doi.org/10.1007/s11130-014-0460-7

34. Pinho, L.; Mesquita, D. S. R.; Sarmento, A. F. & Flávio, E. F. (2015). Enriquecimento de sorvete com amêndoa de baru (*Dipteryx alata* Vogel) e aceitabilidade por consumidores. *Unimontes Científica*, 17(1).

35. Pott, A.; Oliveira, A.K.M.; Damasceno-Junior, G.A. & Silva, J.S.V. (2011). Plant diversity of the Pantanal wetland. *Brazilian Journal of Biology*, 71(1, Suppl. 1), 265-273. Doi: https://doi.org/10.1590/S1519-69842011000200005

36. Ramos, M. I. L., Ramos Filho, M. M., Hiane, P. A., Braga Neto, J. A., Siqueira, E. M. A. Nutritional quality of the pulp of bocaiuva *Acrocomia aculeata* (Jacq.) Lodd. Food Sci. Technol 28 (suppl), Dez 2008 https://doi.org/10.1590/S0101-20612008000500015

37. Rodrigues, I. D.; Santos, M. M. R.; Candido, C. J.; Santos, E. F. Novello, D. (2017). Adição de farinha de bocaiuva em *alfajores*: caracterização físico-química e sensorial entre crianças. *Revista da Universidade Vale do Rio Verde*, 15(2), 721-732. Doi: http://dx.doi.org/10.5892/ruvrd.v15i2.2943

38. Rodrigues, A. A., Macedo, M. N., Silvério, D. V., Maracahipes, L., Coe, M. T., Brando, P. M., Shimbo, J. Z., Rajão, R., Soares-Filho, B., & Bustamante, M. M. C. (2022). Cerrado deforestation threatens regional climate and water availability for agriculture and ecosystems. *Global Change Biology,* 28, 6807–6822. Doi: https://doi.org/10.1111/gcb.16386

39. Sano, S. M., Ribeiro, J. F. & Brito, M. A. (2004). *Baru: biologia e uso.* Planaltina, DF: Embrapa Cerrados, 52 p. Embrapa Cerrados. Documentos, 116 p.

40. Santos, G. G.; Silva, M. R.; Lacerda, D. B. L.; Martins, D. M. O.; & Almeida, R. A. (2012). Acceptability and physicochemical quality of a sweet made from *Dipteryx alata* Vog. nuts. *Pesquisa Agropecuária Tropical*, 42(2), 159-165. Doi: https://doi.org/10.1590/S1983-40632012000200003

41. Schincaglia, R. M., Cuppari, L., Neri, H. F. S, Cintra, D. E., Sant'Ana, M. R. & Mota, J. F. (2020). Effects of baru almond oil (*Dipteryx alata* Vog.) supplementation on body composition, inflammation, oxidative stress, lipid profile, and plasma fatty acids of hemodialysis patients: A randomized, double-blind, placebo-controlled clinical trial. *Complementary Therapies in Medicine*, 52, 102479. doi: http://doi.org/10.1016/j.ctim.2020.102479

42. Schwartz, G. (2018). Jatobá – *Hymenaea courbaril. Exotic Fruits,* 257-261. https://doi.org/10.1016/B978-0-12-803138-4.00033-2

43. Shanley, P. & Medina, G. (2005). *Frutíferas e Plantas Úteis na Vida Amazônica*. CIFOR, Imazon. Belém, 300p.

44. Silva, C. P.; Sousa, M. S. B.; Siguemoto, E. S.; Soares, R. A. M. & Arêas, J. A. G. (2014). Chemical composition and antioxidant activity of jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.) flour. *Food Science and Technology – Campinas*, 34(3), 597-603. Doi: https://doi.org/10.1590/1678-457x.6405

45. Silva, C. P.; Freitas, R. A. M. S.; Sampaio, G. R.; Santos, M. C. B.; Nascimento, T. P.; Cameron, L. C.; Ferreira, M. S. L. & Arêas, J. A. G. (2018). Identification and action of phenolic compounds of Jatobá-do-cerrado (*Hymenaea stignocarpa* Mart.) on α-amylase and

α-glucosidase activities and flour effect on glycemic response and nutritional quality of breads. *Food Research International*, 116, 1076-1083. Doi: https://doi.org/10.1016/j.foodres.2018.09.050

46. Souza, R. G. M., Gomes, A. C., Castro, I. A. & Mota, J. F. (2018). A baru almond-enriched diet reduces abdominal adiposity and improves high-density lipoprotein concentrations: a randomized, placebo-controlled trial. *Nutrition*, 55-56, 154-160. doi: http://doi.org/10.1016/j.nut.2018.06.001

47. Souza, R. G. M., Gomes, A. C., Navarro, A. M., Cunha, L. C. D., Silva, M. A. C., Junior, F. B., Mota, J. F. (2019). Baru Almonds Increase the Activity of Glutathione Peroxidase in Overweight and Obese Women: A Randomized, Placebo-Controlled Trial. *Nutrients*, 11(8), 1750. doi: http://doi.org/10.3390/nu11081750

48. Strassburg, B. B. N.; Brooks, T.; Feltran-Barbieri, R.; Iribarrem, A.; Crouzeilles, R.; Loyola, R.; Latawiec, A. E.; Oliveira Filho, F. J. B.; Scaramuzza, C. A. M.; Scarano, F. R.; Soares-Filho, B. & Balmford, A. (2017). Moment of truth for the Cerrado hotspot. *Nature Ecology and Evolution*, 1, 1-3. Doi: http://doi.org/10.1038/s41559-017-0099

49. Tabela Brasileira De Composição De Alimentos - TACO. (2011). 4. edição revisada e ampliada, Campinas: UNICAMP/NEPA, 161 p.

50. Tan, K. T.; Lee, K. T. & Mohamed, A. R. (2010). Potential of waste palm cooking oil for catalyst-free biodiesel production. *Energy*, 36(4), 2085-2088. Doi: https://doi.org/10.1016/j.energy.2010.05.003

51. Takemoto, E.; Okada, I.A.; Garbelotti, M.L.; Tavares, M. & Aued-Pimentel, S. (2001). Composição química da semente e do óleo de baru (*Dipteryx alata* Vog.) nativo do Município de Pirenópolis, Estado de Goiás. *Revista do Instituto Adolfo Lutz*, 60(2), 113-117.

52. Vieira, P. A. Z.; Scheidt, R. F.; Santos, M. M. R.; Candido, C. J.; Santos, E. F. & Novello, D. (2017). *Cupcakes* adicionados de farinha de bocaiuva: caracterização físico-química e avaliação sensorial entre crianças. *Revista da Universidade Vale do Rio Verde,* 15(2), 501-513. Doi: http://dx.doi.org/10.5892/ruvrd.v15i2.2943

Figure captions

Figure 1. Map showing the six Brazilian biomes: Amazon (Amazônia), Caatinga, Cerrado, Atlantic Forest (Mata Atlântica), Pampa and Pantanal. Source: Biomas e sistema costeiro-marinho do Brasil: compatible with scale 1:250 000 - IBGE, 2019.

Figure 2. Jatobá tree (*Hymenaea* sp.) in the Cerrado and Pantanal region. Original image from Iria Hiromi Ishii

Figure 3. Jatobá (*Hymenaea courbaril*) fruits, its pulp and seeds. Source: Schwartz, 2018.

Figure 4. Cumbaru (*Dipteryx alata* Vogel) tree, dry fruit and almonds. A) cumbaru tree in the Cerrado region, B) cumbaru dry and open fruit showing an almond inside and C) almond packets found in the local market of Campo Grande – Mato Grosso do Sul. Original images from Iria Hiromi Ishii

Figure 5. Bocaiuva palm (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart.) and its intact and open fruits, showing its yellow pulp. Original images from Iria Hiromi Ishii

CHAPTER II

Original Research Article to be submitted to **Food Technology and Biotechnology**

Authors: Carolina Saori Ishii Mauro[1]*, Maria Thereza Carlos Fernandes[1], Milena do Prado Ferreira[2], Lycio Shinji Watanabe[2], Fernanda Silva Farinazzo1, Fernanda Jéssica Mendonça[1], Adriana Lourenço Soares[1], César Ricardo Teixeira Tarley[2], Suzana Nixdorf[2] and Sandra Garcia[1].

[1]Universidade Estadual de Londrina, Centro de Ciências Agrárias, Departamento de Ciência e Tecnologia de Alimentos, Rodovia Celso Garcia Cid, PR-445, Km 380, Londrina, Brasil.
[2]Universidade Estadual de Londrina, Centro de Ciências Exatas, Departamento de Química, Rodovia Celso Garcia Cid, PR-445, Km 380, Londrina, Brasil

*Corresponding author:
Phone: +554333714565
E-mail: carol.saori@gmail.com

Bioaccessibility of total phenolic compounds and antioxidant capacity of fruit flours from the Brazilian Cerrado and Pantanal

Running head: Bioaccessibility of antioxidant compounds in Brazilian fruit flours

ABSTRACT

Background: Some flours from native fruits of the Cerrado and Pantanal are commercialized in the region, such as cumbaru almond flour (*Dipteryx alata* Vogel), jatobá pulp (*Hymenaea courbaril*), bocaiuva pulp (*Acrocomia aculeata*) and bocaiuva almond. There are few scientific works on the chemical characteristics and bioaccessible fraction of these foods.

Experimental approach: Four fruit flours from Mato Grosso do Sul (Brazil) were evaluated for their chemical composition, fatty acid profile, bioaccessibility of antioxidant compounds and the efficiency of five extraction solutions for total phenolic compounds and antioxidant capacity. The Unified BARGE Method for gastrointestinal simulation was used to determine the bioaccessibility of Total Phenolic Compounds (TPC), The ability to scavenge DPPH• radicals (DPPH) and $ABTS^{\bullet+}$ radicals and the Ferric Reducing Antioxidant Power (FRAP).

Results and Conclusion: The fruit flours showed lipids, carbohydrates and proteins as major components of their composition. Oleic acid was the main fatty acid found in cumbaru almond, jatobá pulp and bocaiuva pulp flour, while lauric acid was the main one found in bocaiuva almond flour. Among all solvent systems used, the mixture containing 50% methanol, 70% acetone and water showed the best extraction results. The levels of total phenolic compounds were higher at the end of the gastrointestinal phase for Folin-Ciocalteau method, but not for HPLC method. The bioaccessibility of the antioxidant activity by DPPH method decreased at the end of the gastrointestinal phase (0 – 77.28±17.56%), while the bioaccessibility by the FRAP method varied (56.75±1.24 – 184.26±5.84%). No antioxidant activity was detected by the ABTS method at the end of the simulated digestion.

Novelty and scientific contribution: In this study, through the determination of total phenolic compounds and antioxidant capacity of flours after *in vitro* digestion, relevant data were presented on the bioaccessibility of flours from Brazilian native fruits, since there is still no data on the subject in the literature in relation to the four flours studied.

Keywords: fatty acid profile, *Acrocomia aculeata*, *Dipteryx alata* Vogel, *Hymenaea courbaril*, Unified BARGE Method.

Título: Bioacessibilidade de compostos fenólicos totais e capacidade antioxidante de farinhas de frutos do Cerrado e Pantanal

INTRODUCTION

The Cerrado is the second largest biome in South America, with an area occupying 24% of the Brazilian territory. This is the only biome present in all regions of the country (IBGE, 2020). The Brazilian Cerrado is recognized as the richest savannah in the world, harboring more than 10,000 species of native plants already cataloged (Mendonça et al., 2008). The Pantanal biome is considered one of the largest continuous wetlands on the planet, occupying 1.8% of the Brazilian territory (IBGE, 2020). The vegetation of the Pantanal brings together about 2 thousand species of identified plants (POTT et al., 2011).

In the state of Mato Grosso do Sul, where the Cerrado and Pantanal biomes predominate, several fruits of the region are consumed *in natura* or processed by the population for sale in local commerce, in the form of jams, ice creams, mousses, juices, flours and cakes (Damasceno -Junior et al., 2011). Local population use fruit flour to preserve food for longer and due to the possibility of incorporating the flour into various recipes. Native fruit flours are produced by the settlement community, indigenous community and local artisans (Damasceno-Junior et al., 2010, Santos-Junior et al., 2012, Reis et al., 2012; Silva et al., 2014) . Examples of fruit flour sold in the Mato Grosso do Sul region are cumbaru almond flour (*Dipteryx alata* Vogel), jatoba pulp (*Hymenaea courbaril*), bocaiuva pulp (*Acrocomia aculeata*) and bocaiuva almond.

The cumbaru or baru nut has a high nutritional value and is a source of proteins and fats (Damasceno-Junior et al., 2010). In addition, cumbaru nuts have components with antioxidant capacity and total phenolic compounds, which can bring benefits to consumers (Santiago et al., 2018). Jatobá is a native Brazilian fruit, whose pulp has significant amounts of phenolic compounds, antioxidant compounds and carotenoids, such as β-carotene and lutein (Damasceno-Junior et al., 2010; Silva et al., 2014, Silva et al., 2019). Jatobá pulp is naturally dry and has a high starch content, which is why it is mostly used in the preparation of flour (Schwartz, 2018). This pulp has a high caloric value and has *in vitro* digestion and carbohydrate absorption regulation properties (Silva et al., 2019). The mature pulp of bocaiuva or macaúba has a yellow color and a viscous and fibrous texture. This pulp has a high energy value and can be a source of β-carotene. The pulp flour is an important source of lipids, carbohydrates and fibers (Damasceno-Junior et al., 2010). The bocaiuva fruit contains a round, hard, white almond and can be used to make oily or defatted flour.

However, there are few scientific works on the chemical characteristics and bioaccessible fraction of these foods. Therefore, studies are needed to determine the composition of macronutrients, vitamins and minerals, the bioavailability of these nutrients and the use of fruits in processing for the development of foods with high added value (Silva et al., 2008).

The detection of total phenolic compounds and antioxidant capacity may vary according to the extractor solvent used. The choice of solvent for the extraction of compounds from plant materials is very important because different solvents influence the efficiency of extraction of phenolic components (Kobus-Cisowska et al., 2020). It is necessary to evaluate how much of these compounds are bioaccessible after simulated gastric and gastrointestinal phase conditions. The Bioaccessibility Research Group of Europe (BARGE) has proposed a harmonized *in vitro* bioaccessibility procedure for soils called the Unified BARGE Method (UBM) (Wragg et al., 2011). This method can be used for food, as it simulates the conditions of passage through the gastrointestinal tract.

Therefore, this work aimed to determine the chemical composition of fruit flours from the Brazilian Cerrado and Pantanal, to determine the best solvent for compound extraction and to evaluate the bioaccessibility in the gastric phase (GF) and in the gastrointestinal phase (GI) of phenolic compounds and the antioxidant capacity using the BARGE UBM method.

MATERIAL AND METHODS

Fruit Flours

The artisanal flours used in this study were obtained from the local market in the region of Mato Grosso do Sul, Brazil, between May and July 2019 (Figure 1). The bocaiuva pulp and almond flours were obtained from the local market of Miranda (lat: 20° 14' 34" S, long: 56° 21' 50" W). The flours of jatobá and almond of cumbaru were purchased in the municipal market of Campo Grande (lat: 20° 26' 34” S, long: 54° 38' 47” W). Bocaiuva almond and cumbaru almond flours were processed with the peel, resulting in a product with variable color (Figure 1B and 1D). The bocaiuva almond flour used in this work was subjected to a degreasing process to extract the bocaiuva oil by the manufacturer, and therefore, this flour has a lower lipid content.

To access the Genetic Heritage, this work was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, under

n° A90CEAF). All flours used in this work were packed in dark polyethylene bags to avoid the incidence of UV light during storage and kept at freezer temperature (-18 °C).

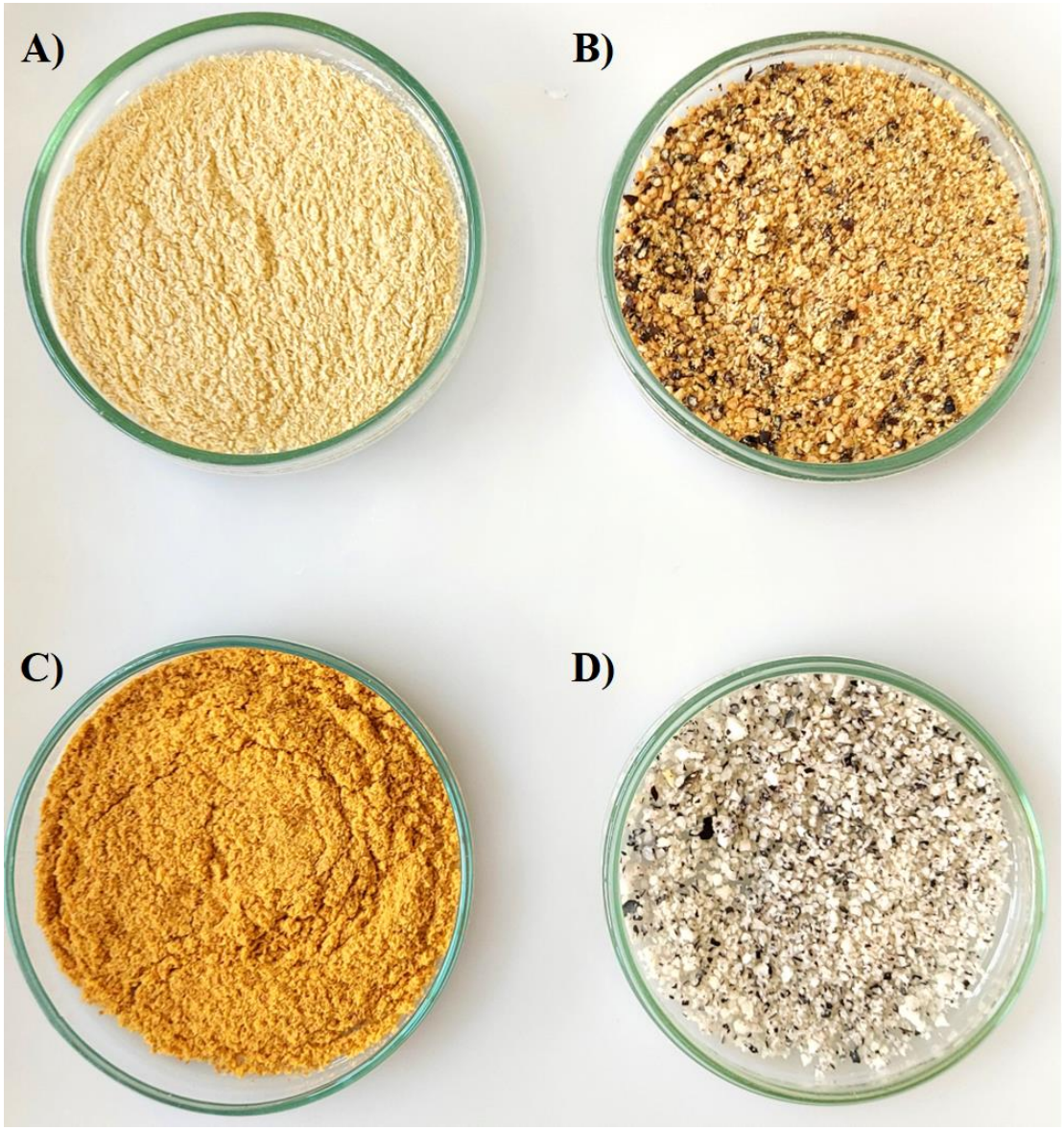


Figure 1. Cerrado and Pantanal fruit flours used in this work. A) jatobá pulp flour, B) cumbaru almond flour, C) bocaiuva pulp flour and D) bocaiuva almond flour

Chemical composition, energy and color

The flour was characterized by physical-chemical analysis (proteins, fat, moisture, ash and carbohydrate) (AOAC, 2006) and by granulometry test or particle size analysis (IAL 377/IV, 2008) . The estimated energy of the sample was determined using the Atwater conversion factors, according to Eq. 1.

$$Energy\ (kcal) = (4 \times protein) + (4 \times carbohydrate) + (9 \times lipid) \quad (1)$$

The color parameters were determined by a colorimeter Konica Minolta® (CM-700D, Tokyo, Japan) using the CIELAB system, with L* representing values from 0 (black) to 100 (white), a* value indicating red (positive) -green (negative) component of a color and b* yellow (positive) and blue (negative) components. As the flour samples presented a heterogeneous color, eight color determinations were made, on different days, to ensure the representativeness of the data.

Fatty acid analysis by Gas Chromatography

The extraction of lipids was carried out cold, following the methodology of Bligh & Dyer (1959), with modifications. The hydrolysis and transesterification of fatty acids was performed according to the ISO 5509:2000 method. The mass of 1 g of oil was weighed and 10 mL of n-heptane was added, followed by homogenization of the mixture by vortexing. Then, 0.5 mL of 2 M NaOH in methanol was added and stirring was performed for 20 s. After phase separation, the supernatant was collected for further analysis by gas chromatography.

Fatty acids were analyzed in a gas chromatograph coupled to a flame ionization detector (GC-DIC) model Shimadzu 17 A, with capillary column of 100 m x 0.25 mm, 0.25 μm of CP-Sil 88 cyanopropylsiloxane. The identification of fatty acids was based on standards of methyl esters or fatty acids (Sigma-Aldrich). The peak area was determined by the integrator coupled to the gas chromatograph. The results were expressed as a relative percentage of identified fatty acids.

Evaluation of the content of total phenolic compounds and antioxidant capacity

The extractor solvents used in this work were: 1) distilled water, 2) acetone 80% (v/v), 3) ethanol 80% (v/v), 4) methanol 80% (v/v) and 5) a mixture of solvents containing 50%

methanol, 70% acetone and water, according to the methodology described by Rufino et al. (2007). To prepare the 50% methanol solution, 50 mL of methanol was added to a volumetric flask and the volume was completed to 100 mL with distilled water. For the 70% acetone solution, 70 mL of acetone was added and the volume was made up to 100 mL with distilled water. To prepare the mixture of solvents 5), in a 100 mL volumetric flask, 40 mL of 50% methanol solution and 40 mL of 70% acetone solution were added. The volume of 100 mL was completed with distilled water.

Falcon tubes containing 1 g of the sample and 10 mL of each solvent were homogenized for 2 minutes in a vortex-type tube shaker and centrifuged at 1928 x *g* for 15 min at 4 °C. This procedure was repeated 2 times. The supernatants obtained were filtered (0.45 μm) and stored at 8 °C for later analysis.

Total Phenolic Compounds (TPC)

To determine the total phenolic compounds in the products, the methodology described by Swain and Hills (1959) was used, using the Folin-Ciocalteau method. Quantification was performed using the standard curve of gallic acid (0.1 to 0.5 mM). The results were expressed in mg gallic acid equivalents per 100 g of sample (mg GAE/100 g), on a wet basis.

Ability to scavenge ABTS•+ free radical

The ability to scavenge ABTS•+ free radical (2,2', azinobis (3-ethylbenzothiazoline-6-sulfonic acid) was performed according to the methodology described by Sanches-Gonzales et al. (2005). Quantification was made based on the trolox standard curve (2.5 to 20 μM) and the results expressed in μmol of Trolox equivalent per gram of sample on a dry basis (μmol TE/g).

Ability to scavenge DPPH• radical

The ability to scavenge DPPH• (2,2-diphenyl-1-picrylhydrazyl) radicals was performed according to the methodology proposed by Brand-Williams et al., (1995). Quantification was performed using a standard Trolox curve (0.5 to 20 μmol/L), with results expressed in μmol of Trolox equivalent per gram of sample on a dry basis (μmol TE/g).

Total Antioxidant Capacity by the Iron Reduction Method - FRAP

The antioxidant power by iron reduction of the flours was estimated using the method described by Benzie and Strain (1996). The Fe^{3+} reducing capacity was expressed in μmol of ferrous sulfate per gram of sample on a dry basis (μmol $FeSO_4$/g).

Bioaccessibility of total phenolic compounds and antioxidant capacity

Reagents

The reagents used for the bioaccessibility assay were prepared according to Wragg et al. (2011). Sigma-Aldrich® enzymes and reagents used were: α-amylase (*Bacillus* species – A6814), porcine type II mucin (M1778), pepsin, from porcine gastric mucosa (P7000), lipase from porcine pancreas (L3126), pancreatin from porcine pancreas (P7545), bovine bile (B3883), bovine albumin (A7906), D-glucuronic acid, glucosamine hydrochloride, uric acid, potassium thiocyanate (≥99%) and sodium chloride (99.5%). The other salts used were potassium chloride (99%, Química Moderna, Brazil), monobasic potassium phosphate (98%) and anhydrous calcium chloride (99-107%) purchased from Nuclear (Nuclear, Brazil), sodium bicarbonate (99.7-100.3 %), ammonium chloride (99.5%), sodium sulfate (99%) and D(+) glucose purchased from Synth (LabSyth, Brazil), magnesium chloride hexahydrate (99-102%, Merck, Germany), monobasic sodium (J. T. Baker, USA) and urea (CAAL, Brazil).

Simulated salivary fluid: KCl, NaH_2PO_4, KSCN, Na_2SO_4, NaCl, NaOH, urea, amylase, mucin and uric acid; simulated gastric fluid: NaCl, NaH_2PO_4, KCl, $CaCl_2$, NH_4Cl, HCl, glucose, glucuronic acid, urea, glucosamine hydrochloride, bovine serum albumin (Sigma), mucin and pepsin; simulated duodenal fluid: NaCl, NaH_2PO_4, KCl, $MgCl_2$, HCl, urea, $CaCl_2$, bovine serum albumin, pancreatin and lipase; simulated biliary fluid: NaCl, $NaHCO_3$, KCl, HCl, $CaCl_2$, bovine serum albumin and bile.

Bioaccessibility test

Bioaccessibility analysis was conducted according to Wragg et al. (2011). The gastrointestinal simulation consisted of three stages: mouth, stomach and small intestine, under fasting conditions. In the salivary phase, 4.5 mL of salivary fluid was added to 0.3 g of sample. The flasks were capped and manually shaken for 30 s. After that, to simulate the gastric phase, 6.75 mL of simulated gastric fluid were added and the flasks were left in a shaking water bath at 37±2 °C for 1 h. After this period, the gastric phase (G) and

gastrointestinal phase (GI) extracts were removed and the pH of the suspension was measured and adjusted until pH 1.2-1.7. The GI extract was carried forward to the intestinal digestion phase. The G extract was centrifuged at 3000 × g for 5 min and filtered. To simulate the gastrointestinal phase, 13.5 mL of duodenal fluid and 4.5 mL of bile fluid were added and the pH was checked to reach 6.3±0.5. After the end of GI, the suspensions were centrifuged for 5 min at 3000 × g and filtered. Both extraction stages were stored at 1–8 °C.

Bioaccessibility (%) was defined as the content of the compound released in the simulated digestion process, called Gastrointestinal Simulation (GIS) in comparison with the content of the compound in the in natura sample and the value was calculated according to Eq. 2 (Leufroy, Noël, Beauchemin, & Guérin, 2012):

Bioaccessibility (%) = (Compound content after GIS ÷ Compound content before GIS) × 100 (2)

Phenolic compounds analysis by HPLC

The analysis of phenolic compounds was performed using *in natura* flours and supernatants after the gastric and gastrointestinal phases, according to the methodology described by Bravo et al. (2007), with modifications.

The developed and validated method was applied to potential quality control of phenolics in different matrices such as yerba mate, coffee and wheat using target and non-target approaches. For this, the flours were extracted by weighing the mass of 10.00 mg in a covered bottle and adding 1.0 mL of methanol (Merck). Samples were sonicated for 15 min at 25°C, filtered through a 0.22 μm PVDF membrane (Merck Millipore), diluted 1:10 (v/v) with water and taken to the chromatographic system for injection.

To determine the composition of phenolic compounds in the samples, a high-performance liquid chromatograph (HPLC) was used in a Shimadzu LC 20 A instrument system (Kyoto, Japan) consisting of a LC-20AT high-pressure pump, SIL-20AC HT automatic injector, RID-10A refractive index detector, SPD-M20A photodiode array detector, CTO-20A column furnace and CBM-20A control module. For the analysis, a Luna C18 chromatographic column (Phenomenex, 250 x 4.6mm, 5μm of particle) was used. Two mobile phases were used: A) Ultrapure Water and B) Methanol (JT Backer, HPLC grade), respectively. The injection volume was 10.0 μL, flow rate 1.0 mL/min, and the separation was performed in the gradient condition using a concentration curve (time 0.00–30.00 min: 95%

of phase A and 5% of phase B; time 30.00–45.0 min: 5% of phase A and 95% of phase B; time 45.10–60.00 min: 95% of phase A and 5% of phase B). The photodiode array detector (SPD-M20A) was used to scan the wavelengths between 190 and 400 nm, and the reading was fixed in the 270 and 320 nm channels. Data acquisition and processing were performed with the aid of the Shimadzu LC-Solution Software (Kyoto, Japan), performed in duplicate (n=2).

To eliminate interference from media components in the gastrointestinal simulation, samples containing only gastric fluid and gastrointestinal fluid were analyzed as blanks and peaks subtracted from the final chromatogram. Therefore, only the peaks referring to the fruit flour sample were analyzed.

Statistical analysis

Data were conducted in triplicate and submitted to Analysis of Variance (ANOVA), with the type of flour as the cause of variation, for comparison of means at a 5% significance level, using the STATISTICA 8.0 program (StatSoftInc, 2007). Graphs were developed in GraphPadPrism 7.

RESULTS AND DISCUSSION

Chemical composition

Table 1 shows the result of the chemical composition analysis of Cerrado and Pantanal fruit flours found in this work. The highest ash content found was 3.84±0.03 g/100g in the AB sample, while the lowest ash content found was 2.31±0.04 g/100g in the CU sample. In bocaiuva almond from Umuarama – Paraná, Machado et al. (2015) found lower values than those described in the present work, with ash content from 1.29±0.16% to 1.54±0.14%. On the other hand, in cumbaru almonds from Goiânia - Goiás, an ash content of 3.01±0.04% was found, a value higher than that found in CU (Fernandes et al., 2015). The determination of the ash content is indicative of the content of minerals since they are inorganic residues that remain after the incineration process of the organic matter.

Table 1. **Chemical composition, Energy (Kcal) and color parameters of cumbaru almond flour (CU), jatobá pulp flour (JA), bocaiuva pulp flour (PB) and bocaiuva almond flour (AB).** Results expressed as mean ±standard deviation. *Different lowercase letters indicate significant differences between lines (P<0.05). **carbohydrates obtained by difference.

Component (g/100g)	Fruit Flour			
	CU	JA	PB	AB
Moisture	4.40^{d}*±0.14	7.95^{a} ±0.11	6.79^{b} ±0.06	5.60^{c} ±0.18
Ash	2.31^{d} ±0.04	3.06^{c} ±0.09	3.44^{b} ±0.01	3.84^{a} ±0.03
Protein	24.20^{b} ±1.74	6.78^{c} ±0.32	3.09^{d} ±0.08	30.29^{a} ±0.96
Fat	39.42^{a} ±1.51	4.89^{c} ±0.29	21.83^{b} ±0.19	22.83^{b} ±0.05
Carbohydrates**	29.55^{d} ±0.63	77.16^{a} ±0.29	64.91^{b} ±0.10	37.05^{c} ±0.64
Energy (Kcal/100 g)	569.78^{a} ±23.07	379.77^{c} ±2.79	468.47^{b} ±2.47	474.83^{b} ±6.85
Color Parameter				
L*	$59.31^{b,c}$ ±3.20	71.99^{a} ±4.67	61.61^{b} ±0.42	55.95^{c} ±4.07
a*	5.36^{b} ±0.53	4.96^{b} ±1.36	10.95^{a} ±1.62	2.24^{c} ±0.63
b*	20.60^{c} ±1.43	25.82^{b} ±1.09	39.03^{a} ±3.64	7.24^{d} ±0.67
Granulometry	Retention in sieve (%)			
1.19 mm	32.1	0.5	0.4	29.8
0.84 mm	9.6	0.5	2.7	12.6
0.60 mm	8.1	2.6	8.8	14.7
0.250 mm	19.6	16.5	34.1	29.7
0.177 mm	5.0	6.6	14.5	4.3
0.149 mm	1.1	0.4	14.5	0.8
< 0.149 mm	19.3	71.1	25.1	8.3

The protein content found varied between 3.09±0.08 and 30.29±0.96 g/100g. The cumbaru and bocaiuva almond flours (CU and AB) had the highest protein contents. The protein isolate present in cumbaru almond has been shown to have high in vitro digestibility and higher protein content than vegetable (soy) and animal (casein and albumin) protein isolates common in the food industry (Nunes et al., 2017). In this work, the protein content found in AB was higher than in CU, which shows that more studies on the proteins of bocaiuva almond are necessary to access the quality of these proteins, and their functional and nutritional properties.

