UNIVERDIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS
Programa de Pós-graduação em Ciências de Alimentos
Área de Bromatologia

Produtos derivados do camu-camu: efeito da secagem sobre elagitaninos e flavonoides, atividades antioxidante e antimicrobiana

Alice Fujita

Tese para obtenção do grau de:
DOUTOR

Orientadora:
Prof. Dr. Maria Inés Genovese

São Paulo
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Versão corrigida da Tese conforme resolução CoPGr 6018.
O original encontra-se disponível no Serviço de Pós Graduação da FCF/USP.

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Ficha Catalográfica
Elaborada pela Divisão de Biblioteca e
Documentação do Conjunto das Químicas da USP.

Fujita, Alice
F961p Produtos derivados do camu-camu : efeito da secagem sobre elagitaninos e flavonoides, atividades antioxidante e antimicrobiana / Alice Fujita. -- São Paulo, 2015.
147p.

Tese (doutorado) - Faculdade de Ciências Farmacêuticas da Universidade de São Paulo. Departamento de Alimentos e Nutrição Experimental
Orientador : Genovese, Maria Inés

1. Alimento: Composto fenólico : Ciência do alimento 2. Secagem de alimentos I. T. II. Genovese, Maria Inés, orientador.

641 CDD

ALICE FUJITA

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São Paulo, _____ de _____.

" Se o ser humano enfrentar tudo com coragem e boa vontade, aprendendo e pesquisando cada coisa, poderá ampliar cada vez mais o seu interesse, a ponto de entender que a vida é um tesouro inesgotável".

(Nidai-sama)

AGRADECIMENTOS

Primeiramente a Supremo Deus e a Meishu-sama pelas inúmeras graças recebidas no decorrer dessa vida e oportunidades de crescimento espiritual, profissional e pessoal.

A Prof. Dr. Maria Inés Genovese pela orientação, dedicação, apoio e principalmente, por ter acreditado em mim.

Ao Prof. Dr. Kalidas Shetty pela orientação durante a doutorado sanduíche na *North Dakota State University* em Fargo, Estados Unidos. Sempre disposto a tirar as dúvidas e incentivar.

Aos professores colaboradores Prof. Dr. Bernadette Franco, Prof. Dr. Roberta Targino, Prof. Dr. Carmen Fávaro-Trindade, Prof. Dr. Heidi Fukumasu e Prof. Dr. Edward Kennelly que permitiram a realização de experimentos essenciais para o desenvolvimento do projeto.

Aos meus amigos e companheiros de laboratório Helena Barros, Daniel Daza, Carlos Donado, Guto, Flavia Beteto, Renata Araújo, Luana, Gabriela, Marcio, Tatyane, Bia e Heloisa que fizeram o dia-a-dia do laboratório mais alegre e divertida. Tivemos muitos cafés na copa e na cantina, momentos de desespero, descontração, crise de risos e choros que serão inesquecíveis. Aos meus amigos mais antigos que me ensinaram as análises no laboratório: Maria Cecília, Gabriela Vieira, Thiago Belchior e Diully. Em especial a Marcela Roquim e Daniela Marques minhas companheiras de quarto, dividimos muitas confidências, desabafos e cervejas!

As técnicas do laboratório Tatiana Garofalo, Luciene e Rosa sempre solícitos em ajudar.

Ao pessoal do Laboratório de Microbiologia de Alimentos, as técnicas, Katia e Lúcia, e os alunos, Verena, Vinícius e Rafael por ensinar a metodologia com muita boa vontade.

Durante o desenvolvimento do projeto conheci muitas pessoas especiais como Katia, Francisco e Juliana em Natal - RS. Volnei e os técnicos Marcelo e Arina em Pirasununga. Dipayan, Ram, Jordan e Jamie que conviveram comigo durante um ano em Fargo - EUA. E também a Suyare com quem compartilhei não só o apartamento, você foi minha companheira de academia, caminhadas, experimentos, compras, além de muitas conversas a altas horas.

Ao pessoal da Secretaria de Alimentos e Nutrição Experimental, Mônica, Cléo, Edilson e Roberta, da Secretaria de Pós-Graduação, Sueli e Irineu e do Centro de Informática pela disposição em ajudar.

As instituições FCF/USP, FZEA/USP (Pirassunga – SP), UFRN (Natal – RN), NDSU (Fargo – ND – EUA) e Lehman College (New York University) pelo uso dos laboratórios.

Não poderia esquecer-me do Sr. Francisco Koga pela doação do fruto camu-camu, Douglas do Sítio de Bello por auxlilar na produção da polpa de camu-camu, à Corn Products pela doação de maltodextrina e à Nexira Brasil pela doação de goma arábica.

À CNPq pela bolsa no período do doutorado e à CAPES pela bolsa sanduiche nos Estados Unidos.

À minha família (mãe, pai, irmã, irmão e sobrinhos) que sempre me incentivaram e apoiaram em tudo que eu fiz. Vocês são meu alicerce e meu porto seguro.

Ao meu noivo Paul, que foi a minha maior alegria durante o doutorado, um presente maravilhoso que a vida me trouxe. Agradeço pela confiança, respeito e companheirismo.

Aos meus amigos Min. Tadeu e Marina que sempre tem uma palavra mágica nos momentos difíceis para me acalmar. Aos meus amigos do coração que me incentivaram a iniciar o doutorado Lyssa, Cristina Lui e principalmente Min. Alexandre Faria que mostraram que tudo é possível quando se acredita.

A todos aqueles que de alguma forma contribuíram para o desenvolvimento deste trabalho.

Muito Obrigada.

RESUMO

Fujita, A. **Produtos derivados do camu-camu: efeito da secagem sobre elagitaninos e flavonoides, e atividades antioxidante e antimicrobiana**. 2015. 147 f. (Tese de Doutorado). Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo 2015.

O camu-camu (*Myrciaria dubia* Mc. Vaugh) tem demonstrado ser um fruto promissor devido ao potencial funcional, principalmente pelo alto teor de vitamina C e compostos fenólicos, em especial elagitaninos. Este trabalho teve como objetivo avaliar os efeitos sobre os compostos fenólicos dos processos de secagem de polpa comercial de camu-camu, por leito de jorro e atomização (spray-drying), em diferentes temperaturas e concentrações de agentes carreadores, comparando-os com os do processo de liofilização. Os pós obtidos foram comparados em relação ao teor total de compostos fenólicos, ácido ascórbico e proantocianidinas. Além disso, avaliou-se o potencial benéfico à saúde através da determinação da capacidade antioxidante *in vitro*, atividade antimicrobiana e inibição das enzimas α-amilase, α-glicosidase e enzima conversora da angiotensina (ECA). Avaliou-se também proteção e regeneração celulares em modelo de planárias (*Dugesia trigrina*). Complementarmente, os pós de camu-camu foram adicionados em leite de soja, que foram fermentados com bactérias produtoras de ácido láctico (*L. helveticus* ATCC 12046 e *L. plantarum* NCDO 1193), para verificar sua funcionalidade quando incorporados como ingredientes em alimentos funcionais. Os resultados mostraram que a secagem da polpa acarretou em perdas significativas de compostos bioativos, na ordem ácido ascórbico>fenólicos totais> proantocianidinas, e spray-drying>leito de jorro>liofilização. Os compostos fenólicos detectados nos pós de camu-camu foram elagitaninos, ácido elágico, derivados de quercetina, ácido siríngico e miricetina, por LC-TOF-MS. A liofilização foi a melhor técnica de secagem para a preservação dos compostos fenólicos, e também da capacidade antioxidante e de inibição enzimática. Além disso, os pós liofilizados e atomizado (contendo 6% goma arábica a 120 °C) foram mais efetivos contra *Staphylococcus aureus* que a ampicilina. Os extratos desses pós demostraram potencial para proteção celular e rejuvenescimento no modelo de planárias. E por último, o leite de soja enriquecido com o pó de camu-camu resultou em um produto com maior teor de fenólicos, alta capacidade antioxidante e propriedades anti-hiperglicêmica e anti-hipertensiva. Portanto, os pós de camu-camu são ricos em compostos fenólicos e tem potencial para serem acrescentados como ingredientes em alimentos para o controle dos estágios iniciais de diabetes tipo 2 e complicações associadas.

Palavras-chave: ácido elágico; liofilização; spray drying; *Staphylococcus aureaus*; Planaria.

ABSTRACT

Fujita, A. **Camu-camu ingredients: effect of drying on ellagitannins and flavonoids, and their antioxidant and antimicrobial activities**. 2015. 147 f. (Tese de Doutorado). Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo 2015.

Camu-camu (*Myrciaria dubia* Mc. Vaugh) has demonstrated promising perspectives as a functional food, mainly due to high vitamin C and phenolic compounds contents, in particular ellagitannins. This study aimed to evaluate the effect of different drying processes (spouted bed drying, spray-drying) at selected temperatures and carrier concentrations, comparing to freeze-drying, on the contents and composition of phenolic compounds. The pulp powders were compared in relation to phenolic profiles, ascorbic acid and proanthocyanidins contents. Further, functional health potential was evaluated such as *in vitro* antioxidant capacity, antimicrobial activity and inhibition of α-amylase, α-glucosidase and angiotensin converting enzyme (ACE). It was also investigated cellular protection and regeneration in planaria (*Dugesia trigrina*) model. Additionally, camu-camu powders were added into soymilk and were fermented by lactic acid bacteria (*L. helveticus* ATCC 12046 and *L. plantarum* NCDO 1193) to verify their functionality as a functional food ingredient. The results showed that drying of the pulp led to significant losses of bioactive compounds, in the order ascorbic acid>total phenolics>proanthocyanidins, and spray-drying>spouted bed drying>freeze-drying. Phenolic compounds, such as ellagitannins, ellagic acid, quercetin derivatives, syringic acid and myricetin were detected in camu-camu by LC-TOF-MS. The freeze-drying was the best technique to preserve phenolic compounds, and also antioxidant capacity and enzyme inhibition. Besides that, freeze-dried and spray-dried (6% arabic gum at 120 °C) powders were more effective against *Staphylococcus aureus* than ampicillin. The extracts of those powders have desmonstrated potential to cellular protection and rejuvenation in planaria model. Finally, soymilk enriched with camu-camu powders resulted in more phenolic contents, high antioxidant capacity and anti-hyperglycemia and anti-hipertension properties product. To sum up, camu-camu powder is rich in phenolic bioactive profiles has potential as part of dietary strategies in the management of early stages of type 2 diabetes and associated complications.

Keywords: ellagic acid; freeze-drying; spray-drying; *Staphylococcus aureaus*; Planaria

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LISTA DE ABREVIATURAS E SÍMBOLOS

AA	Ácido L-ascórbico
AAE	Ascorbic acid equivalents
ACE	Angiotensin converting enzyme
ATP	Adenosina trifosfato
AOAC	Association Official Analitical Chemistry
BHT	Butil hidroxitolueno
BHA	Butil hidroxianisol
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CFU	Colony forming unit
CI50	Concentração inibitória 50%
CLSI	Clinical and Laboratory Standards Institute
DAD	Diode array detector
DE	Equivalência em dextrose
DHA	Ácido L-desidroascórbico
DPPH	2,2-diphenyl-1-picrylhydrazyl radical
DW	Dry weight
ECA	Enzima conversora de angiotensina
ERN	Espécies reativas de nitrogênio
ERO	Espécies reativas de oxigênio
FC	Folin-Ciocalteau reducing capacity
FRAP	Ferric reducing power
GA	Gum arabic /goma arábica
GAE	Gallic acid equivalents
HCA	Hierarchical cluster analysis
HHL	Hippuryl-histidylleucine
HIV	Vírus da imunodeficiência humana
HNO_2	Ácido nitroso
$HO^{\bullet}$	Radical hidroxila
H_2O_2	Peróxido de hidrogênio
HPLC	High performance liquid chromatography
IDF	International Diabetes Federation
IC 50	Half maximal inhibitory concentration

IL-6	Interleucina-6
IL-8	Interleucina-8
INPA	Instituto Nacional de Pesquisas da Amazônia
LC-TOF-MS	Liquid chromatography time-of-flight mass spectrometry
MD	Maltodextrin
MIC	Minimum inhibitory concentration
NCDs	Non-communicable chronic diseases
$NO^{\bullet}$	Óxido nítrico
NO_2	Dióxido de nitrogênio
N_2O	Óxido nitroso
N_2O_3	Trióxido dinitrogênio
O_2	Superóxido
$^1O_2$	Oxigênio singleto
O_3	Ozônio
$ONOO^{\bullet}$	Peróxinitrito
ONOOH	Ácido peróxinitroso
ORAC	Oxygen Radical Absorbance Capacity Assay
OPLS-DA	Orthogonal partial least squares discriminant analysis
PAC	Total proanthocyanidins contents
PCA	Principal component analysis
PG	Galato de propila
rpm	Rotation per minute
$ROO^{\bullet}$	Radical peroxila
ROOH	Hidroperóxido orgânico
RSM	Response surface methodology
SCFas	Ácidos graxos de cadeia curta
SS	Soluble solids
TA	Titrable acidity
TBHQ	Terc-butil hidroquinona
TS	Total sugars
TP	Total phenolics
w/v	Weight/volume
WHO	World health organization

°Brix	Grau brix
° C	Grau celsius

SUMÁRIO

1. INTRODUÇÃO

Pesquisas mostram que dietas ricas em frutas e verduras podem reduzir o risco de doenças cardiovasculares, diabetes e câncer (CROZIER et al., 2009), o que pode ser atribuído, em parte, aos constituintes fitoquímicos, em particular aos compostos fenólicos (ESPÍN et al., 2007). Os compostos fenólicos são encontrados em abundância em alimentos e bebidas de nossa dieta diária, tais como frutas, nozes, verduras, grãos, legumes e condimentos (AMAKURA et al., 2000) e são classificados nos seguintes grupos: ácidos hidroxibenzoicos (gálico, elágico, protocatecuico, vanílico, siríngico), ácidos hidroxicinâmicos (cumárico, caféico, ferúlico, sinápico), estilbenos, lignanas, flavonoides e taninos (SHAHIDI e NACZK, 2004).