The CU sample showed lipids as the major component of its composition. The pulp

and almond flours of bocaiuva (PB and AB) did not differ in terms of the content of this component and JA had the lowest observed content. In contrast to this study, Machado et al. (2015) found lower lipid content in bocaiuva pulp when compared to almond. Likewise, Dias et al. (2013) detected lower lipid content (1.94 ±0.05%) in jatobá pulp than that found in JA.

The JA sample had the highest carbohydrate content among the analyzed flours. This result is similar to that found in jatobá pulp by Rocha et al. (2013), who reported values of 79.8±17.3 g/100g of carbohydrates and Santos et al. (2020), who found 65 g/100g of total carbohydrates (sugars and fibers).

The carbohydrate content found in CU was higher than that described in almonds from the state of Goiás (Santiago et al., 2018). This can be explained due to the growing conditions of the plant, which influence the content of components present in the raw material. Another explanation would be that the peel of the cumbaru nut has higher carbohydrate contents when compared to the nut without the peel (Santiago et al., 2018).

The theoretical energy found in the present study was high for all flour samples, varying between 379.77±2.79 and 569.78±23.07 Kcal/100 g. Cumbaru and bocaiuva almonds are considered sources of calories and rich in protein (Damasceno-Junior et al., 2010). Considering the composition of fruit flours, if popularized these could become important foods in Brazil, or even destined for programs to combat malnutrition.

The color parameters evidenced a higher luminosity (L*) of JA compared to the other samples. The a* component showed positive values for all samples, demonstrating the predominant red color tendency. The b* component also showed positive values, tending towards yellow. The PB flour showed the highest positive values for a* and b*, which can be seen by its intense color in Figure 1.

Fatty Acid Profile

The fatty acid profile of the four Cerrado and Pantanal fruit flours is presented in Table 2. Under the experimental conditions used in this study, up to eleven fatty acids were detected. When analyzing the variety of fatty acids, it was possible to observe palmitoleic acid (C16:1n7) and oleic acid (C18:1n9) as MUFA and the prevalence of linoleic acid (C18:2n6) and α-linolenic acid (C18:3n3) as PUFA. The most representative SFA in CU, JA and PB were palmitic acid (C16:0) and stearic acid (C18:0), however in AB lauric acid (C12:0) was the most present SFA.

Table 2. Fatty acid profile (% w/w) of cumbaru almond flour (CU), jatobá pulp flour (JA), bocaiuva pulp flour (PB) and bocaiuva almond flour (AB). Results expressed as mean ± standard deviation. Different capital letters indicate significant differences between columns and different lowercase letters indicate significant differences between rows (P<0.05). SFA: saturated fatty acids; MCFA: medium chain fatty acids; MUFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids.

Fatty Acid (% w/w)	Fruit flour			
	CU	JA	PB	AB
Caprylic Acid, C8:0	-	-	-	3.58^{EFa} ±0.03
Capric Acid, C10:0	-	-	-	3.37^{EFa} ±0.03
Lauric Acid, C12:0	-	-	0.82^{FGb} ±0.00	38.09^{Aa} ±0.43
Myristic Acid, C14:0	-	-	0.52^{Gb} ±0.00	8.67^{Ca} ±0.00
Palmitic Acid, C16:0	6.06^{Cd} ±0.08	27.68^{Ba} ±0.40	21.18^{Bb} ±0.05	7.51^{Dc} ±0.04
Palmitoleic Acid, C16:1n7	-	-	3.08^{Da} ±0.10	-
Stearic Acid, C18:0	4.82^{Da} ±0.18	4.38^{Ea} ±0.03	2.26^{Ec} ±0.01	3.02^{Fb} ±0.01
Oleic Acid, C18:1n9	56.38^{Ab} ±0.42	48.25^{Ac} ±0.26	66.09^{Aa} ±0.18	31.56^{Bd} ±0.36
Linoleic Acid, C18:2n6	28.27^{Ba} ±0.00	7.58^{Db} ±0.21	5.02^{Cc} ±0.1	3.96^{Ed} ±0.17
α-linolenic Acid, C18:3n3	2.99^{Eb} ±0.10	12.11^{Ca} ±0.10	1.03^{Fc} ±0.04	0.24^{Gd} ±0.00
Arachidonic Acid, C20:0	1.47^{Fa} ±0.26	-	-	-
SFA (%)	12.36	32.28	24.78	64.24
MCFA (%)	0	0	0.82	45.04
MUFA (%)	56.38	48.25	69.17	31.56
PUFA (%)	31.26	20.29	6.06	4.2

The most abundant MUFA in all fruit flours was oleic acid, with values between 31.56±0.36% (AB) and 66.09±0.18% (PB). The consumption of this fatty acid can offer health benefits to consumers. Studies that replaced saturated fats or oils with oils high in oleic acid have shown significant reductions in total cholesterol, LDL cholesterol and apolipoprotein B in controlled clinical trials and therefore may reduce the risk of developing cardiovascular disease and atherosclerosis (Huth et al., 2015, Bowen et al., 2019).

CU had the highest observed PUFA fatty acid content. The fatty acid profile was

similar to that found by Siqueira et al. (2016) in cumbaru seed oil from Rio Verde - Goiás, with a higher content of oleic acid (49.2±0.005%) and linolenic acid (27.3±1.20%). However, the authors did not report the presence of arachidonic acid, which was found in toasted and unpeeled cumbaru almond flour.

JA had a high percentage of oleic acid (48.25±0.26), followed by palmitic acid (27.68±0.40), and in smaller amounts α-linolenic, linoleic and stearic acids. Likewise, in jatobá pulp flour from Nioaque - Mato Grosso do Sul, oleic acid was the main fatty acid found, followed by palmitic acid (Silva et al., 2014). In jatobá pulp oil native to cities in the states of São Paulo and Minas Gerais, Dias et al. (2013) found in descending order of higher content: oleic acid (46.09%) > palmitic acid (25.04%) > α-linolenic acid (14.54%) > linoleic acid (8.54%) > stearic acid (3.09%). The authors also reported the presence of arachidonic and myristic acid SFA, which was not observed in this work.

PB had the highest MUFA fatty acid content. It was observed that oleic acid reached more than 66% of the lipid profile of this flour, followed by palmitic acid (21.18±0.05%). When analyzing the pulp oil of three bocaiuva genotypes from the state of Goiás and the Federal District, Antoniassi et al. (2020) observed that 2 genotypes had a fatty acid profile with a high content of oleic acid (22.1 to 22.8%) and low content of linoleic and linolenic acid. In bocaiuva pulp oil from the region of Lavras – Minas Gerais, there was a predominance of unsaturated fatty acids (65.8%), mainly due to the high content of oleic acid (61.4%) found (De Oliveira, 2017).

AB had higher SFA and MCFA content. The most abundant fatty acid in the pulp oil was lauric acid (38.09±0.43), followed by oleic acid (31.56±0.36). Similar to what was found in the present work, bocaiuva almond oil from Lavras – Minas Gerais proved to be rich in SFA and short and medium-chain unsaturated fatty acids, of which the most abundant were lauric and oleic acids (Magalhães et al., 2020). Due to the high content of the lauric acid present, AB presented 64.24% of SFA. In bocaiuva almond oil from Dourados - Mato Grosso do Sul, a majority of SFA was also reported, with 74.5% (De Oliveira, 2017).

Total carotenoids and beta carotene by HPLC

The determination of total carotenoids, beta carotene and vitamin A (Table 3) was carried out using only flours from fruits made with pulp, which has a yellow to orange color (Figure 1).

Table 3. Analysis of the content of total carotenoids, beta carotene and vitamin A in jatobá pulp flour (JA) and bocaiuva pulp flour (PB). Results expressed as mean ± standard deviation. Vitamin A is expressed as International Units (IU).

Analysis	Fruit Flour	
	JA	PB
Total carotenoids expressed as Beta carotene (mg/100g)	1.56 ±0.07	18.59 ±1.00
Beta carotene (µg/100g)	39.41 ±1.89	133.00 ±6.00
Vitamin A (IU/100g)	11	37

The beta carotene content found in bocaiuva pulp flour was lower than that found in fresh bocaiuva pulp (49.0±2.0 µg/g of whole pulp) (Ramos et al., 2008), mainly due to the drying conditions of flour processing, which may have lost part of the carotenoids present.

The total carotenoid content of jatobá pulp flour found in the present work is higher than that found by Cardoso et al. (2013) in jatobá-do-cerrado pulp, which presented a content of 0.4±0.1 mg/100g.

Extraction using different solvents

Figure 2 shows the results of total phenolic content and antioxidant capacity of extractions with 5 different solvent systems.

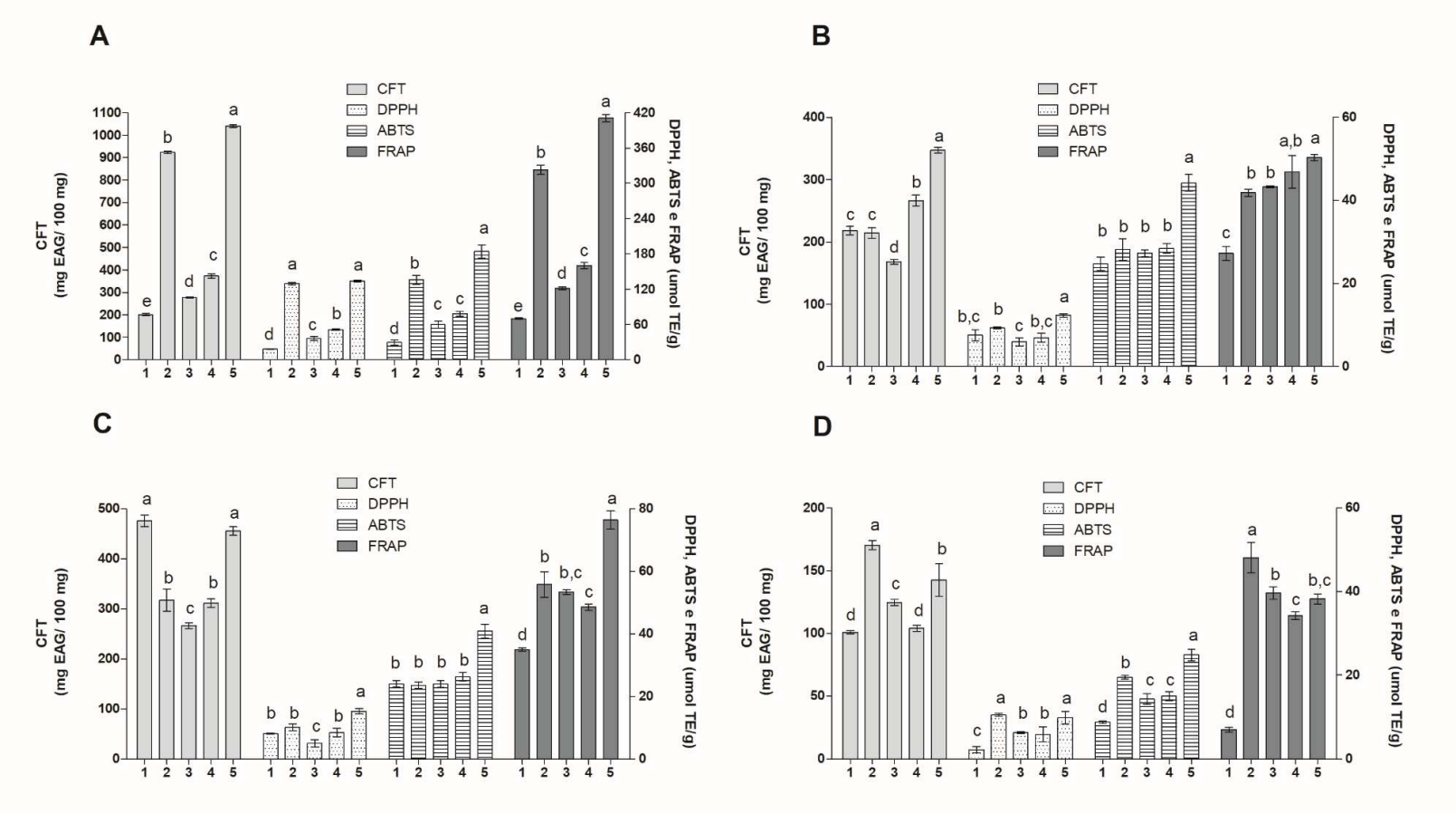


Figure 2. Total phenolic compounds (TPC) content and antioxidant capacity by the DPPH$^{\cdot}$, ABTS$^{\cdot+}$ and FRAP methods of the Cerrado and Pantanal fruit flours A - cumbaru almond flour, B - jatobá pulp flour, C - bocaiuva pulp flour and D - bocaiuva almond flour using different extractor solvents. 1) distilled water, 2) 80% acetone, 3) 80% ethanol, 4) 80% methanol and 5) solvent mixture containing 50% methanol, 70% acetone and water. Different lowercase letters show a significant difference between the same method ($P < 0.05$).

A variation in TPC content and antioxidant activity was observed between the different fruit flour extracts. Cumbaru almond flour showed high levels of TPC, greater scavenging of ABTS and DPPH radicals expressed in Trolox Equivalent and greater iron reducing power (FRAP), when compared to the other analyzed flours. This flour presented solvent mixture 5 as the best extractor for TPC, ABTS and FRAP. Only in the DPPH method, solvent mixture 5 and acetone 80% did not differ from each other.

Santiago et al. (2018) when studying the content of phenolic compounds in toasted cumbaru nuts, found values of 728±41 mg EAG/ 100 g when 50% ethanol was used as extracting solvent. Lemos et al. (2012) found values of 531.8±16.8 mg EAG/100 g of total phenolic compounds in unpeeled cumbaru nuts using methanol extractor solvent. However, it is important to point out that when the extractor solvent used in the present work consisted of a mixture of solvents methanol 50%, acetone 70% and water, the content obtained was 1040.40±5.44 mg EAG/100 g of sample, a result higher than that found in the cited works. The results found for roasted cumbaru nuts according to the DPPH, FRAP and ABTS methods of other authors show lower levels of antioxidant activity when compared to the present work (Lemos et al., 2012; Santiago et al., 2018).

The jatobá pulp flour presented the mixture of solvents 5 as the best extractor in the TPC, ABTS and DPPH methods. Only in the FRAP analysis was it observed that the extraction using a mixture of solvents 5 and methanol 80% did not differ from each other (P<0.05).

When analyzing jatobá pulp from Piauí, Rocha et al. (2013) reported TPC values of 34.1 mg EAG/100 g for the alcoholic extract and 25.19 mg EAG/100 g for the aqueous extract, lower values than those obtained for the jatobá pulp flour, which had a content of 347.12±5.06 mg EAG/ 100 g. In pulp flour from jatobá-da-mata native to the region of Senador Canedo - Goiás, values of 45.5 ± 0.23 µmol TE/g were found in the DPPH analysis, 19.8±0.53 µmol TE/g for ABTS and 36.4±3.19 µmol TE/g for FRAP (Santos et al., 2020). The jatobá flour in the present study showed a higher antioxidant activity detected by the ABTS and FRAP methods, with values of 44.20±1.98 µmol TE/g and 50.24±0.80 µmol TE/g, respectively. However, it showed less antioxidant activity in the DPPH radical scavenging (12.33±0.43 µmol TE/g) than the cited work. On the other hand, Silva et al. (2019) demonstrated that the extraction of TPC from jatobá pulp flour with the combined use of

ethanol and acetone solvents was more efficient in extracting phenolic compounds from this food matrix, with a value of 1753±0.85 mg EAG/100g, higher than that found in this work.

The bocaiuva pulp flour showed mixture 5 as the best extractor solvent by the DPPH., ABTS and FRAP methods, but when analyzing the TPC content, the content found when water was used as solvent did not differ from mixture 5. In this case, water was able to solubilize polar compounds present in the flour in a similar way to the mixture of solvents containing 50% methanol, 70% acetone and water. This result differs from that found by Rocha et al. (2013), who, when analyzing the TPC content in bocaiuva pulp, reported higher concentrations in the alcoholic extract, when compared to the aqueous extract.

According to De Oliveira et al. (2017), bocaiuva pulp and almond flours presented compounds capable of reducing the ABTS radical, with a content of 52±3 μmol TE/g in the pulp and 9±4 μmol TE/g in the almond. This indicates that the fat has antioxidant properties in greater quantity in the pulp. This characteristic was also observed in bocaiuva pulp and almond flours when solvent 5 was used.

Bocaiuva almond flour was the only flour that presented 80% acetone as the best extractor solvent in the TPC and FRAP methods. This could have happened due to the characteristics of the solvent and the polarity of the compounds in the flour. The phenolic compounds present in plant matrices are linked to other biomolecules, such as proteins, polysaccharides, terpenes, chlorophyll, lipids and inorganic compounds. Therefore, the solvent used must be adequate to extract most of them (Kobus-Cisowska et al., 2020).

It has already been suggested that the methanol and water extraction system is more efficient in extracting polyphenols bound to polar fibrous matrices. On the other hand, mixtures of acetone and water are more effective in extracting polyphenols from protein matrices, since they seem to degrade polyphenol-protein complexes (Tabart et al., 2007). Bocaiuva almond flour had the highest protein content among the analyzed flours, and this may explain how the 80% acetone solvent showed a better extraction of these components.

In general, it was observed that the mixture of solvents containing 50% methanol, 70% acetone and water obtained greater prominence in the extraction of the analyzed compounds. This mixture of solvents was originally developed for the determination of the total antioxidant activity in fruits (Rufino et al., 2007), but it presented a satisfactory result for pulp and almond flours of fruits from the Cerrado and Pantanal.

Bioaccessibility of TPC and antioxidant capacity

Table 4 shows the bioaccessibility results obtained for the Cerrado and Pantanal fruit flours before and after the simulated digestion process. The TPC content released after the simulated digestion process was higher than the content found in flours *in natura*, obtaining bioavailability values between 143.85% (CU) and 594.53% (AB). In AB, a significant increase was observed after the gastric phase, becoming more pronounced after the gastrointestinal phase.

Table 4 – Results of Total Phenolic Compounds (TPC) content and antioxidant capacity of Cerrado and Pantanal fruit flours after gastrointestinal simulation. Results presented as mean ± standard deviation; different capital letters show a significant difference in the same line between the same method (P<0.05); CU: Cumbaru almond flour, JA: Jatobá Pulp flour, PB: Bocaiuva pulp flour and AB: Bocaiuva almond flour. ND: not detected.

Fruit Flour Analysis	Fruit Flour *in natura*	After Gastric Phase	After Gastrointestinal Phase	Bioaccessibility (%)
TPC (mg GAE/ 100 mg)				
CU	$1040.40^{B} \pm 5.44$	$586.20^{C} \pm 55.67$	$1428.05^{A} \pm 258.19$	143.85 ± 25.68
JA	$347.12^{B} \pm 5.06$	$346.82^{B} \pm 20.50$	$643.49^{A} \pm 27.25$	187.21 ± 4.97
PB	$475.94^{B} \pm 11.63$	$403.31^{B} \pm 66.01$	$751.69^{A} \pm 28.41$	158.91 ± 8.72
AB	$170.27^{C} \pm 3.60$	$412.08^{B} \pm 9.62$	$997.09^{A} \pm 52.33$	594.53 ± 18.66
DPPH (µmol TE/g)				
CU	$133.92^{A} \pm 1.75$	$29.00^{C} \pm 0.34$	$70.63^{B} \pm 2.91$	52.74 ± 2.04
JA	$12.33^{A} \pm 0.43$	$10.00^{B} \pm 0.58$	ND	0
PB	$15.24^{A} \pm 0.81$	$11.47^{AB} \pm 0.65$	$11.73^{B} \pm 2.38$	77.28 ± 17.56
AB	$10.52^{A} \pm 0.37$	$7.30^{B} \pm 0.25$	$7.23^{B} \pm 1.89$	69.97 ± 17.43
ABTS (µmol TE/g)				
CU	$184.30^{A} \pm 10.82$	$22.16^{B} \pm 1.58$	ND	0
JA	$44.20^{A} \pm 1.98$	$6.81^{B} \pm 0.38$	ND	0
PB	$40.89^{A} \pm 2.23$	$7.05^{B} \pm 0.87$	ND	0
AB	$24.83^{A} \pm 1.38$	$10.49^{B} \pm 0.52$	ND	0
FRAP (µmol $FeSO_4$/g)				
CU	$410.80^{A} \pm 6.37$	$100.52^{C} \pm 1.54$	$233.06^{B} \pm 1.44$	56.75 ± 1.24
JA	$50.24^{A} \pm 0.80$	$48.00^{B} \pm 0.25$	46.62 ± 1.45	92.77 ± 2.01
PB	$76.40^{B} \pm 2.89$	$57.52^{C} \pm 0.70$	$104.27^{A} \pm 0.96$	136.61 ± 5.54
AB	$38.25^{B} \pm 1.23$	$39.28^{B} \pm 2.05$	$70.45^{A} \pm 1.96$	184.26 ± 5.84

The pH of the intestinal phase may have favored the activity of the polyphenols present in the medium. The transition from an acidic to an alkaline environment increases the

antioxidant power of phenolic compounds, causing deprotonation of the hydroxyl moieties present in their aromatic rings (Bouayedi et al., 2011). In addition to the influence of matrix pH, interactions with other dietary constituents released during simulated digestion may occur, which impact the solubility and availability of phenolic compounds, such as some minerals, dietary fibers and proteins (Bouayedi et al., 2011; Velderrain- Rodríguez et al., 2016). Fruit flours come from parts of plants with a different composition of macronutrients, therefore, the differences found in the analysis were expected.

Silva et al. (2019) observed the total phenolic content of the jatobá-do-cerrado extract after in vitro digestion decreased by 42% (1021±0.12 mg GAE/100g). Dutra et al. (2017) characterized the phenolic profile of seriguela, umbu-cajá and mangaba pulps and found that phenolic levels decreased after exposure to simulated gastrointestinal conditions in all frozen pulps. In contrast, Silva et al. (2017) concluded that alkaline hydrolysis and simulated gastrointestinal digestion increased the antioxidant capacity and phenolic fraction present in flaxseeds. Similarly, in a study monitoring the TPC of 11 different fruit seeds, Chen et al. (2016) observed, after the gastrointestinal phase, a significant increase in the TPC of 8 samples compared to the initial total phenolic content. These results agree with what was observed in the present work.

However, the Folin-Ciocalteau analytical method can overestimate the result of phenolic compounds present in the sample, compared to the analysis of individual compounds by HPLC (Cantele et al., 2020). Therefore, the TPC analysis can be complemented by a chromatographic analysis of these components.

The bioaccessibility found for the reduction of the DPPH• radical ranged from 0 to 77.28±17.56% (PB). In the gastric phase there was a significant decrease (P <0.05) of the total phenolic content in the 4 fruit flours. No DPPH• free radical reduction was detected in JA at the end of the gastrointestinal phase. The bioaccessibility of the ability to capture the DPPH• radical was classified as follows: PB > AB > CU > JA (P <0.05).

In the $ABTS^{\bullet+}$ radical capture analysis, no compounds were detected at the end of the gastrointestinal phase, indicating that there was degradation during simulated digestion, in all analyzed flours. A drop in the ABTS content is observed even in the gastric phase. This ABTS analysis did not follow the concentrations found for TPC and this can be explained by the presence of some component of the gastrointestinal simulation medium or even the food itself that may have interfered with the degradation. Some soluble polyphenols in the food

matrix are unstable in gastric and intestinal conditions, due to degradation by enzymes, salts and pH (Cantele et al., 2020).

Pérez-Jiménez et al. (2006) tested the effect of some food constituents in assays of antioxidant capacity and found that all food constituents tested, except cysteine, reduced the value of ABTS, such as galacturonic acid, pectin and tyrosine. Consequently, the components would tend to underestimate the antioxidant capacity of a sample. When food was mixed with polyphenols, it also showed interactions that affected the antioxidant capacity values. Furthermore, the pH of the medium can influence the oxidation of phenolic compounds and their chelating activity and interfere with detection (Bouayedi et al., 2011).

In the present study, bioaccessibility reductions were found for the iron-reducing antioxidant power (FRAP) in CU and JA, which showed final values of 56.75±1.24% and 92.77±2.01%, respectively. However, in the PB and AB flours analyzed by the FRAP method, this value increased. This result indicates that the bioavailable percentage at the end of the simulated digestion was greater than that obtained in the pulp and almond flours of bocaiuva in natura. Bioaccessibility for the FRAP method was classified as follows: AB > PB > JA > CU (P<0.05).

Other authors have described a decrease in the bioaccessibility of the antioxidant capacity of foods. Bouayedi et al. (2011) evaluated apple varieties after simulated gastrointestinal digestion with dialysis and found results 57% and 46% lower in relation to total antioxidants in fresh apples for the FRAP and ABTS methods, respectively. Likewise, after simulating the gastrointestinal digestion of juçara fruits, a species native to the Atlantic Forest, a decrease of 64-78% in DPPH and 55-67% in FRAP was observed (Schulz et al., 2017).

Total phenolic content by HPLC

The HPLC chromatograms of the fruit flours are shown in Figure 3 and the bioaccessibility of total phenolics in Table 5. The samples CU, JA and PB showed more chromatographic peaks in the gastric phase when compared to the gastrointestinal phase, indicating a bioacessibility loss at the end of digestion.

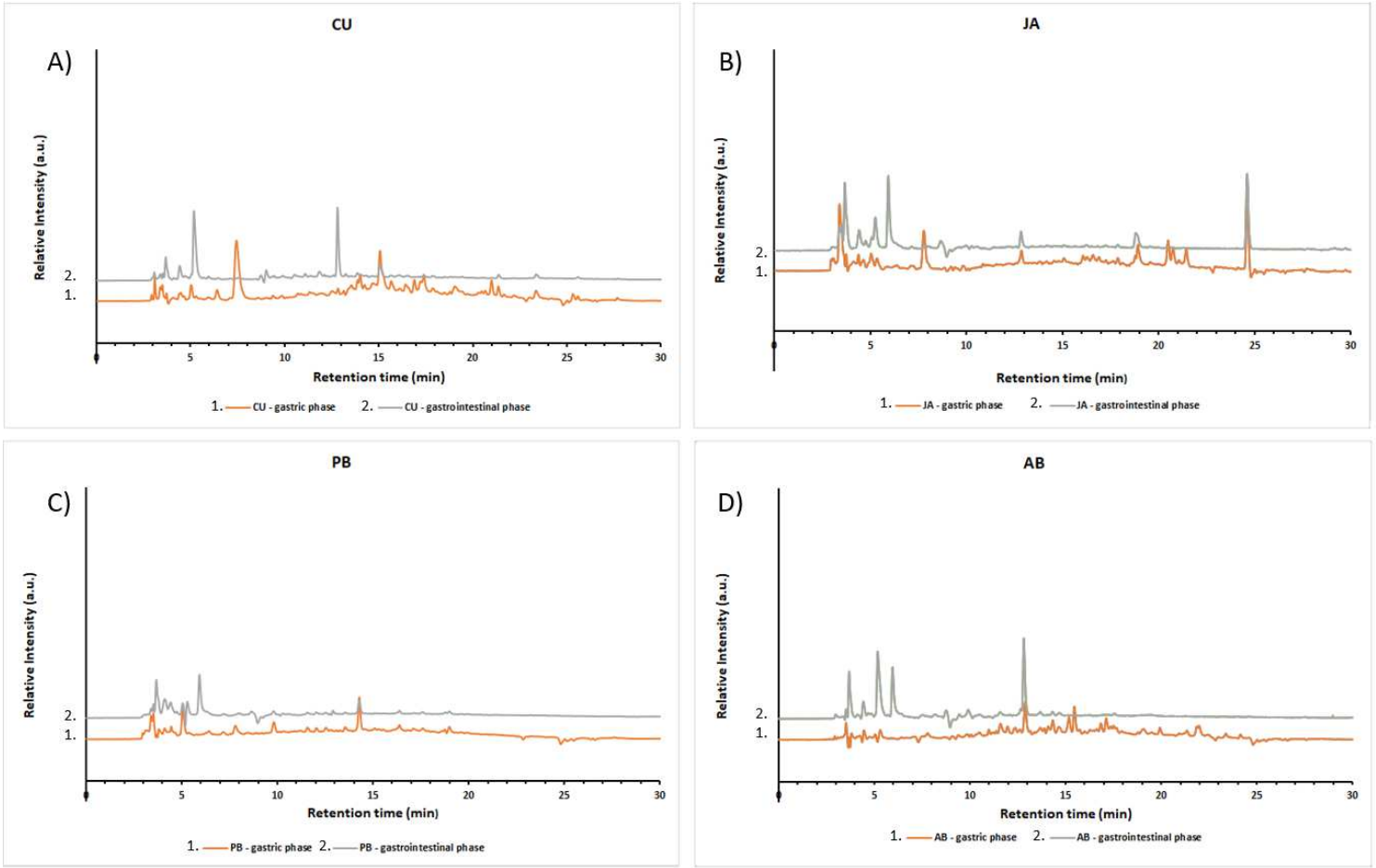


Figure 3. Cromatograms of Cerrado and Pantanal fruit flours at 270 nm after simulated digestion. A: cumbaru almond flour (CU), B: jatobá pulp flour (JA), C: bocaiuva pulp flour (PB) and D: bocaiuva almond flour (AB). a.u.: arbitrary units, 1: gastric phase and 2: gastrointestinal phase.

This can be confirmed by Table 5, as we observed a decrease in the bioaccessibility of total phenolics after the gastrointestinal phase for all flours tested in relation to the *in natura* flour (Supplementary material 1), which is not in accordance with the Folin-Ciocalteu colorimetric analysis used in this work.

According to the chromatogram, AB sample showed an increase in peaks in the gastrointestinal phase, while the percentage of bioaccessibility was 87.96%. This may have happened due to the presence of a large amount of proteins present in AB and to protein-phenolic interactions, which in the gastrointestinal simulation may have been affected by enzymes. Determination of phenolic contents, total antioxidant capacities is affected by protein-phenolic interactions, resulting in changes in bioavailability/ bioaccessibility of phenolics. The parameters that can affect protein–phenolic interactions are temperature, pH, protein type and concentration, and the type and structure of phenolic compounds (Ozdal et al., 2013). Furthermore, due to the presence of bocaiuva almond peel in AB, the heterogeneous flour and the analytical techniques performed, differences in TPC can be observed. The phenolic acids detected in the chromatographic analysis are presented in Supplementary material 2.

Table 5. Bioacessibility of total Phenolic Compounds by HPLC analysis. CU: cumbaru almond flour, JA: jatobá pulp flour, PB: bocaiuva pulp flour and AB: bocaiuva almond flour. Results presented as mean ± standard deviation; different capital letters show a significant difference in the same line (P<0.05).

Fruit Flour	Fruit Flour *in natura*	Bioacessibility (%)	
		After Gastric Phase	After Gastrointestinal Phase
CU	100%	28.25^{A} ±0.20	18.34^{B} ±0.26
JA	100%	28.30^{A} ±0.20	26.63^{B} ±0.19
PB	100%	56.09^{A} ±0.80	45.30^{B} ±0.32
AB	100%	71.97^{B} ±0.51	87.96^{A} ±0.00

Although data obtained after simulated gastrointestinal digestion *in vitro* should not be directly extrapolated to human conditions, this model can be useful to investigate effects such as food matrix and compounds that influence absorption (Bouayedi et al., 2011).