O Brasil está entre os três maiores produtores de frutas do mundo, junto com a Índia e a China. A produção em 2009 foi de 41 milhões de toneladas de frutos frescos (IBRAF, 2010). No entanto, o consumo pela população brasileira é pequeno (57 kg/pessoa/ano), quando comparado com países desenvolvidos (150 kg/pessoa/ano). No entanto, observa-se uma tendência de crescimento do mercado de frutos desidratados, devido facilitar o manuseio, transporte e armazenamento (LESCANO, 2009). Ao mesmo tempo, a vida de prateleira dos frutos é prolongada com a retirada da umidade do produto, e consequentemente diminuição da atividade de água, o que reduz a atividade enzimática e microbiana (AGUIRRE e FILHO, 2002).

Diversos frutos nativos brasileiros têm despertado o interesse da comunidade científica, como: açaí, cupuaçu, umbu, cagaita e camu-camu. O fruto camu-camu, originário da Bacia Amazônica, cresce próximo de rios e lagos, e sua produção é local e baixa. A produção no Brasil não ultrapassa 20 toneladas de polpa por ano. Em contrapartida, o Peru, maior produtor mundial de camu-camu, produz em média 800 toneladas de polpa por ano (INPA, 2011). Quase que a totalidade é exportada para os Estados Unidos, Japão e França, onde são encontrados diversos produtos de camu-camu no mercado, como sucos, sorvetes e balas (ALVES et al., 2002).

Pesquisas mostram que o fruto camu-camu possui grande potencial benéfico à saúde, destacando-se pelo alto teor de vitamina C, flavonoides e elagitaninos, além de possuir elevada capacidade antioxidante, *in vivo* e *in vitro* (GENOVESE et al., 2008; GONÇALVES et al., 2010; MYODA et al., 2010). Estudo realizado no Japão demonstrou os efeitos antioxidante e anti-inflamatório do suco de camu-camu comparado à vitamina C em tabletes em quantidade equivalente, administrados em indivíduos fumantes. O suco da fruta foi

responsável por uma redução significativa dos marcadores do estresse oxidativo, como 8-hidroxi-deoxiguanosina e espécies reativas de oxigênio e dos marcadores de inflamação, como proteína C reativa, interleucina (IL)-6 e IL-8, enquanto nenhum efeito foi observado no grupo que recebeu as mesmas doses de vitamina C (INOUE et al., 2008).

Esses resultados demonstram que não seria a vitamina C a principal responsável pelos efeitos decorrentes do consumo do camu-camu. Além da vitamina C, o camu-camu contém outros compostos antioxidantes como elagitaninos (GENOVESE et al., 2008), antocianinas (ZANATTA et al., 2005) e carotenoides (AZEVEDO-MELEIRO e RODRIGUEZ-AMAYA, 2004), cuja ação, isolada ou conjunta, poderia ser a responsável pelos efeitos observados. Além disso, o camu-camu também contém elevado teor de potássio (JUSTI et al., 2000), o qual é responsável por acelerar a absorção intestinal de vitamina C (STEVENSON, 1974).

Contudo, os frutos maduros de camu-camu são altamente perecíveis, mesmo quando armazenados sob refrigeração.

Diante do exposto, justifica-se o estudo de tecnologias capazes de gerar produtos derivados do camu-camu com maior vida de prateleira e valor agregado, facilitando seu transporte e distribuição. Além disso, disponibilizando os produtos no período de entressafra e atendendo a demanda do comércio, podendo ser introduzidos como ingredientes na formulação de alimentos funcionais e possibilitar sua veiculação na dieta.

Os estudos do fruto camu-camu realizados até agora têm dado ênfase ao teor de vitamina C (SILVA et al., 2005; DIB TAXI et al., 2003). No entanto, o fruto também possui alto teor de compostos fenólicos tais como proantocianidinas e elagitaninos. Portanto, torna-se necessário avaliar o efeito da secagem sobre estes compostos fenólicos, que também contribuem com a capacidade antioxidante e antimicrobiana.

2. REVISÃO BIBLIOGRÁFICA

2.1 Camu-camu

O camu-camu (*Myrciaria dubia* Mc. Vaugh) é um fruto nativo da Bacia Amazônica, que cresce próximo a rios e lagos de águas escuras e ilhas, formando grandes bosques (MCVAUGH, 1963; GOMES, 1983; SOUZA FILHO et al., 2002). A espécie distribui-se abundantemente pelas florestas amazônicas brasileiras, peruanas, colombianas e venezuelanas. No Brasil encontra-se nos Estados do Pará, Amapá, Amazonas, Rondônia, Roraima e Mato Grosso (MCVAUGH, 1963) e é conhecido por diversos nomes como araçá, araçarana, araçazinho, araçá-dágua, araçá-do-lago, caçari, murta, sarão e socoró. O fruto é uma baga esférica (2 a 4 centímetros de diâmetro) de superfície lisa e brilhante de coloração vermelho-arroxeada. Contém entre uma e quatro sementes (**Figura 1 e 2**) (VILLACHICA, 1996; EMBRAPA, 2004).

Figura 1. Frutos do camu-camu (*Myrciaria dubia* Mc. Vaugh).

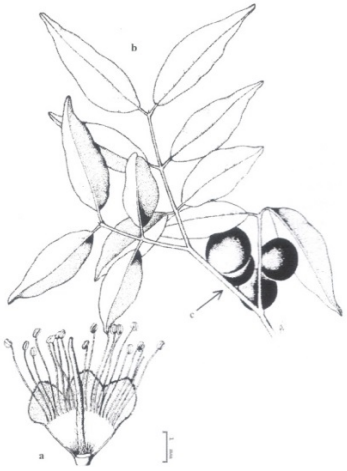


Figura 2. Camu-camu (*Myrciaria dubia*) (a) corte longitudinal e uma flor, (b) ramo frutífero e (c) frutos maduros (Maués & Couturier, 2002).

Esse fruto tem despertado interesse devido o seu alto teor de vitamina C, o qual é superior ao encontrado em acerola ou frutas cítricas já estudadas (GENOVESE et al., 2008). O camu-camu pode conter até 6 g/100 g do fruto (YUYAMA et al., 2002). Comparativamente, a acerola possui em média 1,79 g/100 g do fruto e a laranja 0,08 g/100 g do fruto (SMIDERLE, 2008; VALENTE et al., 2014).

O fruto camu-camu mostrou-se uma excelente fonte de compostos fenólicos, os quais são responsáveis por sua expressiva atividade antioxidante. Gonçalves et al. (2010) analisaram a capacidade antioxidante de várias frutas nativas brasileiras por três métodos diferentes (capacidade redutora de Folin-Ciocalteau, ORAC e sequestro do radical DPPH). O fruto camu-camu apresentou valores 10 vezes maiores que as outras frutas nativas. Os compostos polifenólicos encontrados no fruto camu-camu são: ácido elágico, elagitaninos, quercetina, caempferol e cianidina (GENOVESE et al., 2008; GONÇALVES et al., 2010; CHIRINOS et al., 2010; RUFINO et al., 2010).

O potencial benéfico deste fruto já foi avaliado em estudos *in vivo* e *in vitro*. O suco de camu-camu apresentou propriedades antioxidante e anti-inflamatória em estudo *in vivo*, no qual um grupo de fumantes ingeriu suco de camu-camu (100%) e outro, comprimido de vitamina C em dose equivalente. Somente o grupo que ingeriu o suco apresentou diminuição significativa dos marcadores de estresse oxidativo e inflamatórios, sugerindo que os compostos fenólicos são responsáveis pela ação antioxidante e não apenas a vitamina C (INOUE et al., 2008).

2.1.1. Composição nutricional

A composição físico-química da polpa de camu-camu pode ser observada no **Quadro 1**. Nota-se que é uma fruta com alto teor de umidade, bastante ácida, e que apresenta alto teor de potássio.

Os teores dos compostos bioativos do camu-camu são apresentados no **Quadro 2**. A variação de valores pode ser atribuída a diversos fatores, tais como: região de plantio, variedade, estágio de maturação, teor de acidez e presença de enzimas (YUYAMA *et al*., 2002; VILLANUEVA *et al*., 2010). Verifica-se que o fruto camu-camu possui alto teor de vitamina C e de compostos fenólicos, no entanto, os teores de carotenoides são baixos.

Quadro 1: Comparação da composição físico-química de polpas comercial de camu-camu, expressa em matéria fresca, avaliada por diversos autores

Referência (autores)	Maeda et al. (2006)	Zanatta et al. (2005)		Silva et al. (2005)	Rodrigues et al. (2004)	Alves et al. (2002)	Justi et al. (2000)	Zapata e Dufour (1993)
Procedência	Rio Preto da Eva AM	Iguape SP	Mirandópolis SP	CEAGESP SP	Pará	Belém PA	Curitiba PR	Iquitos Perú
Componentes								
Umidade (g/100 g)	92,65 ± 0,03	na	na	91,42 ± 0,20	na	na	94,1 ± 0,1	na
Lipídio (g/100 g)	0,05 ± 0,01	na	na	na	na	na	0,2	na
Proteína (g/100 g)	0,29 ± 0,0	na	na	na	na	na	0,4	na
Cinzas (g/100 g)	na	na	na	na	na	na	0,3	na
Fibra bruta (g/100 g)	na	na	na	na	na	na	0,1	na
Açúcares redutores (g/100 g)	2,96 ± 0,0	na	na	na	na	na	na	na
Açúcares totais (g/100 g)	4,47 ± 0,03	na	na	na	na	1,28 a 1,48	na	na
Glicose (g/100 g)	na	na	na	na	na	na	na	224 a 816
Frutose (g/100 g)	na	na	na	na	na	na	na	370 a 951
Ácido cítrico (g/100 g)	na	na	na	na	na	na	na	1981 a 2982
Ácido isocitrico (g/100 g)	na	na	na	na	na	na	na	12 a 15
Ácido málico (g/100 g)	na	na	na	na	na	na	na	280 a 598
pH	2,64 ± 0,01	2,4 a 2,8	2,6 a 2,9	2,95 ± 0,01	2,7	2,51 a 2,54	na	2,44 a 2,56
Sólidos solúveis (ºBrix)	6,20 ± 0,0	6,0 a 6,5	6,5 a 8,5	6,5 ± 0,1	6,1 a 6,4	6,36 a 6,40	na	5,5 a 6,8
Acidez (g/100 g)	3,40 ± 0,06	na	na	2,31 ± 0,03	3,5 a 3,9	na	na	3,07 a 3,55
Densidade	na	na	na	na	na	na	na	1,025 a 1,030
Sólidos Totais	na	na	na	na	na	na	na	67,7 a 81
Nitrogênio Total	na	na	na	na	na	na	na	0,568 a 0,735
Micronutrientes (mg/kg)	na	na	na	na	na	na	na	
K	na	na	na	na	na	na	838,8 ± 36,4	532 a 711
Ca	na	na	na	na	na	na	157,3 ± 4,4	62 a 66
Mg	na	na	na	na	na	na	123,8 ± 8,7	47 a 51
Na	na	na	na	na	na	na	111,3 ± 4,3	27 a 49
PO_4	na	na	na	na	na	na	na	245 a 295
SO_4	na	na	na	na	na	na	na	132 a 219
Cl	na	na	na	na	na	na	na	66 a 116

na: não avaliado

Quadro 2: Teores de ácido ascórbico, polifenóis, antocianinas, elagitaninos e carotenoides do camu-camu analisados por diversos autores

Procedência	Ác. Ascórbico (mg/100 g polpa)	Polifenóis (mg ac.gálico/100 g)	Antocianinas Totais (mg/100 g)	Elagitaninos (mg/100 g)	Carotenoides (mg/100 g)	Referência
Belém - PA	1986 ± 75	na	na	na	na	Alves et al. (2002)
Roraima (rio Urubu)	6112 ± 138	na	na	na	na	Yuyama et al. (2002)
Roraima (rio Maú)	3571 ± 12	na	na	na	na	Yuyama et al. (2002)
Careiro-Amazonas	na	978 ± 5	2,12 ± 0,08	na	na	Maeda et al. (2003)
Careiro-Amazonas	na	993 ± 5	3,05 ± 0,93	na	na	Maeda et al. (2003)
São Paulo	1962 ± 27	na	na	na	na	Silva et al. (2005)
São Paulo	1722 ± 16	na	na	na	na	Silva et al. (2006)
Rio Preto da Eva - AM	2585 ± 8	862 ± 64	9,98 ± 0,19	na	na	Maeda et al. (2006, 2007)
Iguape – SP	na	na	54,0 ± 25,9	na	0,35 ± 0,26	Zanatta et al. (2005, 2007)
Mirandópolis – SP	na	na	30,3 ± 6,8	na	1,09 ± 0,24	Zanatta et al. (2005, 2007)
Amazonas	397 ±21	1797 ± 37	30 ± 1	48 ± 2	na	Genovese et al. (2008)
Roraima	2555 ± 35	na	na	na	na	Smiderle e Sousa (2008)
Lima - Peru	2095 ± 185	1270 ± 150	na	na	na	Chirinos et al. (2010)
Belém - PA	4250 ± 626	1637 ± 244	na	na	na	Cohen et al. (2010)
Belém - PA	5183 ± 513	1303 ± 360	na	na	Na	Embrapa Amazônia Oriental (2010)
Belém - PA	1882 ± 43	1176 ± 15	42,2 ± 17,0	na	0,4 ± 0,0	Rufino et al. (2010a, 2010b)
Ucayali – Peru	1378 ± 534	683 ± 87	na	na	na	Villanueva-Tiburcio et al. (2010)
Valencia- Espanha	3510 ± 970	na	19,6 ± 0,6	16 ± 0,33	na	Fracassetti et al. (2013)