CONCLUSION

Cumbaru almond flour (CU), jatobá pulp (JA), bocaiuva pulp (PB) and bocaiuva almond (AB) showed differences in the centesimal composition. Oleic acid (C18:n9) was the main fatty acid found in CU, JA and PB, while lauric acid (C12:0) was the main fatty acid found in AB, followed by oleic acid. The content of total phenolic compounds and antioxidant capacity of the extracts were dependent on the extraction solvent used. The levels of total phenolic compounds were found to be higher at the end of the simulated digestion than in the *in natura* flours through the colorimetric method, however, the HPLC analysis showed that these compounds were in lower concentrations, indicating a method interference. The HPLC method showed that bocaiuva almond flour had a high content of bioaccessible TPC at the end of the gastrointestinal phase. The bioaccessibility of antioxidant capacity by the DPPH method was reduced after the gastrointestinal simulation and for the FRAP method, it varied in relation to the initial value. Compounds were not detected by the ABTS•+ method at the end of the simulated digestion, showing that there was a degradation of antioxidant compounds. These results provide novel information of total antioxidant compounds bioaccessible after simulated digestion of Cerrado and Pantanal fruit flours.

ACKNOWLEDGEMENTS

We would like to thank the National Council for Scientific and Technological Development (CNPq) and Fundação Araucária for the study grant and the State University of Londrina.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTIONS

C.S.I. Mauro: Conceptualization, Investigation, Writing - Original draft preparation, Data curation. M.T.C. Fernandes: Investigation, Writing - review and editing, Visualization. M.P. Ferreira: Formal analysis, Investigation. L.S.W.: Formal analysis, Investigation. F.S. Farinazzo: Writing - review and editing, Visualization. C.R.T. Tarley: Resources. S. L. Nixdorf: Resources. S. Garcia: Conceptualization, Writing - review and editing, Supervision.

ORCID ID

C.S.I. MAURO https://orcid.org/0000-0001-7561-9893

M.T.C. FERNANDES https://orcid.org/0000-0003-3285-2526

M.P. FERREIRA https://orcid.org/0000-0002-6058-746X

F.S. FARINAZZO https://orcid.org/0000-0003-0615-8434

L. S. WATANABE https://orcid.org/0000-0002-7049-9228

F. J. MEDONÇA https://orcid.org/0000-0002-6678-3533

A. L. SOARES https://orcid.org/0000-0002-8515-2796

C.R.T. TARLEY https://orcid.org/0000-0002-8685-1556

S. L. NIXDORF https://orcid.org/0000-0002-6016-8329

S. GARCIA https://orcid.org/0000-0003-3704-9222

REFERENCES

13. Antoniassi R, de Freitas SC, Silva TS, Santiago MCPA, Wilhelm AE, Junqueira NTV. Impact of genotype on fatty acid profile, oil content and nutritional value of the sweet fruits of *Acrocomia aculeata*. Rev Bras Frutic. 2020;42(6):e-796. https://doi.org/10.1590/0100-29452020796

14. AOAC International. Official methods of analysis of AOAC International.18th ed. Gaithersburg, MD, USA: AOAC International; 2006.

15. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70-76. https://doi.org/10.1006/abio.1996.0292

16. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37(8):911-7. https://doi.org/10.1139/o59-099

17. Bouayed J, Hoffmann L, Bohn T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chem. 2011;128(1):14-21. https://doi.org/10.1016/j.foodchem.2011.02.052

18. Bowen KJ, Kris-Etherton PM, West SG, Fleming JA, Connelly PW, Lamarche B et al. Diets Enriched with Conventional or High-Oleic Acid Canola Oils Lower Atherogenic Lipids and Lipoproteins Compared to a Diet with a Western Fatty Acid Profile in Adults with Central Adiposity. J Nutr. 2019;149(3):471-478. doi: https://doi.org/10.1093/jn/nxy307

19. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT – Food Sci Technol. 1995;28:25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

20. Bravo L., Goya L., & Lecumberri E. LC/MS characterization of phenolic constituents of mate (*Ilex paraguariensis,* St. Hil.) and its antioxidant activity compared to commonly consumed beverages. Food Res. Int. 2007, 40, 393-405.

21. Caetano KA, Ceotto JM, Ribeiro APB, Morais FPR, Ferrari RA, Pacheco MTB et al. (2017). Effect of baru (*Dipteryx alata* Vog.) addition on the composition and nutritional quality of cookies. Food Sci Technol (Campinas). 2-17;37(2):239-245. https://doi.org/10.1590/1678-457x.19616

22. Cantele C, Rojo-Poveda O, Bertolino M, Ghirardello D, Cardenia V, Barbosa-Pereira L et al. In Vitro Bioaccessibility and Functional Properties of Phenolic Compounds from Enriched Beverages Based on Cocoa Bean Shell. Foods. 2020;9(6):715. https://doi.org/10.3390/foods9060715.

23. Cardoso, L., Bedetti, S., Ribeiro, S., Esteves, E., & Pinheiro-Sant'ana, H. (2013). 'Jatobá do cerrado' (*Hymenaea stigonocarpa*): Chemical composition, carotenoids and vitamins in an exotic fruit from the Brazilian Savannah. Fruits, 68(2), 95-107. https://doi.org/10.1051/fruits/2013056

24. Carvalho, P.R.N., Collins, C.H. & Rodriguez-Amaya, D.B. (1992). Comparison of provitamin A determination by normal-phase gravity-flow column chromatography and reversed-phase high performance liquid chromatography. *Chromatographia* **33**, 133–137. https://doi.org/10.1007/BF02275893

25. Chen G-L, Chen S-G, Chen F, Xie Y-Q, Han M-d, Luo C-X et al. Nutraceutical potential and antioxidant benefits of selected fruit seeds subjected to an in vitro digestion. J Funct Foods, 2016;20:317-331. https://doi.org/10.1016/j.jff.2015.11.003

26. Damasceno-Junior GA, Souza PR, Bortolotto IM, Ramos MIL, Hiane PA, Braga Neto JÁ et al. Sabores do Cerrado e Pantanal: conhecer para valorizar os frutos nativos receitas e boas práticas de aproveitamento. Campo Grande, MS, Brazil: Editora da Universidade Federal de Mato Grosso do Sul; 2010 (in portuguese).

27. De Oliveira IP, Correa WA, Neves PV, Silva PVB, Lescano CH, Michels FS, Passos WE, Muzzi RM, Oliveira SL, Caires ARL. Optical Analysis of the Oils Obtained from *Acrocomia aculeata* (Jacq.) Lodd: Mapping Absorption-Emission Profiles in an Induced Oxidation Process. Photonics. 2017; 4(1):3. https://doi.org/10.3390/photonics4010003

28. Dias LS, Luzia DMM, Jorge N. Physicochemical and bioactive properties *of Hymenaea courbaril* L. pulp and seed lipid fraction. Ind Crop Prod, 2013;49:610-618.

29. Dutra RLT, Dantas AM, Marques DA, Batista JDF, Meireles BRLA, Cordeiro ÂMTA et al. Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. Food Res International. 2017, 100:650–657. https://doi.org/10.1016/j.foodres.2017.07.047

30. Fernandes DC, Alves AM, Castro GSF, Jordão Junior AA, Naves MMV. Effects of Baru Almond and Brazil Nut Against Hyperlipidemia and Oxidative Stress In Vivo. J Food Res. 2015;4(4) http://dx.doi.org/10.5539/jfr.v4n4p38

31. Huth PJ, Fulgoni VL 3rd, Larson BT. A systematic review of high-oleic vegetable oil substitutions for other fats and oils on cardiovascular disease risk factors: implications for novel high-oleic soybean oils. Adv Nutr. 2015;6(6):674-693. https://doi.org/10.3945/an.115.008979

32. IBGE – Instituto Brasileiro de Geografia e Estatística. Contas de ecossistemas: o uso da terra nos biomas brasileiros: 2000-2018/IBGE, Coordenação de Recursos Naturais e Estudos Ambientais, Coordenação de Contas Nacionais. Rio de Janeiro, RJ, Brazil: IBGE; 2020 (in portuguese).

33. IAL – Instituto Adolfo Lutz. *Métodos Físico-Químicos para análise de Alimentos* (método 377/IV), Edição IV, 1ª, Edição Digital, 2008.

34. Kobus-Cisowska J, Szczepaniak O, Szymanowska-Powałowska D, Piechocka J, Szulc P, Dziedziński M. (2020). Antioxidant potential of various solvent extract from Morus alba fruits and its major polyphenols composition. Ciênc Rural. 2020;50(1):e20190371. https://doi.org/10.1590/0103-8478cr20190371

35. Leufroy A, Noël L, Beauchemin D, Guérin T. Bioaccessibility of total arsenic and arsenic species in seafood as determined by a continuous online leaching method. Anal Bioanal Chem. 2012;402(9):2849-2859. https://doi.org/10.1007/s00216-012-5774-4

36. Magalhães KT, de Sousa Tavares T, Nunes CA. The chemical, thermal and textural characterization of fractions from macauba kernel oil. Food Res Int. 2020;130:108925. doi: http://doi.org/10.1016/j.foodres.2019.108925

37. NAS-NRC Recommended Dietary Allowances, 10th ed., National Academy of Science, Washington, D.C., 1989, p. 78.

38. Nunes ÂA, Favaro SP, Miranda CHB, Neves VA. Preparation and characterization of baru (*Dipteryx alata* Vog) nut protein isolate and comparison of its

physico-chemical properties with commercial animal and plant protein isolates. J Sci Food Agric. 2017;97(1):151-157. https://doi.org/10.1002/jsfa.7702

39. Lemos MRB, Siqueira EMA, Arruda SF, Zambiazi RC. The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [*Dipteryx alata* Vog.]. Food Res Int. 2012;48:592-597. https://doi.org/10.1016/j.foodres.2012.05.027

40. Machado W, Guimarães MF, Lira FF, Santos JVF, Takahashi LSA, Leal AC et al. Evaluation of two fruit ecotypes (*totai* and *sclerocarpa*) of macaúba (*Acrocomia aculeata*). Ind Crop Prod, 2015;63:287-293. https://doi.org/10.1016/j.indcrop.2014.11.002

41. Mendonça RC, Felfili JM, Walter BMT, Silva Júnior MC, Rezende AV, Filgueiras TS et al. Vascular flora of the Cerrado biome: checklist with 12,356 species. In: Sano, SM, Almeida SP, Ribeiro JF, editors. Cerrado: ecology and flora. Vol. II. Brasília, GO, Brazil: Embrapa Cerrados/ Embrapa Informação Tecnológica; 2008. pp. 421-1279 (in Portuguese).

42. Ozdal T, Capanoglu E, Altay F. A review on protein–phenolic interactions and associated changes. Food Res. Int. 2013; 51:954–970. http://dx.doi.org/10.1016/j.foodres.2013.02.009

43. Pérez-Jiménez J, Saura-Calixto F. Effect of solvent and certain food constituents on different antioxidant capacity assays. Food Res Int. 2006;39:791-800. https://doi.org/10.1016/j.foodres.2006.02.003

44. Pott A, Oliveira AKM, Damasceno-Junior GA, Silva JVS. Plant diversity of the Pantanal wetland. Braz J Biol. 2011;71(1):265-273. https://doi.org/10.1590/S1519-69842011000200005

45. Ramos, Maria Isabel Lima et al. Qualidade nutricional da polpa de bocaiuva *Acrocomia aculeata* (Jacq.) Lodd.. Food Science and Technology [online]. 2008, v. 28, pp. 90-94. ISSN 1678-457X. https://doi.org/10.1590/S0101-20612008000500015.

46. Reis RS, Arruda R, Zanella M, Jesus E, Borsato A. 097 - Utilidades da bocaiuva (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart) sob o olhar da comunidade urbana de Corumbá, MS. Cadernos De Agroecologia. 2012;7(2) (in portuguese).

47. Rocha MS, Figueiredo RW, Araujo MAM, Moreira-Araujo, RSR. Caracterização físico-química e atividade antioxidante (*in vitro*) de frutos do cerrado Piauiense. Rev Bras Frutic. 2013;35(4):933-941 (in portuguese). https://doi.org/10.1590/S0100-29452013000400003

48. Rufino MSM, Alves RE, Brito ES, Morais SM, Sampaio CG, Pérez-Jiménez J,

Saura-Calixto FD. Metodologia Científica: Determinação da Atividade Antioxidante Total em Frutas pela Captura do Radical Livre DPPH. Comunicado Técnico 127, Fortaleza: EMBRAPA Agroindústria Tropical, 2007 (in portuguese).

49. Sánchez-González I, Jiménez-Escrig A, Saura-Calixto F. In vitro antioxidant activity of coffees brewed using different procedures (Italian, espresso and filter). Food Chem. 2005;90(1–2):133–139. https://doi.org/10.1016/j.foodchem.2004.03.037

50. Santiago GL, Oliveira IG, Horst MA, Naves MMV, Silva MR. Peel and pulp of baru (*Dipteryx alata* Vog.) provide high fiber, phenolic content and antioxidant capacity. Food Sci Tech (Campinas). 2018;38(2):244-249. https://doi.org/10.1590/1678-457x.36416

51. Santos ACP, Alves AM, Naves MMV, Silva MR. Nutritional profile, bioactive compounds and antioxidant capacity of jatobá-da-mata (*Hymenaea courbaril*, var. *stilbocarpa*) by product. Rev Chil Nutr. 2020;47(3):366-371. https://dx.doi.org/10.4067/S0717-75182020000300366

52. Schwartz G. Jatoba—*Hymenaea courbaril*. In: Rodrigues S, Silva E de O, de Brito ES, editors. Exotic Fruits. Academic Press; 2018. Pp. 257-261. https://doi.org/10.1016/B978-0-12-803138-4.00033-2

53. Schulz M, Biluca FC, Gonzaga LV, Borges GD, Vitali L, Micke GA et al. Bioaccessibility of bioactive compounds and antioxidant potential of juçara fruits (*Euterpe edulis* Martius) subjected to in vitro gastrointestinal digestion. Food Chem. 2017;228:447-454. https://doi.org/10.1016/j.foodchem.2017.02.038

54. Silva MR, Lacerda DBCL, Santos GG, Martins DMO. Caracterização química de frutos nativos do Cerrado. Ciênc Rural. 2008;38(6):1790-1793 (in portuguese). https://doi.org/10.1590/S0103-84782008000600051

55. Silva FGD, Miralles B, Hernández-Ledesma B, Amigo L, Iglesias AH, Reyes F et al. Influence of Protein-Phenolic Complex on the Antioxidant Capacity of Flaxseed (*Linum usitatissimum* L.) Products. Journal Agric Food Chem. 2017;65(4):800–809. https://doi.org/10.1021/acs.jafc.6b04639.

56. Silva CP, Freitas RAMS, Sampaio GR, Santos MCB, Nascimento TP, Cameron LC et al. Identification and action of phenolic compounds of Jatobá-do-cerrado (*Hymenaea stignocarpa* Mart.) on α-amylase and α-glucosidase activities and flour effect on glycemic response and nutritional quality of breads. Food Res Int. 2019;116:1076-1083. https://doi.org/10.1016/j.foodres.2018.09.050

57. Siqueira APS, Castro CFS, Silveira EV, Lourenço MFC. Chemical quality of

Baru almond (*Dipteryx alata* oil). Ciênc. Rural. 2016;46(10):1865-1867. https://doi.org/10.1590/0103-8478cr20150468

58. Swain T, Hills WE. The phenolic constituents of *Prunus domestica*. I.—The quantitative analysis of phenolic constituents. J. Sci Food Agric. 1959;19:63-68.

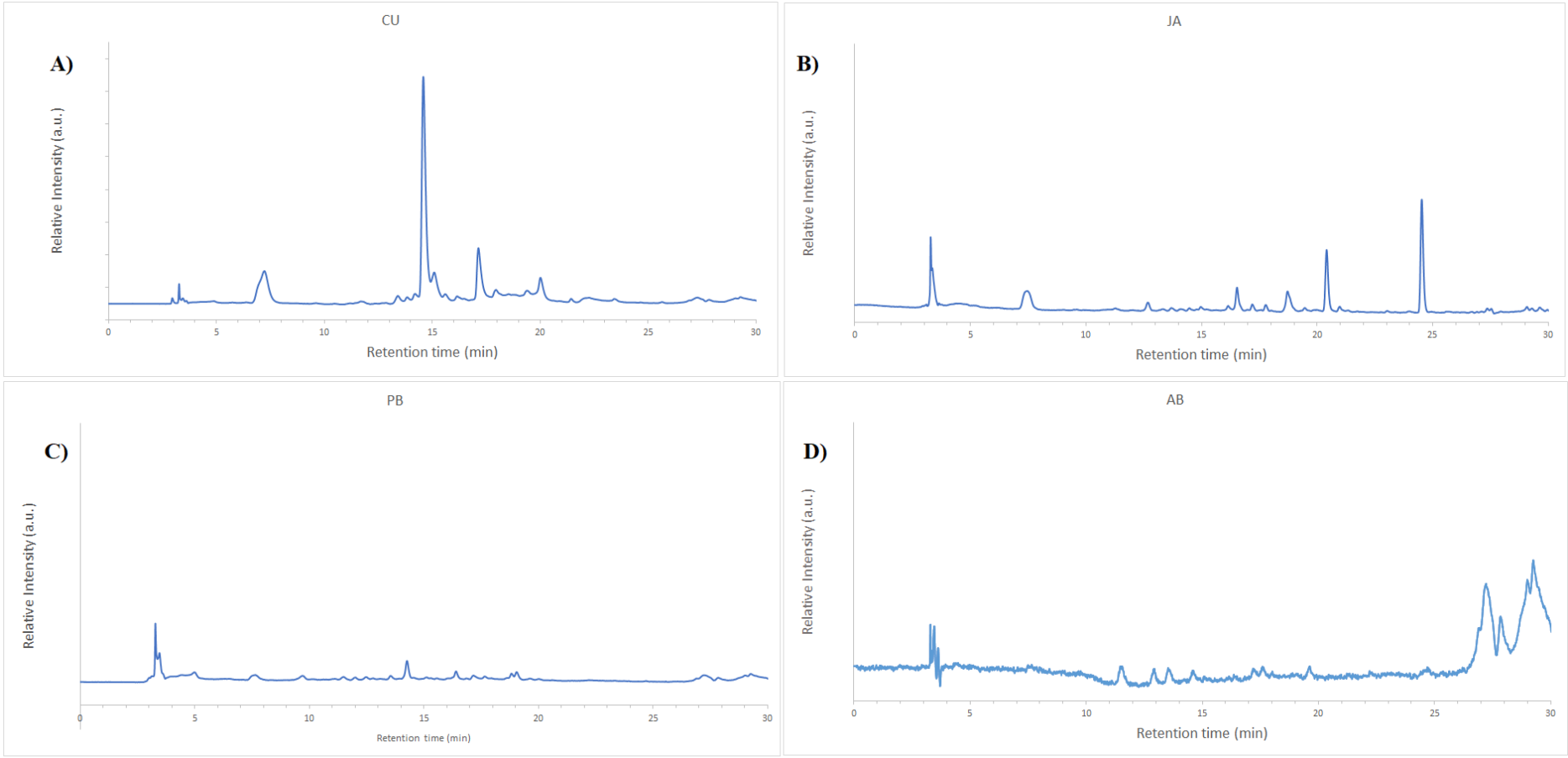
59. Santos-Júnior JF, editor. Bocaiuva, macaúva, macaúba, bocajá: técnicas e dicas de aproveitamento. Campo Grande, MS, Brazil: ECOA; 2012. 44 p. (in portuguese).

60. Tabart J, Kevers C, Sipel A, Pincemail J, Defraigne J-O, Dommes J. Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem. 2007;105(3):1268–1275. https://doi.org/10.1016/j.foodchem.2007.03.005

61. Velderrain-Rodríguez G, Quirós-Sauceda A, Mercado-Mercado G, Ayala-Zavala JF, Astiazarán-García H, Robles-Sánchez RM et al. Effect of dietary fiber on the bioaccessibility of phenolic compounds of mango, papaya and pineapple fruits by an in vitro digestion model. Food Sci Technol (Campinas). 2016;36(2):188-194. https://doi.org/10.1590/1678-457X.6729

62. Wragg J, Cave M, Basta N, Brandon E, Casteel S, Denys S et al. An inter-laboratory trial of the unified BARGE bioaccessibility method for arsenic, cadmium and lead in soil. Sci Total Environ. 2011;409:4016–4030. https://doi.org/10.1016/j.scitotenv.2011.05.019

Supplementary materials



Supplementary Material 1. Cromatograms of Cerrado and Pantanal fruit flours *in natura* at 270 nm. A: cumbaru almond flour (CU), B: jatobá pulp flour (JA), C: bocaiuva pulp flour (PB) and D: bocaiuva almond flour (AB). a.u.: arbitrary units.

Supplementary Material 2. Phenolic acid content (in %) of Cerrado and Pantanal fruit flour *in natura* and after gastrointestinal simulation. CU: cumbaru almond flour, JA: jatobá pulp flour, PB: bocaiuva pulp flour and AB: bocaiuva almond flour. ND: not detected.

		Fruit flour *in natura*			
TR (min)	**Phenolic acid**	**JA (%)**	**CU (%)**	**PB (%)**	**AB (%)**
3.34	Trigonelin	1.38 ±0.05	0.76 ±0.02	0.88 ±0.02	0.41 ±0.02
3.58	Ascorbic acid	12.47 ±1.02	3.15 ±0.21	9.23 ±1.20	1.46 ±0.23
4.79	Citric acid	0.71 ±0.08	ND	ND	ND
7.4	Gallic acid	0.20 ±0.01	0.19 ±0.01	ND	ND
12.54	Teobromin	ND	0.45 ±0.01	ND	ND
14.15	Catechin	ND	0.80 ±0.01	ND	ND
15.492	Chlorogenic acid	ND	0.71 ±0.01	ND	0.67 ±0.05
16.37	Caffeic acid	ND	5.58 ±0.25	ND	ND
18.46	Ferulic acid	0.19 ±0.02	1.07 ±0.02	0.49 ±0.02	ND
18.85	p-Cumaric acid	1.03 ±0.12	0.10 ±0.00	ND	ND
19.234	Rutin	0.38 ±0.02	ND	ND	ND
		After Gastric Phase			
TR (min)	**Phenolic acid**	**JA (%)**	**CU (%)**	**PB (%)**	**AB (%)**
3.34	Trigonelin	0.81 ±0.02	0.26 ±0.01	ND	ND
3.58	Ascorbic acid	ND	ND	26.83 ±2.28	2.23 ±0.44
4.79	Citric acid	ND	ND	ND	ND
7.4	Gallic acid	0.78 ±0.04	4.12 ±0.88	ND	ND
12.54	Teobromin	ND	1.40 ±0.10	ND	ND
14.15	Catechin	ND	0.60 ±0.01	ND	ND
15.492	Chlorogenic acid	ND	1.98 ±0.20	ND	0.67 ±0.04
16.37	Caffeic acid	ND	0.01 ±0.00	ND	0.64 ±0.11
18.46	Ferulic acid	0.20 ±0.00	ND	0.34 ±0.01	ND
18.85	p-Cumaric acid	0.31 ±0.01	0.23 ±0.05	ND	ND
19.234	Rutin	0.26 ±0.05	ND	ND	ND
		After Gastrointestinal Phase			
TR (min)	**Phenolic acid**	**JA (%)**	**CU (%)**	**PB (%)**	**AB (%)**
3.34	Trigonelin	3.66 ±0.42	21.88 ±2.15	19.03 ±2.35	0.41 ±0.02
3.58	Ascorbic acid	11.67 ±1.05	ND	ND	1.46 ±0.05
4.79	Citric acid	ND	ND	ND	ND
7.4	Gallic acid	ND	ND	ND	ND
12.54	Teobromin	ND	ND	ND	ND
14.15	Catechin	ND	ND	ND	ND
15.492	Chlorogenic acid	ND	ND	ND	ND
16.37	Caffeic acid	ND	ND	ND	ND
18.46	Ferulic acid	ND	ND	ND	ND
18.85	p-Cumaric acid	ND	ND	ND	ND
19.234	Rutin	0.89 ±0.15	ND	ND	ND

CHAPTER III

Original Research Article published in the **International Journal of Food Science and Technology**, available at https://doi.org/10.1111/ijfs.16274

Authors: Carolina Saori Ishii Mauro[1], Maryame Kadiri Hassani[2], Monica Barone[3], Maria Teresa Esposito[2], Yolanda Calle[2], Volker Behrends[2], Sandra Garcia[1], Patrizia Brigidi[3], Silvia Turroni[4*], and Adele Costabile[2]

[1]Department of Food Science and Technology, State University of Londrina, Londrina 86057-970, Brazil.
[2]School of Life and Health Sciences, University of Roehampton, London SW15 4JD, UK.
[3]Microbiomics Unit, Department of Medical and Surgical Sciences, University of Bologna, Bologna 40138, Italy.
[4]Unit of Microbiome Science and Biotechnology, Department of Pharmacy and Biotechnology, University of Bologna, Bologna 40126, Italy.

Cerrado and Pantanal fruit flours affect gut microbiota composition in healthy and post-COVID-19 individuals: An *in vitro* pilot fermentation study

Running head: Brazilian fruit flours affect gut microbiota

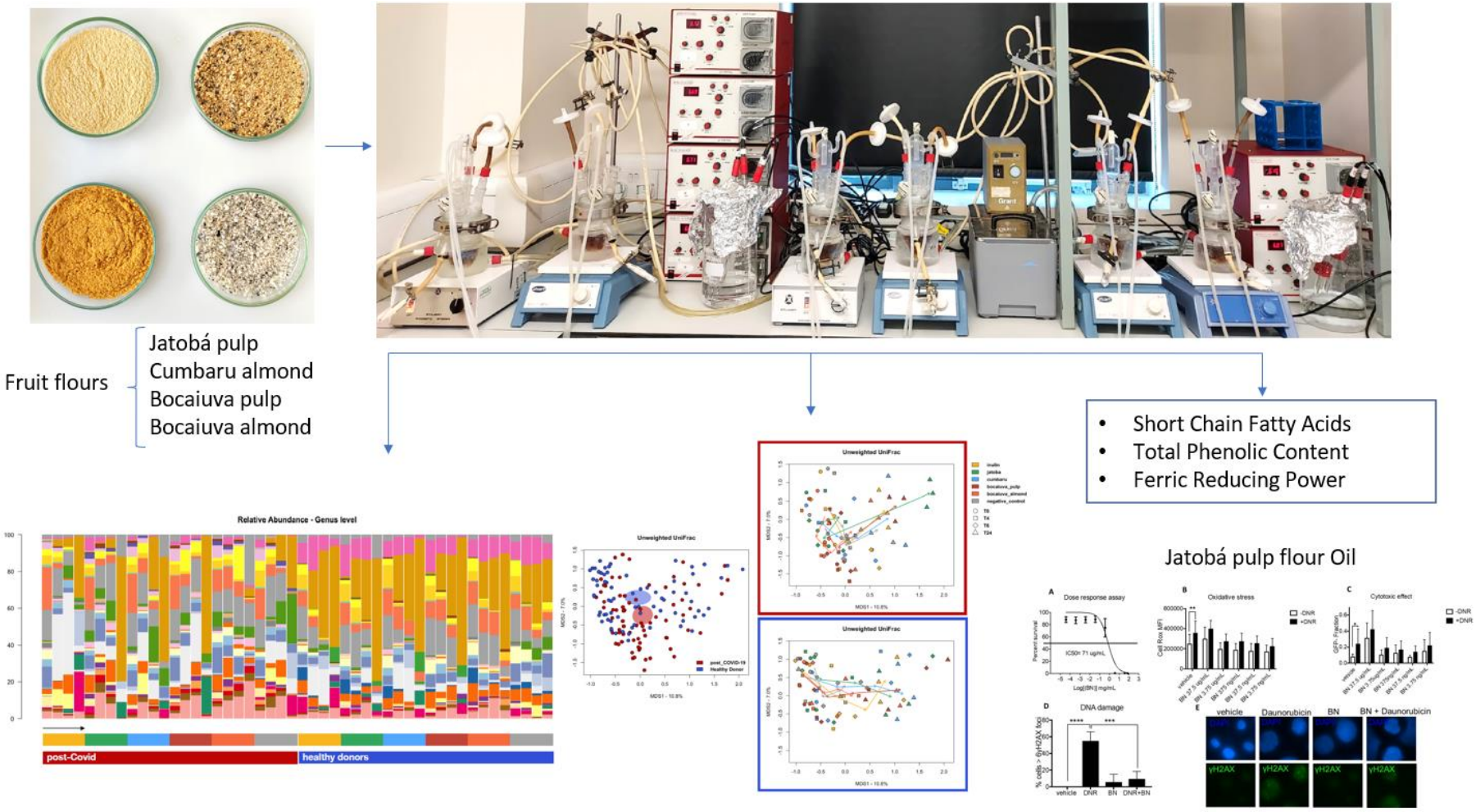
Summary

Brazilian Cerrado and Pantanal plants provides fruits with high nutritional value and antioxidants. This study aims to evaluate four native Brazilian fruit flours (from jatobá pulp, cumbaru almond, bocaiuva pulp and bocaiuva almond) and their effects on the human gut microbiota in healthy (HD) and post-COVID-19 individuals (PC). An *in vitro* batch system was carried out, the microbiota analysed by 16S rRNA amplicon sequencing and the short-chain fatty acids ratio was determined. Furthermore, the effect of jatobá pulp flour oil (JAO) on cell viability, oxidative stress and DNA damage was investigated in a myelo-monocytic cell line. Beyond confirming a gut microbiota imbalance in PC, we identified fruit flour-specific effects including: i) reduction of *Veillonellaceae* with jatobá flour in PC samples; ii) a decrease in *Akkermansia* with jatoba and cumbaru flours; iii) decreasing trend of *Faecalibacterium* and *Ruminococcus* in the presence of all flours tested, with the exception of the bocaiuva almond in HD samples for *Ruminococcus*; and iv) an increase in *Lactobacillus* and *Bifidobacterium* in PC samples with bocaiuva almond flour. JAO displayed antioxidant properties protecting the cells from daunorubicin-induced cytotoxicity, oxidative stress, and DNA damage. The promising microbiota-modulating abilities of some flours and the chemopreventive effects of JAO deserve to be further explored in human intervention studies.

Keywords: *Acrocomia aculeata, Dipteryx alata Vogel, Hymenaea courbaril,* short-chain fatty acids (SCFAs), antioxidants, gut microbiota.

Título: Farinhas de frutos do Cerrado e Pantanal afetam a composição da microbiota intestinal em indivíduos saudáveis e pós-COVID-19: um estudo piloto de fermentação *in vitro*

Graphical abstract of the Original Research Article: Cerrado and Pantanal fruit flours affect gut microbiota composition in healthy and post-COVID-19 individuals: An *in vitro* pilot fermentation study



Introduction

The Cerrado and Pantanal are biomes of South America that gather a great diversity of plant species. Among these plants, we can find many that have edible parts that could enrich the human diet. The fruit pulps and almonds of the Cerrado and Pantanal plants can be consumed raw or processed in the form of flours by local artisan food producers (Damasceno-Junior et al., 2010). The jatobá pulp (*Hymenaea courbaril*), cumbaru almond (*Dipteryx alata* Vogel), bocaiuva almond (*Acrocomia aculeata*) and bocaiuva pulp are examples of materials for the manufacture of fruit flours, which can be sold in the local market, fairs and solidary economy shops. Cerrado and Pantanal fruits have great potential for economic growth due to their great diversity and nutritional content (Bortolotto et al., 2017).

Brazilian Cerrado plants are influenced by climatic and geographical variations such as extreme temperature conditions, high incidence of UV radiation and occurrence of fires. As a result, these plants show adaptations such as high production of antioxidant enzymes and phenolic compounds (Arruda et al., 2022). The nutritional composition of fruit flours may exert beneficial effects after consumption. However, there is no scientific knowledge on the influence of the bioactive compounds on the human gut microbiota.

The human gut microbiota plays an important, multi-layered role in host physiology, including protection from infection and education and modulation of the immune system (Gibson *et al.,* 2017). Imbalances in the gut microbiota (*i.e.*, dysbiosis) can trigger inflammatory pathways in disease and have been linked to several gastrointestinal disorders, such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), and to wider systemic manifestations, such as obesity, type 1 diabetes, and atopy (Buford et al., 2017; Wilmes et al., 2022).