na: não avaliado

2.2. Compostos bioativos

Compostos bioativos são substâncias que promovem benefícios à saúde por reduzirem riscos de desenvolvimento de doenças crônicas não transmissíveis, tais como: doenças cardiovasculares, câncer, diabetes, osteoporose, hipertensão e obesidade (CROZIER et al., 2009, WANG et al., 1996). Esses compostos são encontrados principalmente em vegetais e frutas, e possuem capacidade antioxidante, anti-inflamatória, antimicrobiana, antiproliferativa, entre outros.

Um grupo de compostos bioativos mais conhecidos são os compostos polifenólicos, que são produtos do metabolismo secundário de plantas que exercem efeito protetor. Portanto, quanto maior o estresse sofrido pela planta, maior a produção de compostos fenólicos (GOBBO-NETO e LOPES, 2007).

O teor e a composição dos compostos fenólicos podem ser influenciados por fatores intrínsecos e extrínsecos da planta. Alguns exemplos de fatores intrínsecos são: cultivar, variedade e estágio de maturação. E os fatores extrínsecos são: condições ambientais (índice de precipitação pluviométrica, umidade relativa, exposição à luz), altitude, ataque de herbívoros, bactérias e fungos, cultivo e manejo (BECKMAN, 2000) (**Figura 3**).

Mais de 8000 compostos polifenólicos foram identificados, e esses compostos podem ser subdivididos em três principais classes: flavonoides, ácidos fenólicos e taninos (PIETTA, 2000; KING e YOUNG, 1999). São caracterizados por possuírem um anel aromático com uma ou mais hidroxilas, podendo ser encontrados em forma simples (ácidos fenólicos) ou polimerizados (taninos). No entanto, são normalmente encontrados na forma glicosilada, com uma ou mais moléculas de açúcar ligadas ao grupo hidroxílico. Além disso, os açúcares associados podem ser monossacarídeos, dissacarídeos ou oligossacarídeos. Entre os monossacarídeos, a glicose é comumente encontrada, embora galactose, ramnose, xilose e arabinose também são possíveis. Ainda, compostos fenólicos podem estar conjugados a outros compostos como ácidos carboxílicos e orgânicos, aminas e lipídeos ou até com outros fenóis (BRAVO, 1998).

Os compostos bioativos mais presentes no fruto camu-camu serão discutidos a seguir.

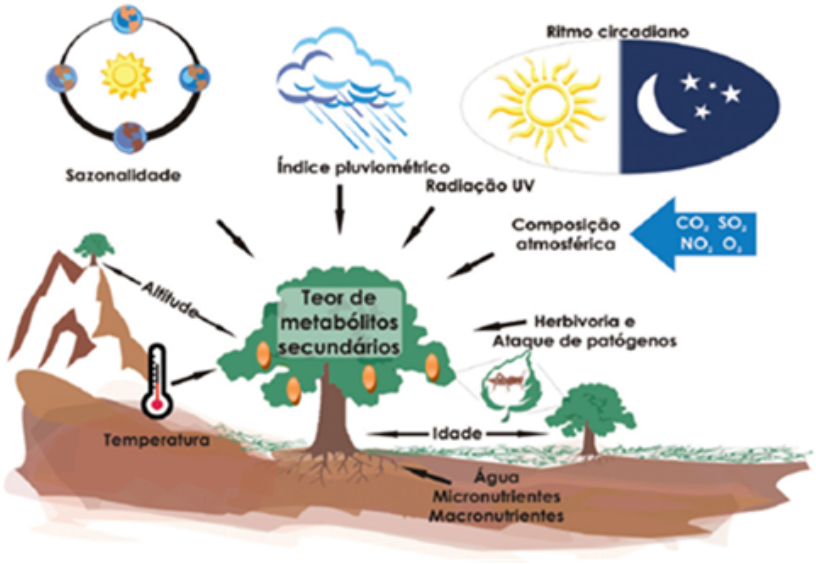


Figura 3. Fatores que influenciam teor e composição dos compostos fenólicos (Fonte: Gobbo-Neto e Lopes, 2007).

2.2.1 Vitamina C

A vitamina C é encontrada em grande quantidade no camu-camu (em média 3 g/100 g b.u.) e é considerada um composto bioativo por apresentar atividade antioxidante. Esta vitamina tem a capacidade de reduzir as espécies reativas de oxigênio como, por exemplo: peróxido de hidrogênio (H_2O_2), superóxido (O_2) e oxigênio singleto ($^1O_2$) (ASARD et al., 1997).

A vitamina C ou ácido L-ascórbico (AA) é um sólido branco, cristalino, bastante solúvel em água. É facilmente oxidado a ácido L-desidroascórbico (DHA), que também apresenta atividade biológica como a vitamina C (**Figura 4**) (HERNÁNDEZ et al., 2006).

Maas (1999) mencionou efeito sinérgico entre vitamina C, carotenoides e compostos fenólicos na capacidade antioxidante total dos alimentos. Outro exemplo do efeito sinérgico entre esses compostos foi observado por Mullen e colaboradores (2002), que investigaram os teores de vitamina C, antocianinas e elagitaninos na framboesa. Foi feita uma comparação da capacidade antioxidante do fruto recém-colhido, na loja e na casa do consumidor, e observou-se redução no teor de vitamina C, manutenção de antocianinas e aumento de elagitaninos, que no final não alterou a capacidade antioxidante do fruto.

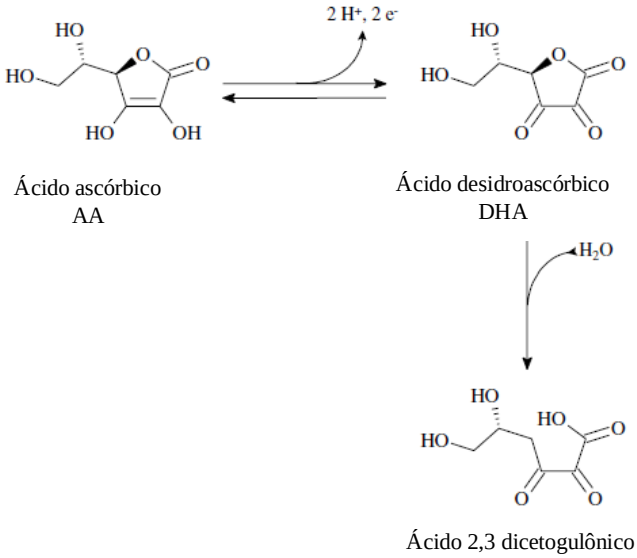


Figura 4. Mecanismo de oxidação do ácido ascórbico (adaptado de HERNÁNDEZ et al., 2006).

2.2.2. Flavonoides

Os flavonoides são compostos polifenólicos constituídos de 15 átomos de carbono e dois anéis aromáticos (A e B) ligados por uma cadeia de três átomos de carbono que pode ou não formar um terceiro anel (C6-C3-C6). Os flavonoides podem ser classificados em flavonas, flavanonas, flavonóis, isoflavonas, flavan-3-óis e antocianidinas. Suas estruturas químicas, sub-classes e principais fontes podem ser visualizados na **Figura 5** e no **Quadro 3** (CROZIER, 2009).

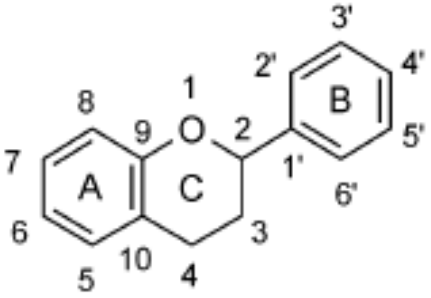
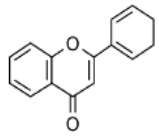
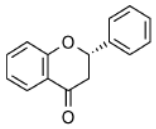
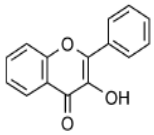
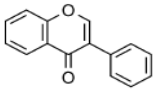
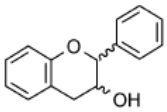
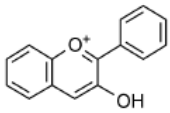


Figura 5. Estrutura química básica de flavonoides (CROZIER, 2009).

Quadro 3: Distribuição das diferentes subclasses de flavonoides entre as fontes alimentares

Compostos	Sub-classes	Fonte
Flavona 	Apigenina 5,7,4'- OH	Salsinha, salsão
	Rutina 5,7,3',4'- OH; 3 – rutinose	Frutos cítricos, pimenta vermelha, vinho tinto, pele do tomate
Flavanona 	Naringenina 5,7,4'- OH	Frutos cítricos
	Hesperidina 3,5,3'- OH, 4'- OMe	Laranja
Flavonol 	Caempferol 3,5,7,4'- OH	Alho-poró, brócolis, chicória, toranja, chá preto
	Quercetina 3,5,7,3',4'- OH	Cebola, alface, brócolis, tomate, chá, berries, maçã, óleo de oliva
	Miricetina 3,5,7,3',4',5'- OH	Tomate cereja, brócolis, mirtilo, damasco, maçã, chá
Isoflavona 	Genisteína 5,7,4'- OH	Soja
	Daidzeína 4',7 – OH	Soja
Flavan-3-ol 	Catequina 3,5,7,3',4'-OH	Chá verde
	Epicatequina 3,5,7,5',4'-OH	Chá verde
	Epigalocatequina 3,5,7,4'-OH	Chá verde
Antocianidina 	Cianidina 3,5,7,4'- OH; 3,5 – OMe	Cereja, framboesa, morango
	Delfinidina 3,5,7,3',4',5'- OH	Frutas roxas
	Peonidina 3,5,7,4'- OH	Frutas roxas

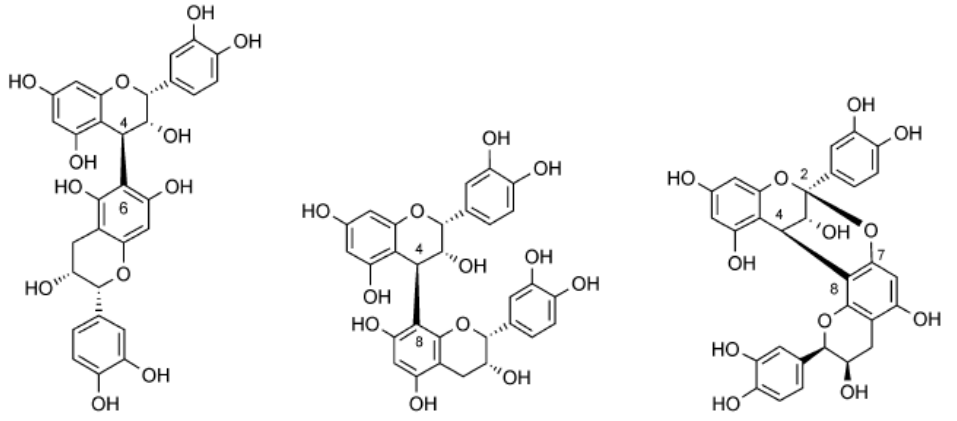
Fonte: CROZIER, 2009; SHAHIDI e NACZK, 2004.

Dentre essas classificações, os flavonoides relevantes para este estudo, pertencem às classes flavonol, flavan-3-ol e antocianidina, pois são encontrados no fruto camu-camu. Os flavonóis mais conhecidos são caempferol, quercetina e miricetina, encontrados em grande quantidade em cebola, alho e brócolis. Flavan-3-ois ou flavanóis (catequina e epicatequina) são encontrados em vários produtos como: chocolate, feijão, damasco, vinho tinto, chá verde, entre outros (MANACH et al., 2004; KING e YOUNG, 1999; PETERSON e DWYER, 1998).

As antocianidinas são pigmentos coloridos que variam de azul, roxo, vermelho, rosa a laranja. Destacam-se a cianidina, pelargonidina, peonidina, delfinidina e malvidina (CASTAÑEDA-OVANDO et al., 2009).

2.2.3 Taninos

Os taninos são compostos fenólicos de alto peso molecular, são produtos do metabolismo secundário de plantas e encontrados em duas formas: condensados e hidrolisáveis. Os taninos condensados ou proantocianidinas (**Figura 6**) são polímeros de flavanol (catequina) e estão frequentemente presentes em nossa dieta. Galotaninos e elagitaninos são subgrupos dos hidrolisáveis que são facilmente hidrolisados por ácidos, bases ou enzimas (DESHPANDE, 2002; KHANBABAEE & VAN REE, 2001) liberando ácidos gálico, cafeico, elágico e uma molécula de açúcar (BOBBIO; BOBBIO, 2003; SGARBIERI, 1996). No que se refere às alterações sofridas pelos taninos durante o processamento, à ingestão e aos efeitos sobre a saúde, os condensados são o grupo mais estudado (BAKKALBASI *et al.*, 2009).