Recently, evidence has suggested distinct dysbiotic features in the gut microbiota of patients with coronavirus disease 2019 (COVID-19), which may persist in the Long Term COVID-19 (Chhibber-Goe, Gopinathan & Sharma, 2021; Gaibani et al., 2021; Lau et al., 2022; Lau et al., 2022). Increases in microorganisms with pathogenic potential (e.g., *Enterococcus* and mucus degraders) and reductions of microorganisms with known immunomodulatory potential (e.g., short-chain fatty acid (SCFA) producers) have been reported up to 6 months after viral clearance (Liu et al., 2022). In this context, the regular consumption of probiotic, prebiotic food and products to

modulate the gut microbiota could have relevant implications for the prevention and mitigation of COVID-19 (Lau et al., 2022).

Prebiotic is 'a substrate that is selectively utilised by host microorganisms conferring a health benefit' (Gibson et al., 2017). Currently established prebiotics are carbohydrates, oligo and polysaccharides, but other substances such as polyphenols and polyunsaturated fatty acids converted to their respective conjugated fatty acids may fit the definition if there is evidence in the target host (Gibson et al., 2017).

The consumption of fruit flours may also have effects on cell protection and oxidative stress prevention due to the content of antioxidants. Jatobá pulp extracts, fractions or compounds have shown antioxidant, anti-inflammatory, myorelaxant activities and anticancer properties (Jayaprakasam 2007; Bezerra, 2013; Keiji et al., 1999).

In the present work, we used a gut model system, which simulated the human colon, to investigate the impact of four Brazilian fruit flours from Cerrado and Pantanal (*i.e.*, jatobá pulp, cumbaru almond, bocaiuva pulp and bocaiuva almond) on gut microbiota composition and metabolic profile of healthy and post-COVID-19 subjects. Furthermore, we investigated the effect of jatobá pulp flour oil (JAO) on oxidative stress and DNA damage induced by a cytotoxic anthracycline (daunorubicin) in a myelo-monocytic cell line.

Material and Methods

Characterization of Cerrado and Pantanal fruit flours

The artisanal flours used in this study were obtained from the local market in the region of Mato Grosso do Sul, Brazil. The bocaiuva pulp and almond flours were obtained from the local market of Miranda (lat: 20° 14' 34" S, long: 56° 21' 50" W). The flours of jatobá and almond of cumbaru were purchased in the municipal market of Campo Grande (lat: 20° 26' 34” S, long: 54° 38' 47” W). To access the Genetic Heritage, this work was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, under n° A90CEAF).

The nutritional profile of the fruit flours was characterised by official reference methods (ashes: UNI ISO 2171; proteins: UNI 10274 831/12/93 and ISO 1871 (15/12/75); total dietary fibre according to Lee et al. (1993). The energy value of the foods was estimated using the Atwater conversion factors (Merril & Watt, 1973).

The amino acid profile analysis was carried out according to AccQ-Tag Ultra Derivatization AminoAcids Kit (SKU: 186003836) UPLC® Amino Acid Analysis Application Solution (Waters, Wilmslow, UK).

pH-controlled batch culture systems

Stool sample collection and preparation

Faecal samples were donated by three individuals up to 10 days after clearance of Covid-19 and by three healthy individuals. The samples were collected in an anaerobic jar (AnaeroJarTM 2.5 L, Oxoid Ltd) including a gas-generating kit (AnaeroGenTM, Oxoid). On the day of the experiment, each faecal sample (20 g) was diluted in 100 mL of anaerobic phosphate buffer solution (0.1 mol/L, pH 7.4, w/w) and homogenised (Stomacher 400, Seward, West Sussex, UK) for 2 min at 240 paddle beats per minute. Samples were added to anaerobic fermenters within 15 minutes of voiding (Costabile et al., 2010).

In vitro batch culture fermentations

The flours were subjected to the simulated gastrointestinal digestion procedure as previously described by Guergoletto et al. (2016). Batch culture fermentation experimental condition, media composition and setting-up were carried out according to Corona et al. (2020).

Jatobá pulp flour (1% w/v), cumbaru almond flour (1% w/v), bocaiuva pulp flour (1% w/v), and bocaiuva almond flour (1% w/v) were fermented, along with inulin (Orafti® Inulin, BENEO, Germany) (1% w/v, positive control) and faecal slurry without any substrate addition (negative control). The plant-derived samples used in this part of the process were lyophilized digested flours, inoculated into the stirring batch-culture vessels containing faecal slurry (1 %).

The experiment was carried out in triplicate, using a stool sample from a different donor each time. Flours or inulin were added to each container immediately before adding 5 mL of faecal inoculum (1:10, w/v), to simulate the conditions of the distal region of the human large intestine (pH 6.7-6.9). Samples (4 mL) were collected at different time points (0, 4, 6 and 24 h) for microbiota profiling and metabolites analysis (see below). Samples were centrifuged for 20 min at 19,000 × *g* and supernatant fractions were removed for SCFAs, ferric reducing antioxidant power assay

(FRAP) and total phenolic content analysis (TPC), whereas the pellet was kept for microbial DNA extraction. All samples were stored at -80°C prior to analysis.

Gut microbiota profiling through 16S rRNA amplicon sequencing

Microbial DNA was extracted from 250 mg of fermentation samples using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The sequencing of the 16S rRNA gene was performed according to Corona et al. (2020). For each sample, the hypervariable V3-V4 regions of the 16S rRNA gene were PCR-amplified using the S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 primers (Klindworth et al., 2013) with Illumina overhang adapter sequences. Sequencing reads were deposited in the National Center for Biotechnology Information Sequence Read Archive (Bioproject ID: PRJNA913064) and sequences were processed using a pipeline combining PANDAseq (Masella et al., 2012) and QIIME 2 (Bolyen et al., 2019). After length and quality filtering, reads were binned into amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). Taxonomy was assigned via the VSEARCH algorithm (Rognes et al., 2016), using the Greengenes database as a reference. Alpha diversity was measured using the Shannon index and the number of observed ASVs. Beta diversity was computed based on weighted and unweighted UniFrac distances and visualised on a Principal Coordinates Analysis (PCoA) plot.

Analysis of short-chain fatty acids (SCFAs)

Acetic acid (C2), propionic acid (C3) and butyric acid(C4) were purchased from Merck (Darmstadt, Germany). All the stock solutions were prepared in water and stored at -20°C. 4-Acetoamido-7-mercapto-2,1,3-benzoxadiazole (AABD-SH) was purchased from Biosynth Carbosynth Ltd (Compton, UK). Triphenylphosphine (TPP), 2,2'-dipyridyl disulfide (DPDS) and mobile phase solvents were from Fisher Scientific UK Ltd. (Loughborough, UK).

Samples taken at 0, 4, 6 and 24 h were analysed for SCFA content. Fermentation supernatant samples were derivatized based on the method described by Song et al. (2019). Briefly, 380 µL of water and 20 µL of internal standard (where appropriate) were added to 200 µL of centrifuged stool samples and mixed well. The solution was transferred to membrane filter (Microcon-30kDa centrifugal filter unit with ultracel-30 membrane, Merck KGaA, Darmstadt, Germany) and centrifuged at 19,000 × *g* for 20

min at 4°C to remove any floating particulates in the supernatant. Afterwards, the supernatants were collected to perform the derivatization.

For derivatization of SCFAs, 20 µL each of 20 mM AABD-SH, 20 mM TPP, and 20 mM DPDS in dichloromethane were added to the supernatant and derivatization was performed at room temperature for 5 min while vortexing. The reaction solution was dried under nitrogen, and then reconstituted with 200 µL methanol prior to UPLC-MS/MS analysis. The calibration curves were generated with standard solutions (100 nM to 1 mM). Derivatives were measured on the UPLC MS/MS system consisting of an Aquity UPLC H-Class coupled to a Xevo TQ-S micro-ESI mass spectrometer (both Waters, Wilmslow, UK). Separation was achieved on HSS T3 column (1.8 µm, 2.1 mm X 100 mm) with a HSS T3 VanGuard column (both Waters) held at 45°C with mobile mobile phase A (water + 0.1 % formic acid) and mobile phase B (acetonitrile + 0.1% formic acid) at a flow rate of 0.6 mL/min and a gradient of initial 99% A for 1 min, followed by a linear decrease to 5% A after 5 min, held at 5% until 6.5 min, switched back to 99% A at 6.55 min, held until 8 min. Mass spectrometry was conducted in positive ion mode with ionisation voltage of 3.5 kV and desolvation gas flow of 650 L/h at a temperature of 450°C. Transitions were taken from Song et al. (2019). Data analysis, including baseline correction and QC batch correction, was carried out in Matlab using in-house scripts (modified from Behrends et al., (2011)).

Total Phenolic Content (TPC) and Ferric Reducing Antioxidant Power (FRAP)

During fermentation, aliquots were taken at 0 and 24 h to access the total phenolic content (TPC) and the *in vitro* antioxidant activity. The TPC was determined spectrophotometrically using the Folin-Ciocalteau assay (Swain and Hills, 1959). The results were expressed as mM gallic acid equivalent (mM GAE). To assess the antioxidant activity, ferric reducing antioxidant power (FRAP) was determined (Benzie and Strain, 1996). The results were expressed as µM ascorbic acid equivalent (µM AAE). Both methods had the volumes adapted to a 96-well plate and read in a microplate reader (Thermo Scientific Multiskan EX).

Effect of the jatobá pulp oil (JAO) on cell viability, oxidative stress and DNA damage

Prior to cell culture analysis, the jatobá pulp flour oil (JAO) was extracted according to Bligh and Dyer (1959).

Cell culture

The acute myeloid leukaemia cell line MV411 cell line (ATCC) was grown in 90% RPMI 1640 medium (Sigma R8758) supplemented with 10% FBS (Fisher 11550356) and 1% pen/strep (Sigma P4333) at 37ºC with 5% CO_2 and 95% humidity. The cells were stably infected with a lentivirus vector expressing the green fluorescent protein (GFP) (Arroyo and Calle, unpublished data). The parental cell line was used for the immunofluorescence experiments whereas the GFP cell line was used for the cytotoxic and oxidative stress experiments.

Cytotoxic, oxidative stress and DNA damage assay

For the cell viability and oxidative stress experiments, MV411-eGFP cells were seeded in 96-well plates (flat bottom) (4×10^4 cells/ 200 µl/well) and incubated at 37ºC with 5% CO_2 and 95% humidity. After 24 h, the cells were divided 1:2 and treated with serial ten-fold dilutions of JAO (from 37.5 µg/mL to 3.75 ng/mL) or vehicle (dimethyl sulfoxide - DMSO) for 24 h. After 24 h, daunorubicin (25 nM) was added to half of the wells and the plate was incubated at 37ºC with 5% CO_2 and 95% humidity for another 24 h. The day after, Cell Rox (250 nM) (Thermofisher) was added to the appropriate wells and the plate was incubated for 1 h at 37ºC with 5% CO_2 and 95% humidity in the dark. The plate was analysed by flow cytometry using a BD Accuri™ C6 Flow cytometer to discriminate GFP- (dead cells) from GFP (alive cells) and detect oxidative stress within the samples by measuring the fluorescence emitted by Cell Rox. For the DNA damage experiments, MV411- cells were seeded in 24-well plates (1.6×10^5 cells/ 1mL/well) and incubated at 37ºC with 5% CO_2 and 95% humidity. After 24 h, the cells were divided 1:2 and treated with JAO (37.5 ng/mL) or vehicle (DMSO) for 24 h. After 24 h, daunorubicin (25 nM) was added to half of the appropriate wells and the plate was incubated at 37ºC with 5% CO_2 and 95% humidity for another 24-48 h, when the cells were collected for analysis of DNA damage.

Flow cytometry

The samples were processed for analysis of apoptosis/viability and oxidative stress by flow cytometry BD Accuri™ C6, using the GFP signal, measured in FL1-A channel (533/30) and Cell Rox, measured in the FL4-A channel (780/605). The cell population was identified and gated based on the forward and side scatter signals profile (FSC/SSC). The combination of these two parameters allows for the discrimination of

the cells by size and internal complexity in single-cell analysis. Among this population, we performed doublets discrimination by plotting area (-A) against the height (-H) for the forward scatter (FSC-A versus FSC-H). Doublets present double the area and width values of single cells whilst the height is roughly the same. Disproportions between height and area are used to identify doublets. This accurate discrimination ensures the exclusion of false fluorescence emission. For each cell population, we recorded a minimum of 10,000 events in medium flow rate in the ungated sample. Results were given in percentage of GFP positive cells (M6 population) and median fluorescence intensity (MFI) for Cell Rox. The cleaning and validations of the flow cytometry were done before each use. Firstly, the cleaning was done with 10% bleach for 2 min, then the rinsing was done with water for 2 min. Finally, the first validation was done with Spherotech 8-Peak validation beads and the second one with Spherotech 6-Peak validation beads.

Analysis of DNA damage by immunofluorescence

Analysis of DNA damage was performed as described (Esposito et al., 2015). Briefly, the cells were transferred from the wells to individual Eppendorf tubes, then centrifuged for 5 min at 500 × *g*. After that, the supernatant was discarded, and the pellet was resuspended in 200 µl of PBS and half of the cells were spun for 5 min at 300 × *g* on a glass slide by using a cytospin cytocentrifuge. Following that, the slides were fixed in 4% paraformaldehyde (PFA) for 15 min and washed three times with PBS 1X for 5 min. The cells were permeabilized and blocked in 10% FBS/1% BSA/0.5% TX-100/TBS 1X for 15 min. Then, the slides were washed three times in cold PBS 1X and incubated with the primary antibody mouse anti yH2AX (1:200) in 10%FBS/1% BSA/TBS 1X and incubated in the dark overnight at 4°C. The day after, the slides were washed three times with PBS 1X for 5 min to remove the primary antibody. The slides were then incubated with the secondary antibody Donkey anti mouse DL 488 Jackson/Stratech 715-485-150 (1:200) in DAPI 0.2 µg/mL/10%FBS/1% BA/TBS 1X in the dark for 1 h. The slides were washed three times for 10 min with PBS 1X/0.05% Tween 2, then mounted with Mowiol-DABCO, by adding 1 drop of mounting medium on the edge of the cells and covered with the coverslip. The slides were stored at room temperature for 24 h and then examined under the microscope. Pictures were captured using Evos FL Digital Inverted Fluorescence Microscope.

242 **Statistical analysis**

243 The fruit flour characterization, TPC and FRAP data were submitted to analysis
244 of variance (ANOVA) and Tukey's test, for comparison of means at the level of 5% of
245 significance, using the STATISTICA 12.0 program (StatSoft Inc., Tulsa, OK). The cell
246 culture data were submitted to a 2-Way Anova Sidak's multiple comparison test for
247 oxidative stress induced by daunorubicin or 2-Way Anova Tukey's multiple comparison
248 test for cytotoxic effect of daunorubicin and DNA damage. The figures were developed
249 in GraphPadPrism 8 (GraphPad Software, San Diego, CA).

250 As for the microbiota data, the statistical analysis was performed using R Studio
251 1.0.44 on R software version 3.3.2 (https://www.r-project.org) implemented with the R
252 packages stats and vegan (https://cran.r-project.org/web/packages/vegan/). The
253 significance of data separation in the PCoA plot was tested by PERMANOVA using the
254 function adonis in vegan. Bar plots were built using the R package made4 (Culhane et
255 al., 2005). Non-parametric tests (Kruskal–Wallis test or Wilcoxon test) were used to
256 assess differences in alpha diversity and relative taxon abundance. A P value ≤ 0.05 was
257 considered statistically significant; a P value between 0.05 and 0.2 was considered a
258 trend.

259

260 **Results**

261

262 **Characterization of Cerrado and Pantanal fruit flours**

263 The tested flours showed differences in terms of macronutrient composition,
264 especially because they were derived either from the almond or from the pulp of fruits
265 (Table 1). All flours showed moisture below 10% (w/w), which is a desirable stability
266 indicator since the low moisture content prevents enzymatic activity and undesirable
267 microbial growth.

268 The Jatobá pulp flour presented 44.49 g/100 g of fibre, a higher value compared
269 to the other flours, which was also reflected in higher values of both soluble and
270 insoluble fibres ($P<0.05$). Cumbaru almond flour presented the highest protein content,
271 with 25.70 g/100 g ($P<0.05$). The flours made of almonds had high lipid content of
272 61.18 g/100 g and 41.21 g/100 g for bocaiuva almond and cumbaru almond flour,
273 respectively. All flours are calorie-dense food, however, bocaiuva almond flour stood
274 out as having the highest energy value, especially due to its high lipid content.

Regarding essential amino acids, cumbaru almond flour, bocaiuva pulp and bocaiuva almond flour presented scores 3.03, 7 and 6.3 times higher, respectively, when compared to jatobá pulp flour. The bocaiuva pulp and bocaiuva almond flours did not differ from each other in terms of essential amino acids, however, bocaiuva almond flour showed higher levels of tryptophan, threonine, and lysine, while bocaiuva pulp flour had higher levels of leucine, isoleucine and phenylalanine.

The amino acid profile showed that jatobá fruit flour has higher content of the non-essential amino acids proline and asparagine. Cumbaru almond flour presented the highest concentrations of the non-essential amino acids aspartate and glutamate ($P<0.05$). Serine and glutamine levels did not differ for all fruit flour samples ($P>0.05$).

Table 1. Proximate composition and amino acid profile of Cerrado and Pantanal fruit flours. Data are presented as mean ± standard deviation (n = 3).

Component (g/100g)	**Jatobá pulp**	**Cumbaru almond**	**Bocaiuva pulp**	**Bocaiuva almond**
Moisture	8.30 ^{A} ±0.11	4.90 ^{B} ±0.70	8.60 ^{A} ±0.08	5.60 ^{B} ±0.10
Ash	3.60 ^{A} ±0.10	2.70 ^{B} ±0.10	3.80 ^{A} ±0.09	1.80 ^{C} ±0.04
Protein	7.40 ^{C} ±0.25	25.70 ^{A} ±0.60	3.20 ^{D} ±0.70	12.50 ^{B} ±0.40
Lipid	3.05 ^{D} ±0.30	41.21 ^{B} ±0.80	19.46 ^{C} ±0.50	61.18 ^{A} ±0.40
Carbohydrate	33.16 ^{B} ±0.57	3.42 ^{C} ±0.85	38.55 ^{A} ±0.90	1.62 ^{C} ±0.60
Total Fibre	44.49 ^{A} ±0.39	22.07 ^{C} ±1.28	26.39 ^{B} ±1.10	17.13 ^{D} ±0.80
Soluble fibre	11.13 ^{A} ±0.27	2.32 ^{C} ±0.37	8.85 ^{B} ±0.70	1.80 ^{C} ±0.38
Insoluble fibre	33.36 ^{A} ±0.60	19.75 ^{B} ±1.47	16.54 ^{C} ±1.00	15.76 ^{C} ±1.40
Energy value (kcal/100g)	189.70 ^{D} ±2.40	487.31 ^{B} ±2.91	342.14 ^{C} ±6.39	607.10 ^{A} ±8.20
Aminoacid Profile (mg/g protein)	**Jatobá pulp**	**Cumbaru almond**	**Bocaiuva pulp**	**Bocaiuva almond**
Glycine	2.83 $^{B.C}$ ±0.78	8.8 $^{A.B}$ ±0.59	1.8 ^{C} ±0.86	10.0 ^{A} ±2.00
Lysine*	2.6 ^{C} ±0.32	36.9 ^{B} ±0.05	3.3 ^{C} ±0.51	49.0 ^{A} ±2.68
Alanine	22.5 ^{B} ±2.51	33.4 ^{B} ±1.55	44.9 ^{B} ±2.45	75.2 ^{A} ±9.53
GABA	1.4 ^{C} ±0.21	5.3 $^{B.C}$ ±0.49	32.5 ^{A} ±1.76	10.9 ^{B} ±0.94
Serine	45.4 ^{A} ±0.52	21.1 ^{A} ±0.70	22.5 ^{A} ±5.33	42.1 ^{A} ±6.83
Proline	345.4 ^{A} ±7.32	23.7 ^{B} ±3.56	14.6 ^{B} ±0.64	10.6 ^{B} ±1.73
Asparagine	446.1 ^{A} ±5.02	73.0 ^{B} ±2.13	18.2 ^{D} ±1.28	48.9 ^{C} ±2.03
Glutamine	1.4 ^{A} ±0.25	0.2 ^{A} ±0.06	2.8 ^{A} ±1.23	2.0 ^{A} ±0.70
Aspartate	27.0 ^{C} ±0.68	128.4 ^{A} ±7.36	67.0 ^{B} ±2.11	45.3 $^{B.C}$ ±3.71
Glutamate	7.0 ^{C} ±0.71	418.8 ^{A} ±4.41	42.8 ^{C} ±5.09	175.2 ^{B} ±10.72
Threonine*	25.1 $^{A.B}$ ±0.29	12.9 ^{B} ±0.11	15.7 ^{B} ±0.22	40.8 ^{A} ±6.49
Homoserine 3	n.d.	0.2 ^{B} ±0.10	1.2 ^{A} ±0.09	0.3 ^{B} ±0.01
Methionine*	n.d.	1.2 ^{B} ±0.07	n.d.	12.2 ^{A} ±0.29
Valine*	23.3 ^{C} ±4.35	49.9 $^{B.C}$ ±1.51	153.9 ^{A} ±28.17	118.6 $^{A.B}$ ±0.43
Leucine*	6.6 ^{C} ±0.25	31.2 ^{C} ±0.57	184.5 ^{A} ±9.52	92.5 ^{B} ±5.29
Isoleucine*	5.1 ^{D} ±0.60	50.4 ^{C} ±1.11	136.9 ^{A} ±4.87	116.7 ^{B} ±4.44
Histidine*	1.2 ^{A} ±0.35	1.6 ^{A} ±0.17	n.d.	0.4 ^{A} ±0.03
aminoadipate	0.9 ^{B} ±0.08	0.1 ^{B} ±0.08	109.5 ^{A} ±3.86	1.5 ^{B} ±0.33
Phenylalanine*	8.4 ^{D} ±0.44	64.0 ^{C} ±4.27	102.6 ^{A} ±5.21	82.3 $^{B.C}$ ±5.42
Arginine	6.5 ^{A} ±0.35	8.5 ^{A} ±0.37	6.0 ^{A} ±0.74	2.1 ^{B} ±0.71
Citrulline	0.3 ^{A} ±0.03	0.5 ^{A} ±0.02	n.d.	0.6 ^{A} ±0.02
Tyrosine	5.7 ^{C} ±1.63	14.3 $^{B.C}$ ±0.61	28.6 ^{A} ±2.05	23.8 $^{A.B}$ ±4.05
Tryptophane*	15.4 ^{B} ±0.87	15.6 ^{B} ±2.37	10.8 ^{B} ±2.99	39.0 ^{A} ±0.71
Essential amino acid score (%)	8.8 ^{C} ±1.1	26.4 ^{B} ±1.4	60.9 ^{A} ±7.3	55.2 ^{A} ±3.6

$^{A-D}$ Different lowercase letters within a row indicate significant differences (P<0.05).

GABA: Gamma-aminobutyric acid.; n.d.: not detected.

*essential amino acids.

Colonic fermentation system

Impact on faecal-derived microbial communities

The 16S rRNA gene-based next-generation sequencing of all fermentation samples yielded a total of 2,142,849 high-quality reads, with an average of 14,881 ± 4,498 sequences per sample, binned into 4,849 amplicon sequence variants (ASVs).

No significant differences were observed in alpha diversity across the entire dataset, regardless of the origin of the faecal sample (post-COVID-19 – PC, or healthy donor – HD), experimental condition (fruit flours, inulin, negative control) and time point (0, 4, 6 and 24 h of fermentation - T0, T4, T6, T24) (P>0.05, Wilcoxon test). However, it should be noted that the diversity of both PC and HD faecal-derived microbial communities tended to decrease over time in all experimental conditions (Supplementary Figure 1).

As for beta diversity, the PCoA of inter-sample variation based on weighted (Figure 1) and unweighted (Supplementary Figure 2) UniFrac distances showed significant segregation between PC and HD faecal-derived microbial communities, regardless of experimental condition and time point (P<0.001, PERMANOVA), thus suggesting the existence of distinctive features of the microbiota at baseline and over time. When focusing on each group (PC and HD), we found that the samples were significantly separated by experimental condition and time point (P<0.001), underlining a differential impact of fruit flours over time. Interestingly, such an impact was overall more marked than that of inulin and negative control.

From the taxonomic standpoint, the differences between the PC and HD faecal-derived microbial communities at baseline were mostly attributable to decreased relative abundances of some Firmicutes members, namely *Christensenellaceae* and *Ruminococcus*, and *Akkermansia* in the former, along with increased proportions of *Faecalibacterium*, *Veillonella*, and unclassified *Lachnospiraceae* (P≤0.05, Wilcoxon test) (Figure 2). Regarding the impact of fruit flours, we observed both common and peculiar microbial signatures of response (Figure 3). Among the features shared between PC and HD, it is worth noting that 24 h of fermentation with the fruit flours resulted in decreased proportions of *Lachnospiraceae* and *Ruminococcaceae*, and increased amounts of *Enterobacteriaceae* (P≤0.2). Although in the absence of statistical significance, these variations were also observed in the control vessels (*i.e.*, with the addition of inulin or without any extract – negative control). When looking for fruit

326 flour-specific effects, we observed: i) a reduction of *Veillonellaceae* with jatobá extract
327 in PC samples; ii) a decrease in *Akkermansia* with jatoba and cumbaru flours; iii) a
328 decreasing trend of *Faecalibacterium* and *Ruminococcus* in the presence of all flours
329 tested, with the exception of the bocaiuva almond in HD samples for *Ruminococcus*;
330 and iv) an increase in *Lactobacillus* and *Bifidobacterium* in PC samples with bocaiuva
331 almond flour ($P \leq 0.2$).
332

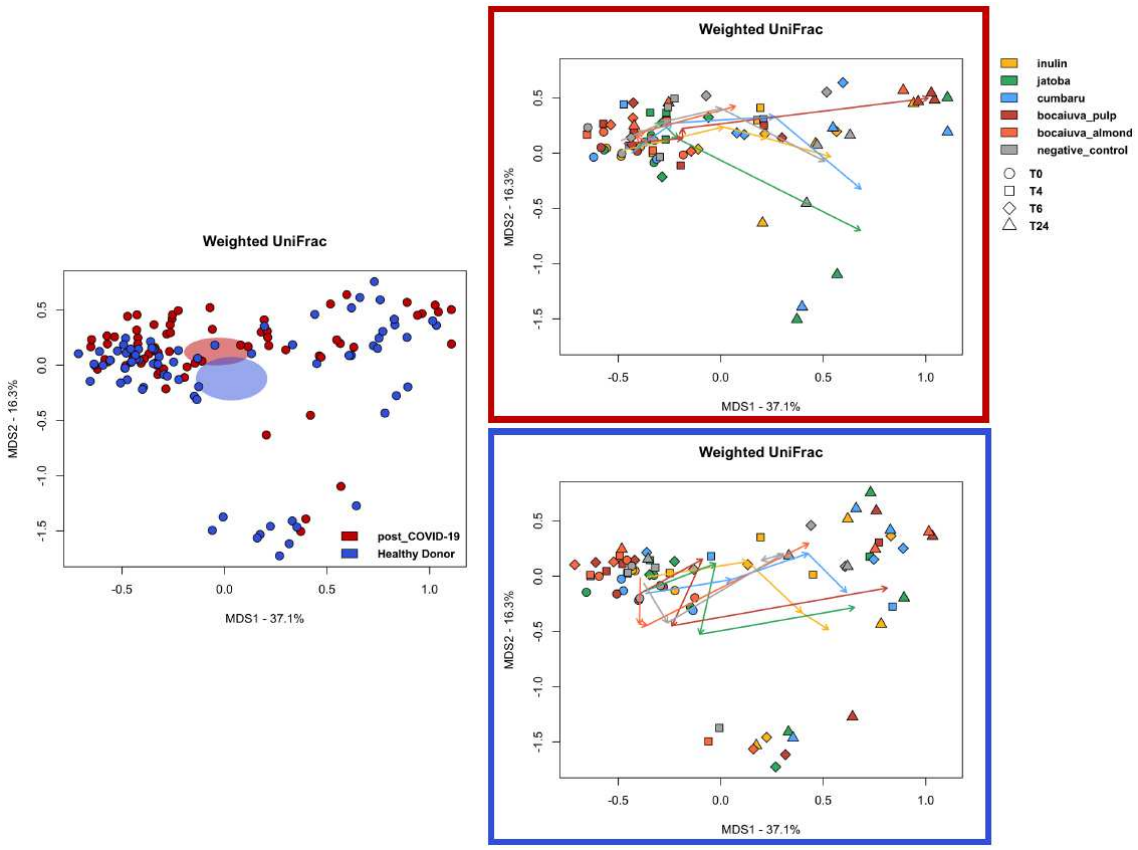


Figure 1. Faecal-derived microbial communities of post-COVID-19 donors separate from those of healthy donors in 24-h fermentation experiments in the presence of fruit flours, inulin or without additions. Left, Principal Coordinates Analysis (PCoA) based on weighted UniFrac distances, showing all fermentation samples, colored by group of donors (post-COVID-19 donors, PC, red vs. healthy donors, HD, blue). A significant separation between groups was found, regardless of experimental condition (fruit flours, inulin and negative control) and time point (0, 4, 6 and 24 h of fermentation - T0, T4, T6 and T24) (P<0.001, PERMANOVA). Right, PCoA plots showing the fermentation samples for PC (top panel) and HD (bottom panel). Within each group of donors, the samples were separated significantly by experimental condition (fruit flours, inulin, negative control) and time point (T0, T4, T6, T24) (P<0.001).

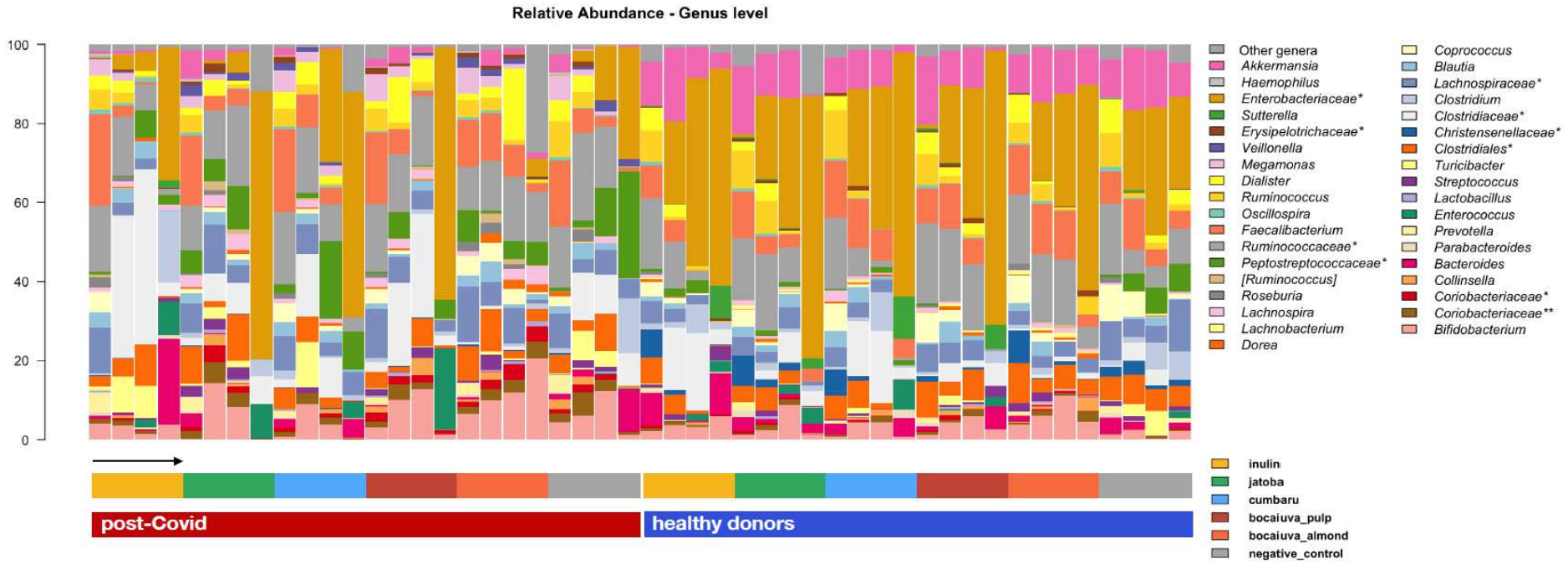


Figure 2. Genus-level relative abundance profiles of faecal-derived microbial communities of post-COVID-19 and healthy donors at 0, 4, 6 and 24 h of fermentation in the presence of fruit flours, inulin or without additions. *, unclassified amplicon sequence variants reported at higher taxonomic level; **, other. For each group of donors (post-COVID-19, PC and healthy donors, HD), the profiles are shown in the following order: samples at 0, 4, 6 and 24 h fermentation (T0, T4, T6 and T24) in the presence of inulin (yellow), jatobá (green), cumbaru (light blue), bocaiuva pulp (red), bocaiuva almond (orange), and negative control (gray). The black arrow below the histograms indicates the temporal succession for each quadruplet of samples (T0, T4, T6 and T24).