Dímero de Proantocianidina B_5 Dímero de Proantocianidina B_7 Dímero de Proantocianidina A_2

Figura 6. Estrutura química de proantocianidinas (adaptado de Crozier et al., 2009).

2.2.3.1 Ácido elágico e derivados

O ácido elágico é encontrado em abundância em frutas vermelhas e nozes (DANIEL et al., 1989). Pode estar na forma livre, no entanto, está normalmente na forma conjugada (elagitaninos) (**Figura 7**) (LARROSA et al., 2010; MAAS, 1991).

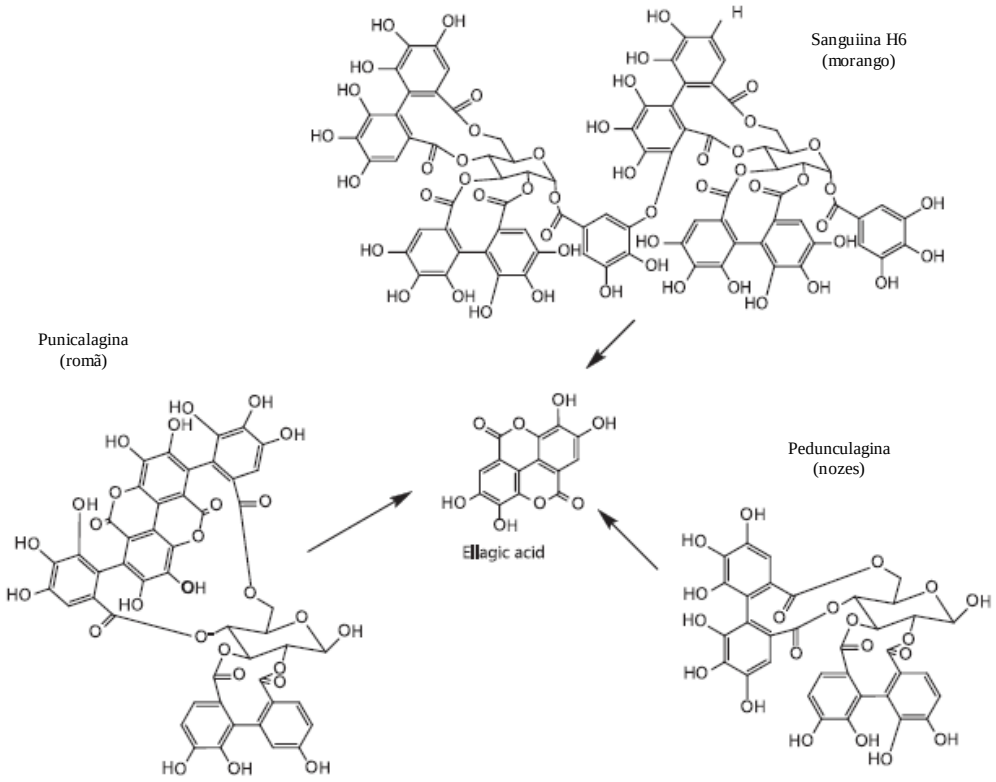


Figura 7. Estrutura química de elagitaninos e ácido elágico (adaptado de LARROSA et al., 2010).

Os elagitaninos são solúveis em água, de alto peso molecular e com capacidade de precipitação de proteínas e alcaloides (SANTOS-BUELGA & SCALBERT, 2000), destacam-se por suas diversas propriedades biológicas tais como: antioxidante, anticancerígena, antiaterogênica, anti-inflamatória, anti-hepatóxica, antibacteriana, antirreplicação de HIV (BAKKALBASI *et al.*, 2009).

Alguns elagitaninos conhecidos são: pedunculagina (nozes), punicalagina (romã), sanguiina H-6, sanguiina H-10, lambertianina C (framboesa e morangos) (LANDETE, 2011).

Sabe-se que o camu-camu contém alto teor de ácido elágico e elagitaninos (REYNERTSON et al., 2008; GENOVESE et al., 2008; GONÇALVES et al., 2010; FRACASSETTI et al. 2013).

O ácido elágico isolado de framboesa, que possui dois grupos dihidroxil, apresenta maior atividade antioxidante que o elagitanino que possui apenas um grupo dihidroxil. Esse ácido elágico mostrou ser tão eficaz quanto catequina e caempferol, no entanto, menos eficaz que a quercetina (ZAFRILLA et al., 2001).

2.3. Atividade biológica dos compostos bioativos

A capacidade antioxidante, antimicrobiana, anti-hiperglicêmica e anti-hipertensiva são alguns exemplos de atividade biológica benéfica a saúde. A seguir será explicada detalhadamente cada propriedade.

2.3.1 Capacidade antioxidante

Quimicamente, a oxidação é a perda de um ou mais elétrons de um átomo para outro, necessária para a aerobiose e para o metabolismo do nosso organismo, produzindo energia (ATP). No entanto, pode produzir também espécies reativas de oxigênio (ERO) ou espécies reativas de nitrogênio (ERN) ilustradas no **Quadro 4** (GÜLÇIN, 2012; DEVASAGAYAM et al., 2004).

Quadro 4: Algumas espécies reativas de oxigênio e nitrogênio conhecidas

Espécies reativas de oxigênio:		**Espécies reativas de nitrogênio :**	
Superóxido	$O_2^{\bullet -}$	Óxido nítrico	$NO^{\bullet}$
Radical hidroxila	$HO^{\bullet}$	Peróxinitrito	$ONOO^{\bullet}$
Peróxido de Hidrogênio	H_2O_2	Dióxido de nitrogênio	NO_2
Radical peroxila	$ROO^{\bullet}$	Ácido peróxinitroso	ONOOH
Hidroperóxido orgânico	ROOH	Óxido nitroso	N_2O
Oxigênio singleto	$^1O_2$	Ácido nitroso	HNO_2
Ozônio	O_3	Trióxido dinitrogênio	N_2O_3

Fonte: GÜLÇIN, 2012; DEVASAGAYAM et al., 2004.

As ERO ou ERN são radicais livres altamente instáveis ou quimicamente reativos, pois reagem com qualquer molécula que entram em contato, produzindo danos oxidativos que

provocam envelhecimento e doenças como câncer, doenças cardíacas, arteriosclerose, diabetes, entre outros (HARBORNE e WILLIAMS, 2000; GUTTERIDGE e HALLIWELL, 2000).

Neste contexto, os antioxidantes têm a função de inibir a oxidação de compostos e retardar a peroxidação lipídica. Basicamente, reduzem os radicais livres, sequestram oxigênio singleto e quelam íons metálicos. Atuam de duas formas: por transferência de elétrons ou transferência de átomos de hidrogênio (PRIOR et al., 2005).

Além disso, nos produtos alimentícios, os antioxidantes aumentam a vida de prateleira e preservam o valor nutricional (HALLIWELL, 2008). Existem antioxidantes sintéticos e naturais. Os sintéticos mais utilizados em alimentos são: butil hidroxitolueno (BHT), butil hidroxianisol (BHA), terc-butil hidroquinona (TBHQ) e galato de propila (PG) (**Figura 8**) (GÜLCIN, 2012).