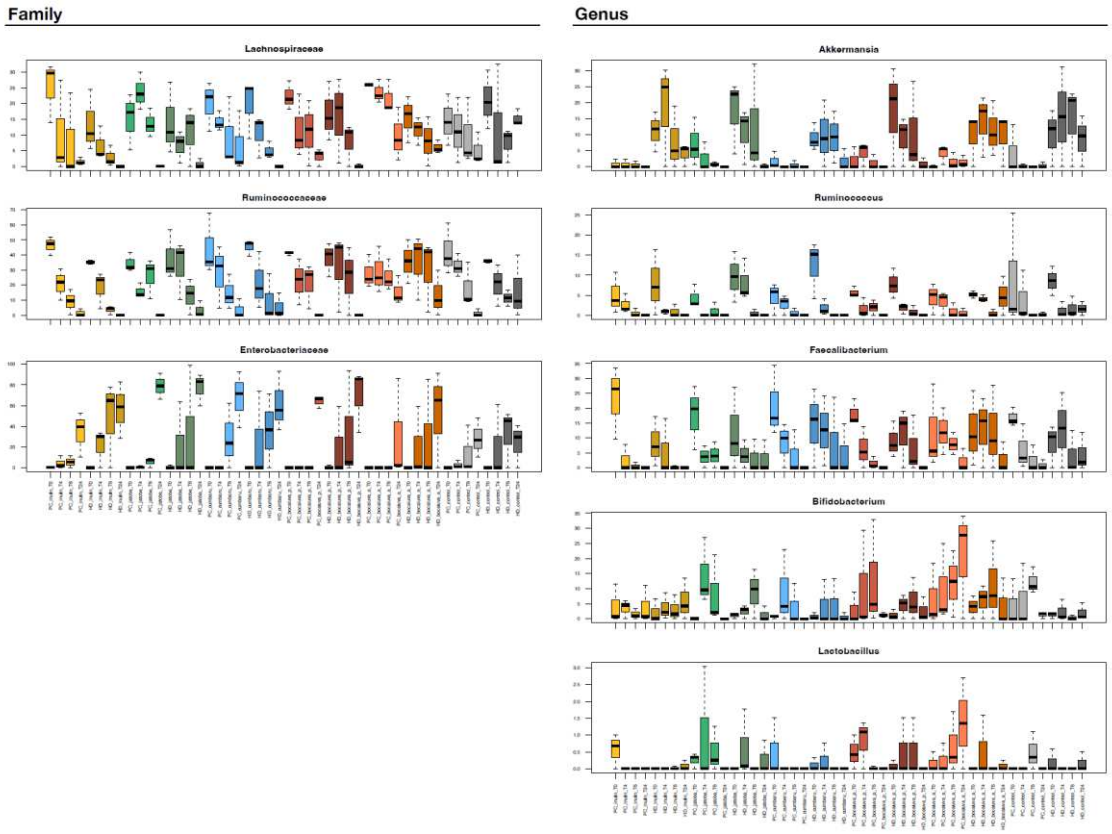


Figure 3. Families and genera of faecal-derived microbial communities of post-COVID-19 (PC) and healthy donors (HD), differing over time in 24-h fermentation experiments in the presence of fruit flours, inulin or without additions. Boxplots showing the relative abundance distribution of bacterial taxa in the different study groups at 0, 4, 6, and 24 h fermentation (T0, T4, T6 and T24). Only significantly different taxa or trends are shown ($P \leq 0.2$, Wilcoxon test). Bocaiuva_p, bocaiuva pulp; bocaiuva_a, bocaiuva almond; control, without any additions.

Analysis of short-chain fatty acids (SCFAs)

Table 2 shows the concentrations of the major SCFAs at 0, 4, 6 and 24 h of fermentation with fruit flours, inulin, and negative control. Overall, SCFA concentrations tended to increase after 24-h fermentation due to substrate utilisation by gut bacteria. Jatobá pulp flour generated higher butyrate production when compared to the other flours in HD ($P<0.05$), whereas no changes were observed in PC. None of the flours generated major changes in propionate production in both donors.

Table 2. Concentrations of major short-chain fatty acids (acetate, propionate and butyrate) during 0, 4, 6 and 24 h of fermentation of Brazilian fruit flours, inulin, and negative control in stirred pH-controlled batch culture systems with stool samples from healthy (HD) and post-COVID-19 donors (PC). Data are presented as mean ± standard deviation (n = 3).

Treatment	Time point (h)	HD			PC		
		Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate
Inulin (positive control)	0	4.28 ± 0.25	3.77 ± 0.05	4.74 ± 0.28	4.12 ± 0.48	3.75 ± 0.29	5.26 ± 0.06
	4	4.28 ± 0.46	3.61 ± 0.11	4.85 ± 0.25	4.93 ± 0.44	4.54* ± 0.24	5.65 ± 0.26
	6	4.63 ± 0.15	3.58 ± 0.27	4.69 ± 0.50	4.95* ± 0.17	4.40* ± 0.11	5.59* ± 0.16
	24	5.40*^{A} ± 0.24	4.98*^{A} ± 0.23	7.21*^{A} ± 0.43	5.15*^{A,B} ± 0.24	4.73*^{A} ± 0.02	6.68*^{A} ± 0.20
Jatobá pulp flour	0	4.10 ± 0.0	3.41 ± 0.00	4.79 ± 0.14	3.74 ± 0.05	4.19 ± 0.01	5.72 ± 0.39
	4	3.87 ± 0.43	3.55 ± 0.43	4.89 ± 0.30	4.25* ± 0.27	4.43* ± 0.11	5.61 ± 0.36
	6	3.99 ± 0.36	3.43 ± 0.08	5.07 ± 0.45	4.50* ± 0.31	3.91 ± 0.71	5.60 ± 0.10
	24	4.78^{A,B} ± 0.52	4.01^{A,B,C} ± 0.59	6.38*^{A,B} ± 0.19	4.59*^{C} ± 0.44	3.99^{A} ± 0.34	5.74^{A,B} ± 0.16
Cumbaru almond flour	0	3.61 ± 0.01	2.81 ± 0.00	4.55 ± 0.24	3.08 ± 0.33	3.03 ± 0.42	4.70 ± 0.12
	4	4.01 ± 0.42	2.96*± 0.04	4.58 ± 0.41	3.20 ± 0.18	2.63 ± 0.10	4.43 ± 0.41
	6	3.74 *± 0.02	2.94 ± 0.15	4.44 ± 0.19	4.20* ± 0.26	3.83* ± 0.63	5.46* ± 0.29
	24	3.90^{B} ± 0.40	3.39*^{B,C} ± 0.33	5.36*^{B,C} ± 0.61	4.21*^{B,C} ± 0.27	4.01*^{A} ± 0.42	4.81^{B} ± 0.03
Bocaiuva pulp flour	0	4.29 ± 0.02	3.32 ± 0.40	4.80 ± 0.20	4.28 ± 0.19	3.07 ± 0.06	4.82 ± 0.25
	4	4.03 ± 0.28	3.49 ± 0.05	4.70 ± 0.24	4.36 ± 0.32	3.94* ± 0.34	5.19 ± 0.45
	6	4.05 * ± 0.18	3.38 ± 0.25	4.59 ± 0.06	4.18 ± 0.34	3.53 ± 0.47	5.33 ± 0.36
	24	4.5^{A,B} ± 0.30	3.30 ^{B,C} ± 0.27	5.5*^{B,C} ± 0.42	4.55^{B,C} ± 0.08	3.70*^{A} ± 0.19	5.07^{B} ± 0.30
Bocaiuva almond flour	0	3.05 ± 0.20	3.27 ± 0.27	4.52 ± 0.15	4.77 ± 0.75	4.19 ± 0.22	5.09 ± 0.13
	4	4.34* ± 0.29	3.49 ± 0.37	4.73* ± 0.15	5.60 ± 0.05	4.08 ± 0.68	4.83 ± 0.30
	6	4.36* ± 0.32	3.38 ± 0.50	4.59 ± 0.30	5.36 ± 0.12	4.22 ± 0.17	5.14 ± 0.32
	24	4.27*^{B} ± 0.22	3.15^{C} ± 0.13	4.56^{C,D} ± 0.27	5.67^{A} ± 0.17	4.68^{A} ± 0.94	5.56^{A,B} ± 1.19
Negative control	0	4.10 ± 0.34	3.45 ± 0.01	4.57 ± 0.01	3.43 ± 0.49	3.51 ± 0.69	4.57 ± 0.09
	4	3.90 ± 0.01	3.36 ± 0.09	4.80 ± 0.08	4.35* ± 0.22	3.90 ± 0.04	5.43* ± 0.04
	6	4.33 ± 0.26	4.37* ± 1.05	4.09 ± 0.90	4.54 ± 0.28	4.08 ± 0.24	5.56* ± 0.12
	24	4.79*^{A,B} ± 0.54	4.49*^{A,B} ± 0.84	4.27*^{D} ± 0.24	4.63*^{B,C} ± 0.31	4.42^{A} ± 0.51	5.24*^{B} ± 0.10

* Significant differences compared to baseline (0h) within the same substrate (using t-test, $P < 0.05$). $^{A-D}$ significant differences of each SCFAs at 24 h of fermentation using one-way ANOVA with Tukey's post-hoc comparison ($P<0.05$)

Total Phenolic Content (TPC) and Ferric Reducing Power (FRAP)

In order to measure the antioxidant activity during colonic fermentation, the TPC content and FRAP were monitored (Figures 4 and 5). As regards the fermentation process, no differences were found for TPC over time. On the other hand, FRAP analysis showed an increase in antioxidant activity after 24 h for jatobá pulp and bocaiuva pulp flours exclusively in PC (P<0.05). The other treatments did not result in significant differences in FRAP for either HD or PC (P<0.05).

However, it was possible to observe an increase in TPC at T0 in the treatments with the fruit flour samples when compared to the negative control and even to the inulin treatment (positive control). This was due to the presence of phenolic compounds in these fruit flours.

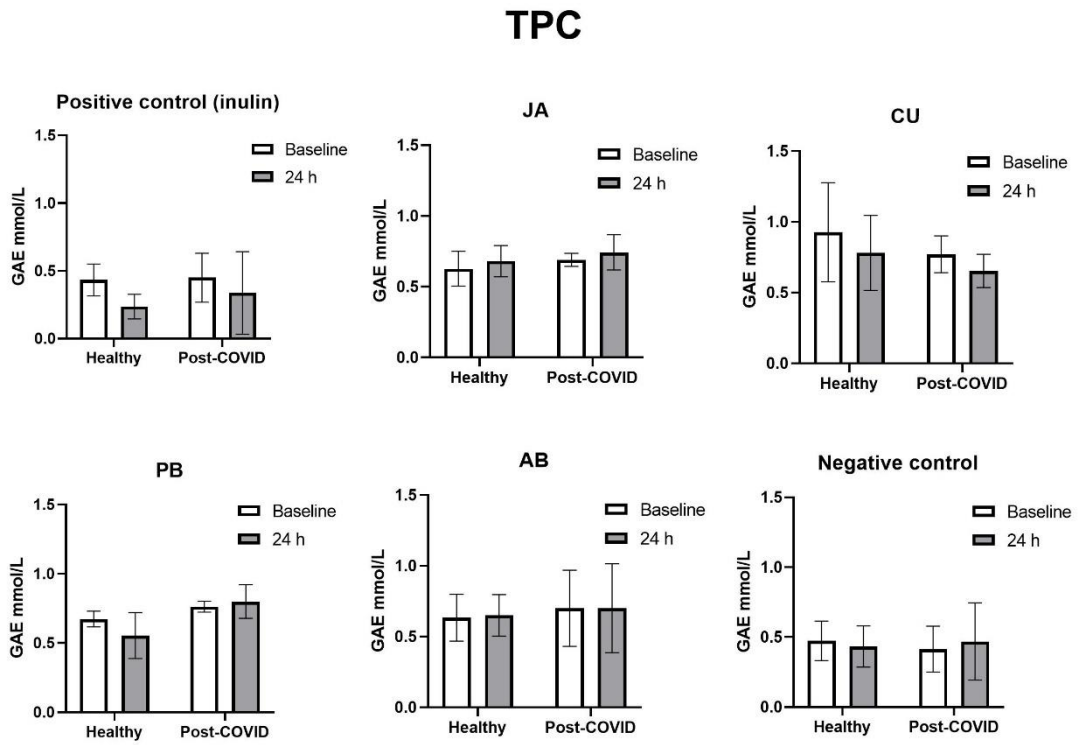


Figure 4. Total phenolic content (mM gallic acid equivalent - GAE) of the supernatants collected at baseline and after 24 h of in vitro colonic fermentation of Brazilian fruit flours, inulin, and negative control with stool samples from healthy and post-COVID-19 donors. JA: jatobá pulp flour, CU: cumbaru almond flour, PB: bocaiuva pulp flour, AB: bocaiuva almond flour, GAE: gallic acid equivalent.

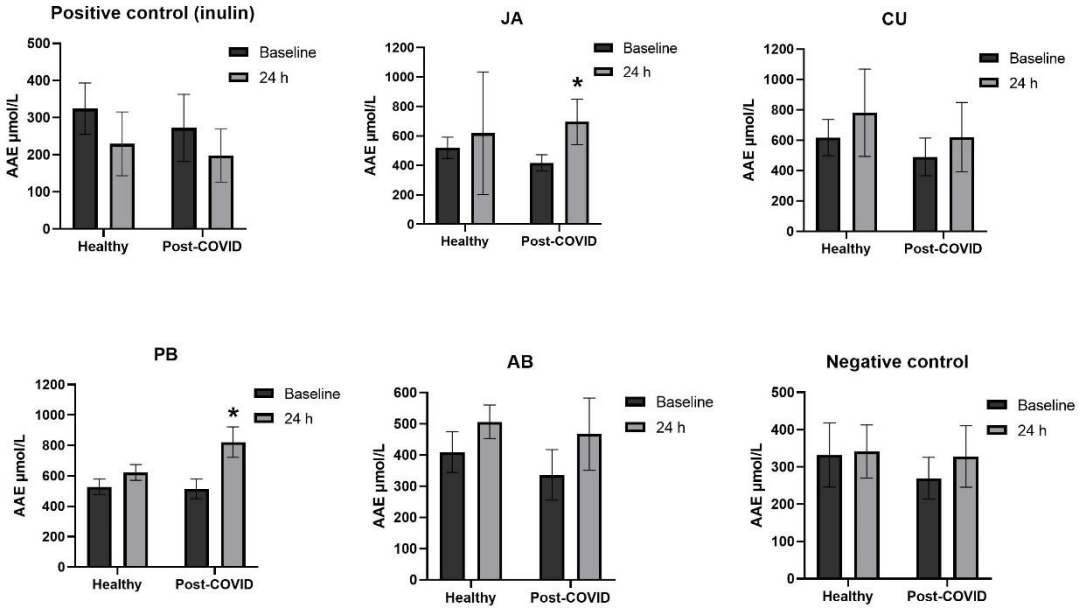
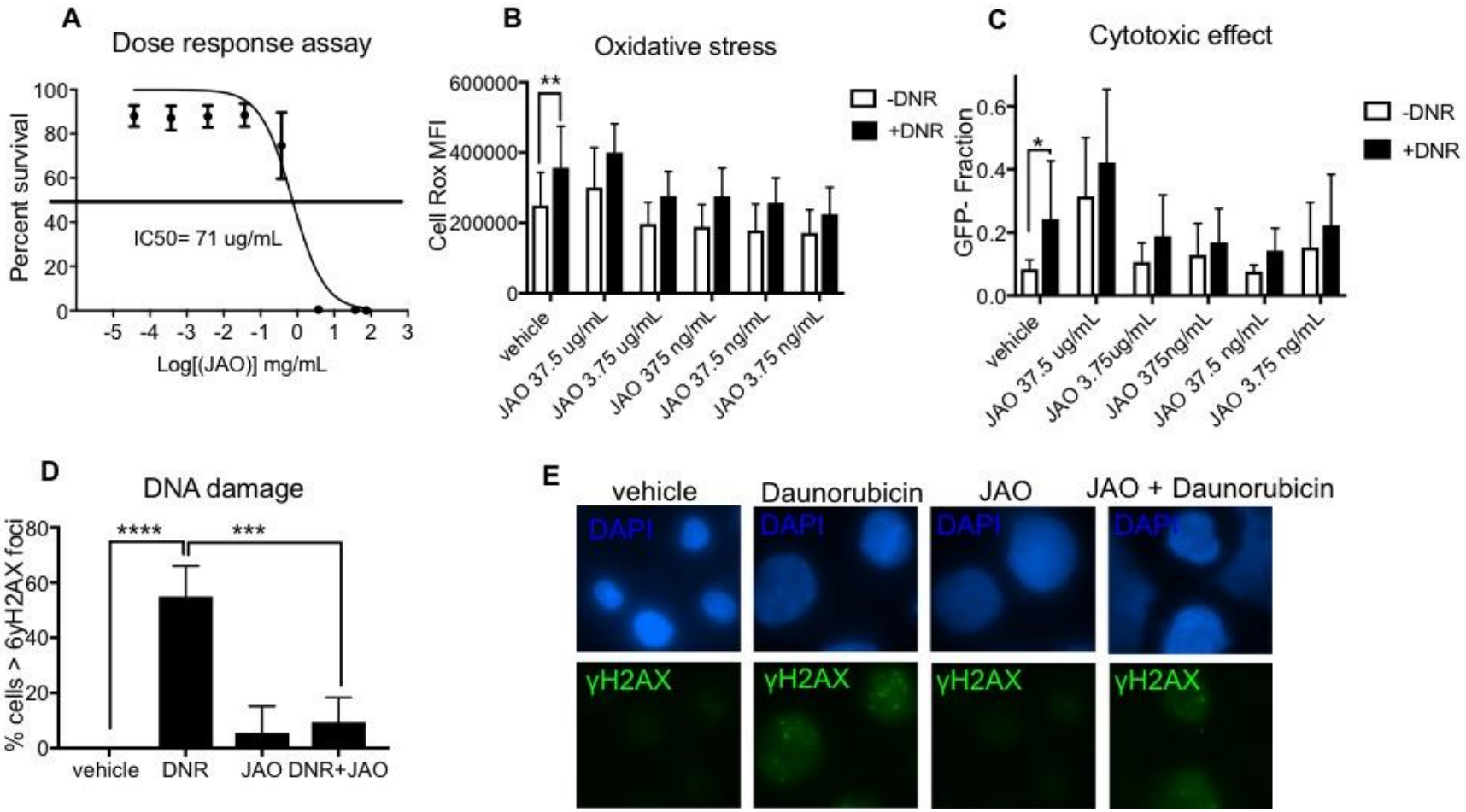


Figure 5. FRAP results (µM ascorbic acid equivalent - AAE) of the supernatants collected at baseline and after 24 h of in vitro colonic fermentation of Brazilian fruit flours, inulin, and negative control with stool samples from healthy and post-COVID-19 donors. JA: jatobá pulp flour, CU: cumbaru almond flour, PB: bocaiuva pulp flour, AB: bocaiuva almond flour, AAE: ascorbic acid equivalent. *Statistical differences after 24 h in the same donor (P<0.05).

Effect of the jatobá pulp oil (JAO) on cell viability, oxidative stress, and DNA damage

We tested the antioxidant properties of JAO in a myelo-monocytic cell line (MV411-eGFP), engineered to express enhanced green fluorescent protein (eGFP), as described in the materials and methods. We first determined the half-maximal inhibitory concentration (IC50) for JAO by incubating the cells with escalating concentrations of JAO extract and measuring the percentage of eGFP positive (alive) and negative cells (dead). The results indicated that the IC50 for JAO in MV411 is 71 µg/mL (Figure 6A). We then decided to assess the effect of non-toxic and non-pharmacological concentrations of JAO (ranging from 3.75 ng/mL to 37.5 µg/mL) on oxidative stress and DNA damage induced by daunorubicin, an anthracycline antibiotic widely used in chemotherapy, which possesses several mechanisms of action, including intercalation into DNA strands, topoisomerase II inhibition, formation of DNA double strand breaks and production of reactive oxygen species (ROS) (Al-Amri et al., 2019). Following exposure of MV411 cells to daunorubicin, a significant increase in oxidative stress,

419 measured as fluorescence emitted by Cell ROX, was observed (MFI Cell ROX in
420 vehicle vs daunorubicin, 249912.292± 93116.016 vs 356684.786±117841.971, P<0.05;
421 fold increase, 1.42±0.47) (Figure 6B and Supplementary Figure 3). This was
422 accompanied by a cytotoxic effect (fraction of eGFP- cells in vehicle vs daunorubicin,
423 0.084± 0.028 vs 0.242±0.185, P<0.05; fold increase, 2.88±2.2) (Figure 6C and
424 Supplementary Figure 3). 24-h pre-exposure with JAO extract reduced daunorubicin-
425 induced oxidative stress and cytotoxic effect to levels comparable to vehicle (DMSO)
426 (Figure 6B-C and Supplementary Figure 3), demonstrating a chemopreventive effect
427 against daunorubicin-induced cytotoxic effect and oxidative stress.
428 Incubation with daunorubicin also induced a significant increase in DNA
429 damage (double strand breaks), measured as percentage of cells showing more than 6
430 γH2AX foci (% cells with > 6γH2AX foci in vehicle vs daunorubicin after 48-h
431 exposure, 0±0 vs 54.95±11.07, P<0.05) (Figure 6D-E). 24-h pre-exposure with JAO (37
432 ng/mL) reduced daunorubicin-induced DNA damage to levels comparable to vehicle
433 (DMSO) (% cells with > 6γH2AX foci in vehicle vs JAO after 24-h exposure, 0±0 vs
434 5.55±9.62, P=0.34; daunorubicin vs dauorubicin+JAO after 24-h exposure, 54.95±11.07
435 vs 9.34±8.94, P<0.01) (Figure 6D-E and Supplementary Figure 4), demonstrating that
436 JAO pulp exerted a chemopreventive effect even against daunorubicin-induced DNA
437 damage.
438



439

440 **Figure 6. Effect of Jatobá pulp oil (JAO) on daunorubicin-induced oxidative stress and DNA damage.** A) IC50 of Jatobá pulp oil (JAO)
441 in macrophage cell line MV411. Non-linear regression concentration/response curve reporting cell viability, determined by Trypan blue exclusion upon
442 treatment with JAO for 72 h. Data are shown as average +/-SD of triplicate wells and are representative of four independent experiments. The IC50 was
443 calculated using GraphPad Prism software. B) JAO protects from the oxidative stress induced by daunorubicin. The bar chart reports the oxidative stress

as Median Fluorescence Intensity (MFI) of Cell ROX for MV411 pre-treated with either vehicle (DMSO) or increasing concentrations of JAO for 24 h before incubation with daunorubicin (DNR 25 nM) for additional 24 h. Data show mean +/-SD of triplicate wells and are representative of four independent experiments. 2-Way Anova Sidak's multiple comparison test ** $P<0.01$. C) JAO protects from the cytotoxic effect of daunorubicin. The bar chart reports the cytotoxic effect as a fraction of GFP- cells for MV411 pre-treated with either vehicle (DMSO) or increasing concentrations of JAO for 24 h before incubation with 25 nM DNR for additional 24 h. Data show mean +/-SD of triplicate wells and are representative of four independent experiments. 2-Way Anova Sidak's multiple comparison test * $P<0.05$. D) JAO protects from the DNA damage induced by daunorubicin. The bar chart reports the DNA damage as percentage of MV411 cells with more than 6 γH2AX foci. The cells were pre-treated with either vehicle (DMSO) or 37 ng/mL of JAO for 24 h before incubation with 25 nM DNR for additional 48 h. Data show mean +/-SD of triplicate wells and are representative of four independent experiments. 2-Way Anova Tukey's multiple comparison test *** $P<0.001$. **** $P<0.0001$. E) Digital microscope images showing DNA damage γH2AX foci in MV411 cells pre-treated with either vehicle (DMSO) or 37 ng/mL of JAO for 24 h before incubation with 25 nM DNR for additional 48 h. Images were captured using Evos FL Digital Inverted Fluorescence Microscope (a particular from magnification 40X).

Discussion

The Brazilian pulp and almond fruit flours tested in this study presented important nutritional values, with proteins, sugars, fibre, and lipids. Total fibre in jatobá pulp flour was present in greater quantity than in other flours, which may contribute to a beneficial activity for use by gut bacteria (Gibson et al., 2017). Bocaiuva almond flour had the highest lipid content, even when compared to *in natura* bocaiuva almond in the literature (Hiane et al., 2006). Bocaiuva pulp and almond flour were also potentially good sources of some essential amino acids, as they had a higher essential amino acid score than other flours. However, for a protein to be considered of good quality, it must be well absorbed after digestion. To assess this, direct study by measuring nitrogen balance along with body weight and body composition are required (Joint WHO/FAO/UNU, 2007). Protein content and amino acid profile of foods are important substrates for gut fermentation, as they provide nitrogen sources for the proliferation of bacteria. It has been suggested that specific amino acid mixtures are likely to be of benefit to the human colonic microbiota, in addition to dietary fibre and prebiotics (Bifari et al., 2017).

The small intestine is highly specialised in the breakdown, emulsification and absorption of nutrients and few nutrients escape the digestive process. The human body lacks the enzymes necessary to digest the wide range of complex carbohydrates allowing them to escape digestion in the small intestine. In the colon, however, they can be used as an energy source by specific resident bacteria (Louis and Flint, 2009; de Vos et al., 2022). In fact, various gut microbes contribute to the metabolization of these non-digestible polysaccharides in several endpoints of anaerobic fermentation, including SCFAs. These compounds regulate numerous metabolic pathways both in the intestine and at distance, *i.e.*, at the level of the liver, adipose tissue, muscles, and brain. It is in fact well known that SCFAs contribute to numerous physiological effects, ranging from the modulation of energy homeostasis, glucose/lipid metabolism, inflammation and even immunity and cancer (Pascale et al., 2018; Silva et al., 2020; Marrocco et al., 2022).

Colonic fermentation models provide insights into the processes in the gut and are considered screening tools of many dietary ingredients. In this study, we chose to ferment fruit flours of pulp and almonds from Cerrado and Pantanal to observe the effect of these whole foods on microbiota modulation and production of SCFAs. Under these circumstances, the intention of these experiments was to use the food as it is sold

in the local market and ready for consumption, considering all its characteristics of macronutrients that can positively impact the health of consumers. However, we are aware that it has been documented in the literature that the proximate composition of foods can directly affect the composition of the microbiota (Leeming et al., 2021).

As for stool donors, we chose subjects who had COVID-19 (PC) and healthy volunteers (HD). Indeed, it has recently been hypothesised that the gut microbiota plays a key role in the context of COVID-19 and also contributes to its long-term effects after viral clearance (Lau et al., 2022; Sun et al., 2022; Groves et al., 2020). In particular, reduced bacterial diversity, together with a reduction in the relative abundance of health-associated bacteria such as SCFA producers belonging to the *Lachnospiraceae* and *Ruminococcaceae* families, has been observed in patients with COVID-19 (Yeoh et al., 2021; Gaibani et al., 2021; Ren et al., 2021). In our dataset, although the reduction in biodiversity is consistent with what is commonly observed in *in vitro* models, it was possible to confirm the depletion of some taxa generally considered health-promoting in PC, such as *Ruminococcus, Christensenellaceae and Akkermansia* (Lau et al., 2022; Gaibani et al., 2021; Gu et al., 2020; Tao et al., 2020; Nashed et al., 2022). We also confirmed PC-related enrichment in potential opportunistic pathogens, including *Veillonellaceae* members (Gu et al., 2020; Tao et al., 2020).

Taking into account the impact of fruit extracts on the faecal-derived microbial communities, it is interesting to note that the jatobá extract counteracted the increase of *Veillonellaceae* in PC while that of bocaiuva almond the further decrease of *Ruminococcus*. Moreover, the fermentation of the bocaiuva almond led to an enrichment of *Lactobacillus* and *Bifidobacterium*, underlining a relevant prebiotic potential.

TPC and FRAP analyses were conducted to analyse the content of total phenolic compounds and antioxidants during colonic fermentation, considering that gut bacteria may be involved in their biotransformation, as reported in some simulated *in vitro* models (Gibson et al., 2017). However, it was not possible to observe differences in TPC between baseline and after 24 h, whereas in the FRAP analysis only for jatobá and bocaiuva pulp flours (PC donors), an increase in antioxidant activity was observed. It is estimated that 90-95% of polyphenols contained in food are not absorbed by the small intestine and, therefore, can reach the colon (Gibson et al., 2017). However, the amount of phenolic compounds and antioxidants that reach the colon and the bloodstream is limited compared to the content found in food right after consumption, as these

substances are highly reactive to alkaline conditions and can be easily oxidised, resulting in degradation or failure to detect (Lafarga et al., 2019). Furthermore, the basal medium used in the colonic fermentation simulation contains microorganisms that degrade and convert components in the medium, such as phenolic compounds into phenolic acid or lactone metabolites (Aura & Maukonen, 2015). In juçara pulp, a fruit with a high content of phenolic compounds and anthocyanins, Guergoletto et al. (2016) found a decrease in the amount of these components after simulated digestion, indicating degradation by gut bacteria during the fermentation process.

Phenolic compounds have gained a lot of attention as a dietary intervention to augment endogenous oxidative defenses. This is important because ROS can damage DNA, proteins and lipids and trigger pathogenetic processes. Most importantly, ROS are not only the result of exposure to genotoxic substances of environmental origin but are continuously produced in aerobic organisms both as by-products of normal oxygen metabolism and as bactericidal agents by activated phagocytic cells. Therefore, a correct redox balance is considered very important for the prevention and treatment of various degenerative diseases, including cancer (Schieber and Chandel, 2014; Robinson et al., 2021).

Finally, we chose to test the antioxidant properties of jatobá pulp in a cell model because JAO contains bioactive substances such as g-tocopherol, b-sitosterol, campesterol, stigmasterol, and stigmastanol (Oliveira et al., 2018), which could protect cells by hindering the harmful action of free radicals. Cell models are extremely useful and well characterised experimental models that can be employed for investigating the effect of genetic and pharmacological manipulations, as well as the delicate balance between pro- and antioxidant activities. The oxidative response is particularly prevalent in cells of the innate immune system, including granulocytes, monocytes, and macrophages, as it is central to the antimicrobial action of these cells. Therefore, myelo-monocytic cell lines such as MV411 and THP1 represent gold standard experimental models for studying the mechanisms of redox homeostasis (Karwaciak et al., 2017).

After determining the IC50 of JAO in the myelo-monocytic cell line MV411, we decided to further test non-toxic, nutritional concentrations (from 3.75 ng/mL to 37.5 µg/mL), which could be easily reached in the bloodstream and other tissues with an ordinary intake of fruits. In our study, JAO showed a protective antioxidant and antigenotoxic effect as it was able to reduce both oxidative stress and DNA damage induced by daunorubicin. Our data are in agreement with Spera *et al.* (2019) that

showed that *H. courbaril* seed extract possessed antioxidant and antigenotoxic properties. However, the authors also reported that high concentrations of *H. courbaril* extracts (50-100 µg/mL) possessed a cytotoxic effect against B16F10 murine melanoma cells. More studies are therefore needed to evaluate the effects of non-nutritional concentrations of JAO in cancer cells and explore JAO as a pharmacological tool (Halliwell, 2012), as it has been reported for resveratrol and hydroxytyrosol, polyphenols abundant in edible plants such as grapes, olives and annurca apples (Borska et al., 2016; Corona et al., 2009; Martino et al., 2019).

Conclusions

To our knowledge, this is the first *in vitro* study to evaluate the impact of native fruit flours from the Brazilian Cerrado and Pantanal on gut microbiota composition and metabolic profile. In addition to their high nutritional value, some of these fruit flours showed promising ability to modulate gut microbiota imbalances, which could be relevant in post-COVID-19. We also showed that JAO has chemopreventive effects as it reduces daunorubicin-induced oxidative stress, DNA damage and cytotoxic effect. Taken together, these preliminary data demonstrate all the strengths and limitations of our study and lay the foundations for the design of an *in vivo* human intervention study to confirm the promising trends herein observed, along with studies in animal models to unravel the underlying mechanisms and evaluate the potential role of these flours in other contexts as well.

Acknowledgements

We would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES - Brazil), for the scholarship granted to C.S.I. Mauro (process number 88881.624468/2021-01), and the Department of Life Sciences at University of Roehampton (London, UK).

Author Contributions

Carolina Saori Ishii Mauro: Conceptualization (equal); formal analysis (equal); investigation (equal); writing – original draft (equal). **Maryame Kadiri Hassani:** Formal analysis (equal); investigation (equal); writing – original draft (equal). **Monica Barone:** Formal analysis (equal); writing – review and editing (equal). **Maria Teresa Esposito:** Formal analysis (equal); writing – review and editing (equal).

Yolanda Calle: Writing – review and editing (equal). **Volker Behrends:** Formal analysis (equal); writing – review and editing (equal). **Sandra Garcia:** Writing – review and editing (equal). **Patrizia Brigidi:** Writing – review and editing (equal). **Silvia Turroni:** Formal analysis (equal); writing – review and editing (equal). **Adele Costabile:** Conceptualization (equal); formal analysis (equal); writing – review and editing (equal).

Conflict of Interest

The authors declare no conflict of interests.

Ethical Guidelines

The University of Roehampton Research Ethics Committee (LSC 18-241) approved this study in accordance with the Declaration of Helsinki.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.16274.

Data Availability Statement

Sequence reads were deposited in the National Center for Biotech

nology Information Sequence Read Archive (NCBI SRA; BioProject ID PRJNA913064).

References

Al-Aamriri, H. M. *et al.* (2019). Time dependent response of daunorubicin on cytotoxicity, cell cycle and DNA repair in acute lymphoblastic leukaemia. *BMC Cancer*, 19, 179. https://doi.org/10.1186/s12885-019-5377-y

Arruda, H. S., Araújo, M. V. L. and Marostica Junior, M. R. (2022). Underexploited Brazilian Cerrado fruits as sources of phenolic compounds for diseases management: A review. *Food Chemistry: Molecular Sciences*, 100148. https://doi.org/10.1016/j.fochms.2022.100148

Aura, A. M. and Maukonen, J. (2015). One Compartment Fermentation Model. In: *The Impact of Food Bioactives on Health*. Pp. 281–292. Springer. https://doi.org/10.1007/978-3-319-16104-4_25

626 Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a
627 measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-
628 6. https://doi.org/10.1006/abio.1996.0292

629 Behrends, V., Tredwell, G. D. and Bundy, J. G. (2011). A software complement to
630 AMDIS for processing GC-MS metabolomic data, *Analytical Biochemistry*, 415(2),
631 206-208. https://doi.org/10.1016/j.ab.2011.04.009

632 Bifari et al. (2017). Amino acid supplements and metabolic health: a potential interplay
633 between intestinal microbiota and systems control. *Genes & Nutrition*, 12, 27.
634 https://doi.org/10.1186/s12263-017-0582-2

635 Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and
636 purification. *Canadian Journal of Biochemistry and Physiology*, 37 (8).
637 https://doi.org/10.1139/o59-099

638 Bolyen, E. *et al.* (2019). Reproducible, interactive, scalable and extensible microbiome
639 data science using QIIME 2. *Nature Biotechnology*, 37, 852–857.
640 https://doi.org/10.1038/s41587-019-0209-9

641 Borska, S. et al. (2016). Classical and atypical resistance of cancer cells as a target for
642 resveratrol. *Oncology Reports*, 36, 1562-1568. https://doi.org/10.3892/or.2016.4930

643 Bortolotto et al. (2017). A knowledge network to promote the use and valorization of
644 wild food plants in the Pantanal and Cerrado, Brazil. *Regional Environmental Change*,
645 17, 1329–1341. https://doi.org/10.1007/s10113-016-1088-y

646 Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995) Use of a free radical method
647 to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28 (1), 25-30.
648 https://doi.org/10.1016/S0023-6438(95)80008-5

649 Buford, T. W. (2017). (Dis)Trust your gut: the gut microbiome in age-related
650 inflammation, health, and disease. *Microbiome*, 5, 80. https://doi.org/10.1186/s40168-
651 017-0296-0

652 Callahan, B. J., *et al*. (2016). DADA2: High-resolution sample inference from Illumina
653 amplicon data. *Nature Methods*, 13(7), 581-583. https://doi.org/10.1038/nmeth.3869

654 Chhibber-Goel, J., Gopinathan, S. & Sharma, A. (2021). Interplay between severities of
655 COVID-19 and the gut microbiome: implications of bacterial co-infections? *Gut*
656 *Pathogens*, 13, 14, 2021. https://doi.org/10.1186/s13099-021-00407-7

657 Corona *et al.* (2009). Hydroxytyrosol inhibits the proliferation of human colon
658 adenocarcinoma cells through inhibition of ERK1/2 and cyclin D1. *Molecular Nutrition*
659 *& Food Research*, 53, 897-903. https://doi.org/10.1002/mnfr.200800269

660 Corona, G. *et al*. (2019). Impact of lignans in oilseed mix on gut microbiome
661 composition and enterolignan production in younger healthy and premenopausal
662 women: an in vitro pilot study. *Microbial Cell Factories*, 19, 1–14.
663 https://doi.org/10.1186/s12934-020-01341-0

664 Costabile, A. *et al.* (2010). A double-blind, placebo-controlled, cross-over study to
665 establish the bifidogenic effect of a very-long-chain inulin extracted from globe
666 artichoke (*Cynara scolymus*) in healthy human subjects. *British Journal of Nutrition*,
667 104, 1007-1017. https://doi.org/10.1017/S0007114510001571

668 Costabile, A. *et al*. (2015). Effects of orange juice formulation on prebiotic functionality
669 using an in vitro colonic model system. *PLoS One*, 10(3), e0121955.
670 https://doi.org/10.1371/journal.pone.0121955

671 Culhane, A. C. *et al. (*2005). MADE4: an R package for multivariate analysis of gene
672 expression data. *Bioinformatics*, 21 (11), 2789–2790.
673 https://doi.org/10.1093/bioinformatics/bti394

674 Damasceno-Junior, G. A. *et al.* (2010). *Sabores do Cerrado e Pantanal: conhecer para*
675 *valorizar os frutos nativos; receitas e boas práticas de aproveitamento*. Pp. 142. Campo
676 Grande, Brazil: Editora da Universidade Federal de Mato Grosso do Sul.

677 de Vos, W. M., et al. (2022). Gut microbiome and health: Mechanistic insights. *Gut*,
678 71(5), 1020–1032. https://doi.org/10.1136/gutjnl-2021-326789

679 Espín, J. C., González-Sarrías, A. and Tomás-Barberán, F. A. The gut microbiota: A
680 key factor in the therapeutic effects of (poly)phenols. *Biochemical Pharmacology*, 139,
681 82-93. https://doi.org/10.1016/j.bcp.2017.04.033

682 Esposito *et al.* (2015). Synthetic lethal targeting of oncogenic transcription factors in
683 acute leukemia by PARP inhibitors. *Nature Medicine*, 21, 1481–1490.
684 https://doi.org/10.1038/nm.3993

685 Gaibani, P. *et al.* (2021). The Gut Microbiota of Critically Ill Patients With COVID-19.
686 *Frontiers in Cellular and Infection Microbiology*.
687 https://doi.org/10.3389/fcimb.2021.670424

688 Gibson, G. R. *et al.* (2017). Expert consensus document: The International Scientific
689 Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition
690 and scope of prebiotics. *Nature Review Gastroenterology and Hepatology*, 14(8), 491-
691 502. https://doi.org/10.1038/nrgastro.2017.75

692 Groves, H. T. *et al.* (2020). Respiratory viral infection alters the gut microbiota by
693 inducing inappetence. *mBio*, 11, e03236-19. https://doi.org/10.1128/mBio.03236-19

694 Gu, S. *et al.* (2020). Alterations of the Gut Microbiota in Patients With Coronavirus
695 Disease 2019 or H1N1 Influenza. *Clinical Infectious Diseases*, 71(10), 2669-2678.
696 https://doi.org/%2010.1093/cid/ciaa709

697 Guergoletto, K. B. *et al.* (2016) *In vitro* fermentation of juçara pulp (*Euterpe edulis*) by
698 human colonic microbiota. *Food Chemistry*, 196, 251-258.
699 https://doi.org/10.1016/j.foodchem.2015.09.048

700 Halliwell, B. (2013). The antioxidant paradox: less paradoxical now? *British Journal of*
701 *Clinical Pharmacology*, 75(3), 637-44. https://doi.org/10.1111/j.1365-
702 2125.2012.04272.x

703 Hiane, P. A. *et al.* (2006). Chemical and nutritional evaluation of kernels of bocaiuva,
704 *Acrocomia aculeata* (Jacq.) Lodd. *Food Science and Technology*, 26 (3).
705 https://doi.org/10.1590/S0101-20612006000300031

706 Hooper *et al.* (2012). Reduced or modified dietary fat for preventing cardiovascular
707 disease. *Cochrane Database of Systematic Reviews*, 5, Art. No.: CD002137.
708 https://doi.org/10.1002/14651858.CD002137.pub3

709 Bezerra *et al.* (2013). Phytochemical study guided by the myorelaxant activity of the
710 crude extract, fractions and constituent from stem bark of *Hymenaea courbaril* L.
711 *Journal of Ethnopharmacology*, 149, 62-69. https://doi.org/10.1016/j.jep.2013.05.052

712 Jayaprakasam, B. *et al.* (2007). Terpenoids from Stinking toe (*Hymneae courbaril*)
713 fruits with cyclooxygenase and lipid peroxidation inhibitory activities. *Food Chemistry*,
714 105(2), Pages 485-490. https://doi.org/10.1016/j.foodchem.2007.04.004

715 Joint WHO/FAO/UNU Expert Consultation. (2007). Protein and amino acid
716 requirements in human nutrition. World Health Organ technical report series. (935):1-
717 265.

718 Karwaciak, I., *et al.* (2017). TLR2 activation induces antioxidant defence in human
719 monocyte-macrophage cell line models. *Oncotarget*, 8(33), 54243–54264.
720 https://doi.org/10.18632/oncotarget.17342

721 Keiji, T. *et al.* (1999). Tyrosinase Inhibitors from the Pericarp of Jatoba (*Hymenaea*
722 *courbaril* L.). *Natural Medicine*s, 53, 15-21.

723 Klindworth, A. *et al.* (2013). Evaluation of general 16S ribosomal RNA gene PCR
724 primers for classical and next-generation sequencing-based diversity studies. *Nucleic*
725 *Acids Research*, 41(1), e1. https://doi.org/10.1093/nar/gks808

726 Lafarga, T. *et al.* (2019). Effect of ultrasound processing on the bioaccessibility of
727 phenolic compounds and antioxidant capacity of selected vegetables. *Food Science and*
728 *Biotechnology*, 28, 1713–1721. https://doi.org/10.1007/s10068-019-00618-4

729 Lakshmanan, A. P. *et al.* (2022). Increased Relative Abundance of *Ruminoccocus* is
730 Associated with Reduced Cardiovascular Risk in an Obese Population. *Frontiers in*
731 *Nutrition*, 9, 849005. https://doi.org/10.3389/fnut.2022.849005

732 Lau, R. I. *et al.* (2022). Gut microbiota in COVID-19: key microbial changes, potential
733 mechanisms and clinical applications. *Nature Reviews Gastroenterology & Hepatology*.
734 https://doi.org/10.1038/s41575-022-00698-4

735 Lee, S. C., Prosky, L. and Devries, J. W. (1992). Determination of total, soluble and
736 insoluble dietary fiber in foods. Enzymatic-gravimetric method, MES-TRIS buffer:
737 collaborative study. *Journal of the Association of the Official Analytical Chemists*
738 (AOAC) *International*, 75, 395-416. https://doi.org/10.1093/jaoac/75.3.395

739 Leeming, E. R. *et al.* (2021). The complexities of the diet-microbiome relationship:
740 advances and perspectives. *Genome Medicine*, 13, 10. https://doi.org/10.1186/s13073-
741 020-00813-7

Liu, Q. *et al.* (2022). Gut microbiota dynamics in a prospective cohort of patients with post-acute COVID-19 syndrome. *Gut*, 71, 544-552. https://doi.org/10.1093/jaoac/75.3.39510.1136/gutjnl-2021-325989

López de Felipe, F., de las Rivas, B. & Muñoz, R. (2022). Molecular Responses of Lactobacilli to Plant Phenolic Compounds: A Comparative Review of the Mechanisms Involved. *Antioxidants*, 11, no. 1: 18. https://doi.org/10.3390/antiox11010018

Louis, P., and Flint, H. J. (2009). Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiology Letters*, 294(1), 1–8. https://doi.org/10.1111/j.1574-6968.2009.01514.x

Marrocco, F., *et al*. (2022). Short-chain fatty acids promote the effect of environmental signals on the gut microbiome and metabolome in mice. *Communication Biology*, 5, 517 https://doi.org/10.1038/s42003-022-03468-9

Martino, E. *et al.* (2019). Annurca apple polyphenol extract selectively kills MDA-MB-231 cells through ROS generation, sustained JNK activation and cell growth and survival inhibition. *Scientific Reports*, 9, 13045. https://doi.org/10.1038/s41598-019-49631-x

Masella, A. P. *et al*. (2012). PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics*, 13, 31. https://doi.org/10.1186/1471-2105-13-31

Mente *et al.* (2009). A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Archives of Internal Medicine*, 169(7), 659-669. https://doi.org/10.1001/archinternmed.2009.38

Merril, A. L. and Watt, K. B. (1973). *Energy Value of Foods: Basis and Derivation*, United States Department of Agriculture, Washington.

Nashed *et al.* (2022). Gut microbiota changes are detected in asymptomatic very young children with SARS-CoV-2 infection. *Gut*, 71(11), 2371-2373. https://doi.org/10.1136/gutjnl-2021-326599

Oliveira, F. G. S. *et al.* (2018). *The genus Hymenaea: A chemical and pharmacological review.* In F.R.S. Atta-ur-Rahman (Ed.), Studies in natural products chemistry (Chap. 12, pp. 339-388). Amsterdam: Elsevier.

Ozdal *et al.* (2016). The Reciprocal Interactions between Polyphenols and Gut Microbiota and Effects on Bioaccessibility. *Nutrients*, 8(2), 78. https://doi.org/10.3390/nu8020078

Parkar, S. G., Stevenson, D. E. and Skinner, M. A. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. *International Journal of Food Microbiology,* 3(10), 295-298. https://doi.org/10.1016/j.ijfoodmicro.2008.03.017

Pascale, A., et al. (2018). Microbiota and metabolic diseases. *Endocrine*, 61, 357–371. https://doi.org/10.1007/s12020-018-1605-5

Ren, Z. *et al.* (2021). Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. *Gut*, 70(7), 1253-1265.https://doi.org/10.1136/gutjnl-2020-323826

781 Robinson *et al.* (2021). Reactive Oxygen Species Rewires Metabolic Activity in Acute
782 Myeloid Leukemia. *Frontiers in Oncology*, 11.
783 https://doi.org/10.3389/fonc.2021.632623

784 Rognes, T. *et al*. (2016). VSEARCH: a versatile open source tool for metagenomics.
785 *PeerJ*, 4, e2584. https://doi.org/10.7717/peerj.2584

786 Schieber, M. and Chandel, N. S. (2014). ROS Function in Redox Signaling and
787 Oxidative Stress. *Current Biology*, 24(10), R453-
788 R462.https://doi.org/10.1016/j.cub.2014.03.034

789 Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids
790 from gut microbiota in gut-brain communication. *Frontiers in Endocrinology*, 11, 25.
791 https://doi.org/10.3389/fendo.2020.00025

792 Skeaff, C. M. and Miller, J. (2009). Dietary Fat and Coronary Heart Disease: Summary
793 of Evidence from Prospective Cohort and Randomised Controlled Trials. *Annals of*
794 *Nutrition and Metabolism*, 55, 173-201. https://doi.org/10.1159/000229002

795 Song, H. E. *et al.* (2019). A Facile Profiling Method of Short Chain Fatty Acids Using
796 Liquid Chromatography-Mass Spectrometry *Metabolites*, 9, 173.
797 https://doi.org/10.3390/metabo9090173

798 Spera, K. D. *et al.* (2019). Genotoxicity, anti-melanoma and antioxidant activities of
799 *Hymenaea courbaril* L. seed extract. *Annals of the Brazilian Academy of Sciences,* 91:
800 e20180446. https://doi.org/10.1590/0001-3765201920180446

801 Sun, Z. *et al.* (2022). Gut microbiome alterations and gut barrier dysfunction are
802 associated with host immune homeostasis in COVID-19 patients. *BMC Medicine*, 20,
803 24. https://doi.org/10.1186/s12916-021-02212-0

804 Swain, T. and Hills, W. E. (1959). The phenolic constituents of *Prunus domestica*.
805 *Journal of the Science of Food and Agriculture*, 10, 63-69.
806 https://doi.org/10.1002/jsfa.2740100110

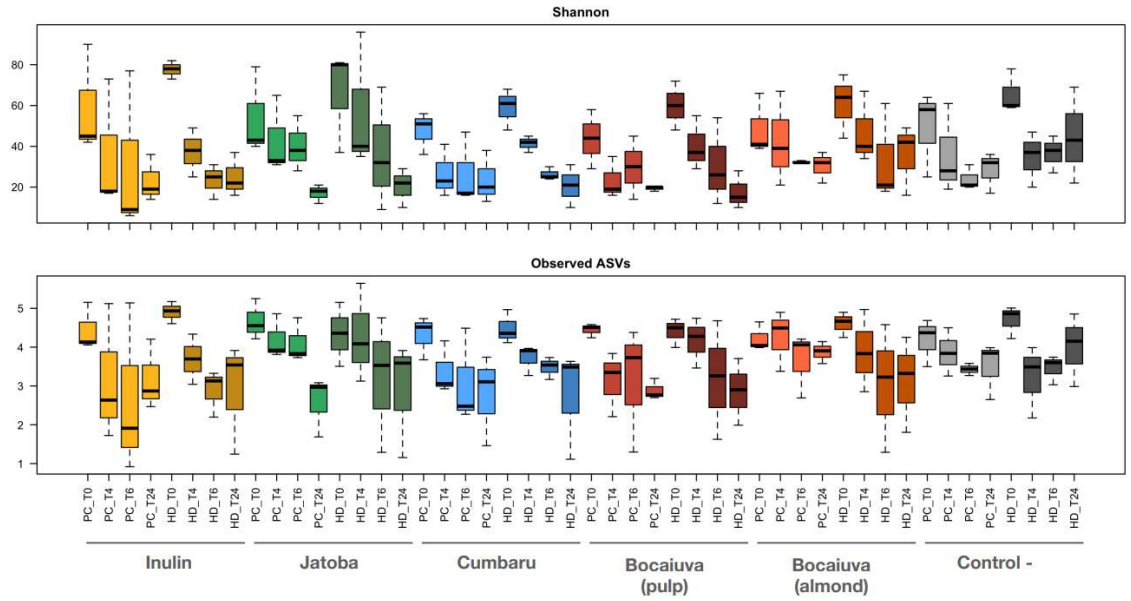
807 Tao, W. *et al.* (2020). Analysis of the intestinal microbiota in COVID-19 patients and
808 its correlation with the inflammatory factor IL-18. *Medicine in Microecology*, 5.
809 https://doi.org/10.1016/j.medmic.2020.100023

810 Wilmes, P. *et al.* (2022). The gut microbiome molecular complex in human health and
811 disease. *Cell Host and Microbe*, 30(9), 1201-1206.
812 https://doi.org/10.1016/j.chom.2022.08.016

813 Yeoh, K. Y. *et al.* (2021). Gut microbiota composition reflects disease severity and
814 dysfunctional immune responses in patients with COVID-19. *Gut*, 70(4), 698-706.
815 https://doi.org/10.1136/gutjnl-2020-323020
816

817 ***Supplementary materials***

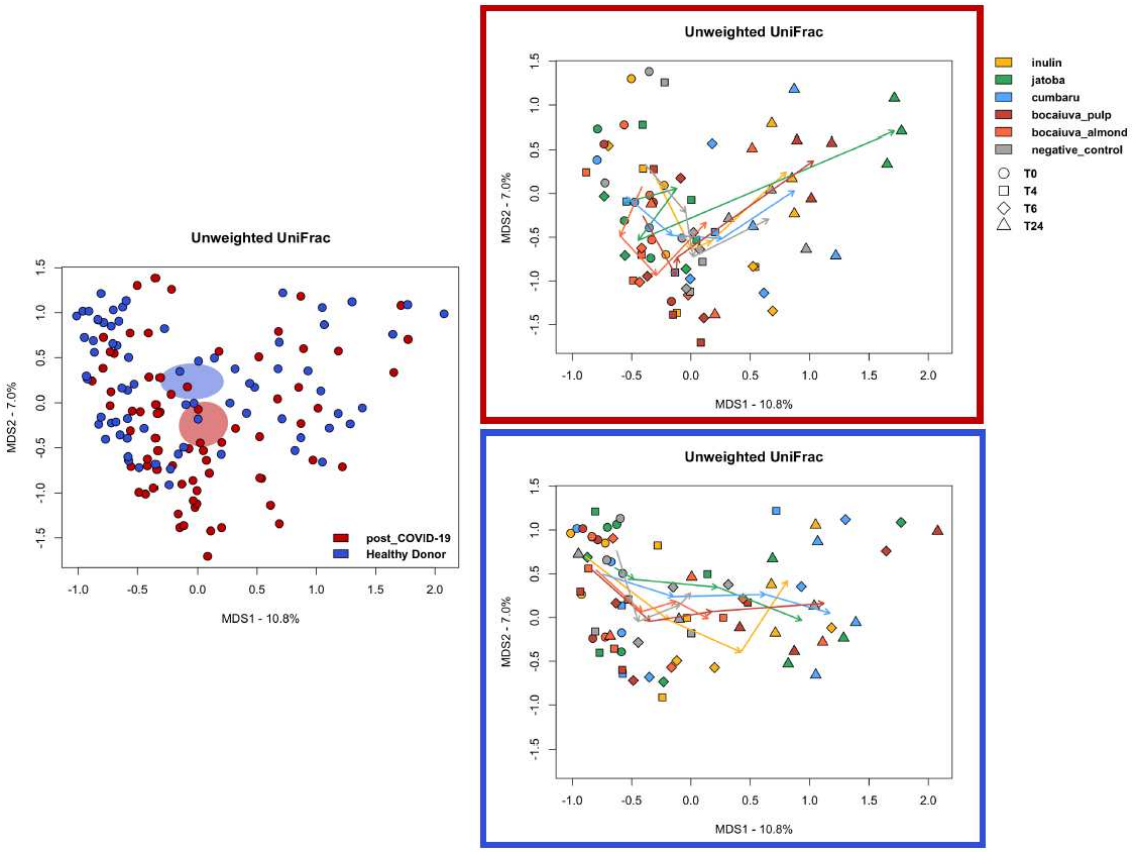
818



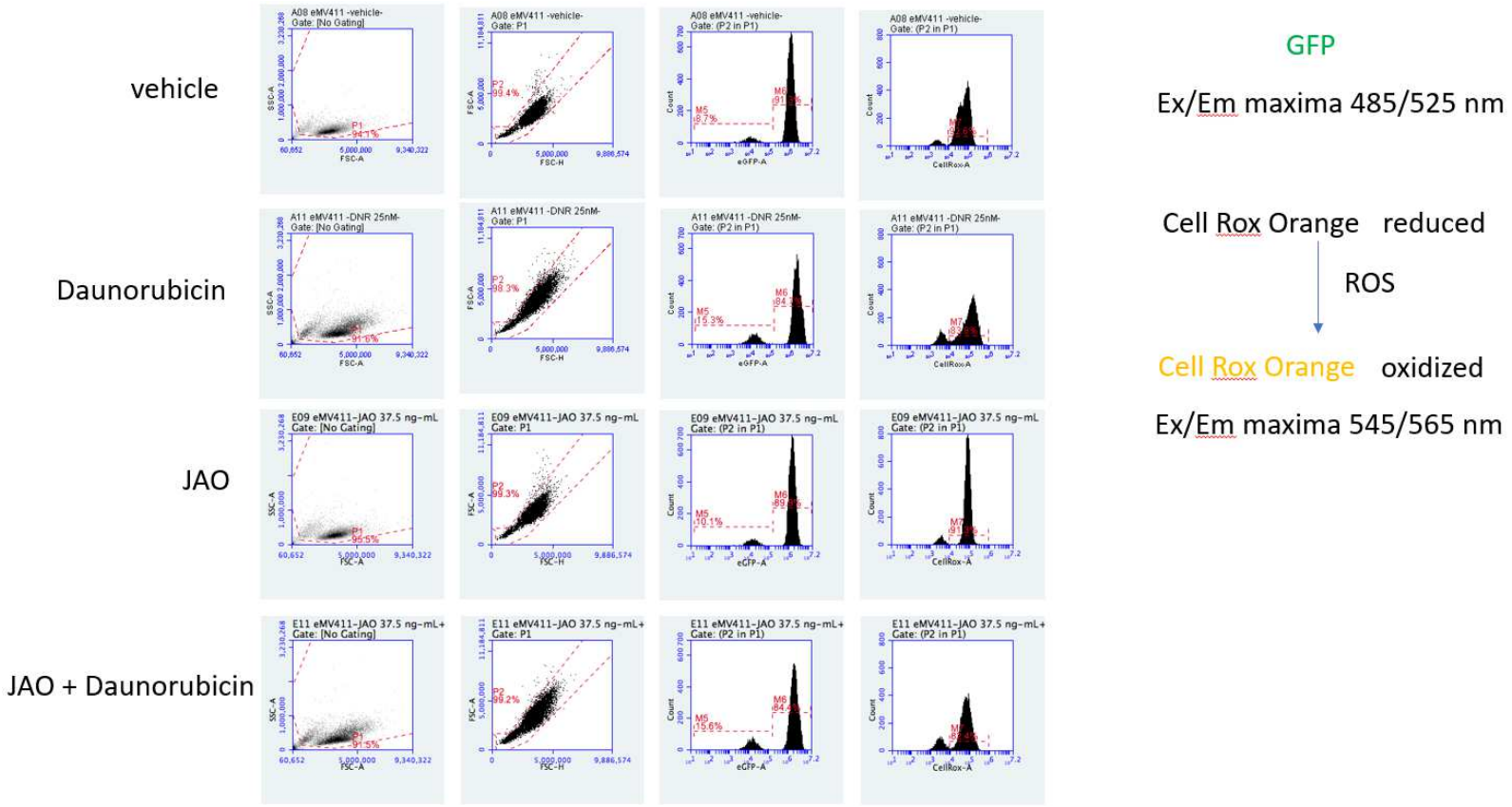
819

820 **Supplementary Figure 1. The alpha diversity of faecal-derived microbial**
821 **communities from post-COVID-19 and healthy donors tends to decrease over time**
822 **in 24-h fermentation experiments in the presence of fruit flours, inulin or without**
823 **additions.** Boxplots showing the distribution of alpha diversity values, according to the
824 Shannon index (upper panel) and the number of observed amplicon sequence variants
825 (ASVs, lower panel), for the faecal-derived microbial communities from post-COVID-
826 19 (PC) and healthy (HD) donors at time point 0, 4, 6 and 24 h of fermentation (T0, T4,
827 T6 and T24) in the presence of fruits flours (jatobá, cumbaru, bocaiuva pulp, bocaiuva
828 almond), inulin or without additions (control -).

829

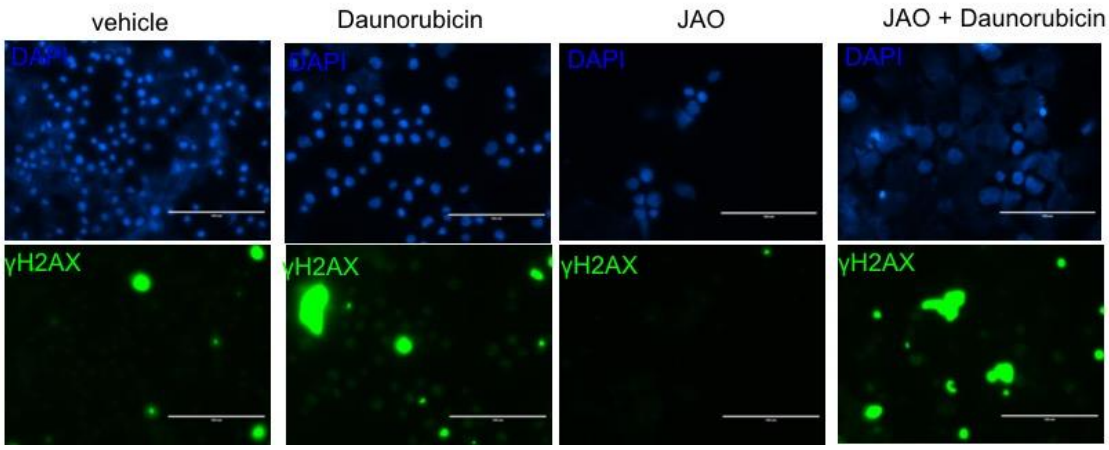


Supplementary Figure 2. Unweighted UniFrac-based Principal Coordinates Analysis (PCoA) of faecal-derived microbial communities of post-COVID-19 and healthy donors over 24 h of fermentation in the presence of fruit flours, inulin or without additions. Left, PCoA plot showing all fermentation samples, colored by group of donors (post-COVID-19 donors, PC, red vs. healthy donors, HD, blue). A significant separation between groups was found, regardless of experimental condition (fruit flours, inulin and negative control) and time point (0, 4, 6 and 24 h of fermentation - T0, T4, T6 and T24) (P<0.001, PERMANOVA). Right, PCoA plots showing the fermentation samples for PC (top panel) and HD (bottom panel). Within each group of donors, the samples were separated significantly by experimental condition (fruit flours, inulin, negative control) and time point (T0, T4, T6, T24) (P<0.001).



844

845 **Supplementary Figure 3. Effect of Jatobá pulp oil (JAO) alone or in combination with daunorubicin on oxidative stress and**
846 **viability.** MV411 cells were infected with a lentiviral vector expressing eGFP and positive cells were isolated by cell sorting. The GFP
847 fluorescence emission is proportional to the number of cells. Representative FACS plots indicating the Forward and Side scatter of MV411
848 cells included in the analysis (population P1), the gating strategy to exclude duplets from the analysis (population P2), the gates M1 and
849 M2 to discriminate GFP- from GFP+ cells and the gate M7 that reports the intensity of oxidative stress.



Supplementary Figure 4. Effect of Jatobá pulp oil (JAO) alone or in combination with daunorubicin on DNA damage. Digital microscope images showing DNA damage γH2AX foci in MV411 cells pre-treated with either vehicle (DMSO) or 37 ng/mL of JAO for 24 h before incubation with 25 nM DNR for additional 48 h. Images were captured using Evos FL Digital Inverted Fluorescence Microscope (magnification 40X).

CHAPTER IV

Original Short Communication to be submitted to the **Brazilian Archives of Biology and Technology**

Authors: Carolina Saori Ishii Mauro[1]*, Maria Thereza Carlos Fernandes[1], Fernanda Silva Farinazzo[2], Lycio Shinji Watanabe[3], Suzana Lucy Nixdorf[3] and Sandra Garcia[1].

[1]State University of Londrina (UEL), Departament of Food Science and Technology.
[2]State University of Mato Grosso do Sul (UEMS) - Campus Naviraí, Departament of Food Engineering.
[3]State University of Londrina (UEL), Departament of Chemistry.

*Corresponding author. E-mail: carol.saori@gmail.com

Analysis of the chemical composition of jatobá pulp flour and effect on the growth of probiotic bacteria

ABSTRACT

This work aimed to determine the chemical composition of jatobá flour and evaluate its effect on the growth of the probiotics *Lacticaseibacillus casei* (LC-01), *Limosilactobacillus reuteri* (LR 92) and *Lactobacillus acidophillus* (LA-5). Furthermore, the growth of *Escherichia coli* (ATCC 25922) in the presence of jatobá pulp flour and glucose after 24 hours was analyzed. Carbohydrates were the major macronutrient found in jatobá pulp flour. The flour had 9.26 ±3.66 g/100g of soluble fibers and 43.93 ±3.20 g/100g of insoluble fibers, and therefore, according to the Brazilian legislation this food is considered a food high in fibers. The sugar profile by HPLC showed that jatobá pulp flour has 123.26 ±0.36, 31.21 ±0.01 and 18.60 ±0.09 mg/g of sucrose, glucose and fructose, respectively. The effect on the growth of beneficial bacteria showed that jatobá pulp flour is a suitable substrate for *L. casei, L. reuteri* and *L. acidophilus* fermentation, promoting after 24 h glucose-like counts for *L. casei* (9.01 ±0.15 log CFU/mL) and higher than that of fructooligosaccharides (FOS) for *L. reuteri* (8.65 ±0.13 log CFU/mL). *L. acidophilus* showed counts of 8.64 ±0.05 log CFU/mL in the medium containing jatobá pulp flour, a result higher than glucose and that did not differ from FOS. The flour fiber content should have promoted the growth of the probiotic bacteria used in this work, together with other nutrients and sugars naturally present. The growth of *E. coli* in the presence of jatobá pulp flour was lower than that observed in the positive control (glucose). However, we suggest that more tests are needed to confirm the prebiotic potential of this fruit flour and if it is strain specific.

Keywords: *Escherichia coli,* Fermentation, *Hymenaea* spp., *Lacticaseibacillus casei*, *Limosilactobacillus reuteri*, *Lactobacillus acidophilus.*

Título: Análise da composição química de farinha de polpa de jatobá e efeito no crescimento de bactérias probióticas

INTRODUCTION

Jatobá is a tree species of the *Fabaceae* family that occurs from Mexico to southern Brazil and its trees can reach more than 50 m in height and 2 m in diameter (Schwartz, 2018). The jatoba found in the Cerrado and Pantanal of the state of Mato Grosso do Sul may be of the species *Hymenaea stignocarpa* Mart. ex Hayne (jatobá, jatobá-do-cerrado) and *Hymenaea courbaril* L. (jatobá-mirim) (Damasceno-Junior et al., 2010).

Jatobá fruit has a slightly sweet aroma and flavor, with yellow, fibrous pulp and a high fiber content (Damasceno-Junior et al., 2010). The pulp is naturally dry, can be consumed fresh or used in the preparation of flour for application in cakes, breads, biscuits, and other culinary preparations (Schwartz, 2018). Jatobá pulp has chemical characteristics that may favor the growth of bacteria beneficial to the human intestinal microbiota, such as probiotics.

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. As a common feature, probiotics are responsible for promoting a healthy digestive tract and immune system protection (Hill et al., 2014). The bacterium *Lacticaseibacillus casei* LC-01 (formerly denominated *Lactobacillus casei* (Zheng et al., 2020)) was able to improve lipid profiles in humans and promote a positive impact on blood pressure values (Sperry et al., 2018), while some strains of *Limosilactobacillus reuteri* (formerly denominated *Lactobacillus reuteri* (Zheng et al., 2020)) promoted a reduction in gastrointestinal discomfort, and can be used as an effective treatment for infantile colic (Zermiani et al., 2021). In the same context, *Lactobacillus acidophilus* have shown strong evidence of an active role in immune regulation of humans (Zhao et al., 2020).

A prebiotic ingredient is defined as a substrate that is selectively utilized by host microorganisms, conferring a health benefit (Gibson et al., 2017). Fructooligosaccharides (FOS) are non-digestible fructans that promote the growth of beneficial bacteria and may play an important role in promoting human health (Gibson et al., 2017). Prebiotics have the ability to metabolize prebiotic sugars, that provides for their selective enrichment in the gastrointestinal tract (Huebner et al., 2007) Preferably, a prebiotic should not promote the growth of commensal or pathogenic bacteria present in the gut.

In this context, this work aimed to determine the chemical composition of jatobá flour and evaluate its effect on the growth of the beneficial bacteria *Lacticaseibacillus*

casei (LC-01), *Limosilactobacillus reuteri* (LR 92) and *Lactobacillus acidophillus* (LA-5). Furthermore, we compared the growth of *Escherichia coli* in the presence of jatoba pulp flour.

MATERIAL AND METHODS

Jatobá pulp flour

The artisanal jatobá pulp flour used in this work was obtained from the local market of Campo Grande, Mato Grosso do Sul (20° 26' 37" South; 54° 38' 52" West), with a jatobá fruit harvest of July 2019. The flour was packed in dark polyethylene bags to avoid the incidence of UV light during storage and kept at freezer temperature (-18 °C).

Chemical composition and energy

The protein, mineral, lipid, moisture, ash and fiber contents were determined by methods described by the AOAC (2006) and the carbohydrate content was obtained by difference. The estimated total caloric value of the sample was determined according to the Atwater factor (Atwater, 1902), as shown in the following Equation:

Total caloric value/energy (Kcal) = (4 x protein) + (4 x carbohydrate) + (9 x lipid)

Sugar profile by HPLC

For the determination and quantification of sugars, glucose, fructose and sucrose standards were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany, ≥99% purity). For sample preparation, 0.1 g of jatobá pulp was homogenized in 1 mL of water, centrifuged at 9056 x g for 15 min and the supernatant was collected and filtered through a 0.22 μm PVDF membrane (Millipore, Ireland). Analyzes were performed in duplicate (n = 2). A Shimadzu LC 20A high performance liquid chromatograph (Shimadzu, Kyoto, Japan) composed of a high-pressure pump (LC-20AT), automatic injector (SIL-20AC HT), column oven (CTO-20A) and series-coupled photodiode array (SPD-M20A) and refractive index (RID-10A) detectors (HPLC-PDA-RID). Chromatographic analyzes were isocratic with pure water as mobile phase. An oven temperature of 85 °C was used, with an Aminex HPX-87P ion exchange column (7.8 x 300 mm in Pb^{2+} ionic form, Biorad, USA) previously equilibrated with 100% ultrapure water (MiliQ®) as a mobile phase at a flow rate of 1.0 mL/min and RID cell

temperature maintained at 40 °C. The PDA wavelength was set at 215nm, in the scan mode from 200 to 400nm (Pauli et al., 2011). Data acquisition and integration of chromatographic peaks were performed using LC Solutions software (Shimadzu Co., Japan) and results were expressed in mg/g (dry basis).

Bacterial strains

The probiotic strains used in this study were *Lacticaseibacillus casei* (LC 01), *Lactobacillus acidophilus* (LA-5) donated from Chr.Hansen®, (Chr. Hansen Holding A/S, Denmark). and *Limosilactobacillus reuteri* (LR 92) donated from Sacco® (Sacco Brasil, Brazil). The lyophilized powder containing the strains was added at 0.01% (w/v) to DeMan, Rogosa, and Sharpe (MRS) broth (Acumedia, Brazil) containing 20% (v/v) of sterile glycerol (Synth, Brazil) and the stock cultures were kept frozen (-20 °C). For activation before use in the experiments, the strains were activated twice in MRS broth at 37 °C for 24 hours. The *Escherichia coli* ATCC 25922 strain were donated by the Microbiology Department of the State University of Londrina, Brazil. The inoculum was prepared from overnight cultures grown on Brain Heart Infusion (BHI) broth (HiMedia, Brazil) at 37 °C for 24 h.

Inoculum preparation

The inoculum preparation was made according to Melo et al. (2020), with modifications. For the probiotic bacteria, MRS broth containing activated strains was centrifugated (4500 × *g*, 15 min, 4 °C), washed twice with sterile saline solution (NaCl 0.85 g/100mL), resuspended in saline solution and homogenized for 10 s using a vortex. Cell suspensions of optical density 0.8 measured at a wavelength of 660 nm (OD_{660}=0.8) provided viable counts of approximately 8 log CFU/mL.

For *E. coli*, streaking technique was first performed on a Petri dish containing nutrient agar, then after incubation at 37 °C for 24 h one colony was removed, added to the sterile saline solution and homogenized for 10 s using a vortex. From this *E. coli* solution, a dilution with saline solution was made until it reached a cell suspension of 0.1 at 625 nm (OD_{625}=0.1), which provided viable counts of approximately 8 log CFU/mL.

These cell suspensions were inoculated in the broths at 1% (v/v), therefore, the initial viable cell concentration ranged between 4.76 and 6.0 log UFC/mL at time 0 of fermentation.

Effect of Jatobá pulp flour on probiotic growth

Analyzes were performed according to Melo et al. (2020), with modifications. The *L. casei*, *L. reuteri* and *L. acidophilus* strains were cultivated in MRS broth without added sugars (10 g/L of tryptone, 8 g/L of meat extract, 4 g/L of yeast extract, 2 g/L of dipotassium phosphate, 1 g/L of Tween 80, 5 g/L of sodium acetate, 2 g/L of dibasic ammonium citrate, 0.2 g/L of magnesium sulfate and 0.04 g/L of sodium sulfate manganese).

To monitor the growth of the strains, media containing 2% (w/v) of: 1) jatobá pulp flour, 2) FOS (recognized prebiotic fiber) and 3) glucose (positive control) were prepared. In addition, a negative control was analyzed, with medium without the addition of a carbon source, totaling four treatments. Bacterial strains were inoculated at 1% (v/v), separately. Fermentations were carried out at 37 ºC for 24 h in aerobic conditions. Samples were collected at 0h and 24h for cell viability analysis.

Effect of Jatobá pulp flour on E. coli growth

E. coli ATCC 25922 suspension was added to M9 broth (Sigma-Aldrich, St. Louis, MO, USA) alone or M9 broth containing 1% (w/v) tryptone. Tryptone added to one of the broths was intended to provide a greater supply of proteins for bacterial growth, for comparison of results with the medium without the addition of this component. To access the fermentation effect, the substrate added was 1) 2% (w/v) jatobá pulp flour or 2) 2% (w/v) glucose (positive control). Samples were collected at 0h and 24h for cell viability analysis.

Cell viability of bacteria

For probiotic bacteria viability analysis, decimal dilutions were made in sterile 0.1% (w/v) peptone water and distributed in depth on MRS agar (Acumedia, Brazil) followed by incubation at 37 ºC for 48 hours. For *E. coli* viability, decimal dilutions were made in sterile saline solution (NaCl 0.85 g/100mL) and surface plating with the Drigalski-glass spatula technique in nutrient agar, followed by incubation 37 ºC for 24 hours. The analyzes were conducted in five replicates.

Statistical analysis

Data from the cell viability analysis were submitted to Analysis of Variance (ANOVA) to compare means at a 5% significance level according to Tukey's test, using the STATISTICA 8.0 program (StatSoftInc, 2007).

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of jatobá flour, energy and sugar content. This flour for culinary purposes showed low moisture content (<10%), which is an important feature for conservation during storage and commercialization. The maximum allowed moisture limit for wheat flour is 15%, according to Brazilian legislation (Brazil, 2005).

Table 1. Chemical composition, energy (Kcal) and sugar content of jatobá pulp flour (2019 harvest). *Carbohydrates obtained by difference**.** Results expressed as mean ± standard deviation.

Component	Content in g/100g
Moisture	7.95 ±0.11
Ash	3.06 ±0.09
Proteins	6.78 ±0.32
Fat	4.89 ±0.29
Carbohydrates*	77.16 ±0.29
Soluble fiber	9.26 ±3.66
Insoluble fiber	43.93 ±3.20
Energy (Kcal/100 g)	379.77 ±2.79
Sugars	**Content in mg/g**
Sucrose	123.26 ±0.36
Glucose	31.21 ±0.01
Fructose	18.60 ±0.09

The main macronutrient found was carbohydrate, which had a content similar to that found in jatobá pulp by Rocha et al. (11), who reported values of 79.8 ±17.3 g/100g. Most carbohydrates found was fibers, which had a sum of soluble and insoluble fibers of 53.29 g/100g. Likewise, Dias et al. (2013) found total fiber values of 50.02 ±0.28 g/100g for jatobá pulp. The Brazilian Resolution RDC 54/2012 (Brazil, 2012) establishes that as a condition for declaring supplementary nutritional information

(declarations related to nutrient content), the minimum amount of fiber for a solid food to be considered as having a high fiber content is 6 g/100 g. Therefore, according to current Brazilian legislation, jatobá pulp flour is a food with a high fiber content. Other authors have already confirmed this allegation of jatobá pulp flour (Damasceno-Junior et al., 2010, Dias et al., 2013, Borges et al., 2022).

The sugar profile showed that sucrose was the most abundant sugar in jatobá pulp flour, followed by glucose and fructose. This confirms that in addition to having a high fiber content in its composition, this flour has sugars that can promote the growth of probiotic microorganisms.

Table 2 shows the cell viability of lactic acid bacteria at 0 and 24 hours. All fermentation media started with a viability within the range of 5 log CFU/mL.

Table 2. Cell viability presented in log CFU/mL of *L. casei* (LC-01), *L. reuteri* (LR92) and *L. acidophilus* (LA-5) in media containing different carbon sources at fermentation times 0 and 24 hours. Results expressed as mean ±standard deviation.[a-d] Means followed by different lowercase letters within a column indicate significant differences between the treatments at same time point (Tukey test, $P<0.05$).

	Probiotic strain					
	L. casei (LC-01)		*L. reuteri* (LR92)		*L. acidophilus* (LA-5)	
Treatment	0 h	24 h	0 h	24 h	0 h	24 h
Jatobá Pulp Flour	4.98^{a} ±0.07	9.01^{a} ±0.15	4.85^{a} ±0.13	8.65^{b} ±0.13	5.58^{a} ±0.05	8.64^{a} ±0.05
FOS	5.31^{a} ±0.63	8.60^{b} ±0.02	4.76^{a} ±0.22	7.64^{c} ±0.03	5.62^{a} ±0.02	8.63^{a} ±0.05
Glucose	5.31^{a} ±0.60	9.21^{a} ±0.10	5.10^{a} ±0.17	9.13^{a} ±0.03	5.56^{a} ±0.04	8.42^{b} ±0.10
Negative Control	5.37^{a} ±0.57	8.13^{c} ±0.23	4.97^{a} ±0.19	7.39^{d} ±0.02	5.62^{a} ±0.02	7.88^{c} ±0.04

In the medium containing jatobá pulp flour, *L. acidophilus* showed counts higher than glucose and that did not differ from FOS after 24 h of fermentation ($P<0.05$). This result confirms that jatobá pulp flour is able to promote the growth of this specific strain, up to counts similar to the commercial prebiotic FOS. The medium supplemented

with glucose promoted the greatest cell growth for *L. reuteri*, followed by the treatment containing jatobá flour and FOS. *L. casei*, on the other hand, showed greater growth in the medium supplemented with jatobá flour and glucose, which did not differ from each other. These results indicates that the flour was able to provide the necessary substrate for the growth of this microorganism up to a count of 9 log CFU/mL. This can be explained by the jatobá flour sample having a high carbohydrate content (Table 1), which may have benefited the growth of these bacteria.

FOS is an ingredient that can promote the growth of bacteria beneficial to human health (Gibson et al., 2017). However, it was possible to observe that the growth of *L. casei* and *L. reuteri* was lower for the treatment with FOS when compared to the treatment containing glucose. It has already been documented that strains of *L. casei* prefer to use glucose compared to other carbon sources such as lactose, galactose, maltose or ribose (Viana et al., 2000). In the literature, several fermentable carbohydrates have been reported to promote a prebiotic effect, however, the dietary prebiotics most extensively documented as having health benefits in humans have been non-digestible oligosaccharides such as FOS (Gibson et al., 2017). Therefore, more tests need to be conducted to prove the potentially prebiotic effect of jatobá pulp flour and if these effects are strain specific.

In the negative controls, all prebiotics tested showed growth after 24 h, although lower than that observed in the other treatments ($P<0.05$). The peptone used in the growth medium is a semi-digested protein that serves as a source of nitrogen and carbon, while the yeast extract has carbohydrates in its composition, factors that may have promoted the growth of these bacteria. Furthermore, lactic acid bacteria are capable of hydrolyzing proteins present in the medium, generating free amino acids necessary for cell proliferation (Raveschot et al., 2018).

Table 3 shows the viability of *E. coli* at 0 h and after 24 h of fermentation in broth with addition of glucose only and broth with addition of glucose and tryptone. We can observe that the *E. coli* viability in the M9 broth containing only glucose as a carbon source, the bacteria was not able to reach an adequate growth, remaining at 6.77 log CFU/mL after 24 h. However, when the medium was supplemented with glucose and tryptone, *E. coli* reached counts of 9.43 log CFU/mL. This evidenced that the bacteria need a source of carbon and nitrogen to achieve greater growth in the culture broth.

Table 3. Cell viability presented in log CFU/mL of *E. coli* in media (M9 broth) containing 1) 2% (w/v) carbon source and 2) 2% (w/v) carbon source and 1% (w/v) tryptone in fermentation times 0 and 24 hours. Results expressed as mean ±standard deviation.[a-c] Means followed by different lowercase letters within a column indicate significant differences between the treatments at same time point (Tukey test, $P<0.05$).

Media	Treatment	Fermentation time	
		0 h	24 h
1) M9 broth + Carbon source	Jatobá Pulp Flour	6.02[a] ±0.04	8.68[b] ±0.16
	Glucose	6.03[a] ±0.03	6.77[c] ±0.18
2) M9 broth + Carbon source + tryptone	Jatobá Pulp Flour	6.04[a] ±0.04	8.74[b] ±0.04
	Glucose	6.05[a] ±0.03	9.43[a] ±0.13

As for the jatobá flour, which is food with several other nutrients such as proteins, fat, minerals and vitamins for bacterial growth, the broth containing only glucose and the medium containing glucose and tryptone did not differ from each other in relation to the growth of *E .coli* after 24 hours of fermentation. We can highlight that when broth 2) was used, in which tryptone was added, the growth of *E. coli* in the presence of jatobá pulp flour was lower than that observed in the positive control (glucose).

To be considered a prebiotic ingredient, jatobá flour should promote the growth of probiotic bacteria, but it should not promote the growth of *E. coli.* To be effective, a prebiotic ingredient should have the ability to be selectively fermented by and to support growth of specific beneficial microorganisms (Huebner et al., 2007). This was not observed in the present work using the whole pulp flour. Therefore, further studies are needed to confirm this effect, preferably using the isolated or purified jatobá pulp fiber.

CONCLUSION

The effect on the growth of beneficial bacteria shows that jatobá pulp flour is a suitable substrate for the growth of *L. casei, L. reuteri* and *L. acidophilus*, promoting glucose-like counts for *L. casei* and *L. acidophilus* and higher than that of

fructooligosaccharides for *L. reuteri.* The chemical composition shows that carbohydrates are the macronutrient with the highest content in jatobá pulp flour. This component promoted the growth of the probiotic bacteria used in this work, together with other nutrients naturally present. However, more tests are needed to confirm the prebiotic potential of this flour.

ACKNOWLEDGEMENTS

We would like to thank the National Council for Scientific and Technological Development (CNPq) and Fundação Araucária for the study grant and the State University of Londrina.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR'S CONTRIBUTIONS

C.S.I. Mauro: Conceptualization, Investigation, Writing - Original draft preparation, Data curation. M.T.C. Fernandes: Investigation, Writing - review and editing. F.S. Farinazzo: Writing - review and editing, Visualization. S. Garcia: Writing - review and editing, Supervision.

REFERENCES

AOAC International. Official methods of analysis of AOAC International.18th ed. Gaithersburg, MD, USA: AOAC International; 2006.

ATWATER, W. O. Principles of nutrition and nutritive value of food. Farmer´s Bulletin, n.142, 1902.

BORGES, A. V. C.; ROMÃO, B.; ZANDONADI, R. P. Jatobá (*Hymenaea* sp.) as a wheat substitute in plant-based cookies to improve its nutritional quality. Research, Society and Development, [S. l.], v. 11, n. 17, p. e190111739085, 2022. https://doi.org/10.33448/rsd-v11i17.39085

BRAZIL -Ministry of Agriculture, Livestock and Food Supply (MAPA). Normative Instruction No. 8, of June 2, 2005. Technical regulation of identity and quality of wheat flour. Official Gazette of the Federal Government, Brasília, DF, n. 105, p. 91.

BRAZIL – Brazilian Health Regulatory Agency (ANVISA). RDC Resolution No. 54, of November 12, 2012. Provides for the Technical Regulation on Complementary Nutritional Information.

DAMASCENO-JUNIOR, G. A.; SOUZA, P. R.; BORTOLOTTO, I. M.; RAMOS, M. I. L.; HIANE, P. A.; BRAGA NETO, J. A.; ISHII, I. H.; COSTA, D. C.; RAMOS FILHO, M. M.; GOMES, R. J. B.; BARBOSA, M. M.; RODRIGUES, R. B. Sabores do Cerrado e Pantanal: Receitas e boas práticas de aproveitamento. 1. ed. Campo Grande: Editora UFMS. 2010. V. 1. 141p.

DIAS, L. S.; LUZIA, D. M. M.; JORGE, N. Physicochemical and bioactive properties of *Hymenaea courbaril* L. pulp and seed lipid fraction. Ind Crop Prod, 49, 610-618, 2013. https://doi.org/10.1016/j.indcrop.2013.06.005

GIBSON, G. R.; HUTKINS, R.; SANDERS, M. E., PRESCOTT, S. L.; REIMER, R. A.; SALMINEN, S. J.; SCOTT, K.; STANTON, C.; SWANSON, K. S.; CANI, P. D.; VERBEKE, K.; REID, G. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev. Gastroenterol. Hepatol. 14, 491–502, 2017. https://doi.org/10.1038/nrgastro.2017.75

HILL, C.; GUARNER, F.; GIBSON, G. R.; MERENSTEIN, D. J.; POT, B.; MORELLI, L.; CANANI, R. B.; FLINT, H. J.; SALMINEN, S.; CALDER, P. C.; SANDERS, M. E. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat. Rev. Gastroenterol. Hepatol., 11, 506, 2014. https://doi.org/10.1038/nrgastro.2014.66

HUEBNER, J.; WEHLING, R.L.; HUTKINS, R.W. Functional activity of commercial prebiotics, International Dairy Journal, 2007, v. 17(7), pp. 770-775. https://doi.org/10.1016/j.idairyj.2006.10.006

MELO, F. H. C.; MENEZES, F. N. D. D.; SOUSA, J. M. B.; LIMA, M. S.; BORGES, G. S. C.; SOUZA, E. L.; MAGNANI, M. Prebiotic activity of monofloral honeys produced by stingless bees in the semi-arid region of Brazilian Northeastern toward *Lactobacillus acidophilus* LA-05 and *Bifidobacterium lactis* BB-12. Food Res Int. 128, 2020. https://doi.org/10.1016/j.foodres.2019.108809

PAULI, E. D.; CRISTIANO, V. & NIXDORF, S. L. Método para determinação de carboidratos empregado na triagem de adulterações em café. Química Nova, 34(4), 689-694, 2011. https://doi.org/10.1590/S0100-40422011000400023

RAVESCHOT, C.; CUDENNEC, B.; COUTTE, F.; FLAHAUT, C.; FREMONT, M.; DRIDER, D.; DHULSTER, P. Production of Bioactive Peptides by *Lactobacillus* Species: From Gene to Application. Front. Microbiol., 9, 2018. https://doi.org/10.3389/fmicb.2018.02354

ROCHA, M. S.; FIGUEIREDO, R. W.; ARAUJO, M. A. M.; MOREIRA-ARAUJO, R. S. R. Caracterização físico-química e atividade antioxidante (in vitro) de frutos do cerrado Piauiense. Rev Bras Frutic. 35(4), 933-941, 2013. https://doi.org/10.1590/S0100-29452013000400003

SCHWARTZ, G. Jatoba—*Hymenaea courbaril*. Exotic Fruits Reference Guide. Elsevier Inc, 2018. pp. 257-261. https://doi.org/10.1016/B978-0-12-803138-4.00033-2

SPERRY, M. F; SILVA, H. L. A.; BALTHAZAR, C. F.; ESMERINO, E. A.; VERRUK, S.; PRUDENCIO, E. S.; NETO, R. P. C.; TAVARES, M. I.; PEIXOTO, J. C.; NAZZARO, F.; ROCHA, R. S.; MORAES, J; GOMES, A. S. G.; RAICES, R. S. L.; SILVA, M. C.; GRANATO, D.; PIMENTEL, T. C.; FREITAS, M. Q.; CRUZ, A. G. Probiotic Minas Frescal cheese added with *L. casei* 01: Physicochemical and bioactivity characterization and effects on hematological/biochemical parameters of hypertensive overweighted women – A randomized double-blind pilot trial. J. Funct. Foods, 45, 435-443, 2018. https://doi.org/10.1016/j.jff.2018.04.015

VIANA, R.; MONEDERO, V.; DOSSONET, V.; VADEBONCOEUR, C.; PÉREZ-MARTÍNEZ, G.; DEUTSCHER, J. Enzyme I and HPr from *Lactobacillus casei*: their role in sugar transport, carbon catabolite repression and inducer exclusion. Mol. Microbiol. 36(3), 570-584, 2000. https://doi.org/10.1046/j.1365-2958.2000.01862.x

ZERMIANI, A. P. R. B.; SOARES, A. L. P. P. P.; MOURA, B. L. S. G.; MIGUEL, E. R. A.; LOPES, L. D. G.; SANTANA, N. C. S.; SANTOS, T. S.; DEMARCHI, I. G.; TEIXEIRA, J. J. Evidence of *Lactobacillus reuteri* to reduce colic in breastfed babies: Systematic review and meta-analysis. Complement. Ther. Med. 63, 2021. https://doi.org/10.1016/j.ctim.2021.102781

ZHAO, W.; LIU, Y; KWOK, L.; CAI, T.; ZHANG, W. The immune regulatory role of *Lactobacillus acidophilus*: An updated meta-analysis of randomized controlled trials. Food Bioscience, 2020, v. 36. https://doi.org/10.1016/j.fbio.2020.100656

ZHENG, J.; WITTOUCK, S.; SALVETTI, E.; FRANZ, C. M. A. P.; HARRIS, H. M. B.; MATARELLI, P.; O'TOOLE, P. W.; LEBEER, S. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerink 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int. J. Syst. Evol. Microbiol., 70, 2782–2858. 2020. https://doi.org/10.1099/ijsem.0.004107

CONCLUSÃO GERAL

O Brasil possui uma grande diversidade de plantas nativas que não são conhecidas ou que não são comumente utilizadas na culinária. Parte do problema está relacionado à monotonia alimentar, em que os mesmos alimentos são consumidos com frequência, limitando a variedade da dieta. A rica biodiversidade brasileira oferece alimentos saborosos e nutritivos que ocorrem naturalmente nas regiões do país. Nesse contexto, são necessários estudos sobre as características das frutas nativas brasileiras e os efeitos metabólicos ou fisiológicos benéficos ao consumidor. Assim, uma iniciativa que promova uma maior compreensão dos benefícios das frutas nativas à saúde por meio de pesquisas é fundamental para estimular o consumo desses alimentos. Portanto, este é o primeiro trabalho de fermentação *in vitro* com o objetivo de estudar os efeitos de farinhas de frutas brasileiras na composição da microbiota intestinal humana e metabólitos.

Nossas descobertas mostram como essas frutas podem levar a melhorias relevantes no ecossistema microbiano intestinal e alterar a atividade metabólica para uma configuração que pode conferir benefícios à saúde do hospedeiro. Nosso estudo piloto estabelece as bases para a concepção de um estudo de intervenção humana *in vivo* que pode confirmar as tendências promissoras aqui observadas.

Além disso, o trabalho visou a investigação dos compostos antioxidantes presentes nas farinhas de frutos. Foi observado que o solvente extrator influenciou na eficiência da extração dos compostos fenólicos totais e na capacidade antioxidante. Após condições simuladas de passagem pelo trato gastrointestinal, foi observada variação na bioacessibilidade dos compostos antioxidantes, apresentando diminuição ou aumento. Porém, foi constatado que o método colorimétrico de Folin-Ciocalteu pode superestimar o conteúdo de compostos fenólicos totais, quando comparado ao método de detecção por cromatógrafo HPLC. O óleo extraído da farinha de polpa de jatobá em ensaios utilizando células humanas apresentou proteção ao estresse oxidativo induzido por daunorrubicina e ao dano ao DNA, enquanto a farinha de jatobá apresentou-se um substrato capaz de estimular o crescimento de bactérias probióticas.

As farinhas do Cerrado e Pantanal ainda possuem um grande potencial a ser abordado. O incentivo ao consumo destes alimentos é uma importante etapa para o valor social da pesquisa, visando o combate à fome, ao desperdício de alimentos e aproveitamento de recursos naturais da região. Uma popularização destes frutos nativos

pode gerar benefícios econômicos e sociais, promover a conservação de espécies e o desenvolvimento sustentável de regiões brasileiras.

Sugestões para trabalhos futuros são:

- Investigar as fibras das farinhas de frutos isoladamente e sua capacidade prebiótica;
- Investigar as proteínas presentes nas amêndoas dos frutos do Cerrado e Pantanal, a qualidade e fração biodisponível;
- Desenvolvimento de novos produtos alimentícios utilizando as farinhas de frutos e investigação da aceitação sensorial pela população;
- Estudo de intervenção humana com o consumo destes frutos e investigação da alteração de marcadores biológicos, principalmente porque não existem estudos clínicos em humanos com frutos de bocaiuva;
- Iniciativas para popularizar o resultado de pesquisas científicas para o acesso da população e valorização destes alimentos nativos.

GENERAL CONCLUSION

Brazil has a great diversity of native plants that are not known or that are not commonly used in cooking. Part of the problem is related to food monotony, in which the same foods are consumed frequently, limiting the variety of the diet. The rich Brazilian biodiversity offers tasty and nutritious foods that occur naturally in the regions of the country. In this context, studies on the characteristics of native Brazilian fruits and the beneficial metabolic or physiological effects to the consumer are necessary. Therefore, an initiative that promotes a greater understanding of the benefits of native fruits to health through research is fundamental to stimulate the consumption of these foods. Therefore, this is the first *in vitro* fermentation work aiming to study the effects of Brazilian fruit flours on the human intestinal microbiota composition and metabolites.

Our findings show how these fruits might lead to relevant improvements in the intestinal microbial ecosystem and alter metabolic activity towards a configuration that might confer health benefits to the host. Our pilot study lays the foundations for the design of an *in vivo* human intervention study that can confirm the promising trends herein observed.

In addition, the work aimed to investigate the antioxidant compounds present in fruit flours. It was observed that the extractor solvent influenced the extraction efficiency of total phenolic compounds and the antioxidant capacity. After simulated conditions of passage through the gastrointestinal tract, variation in the bioaccessibility of antioxidant compounds was observed, showing a decrease or increase. However, it was found that the Folin-Ciocalteu colorimetric method can overestimate the content of total phenolic compounds when compared to the HPLC chromatograph detection method. The oil extracted from the jatobá pulp flour in assays using human cells showed protection against oxidative stress induced by daunorubicin and DNA damage, while the jatobá pulp flour proved to be a substrate capable of promoting the growth of probiotic bacteria.

Cerrado and Pantanal flours still have great potential to be addressed. Encouraging the consumption of these foods is an important step towards the social value of research, aimed at combating hunger, food waste and utilizing the region's natural resources. A popularization of these native fruits can generate economic and

social benefits, promote the conservation of species and the sustainable development of Brazilian regions.

Suggestions for future work are:

- Investigation of the fibers present in fruit flours separately and their prebiotic capacity;
- Investigation of the proteins present in almonds and fruits, its quality and bioavailable fraction;
- Development of new food products using fruit flours and investigation of sensory acceptance by the population;
- Human intervention studies with the consumption of these fruits and investigation of biological markers, especially since there are no clinical human trials regarding bocaiuva fruits
- Popularization of scientific researches' results to increase population's access and appreciation of these native foods.

ANNEXES

REGISTRATION IN THE NATIONAL SYSTEM FOR THE MANAGEMENT OF GENETIC HERITAGE AND ASSOCIATED TRADITIONAL KNOWLEDGE (SISGEN), N° A90CEAF

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Cadastro de Acesso Nº A90CEAF

Tipo de Usuário:	**UEL**
Responsável pelo cadastro:	**02472614837**
Objeto do Acesso:	**Patrimônio Genético e Conhecimento Tradicional Associado**
O acesso foi realizado antes de 17/11/2015 ou obteve autorização de acesso antes de 17/11/2015?	**Não, sem solicitação de autorização em tramitação**
Finalidade do Acesso:	**Pesquisa e Desenvolvimento Tecnológico**
Estas atividades são baseadas em acesso realizado anteriormente?:	**Não**
Este cadastro está vinculado a cadastro anterior de remessa?	**Não**

Patrimônio Genético e Conhecimento Tradicional Associado

Título da Atividade:	**Estudo do potencial prebiótico de farinhas de frutos do cerrado e pantanal**
Título da Atividade em inglês:	**Study of the prebiotic potential of Brazilian Cerrado and Pantanal fruit flours**
Resumo da atividade (incluindo objetivos e resultados esperados ou obtidos, conforme o caso)	**Os frutos da região do Cerrado e Pantanal são consumidos in natura ou processados pela população para venda no comércio local, entre eles as farinhas de frutos e sementes. As farinhas de polpa de jatobá, amêndoa de cumbaru, polpa de bocaiuva e amêndoa de bocaiúva são exemplos de farinhas comercializadas no estado de Mato Grosso do Sul. Entretanto, existe pouco conhecimento científico a respeito de seus compostos bioativos e funcionais. Os prebióticos são ingredientes fermentáveis capazes de estimular seletivamente o crescimento de microrganismos intestinais que conferem benefícios à saúde do hospedeiro. Os frutos do Cerrado e Pantanal possuem propriedades ainda não estudadas quanto a quantidade de fibras funcionais prebióticas e compostos bioativos benéficos à saúde. Com isso, o objetivo da pesquisa será investigar o potencial prebiótico e funcional de farinhas de frutos do Cerrado e Pantanal através de análises in vitro, caracterizar as farinhas através de análises físico-químicas, quantificar o teor de compostos bioativos e determinar a capacidade antioxidante. A pesquisa pode oferecer uma diversificação do uso dos alimentos e o acesso pela população através do conhecimento gerado, utilizando os recursos naturais locais. Sendo assim, o presente estudo contribuirá para a investigação da qualidade nutricional e efeitos funcionais de farinhas feitas com frutos e sementes do Cerrado e Pantanal.**
Resumo não sigiloso da Atividade em Inglês	**The fruits of the Brazilian Cerrado and Pantanal are consumed raw or processed by the population for sale in the local trade, including flours made of fruits and seeds. Jatobá pulp flour,**

Equipe

Sandra GARCIA	**UEL**
CAROLINA SAORI ISHII MAURO	**UEL**

Parceiras no Exterior

University of Roehampton

Envios de Amostra

Espécie:	**Acrocomia aculeata**
Tipo do Patrimônio Genético:	**-**
Forma do Patrimônio Genético:	**Amostra sólida em tubo de microcentrífuga (eppendorf)**
Instituição Destinatária:	**University of Roehampton**
Sede da Instituição Destinatária:	**Roehampton Ln, London SW15 5PU, Reino Unido, Londres, Grande Londres, SW15 4JD, Rein**
Espécie:	**Hymenaea sp.**
Tipo do Patrimônio Genético:	**-**
Forma do Patrimônio Genético:	**Amostra sólida em tubo de microcentrífuga (eppendorf)**
Instituição Destinatária:	**University of Roehampton**
Sede da Instituição Destinatária:	**Roehampton Ln, London SW15 5PU, Reino Unido, Londres, Grande Londres, SW15 4JD, Rein**
Espécie:	**Dipteryx alata**
Tipo do Patrimônio Genético:	**-**
Forma do Patrimônio Genético:	**Amostra sólida em tubo de microcentrífuga (eppendorf)**
Instituição Destinatária:	**University of Roehampton**
Sede da Instituição Destinatária:	**Roehampton Ln, London SW15 5PU, Reino Unido, Londres, Grande Londres, SW15 4JD, Rein**

Data do Cadastro:	**21/01/2021 10:59:11**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **11:00** de **21/01/2021**.

SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - **SISGEN**

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A90CEAF

A atividade de acesso ao Patrimônio Genético/CTA, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A90CEAF**
Usuário:	**UEL**
CPF/CNPJ:	**78.640.489/0001-53**
Objeto do Acesso:	**Patrimônio Genético/CTA**
Finalidade do Acesso:	**Pesquisa e Desenvolvimento Tecnológico**

Espécie
Hymenaea spp.
Dipteryx alata
Acrocomia aculeata
Hymenaea spp.
Dipteryx alata
Acrocomia aculeata

Fonte do CTA
CTA de origem não identificável
CTA de origem não identificável
CTA de origem não identificável

Título da Atividade:	**Estudo do potencial prebiótico de farinhas de frutos do cerrado e pantanal**

Forma de Acondicionamento	**Amostra sólida em tubo de microcentrífuga (eppendorf)**
Quantidade Recipiente	**2**
Volume a ser enviado	**0100 gramas**
Método do Envio	**Em mãos**
Número do Conhecimento de Carga	

Informações do Projeto

Especificação das atividades a serem realizadas no exterior	**O objetivo do trabalho será avaliar os efeitos in vitro de quatro farinhas de frutos brasileiros (polpa de jatobá, amêndoa de**
O envio de amostra tem por finalidade o sequenciamento genético?	**Não**

Instituição Destinatária no Exterior

Nome Institucional	**University of Roehampton**
País	**Reino Unido**
Região	**Grande Londres**
Município	**Londres**
Código Postal	**SW15 4JD**
Endereço	**Roehampton Ln, London SW15 5PU, Reino Unido**
Contato	**Adele Costabile**
Telefone	**+44 (0) 20 8392 3571**
Email	**adele.costabilel@roehampton.ac.uk**
Representante Legal	**Adele Costabile**

Data do Cadastro:	**21/01/2021 10:59:11**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situacão cadastral conforme consulta ao SisGen em **11:03** de **21/01/2021**.

SISTEMA NACIONAL DE GESTÃO
DO PATRIMÔNIO GENÉTICO
E DO CONHECIMENTO TRADICIONAL
ASSOCIADO - SISGEN

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**
Fonte de obtenção do Conhecimento Tradicional Associado:	**CTA de origem não identificável**
Conhecimento Tradicional Associado ao Patrimônio Genético Acessado:	**O processamento de frutos do Cerrado e Pantanal para a forma de farinha para fins alimentícios é proveniente de informação ou**
Patrimônio Genético:	**Dipteryx alata**

Sobre o provedor do Conhecimento Tradicional Associado

Houve ingresso em Terra Indígena?	**Não**

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**
Fonte de obtenção do Conhecimento Tradicional Associado:	**CTA de origem não identificável**
Conhecimento Tradicional Associado ao Patrimônio Genético Acessado:	**O processamento de frutos do Cerrado e Pantanal para a forma de farinha para fins alimentícios é proveniente de informação ou**
Patrimônio Genético:	**Acrocomia aculeata**

Sobre o provedor do Conhecimento Tradicional Associado

Houve ingresso em Terra Indígena?	**Não**

Parceria com instituição sediada no exterior

Nome	**University of Roehampton**						
Estado	**Grande Londres**	Município	**Londres**	CEP	**SW15 4J**	Endereço	**Roehampton Ln, London SW1**
Contato	**Adele Costabile**	Telefone	**+44 (0) 20 8392 3**	Email	**adele.costabilel@roehampton.ac.uk**		

Envio de Amostra que Contenha Patrimônio Genético ao Exterior

Sobre o Patrimônio Genético

Tipo do PG a ser enviado	**Amostra vegetal, fúngica ou animal (organismos inteiros ou partes destes)**
Patrimônio Genético	**Hymenaea spp.**
Forma de Acondicionamento	**Amostra líquida em tubo de microcentrífuga (eppendorf)**
Quantidade Recipiente	**2**
Volume a ser enviado	**0100 gramas**
Método do Envio	**Em mãos**
Número do Conhecimento de Carga	

UF:	**MS**
Município:	**Campo Grande**
Data de obtenção	**01/07/2019**

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**
Tipo de Componente:	**Flora (exceto algas)**
Nome Científico:	**Acrocomia aculeata**
Reino:	**Plantae**
Filo/Divisão:	**Magnoliophyta**
Classe:	**Liliopsida**
Ordem:	**Arecales**
Família:	**Arecaceae**
Nome(s) popular(es):	**macaúba, macaúva, bocaiúva, coco-de-catarro, coco-de-espinho, coco**
Trata-se de variedade tradicional local ou crioula ou raça localmente adaptada ou crioula?	**Sim**

Sobre a Procedência Do Patrimônio Genético

Procedência da amostra:	**Ex situ**
Tipo de fonte ex situ:	**Comércio**
Nome do estabelecimento Comercial de Aquisição:	**SR Ouro Verde**
UF:	**MS**
Município:	**Miranda**
Data de obtenção	**02/12/2019**

Sobre o Conhecimento Tradicional Associado

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**
Fonte de obtenção do Conhecimento Tradicional Associado:	**CTA de origem não identificável**
Conhecimento Tradicional Associado ao Patrimônio Genético Acessado:	**O processamento de frutos do Cerrado e Pantanal para a forma de farinha para fins alimentícios é proveniente de informação ou**
Patrimônio Genético:	**Hymenaea spp.**

Sobre o provedor do Conhecimento Tradicional Associado

Houve ingresso em Terra Indígena?	**Não**

Tipo de Componente:	**Flora (exceto algas)**
Nome Científico:	**Hymenaea spp.**
Reino:	**Plantae**
Filo/Divisão:	**Magnoliophyta**
Classe:	**Magnoliopsida**
Ordem:	**Fabales**
Família:	**Fabaceae**
Nome(s) popular(es):	**jatobá, jataí, jatobá-da-mata, jatobá-do-cerrado, jatobá-mirim , jutaí-açu**
Trata-se de variedade tradicional local ou crioula ou raça localmente adaptada ou crioula?	**Sim**

Sobre a Procedência Do Patrimônio Genético

Procedência da amostra:	**Ex situ**
Tipo de fonte ex situ:	**Comércio**
Nome do estabelecimento Comercial de Aquisição:	**Frutos do Mato**
UF:	**MS**
Município:	**Campo Grande**
Data de obtenção	**08/07/2019**

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**

Tipo de Componente:	**Flora (exceto algas)**
Nome Científico:	**Dipteryx alata**
Reino:	**Plantae**
Filo/Divisão:	**Magnoliophyta**
Classe:	**Magnoliopsida**
Ordem:	**Fabales**
Família:	**Fabaceae**
Nome(s) popular(es):	**cumbaru, cumaru, baru, barujo, coco-feijão, cumarurana, emburena-br**
Trata-se de variedade tradicional local ou crioula ou raça localmente adaptada ou crioula?	**Sim**

Sobre a Procedência Do Patrimônio Genético

Procedência da amostra:	**Ex situ**
Tipo de fonte ex situ:	**Comércio**
Nome do estabelecimento Comercial de Aquisição:	**Frutos do Mato**

	cumbaru almond flour, bocaiuva pulp flour and bocaiuva almond flour are examples of flours commercialized in the region of Mato Grosso do Sul. However, there is little scientific knowledge about its bioactive and functional compounds. Prebiotics are fermentable ingredients capable of selectively stimulating the growth of intestinal microorganisms that confer benefits to the host. Cerrado and Pantanal fruits have properties not yet studied in terms of the amount of prebiotic functional fibers and bioactive compounds beneficial to health. With this, the objective of the research will be to investigate the prebiotic and functional potential of fruit flours from the Cerrado and Pantanal through in vitro analyzes, characterize the flours through physical-chemical analyzes, quantify the content of bioactive compounds and determine the antioxidant capacity. The research can offer a diversification in the use of food and access by the population through the knowledge generated, valuing local natural resources. Thus, the present study will contribute to the investigation of the nutritional quality and functional effects of flours made with fruits and seeds from the Cerrado and Pantanal.
Palavra(s)-chave:	**alimentos funcionais, antioxidantes, compostos bioativos, frutooligossacarídeos, microbiota**
Palavra(s)-chave em inglês:	**functional foods, antioxidants, bioactive compounds, fructooligosaccharides, microbiota**
Setor de Aplicação	
Seção:	**ATIVIDADES PROFISSIONAIS, CIENTÍFICAS E TÉCNICAS**
Divisão:	**PESQUISA E DESENVOLVIMENTO CIENTÍFICO**
Grupo:	**Pesquisa e desenvolvimento experimental em ciências físicas e naturais**
Classe:	**Pesquisa e desenvolvimento experimental em ciências físicas e naturais**
Subclasse:	**Pesquisa e desenvolvimento experimental em ciências físicas e naturais**
Período das Atividades:	**01/07/2018** **01/07/2022**

Equipe

Nome Completo	Documento	Instituição	Nacionalidade
Sandra GARCIA	***.726.148-**	UEL	Brasil
CAROLINA SAORI ISHII MAURO	***.648.581-**	UEL	Brasil

Sobre o Componente do Patrimônio Genético Acessado

O acesso ao patrimônio genético será realizado em área indispensável à segurança nacional ou águas jurisdicionais brasileiras, plataforma continental e zona econômica exclusiva:	**Não**

AGREEMENT FOR SENDING GENETIC HERITAGE

Universidade Estadual de Londrina

AGREEMENT FOR SENDING GENETIC HERITAGE

The undersigned (Sender and recipient) resolve to formalize this Term under the following conditions:

1. This Agreement targets the use of Genetic Heritage *Hymenaea* sp., *Acrocomia aculeata* and *Dipteryx alata* Vogel for *in vitro* evaluation on the human intestinal microbiota.
2. The Genetic Heritage referred to in item 1 has the features described below:
a) Sample type and preparation: four Brazilian fruit flours (jatobá pulp – *Hymenaea* sp., cumbaru almond – *Dipteryx alata* Vogel, bocaiuva almond and bocaiuva pulp – *Acrocomia aculeata*) from the Cerrado and Pantanal.
b) Quantity of containers, volume or weight: 4 plastic bags of 100 g each.
3. The Genetic Heritage may not be used for any other purposes beyond that provided for in item 1, as well as for any commercial purpose. No other right or license is granted or implied by this Agreement.
4. The Genetic Heritage, as well as the information of genetic origin of the taxon being sent, including any substances deriving from their metabolism, must not be passed on to third parties without the prior consent of the sender.
5. The Genetic Heritage and any related intellectual property rights are and shall remain the property of the Sender. The recipient is strictly prohibited from requesting any type of intellectual property right related directly or indirectly to the Genetic Heritage.
6. Any research output (such as publication) resulting from access to the Genetic Heritage and the decision of authorship should be agreed between the parties.
7. The recipient assumes full responsibility for any damage that may occur from the use, storage or disposal of the Genetic Heritage.
8. The recipient agrees to use the Genetic Heritage in accordance with all applicable laws, governmental regulations and guidelines in force that may apply.
9. The sender shall be entitled to terminate this Agreement at any time if the recipient violates any of the terms, provisions or conditions of this Agreement.
10. The law that applied to this Agreement is Brazilian law and disputes arising out of this Agreement should be resolved in good faith between the parties.

Recognized, agreed and signed by:

- Signature of the employees at State University of Londrina (Sender):

Carolina Saori Ishii Mauro	Sandra Garcia
Ms. Carolina Saori Ishii Mauro PhD student in Food Science State University of Londrina	Dr. Sandra Garcia PhD Advisor in Food Science State University of Londrina

Date: 13/09/2021

- Signature of the recipient:

Adele Costabile
Dr. Adele Costabile Associate Professor in Nutrition University of Roehampton

Date 05/10/21

SCIENTIFIC PRODUCTION

SCIENTIFIC PRODUCTION OF THE PHD STUDENT'S THESIS PROJECT (2018-2023)

Thesis: Brazilian Cerrado and Pantanal Fruit Flours: Bioaccessibility of Phenolic Compounds, Antioxidant Capacity and Effect on Human Colonic Microbiota

Original Research Article

Mauro, Carolina Saori Ishii; Hassani, Maryame Kadiri; Barone, Monica; Esposito, Maria Teresa; Calle, Yolanda; Behrends, Volker; Garcia, Sandra; Brigidi, Patrizia; Turrroni, Silvia and Costabile, Adele. Cerrado and Pantanal fruit flours affect gut microbiota composition in healthy and post-COVID-19 individuals: An *in vitro* pilot fermentation study. **International Journal of Food Science and Technology**, 2023. DOI: https://doi.org/10.1111/ijfs.16274

Book Chapter

Mauro, Carolina Saori Ishii; Fernandes, Maria Thereza Carlos; Farinazzo, Fernanda Silva; Silva, José Renato da; Garcia, Sandra. Avaliação de Fenólicos Totais e Capacidade Antioxidante de Farinha de Cumbaru Após Extração com Diferentes Solventes In: **Avanços em Ciência e Tecnologia de Alimentos**- Volume 3.1, 2021, v.3, p. 63-70.

Summaries published in Congress Annals

Carolina Saori Ishii Mauro; Maria Thereza Carlos Fernandes; Fernanda Silva Farinazzo; José Renato Silva; Sandra Garcia. Avaliação do teor de compostos fenólicos totais e atividade antioxidante de farinha de cumbaru após extração com diferentes solventes. In: **1° Simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA 2020**. Anais online do 1° simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA2020. Itapetinga - BA: Canal INECA 2020 (YouTube), 2020.

Carolina Saori Ishii Mauro; Maria Thereza Carlos Fernandes; Milena do Prado Ferreira; Fernanda Silva Farinazzo; Cesar Ricardo Teixeira Tarley; Sandra Garcia. Bioacessibilidade de compostos fenólicos totais e atividade antioxidante de farinha de cumbaru após condições simuladas de passagem pelo trato gastrointestinal. In: **1° Simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA**

2020. Anais online do 1° simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA2020. Itapetinga - BA: Canal INECA 2020 (YouTube), 2020.

Expanded summaries presented

Carolina Saori Ishii Mauro, Maria Thereza Carlos Fernandes, Fernanda Silva Farinazzo, Sandra Garcia. Análise da composição centesimal de farinha de polpa de jatobá e efeito no crescimento de *Lacticaseibacillus casei* e *Limosilactobacillus reuteri.* IV Congresso Internacional de Gastronomia e Ciência de Alimentos/ **IV International Congress of Gastronomy and Food Science, 2022.**

Seminar Series Participation

On the 21st of January 2022 Ms. Carolina Saori Ishii Mauro, a visiting PhD student in Food Science from Londrina State University-UEL, Brazil (Award Internship CAPES-Brazilian Scholarship) and working under the supervision of Dr. Adele Costabile) gave a talk for the Research Theme: Health, Performance, and Physiology Seminar Series at the University of Roehampton, UK.

Title of the Seminar: *In vitro* study of fruit flours from the Brazilian Cerrado and Pantanal on human health.

SCIENTIFIC PRODUCTION OF THE PHD STUDENT'S RESEARCH GROUP (2018-2023)

Original Research Articles

Mauro, Carolina Saori Ishii; Fernandes, Maria Thereza Carlos; Farinazzo, Fernanda Silva; Garcia, Sandra. Characterization of a fermented coconut milk product with and without strawberry pulp. **Journal of Food Science and Technology-Mysore**, v. 59, p. 2804-2812, 2022. https://doi.org/10.1007/s13197-021-05303-1

Farinazzo, Fernanda Silva; Fernandes, Maria Thereza Carlos; Mauro, Carolina Saori Ishii; Garcia, Sandra. Statistical optimization of exopolysaccharide production by JF17 from native Atlantic Forest juçara fruit. **Preparative Biochemistry & Biotechnology**, v. 1, p. 1-8, 2021. https://doi.org/10.1080/10826068.2021.1931880

Farinazzo, Fernanda Silva; Valente, Leticia Juliani; Almeida, Mariana Bortholazzi; Simionato, Ane Stéfano; Carlos Fernandes, Maria Thereza; Ishii Mauro, Carolina Saori; Bosso Tomal, Adriana Aparecida; Garcia, Sandra. Characterization and antioxidant activity of an exopolysaccharide produced by *Leuconostoc pseudomesenteroides* JF17 from juçara fruits (*Euterpe edulis* Martius). **Process Biochemistry**, v. 91, p. 141-148, 2020. https://doi.org/10.1016/j.procbio.2019.12.005

Machado, Cíntia Cristina Da Silva; Fernandes, Maria Thereza Carlos; Mauro, Carolina Saori Ishii; Farinazzo, Fernanda Silva; Prudencio, Sandra Helena; Garcia, Sandra. Probiotic Juçara and Banana Sorbet: Cell Viability, Antioxidant Activity during Storage and Sensory Acceptability by Children. **Journal of Culinary Science & Technology**, v. 1, p. 1-15, 2020. https://doi.org/10.1080/15428052.2020.1787287

Kiesling, Yolanda Graciela; Farinazzo, Fernanda Silva; Fernandes, Maria Thereza Carlos; Mauro, Carolina Saori Ishii; Iorio, Adriana Beatriz Di; Garcia, Sandra. Coconut Water Fermented by *Lactobacillus plantarum* with Inulin Addition: Development of a Potentially Synbiotic Beverage. **Brazilian Journal of Development**, v. 6, p. 42324-42337, 2020. http://doi.org/10.34117/bjdv6n7-009

Silva Farinazzo, Fernanda; Bervelieri Madeira, Tiago; Carlos Fernandes, Maria Thereza; Ishii Mauro, Carolina Saori; Bosso Tomal, Adriana Aparecida; Nixdorf, Suzana Lucy; Garcia, Sandra. Organic and conventional apple fermented by *Saccharomyces boulardii* - The effect of the antioxidant quercetin on cellular oxidative stress. **British Food Journal**, v. 123, p. 520-534, 2020. https://doi.org/10.1108/BFJ-07-2019-0564

Mauro, Carolina Saori Ishii; Garcia, Sandra. Coconut milk beverage fermented by *Lactobacillus reuteri*: optimization process and stability during refrigerated storage. **Journal of Food Science and Technology-Mysore**, v. 56, p. 854-864, 2019. https://doi.org/10.1007/s13197-018-3545-8

Book Chapters

Fernandes, Maria Thereza Carlos; Mauro, Carolina Saori Ishii; Farinazzo, Fernanda Silva; Guergoletto, Karla Bigetti; Garcia, Sandra. Bioacessibilidade de Compostos Fenólicos e Flavonoides em Polpa de Juçara. In: Silvani Verruck. (Org.). **Avanços em Ciência e Tecnologia de Alimentos - Volume 3**. 1ed., 2021, v. 3, p. 71-78.

Garuz, Diana Melina Jované; Mauro, Carolina Saori Ishii; Fernandes, Maria Thereza Carlos; Farinazzo, Fernanda Silva; Basso, Juliana Morilha; Amancio, Rayssa da Rocha; Ferreira, Débora Pinhatari; Tomal, Adriana Aparecida Bosso; Garcia, Sandra. Inibição de Bactérias Patogênicas por Extrato Contendo Produtos do Metabolismo de *Lactobacillus reuteri* E Aplicação Em Iogurte. **As Ciências Biológicas e a Construção de Novos Paradigmas de Conhecimento - Volume 2. 1ed**.: Atena Editora, 2020, p. 28-36.

Guergoletto, Karla Bigetti; Farinazzo, Fernanda Silva; Mauro, Carolina Saori Ishii; Fernandes, Maria Thereza Carlos; Alves, Gilberto; Prudencio, Sandra Helena; Garcia, Sandra. Nondairy Probiotic and Prebiotic Beverages: Applications, Nutrients, Benefits, and Challenges. **Nutrients in Beverages**. 1ed.: Elsevier, 2019, v. 12, p. 277-314.

Fernanda Silva Farinazzo; Paulo Terumitsu Saito; Maria Thereza Carlos Fernandes; Carolina Saori Ishii Mauro; Marsilvio Lima de Moraes Filho; Marli Busanello; Karla Bigetti Guergoletto; Sandra Garcia. Comparative Study of the Development and Probiotic Protection in Food Matrices. In: Deepak Kumar Verma; Ami R. Patel; Prem Prakash Srivastav; Balaram Mohapatra; Alaa Kareem Niamah. (Org.). **Microbiology**

for Food and Health - Technological Developments and Advances. 1ed.Florida: Apple Academic Press Inc., 2019, v. 1, p. 367-.

Fernandes, Maria Thereza Carlos; Farinazzo, Fernanda Silva; Mauro, Carolina Saori Ishii; Basso, Juliana Morilha; Valente, Leticia Juliani; Tomal, Adriana Aparecida Bosso; Bosso, Alessandra; Pacheco, Camilla de Andrade; Garcia, Sandra. Study of Cell Viability and Physical-Chemical Characteristics of Probiotic Juice From Cashew and Tangerine. **Inovação em Ciência e Tecnologia de Alimentos**. 1ed.Ponta Grossa: Atena Editora, 2019, v. 1, p. 263-272.

Trends and Technological Advances in Brazilian Fruit Fermentation. Carolina Saori Ishii Mauro, Maria Thereza Carlos Fernandes, Paulo Terumitsu Saito, Fernanda Silva Farinazzo, Deepak Kumar Verma and Sandra Garcia. – Submission accepted awaiting publication

Summaries published in Congress Annals

Milena P. Ferreira, Carolina Saori Ishii Mauro, Sharise B. R. Berton, Adriana A. B. Tomal, Thais S. Rocha. Aceitação sensorial de um patê elaborado com farinha de berinjela com potencial antioxidante. IV Congresso Internacional de Gastronomia e Ciência de Alimentos/ **IV International Congress of Gastronomy and Food Science, 2022.**

Maria Thereza Carlos Fernandes; Carolina Saori Ishii Mauro; Fernanda Silva Farinazzo; Karla Bigetti Guergoletto; Sandra Garcia. Bioacessibilidade de compostos fenólicos e flavonoides em polpa de juçara. In: **1° Simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA**. Anais online do 1° simpósio nacional sobre inovação em engenharia e ciência de alimentos – INECA2020. Itapetinga - BA: Canal INECA 2020 (YouTube), 2020.

Fernanda Silva Farinazzo; Maria Thereza Carlos Fernandes; Carolina Saori Ishii Mauro; Talita Szlapak Franco; Sandra Garcia. Triagem de potenciais propriedades probióticas de *Lactobacillus plantarum* isolado de gérmen de trigo. In: **1° Simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA**. Anais online do 1° simpósio nacional sobre inovação em engenharia e ciência de alimentos - INECA2020. Itapetinga - BA: Canal INECA 2020 (YouTube), 2020.

Mauro, C. S. I.; Fernandes, M. T. C.; Farinazzo, F. S.; Tomal, A. A. B.; Garcia, S. Aceitação sensorial de bebida de coco fermentada por *Lactobacillus reuteri*. In: **Encontro Brasileiro de Alimentos Funcionais - ENBRAF**, Maringá - PR. Anais do Encontro Brasileiro de Alimentos Funcionais, 2019. v. 1.

Farinazzo, F. S.; Mauro, C. S. I.; Fernandes, M. T. C.; Valente, L. J.; Tomal, A. A. B.; Garcia, S. In vitro evaluation of potential probiotic properties of *Lactobacillus casei* V5 isolated from viili. In: **30º Congresso Brasileiro de Microbiologia**, Maceió - AL. Anais do 30º Congresso Brasileiro de Microbiologia, 2019.

Fernandes, M. T. C.; Mauro, C. S. I.; Farinazzo, F. S.; Simionato, A. S.; Guergoletto, K. B.; Garcia, S. Effect of processing conditions on the juçara pulp for in vitro

fermentation. In: **30º Congresso Brasileiro de Microbiologia**, Maceió - AL. Anais do 30º Congresso Brasileiro de Microbiologia, 2019.

Farinazzo, F. S.; Fernandes, M. T. C.; Mauro, C. S. I.; Valente, L. J.; Tomal, A. A. B.; Garcia, S. Isolation, production and characterization of extracellular polysaccharides by lactic acid bacteria isolated from *Euterpe edulis* Martius. In: **30º Congresso Brasileiro de Microbiologia**, Maceió - AL. Anais do 30º Congresso Brasileiro de Microbiologia, 2019.

Fernandes, M. T. C.; Farinazzo, F. S.; Mauro, C. S. I.; Valente, L. J.; Tomal, A. A. B.; Guergoletto, K. B.; Garcia, S. Effect of juçara pulp addition on the in vitro fermentation by *Lactobacillus reuteri* and *Bifidobacterium* spp. In: **30º Congresso Brasileiro de Microbiologia**, Maceió - AL. Anais do 30º Congresso Brasileiro de Microbiologia, 2019.

Jovane-Garuz, D. M.; Mauro, C. S. I.; Fernandes, M. T. C.; Farinazzo, F. S.; Garcia, S. In vitro inhibition of *Escherichia coli*, *Salmonella* spp. and *Staphylococcus aureus* by *Lactobacillus reuteri* metabolites. In: **30º Congresso Brasileiro de Microbiologia**, Maceió - AL. Anais do 30º Congresso Brasileiro de Microbiologia, 2019.

Valente, L. J.; Mauro, C. S. I.; Fernandes, M. T. C.; Farinazzo, F. S.; Tomal, A. A. B.; Garcia, S. Shelf life of a potentially symbiotic beverage made with tangerine juice fermented by *Lactobacillus plantarum* BG 112 and galactooligosaccharides. In: **30º Congresso Brasileiro de Microbiologia**, Maceió - AL. Anais do 30º Congresso Brasileiro de Microbiologia, 2019.

Carolina Saori Ishii Mauro; Maria Thereza Carlos Fernandes; Fernanda Silva Farinazzo; Sandra Garcia. Fermented Coconut Beverage: Analysis of Syneresis And Cell Viability During Refrigerated Storage. In: **XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA**, Belém - PA. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA, 2018.

Yolanda Graciela Kiesling; Carolina Saori Ishii Mauro; Fernanda Silva Farinazzo; Maria Thereza Carlos Fernandes; Sandra Garcia. Optimization of *Lactobacillus plantarum* BG 112 Cellular Production in Coconut ater With Addition of Agave Inulin. 2018. In: **XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA**, Belém - PA. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA, 2018.

Fernanda Silva Farinazzo; José Renato Silva; Carolina Saori Ishii Mauro; Maria Thereza Carlos Fernandes; Sandra Garcia. Optimization of Exopolysaccharide Produced by Leuconostoc Pseudomesenteroides Isolated From Juçara Fruits. 2018. In: **XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA**, Belém - PA. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA, 2018.

Maria Thereza Carlos Fernandes; Cíntia Cristina da Silva Machado; Fernanda Silva Farinazzo; Carolina Saori Ishii Mauro; Sandra Garcia. Probiotic Frozen Juçara Pulp with Addition of Linseed Mucilage. In: **XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA**, Belém - PA. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA, 2018.

Fernanda Silva Farinazzo; Maria Thereza Carlos Fernandes; Carolina Saori Ishii Mauro; Sandra Garcia. Spontaneous Fermentation of The Juçara Pulp: Characterization and Microbiota Identification. In: **XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA**, Belém - PA. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA, 2018.

Maria Thereza Carlos Fernandes; Fernanda Silva Farinazzo; Carolina Saori Ishii Mauro; Sandra Garcia. Stability to Storage of Probiotic Cashew And Tangerine Juice. In: **XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA**, Belém - PA. Anais do XXVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos - CBCTA, 2018.

Workshop

Via Rural Fazendinha: Partnership SEAB - Secretary of Agriculture and Supply of Paraná, Sociedade Rural Do Paraná, UEL, EMATER and IAPAR. **Unconventional Food Plants: Concept and Application (Juçara Jam)**. 2019.