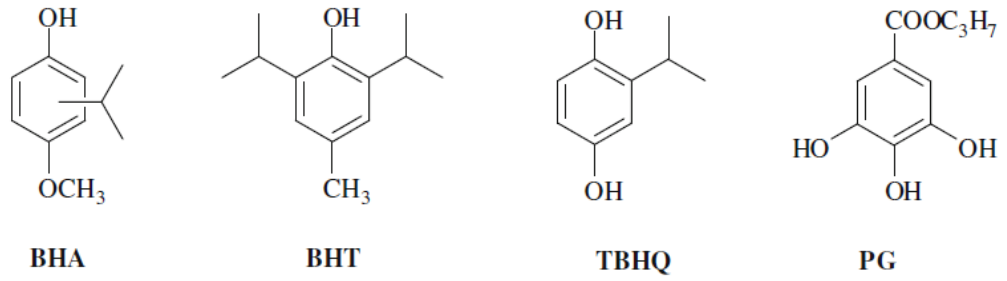


Figura 8. Antioxidantes sintéticos utilizados na indústria de alimentos (GÜLCIN, 2012).

Esses antioxidantes sintéticos têm seu uso limitado devido à possibilidade de apresentarem efeito cancerígeno. Na Europa e no Canadá, o TBHQ é proibido. No Brasil, a legislação permite o uso de até 200 mg/kg de TBHQ, BHA e PG, e de 100 mg/kg de BHT, em óleos e gorduras (Lei n° 363/98 de 19 de novembro de 1998). Os antioxidantes BHT e BHA, normalmente são usados combinados, por apresentar efeito sinérgico (BOBBIO e BOBBIO, 2003).

Dessa forma, os antioxidantes naturais têm sido cada vez mais pesquisados para serem usados em substituição aos sintéticos. Os antioxidantes naturais mais representativos da dieta são: vitamina C, tocoferóis, carotenoides e flavonoides.

2.3.2. Atividade antimicrobiana

Outra propriedade dos compostos bioativos é possuir atividade antimicrobiana, ou seja, inibem o crescimento de micro-organismos patogênicos.

A atividade antimicrobiana pode ser bactericida ou bacteriostática. As substancias de ação bacteriostática bloqueiam a síntese dos ácidos nucleicos ou das proteínas de forma reversível.

Alguns alimentos possuem substancias naturais com ação antimicrobiana bactericida. Estudos com o camu-camu mostraram que resíduos do processamento de suco do fruto, casca e as sementes, são capazes de inibir *Staphylococcus aureus* ATCC11522 (MYODA *et al*., 2010). Estudos mostram que os elagitaninos inibem micro-organismos patogênicos como *Vibrio cholerae*, *Shigella dysenteriae* e *Campylobacter* spp (CLIFFORD & SCALBERT, 2000). A romã (*Punica granatum*), rica em elagitaninos, tem efeito inibitório sobre *S. aureus* resistentes à meticilina *in vitro* (MACHADO *et al*., 2002) e os extratos da sua casca apresentam atividade antimicrobiana contra *Salmonella typhimurium* em ratos infectados (CHOI *et al*., 2009). Extratos metanólicos de romã também exibem considerável atividade antimicrobiana contra *Bacillus subtilis* (ATCC 441), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615) e *Candida albicans* (MTCC 227) *in vitro* (DURAIPANDIYAN *et al*., 2006). E contra *Salmonella typhimurium e Shigella flexneri* mais efetiva que os antibióticos ciprofloxacina, ofloxacina e tetraciclina (PRADEEP *et al*., 2008).

De acordo com Puupponen-Pimia e colaboradores (2005), os compostos bioativos em frutos vermelhos apresentam capacidade de inibir o crescimento de bactérias patogênicas gram negativas e gram positivas, especificamente, os elagitaninos inibem *Staphylococcus*; ácidos orgânicos inibem *Salmonella* e ácidos hidroxicinâmicos a *Listeria monocytogenes*.

2.3.3. Atividade anti-hiperglicêmica

A Diabetes mellitus é uma doença crônica caracterizada pela desordem metabólica causada pela hiperglicemia crônica e deficiência herdada ou adquirida na produção de insulina pelo pâncreas (ARNOLDI, 2004). Atualmente, existem 382 milhões de diabéticos no mundo, e estima-se que atingirá mais de 592 milhões de casos até 2035 (IDF, 2013, WHO, 2014).

De acordo com WHO (2015) existem três tipos de diabetes. A diabetes tipo 1 que geralmente aparece na infância ou adolescência, caracteriza-se pela destruição das células beta do pâncreas, que produz a insulina. Portanto, requer a administração diária de insulina.

Por outro lado, a diabetes tipo 2 se desenvolve, geralmente, em indivíduos adultos e caracteriza-se pela resistência à insulina ou deficiência na secreção da insulina. Este tipo corresponde a 90% dos casos, por ser resultado do sobrepeso corporal e vida sedentária, porém pode ser medicada. E por último, a diabetes gestacional que é a hiperglicemia detectada pela primeira vez durante a gravidez.

Uma das alternativas de tratamento para pacientes de diabetes tipo 2 é retardar a absorção de glicose pelo organismo através da redução da hidrólise de carboidratos pela inibição da α-amilase pancreática e α-glicosidase intestinal. Inibidores de α -amilase e α-glicosidase naturalmente presente em frutas e vegetais podem oferecer uma estratégia de controle da hiperglicemia pós-prandial sem os efeitos colaterais normalmente causados pelos medicamentos, como distensão abdominal, flatulência e possibilidade de diarreia (KWON, VATTEM e SHETTY, 2006; MCDOUGALL et al., 2005; KOTOWAROO et al. 2006).

Algumas frutas vermelhas como morango (PINTO et al., 2010; CHEPLICK et al., 2010) e framboesa (CHEPLICK et al., 2007) mostraram maior inibição da α-glicosidase do que da α-amilase. Esta característica é desejável, pois excesso de inibição de α-amilase resulta em amido não digerido no cólon e consequente desconforto intestinal (PINTO et al., 2010).

Os extratos ricos em polifenois são responsáveis pela inibição das enzimas α-amilase e α-glicosidase (OBOH et al., 2012, GONCALVES et al., 2010). Especificamente, em frutas vermelhas, as proantocianidinas são as responsáveis por inibir α-amilase (GRUSSU et al., 2011). Ácido elágico e quercetina mostraram alta capacidade inibitória de α-glicosidase com concentração inibitória média (CI50) de 2,18 e 15,20 µg/mL, respectivamente (YOU et al, 2012). As antocianinas, em estudos *in vivo* com ratos hiperglicêmicos, inibiram a α-glicosidase e reduziram a taxa de glicose sanguínea (MATSUI et al, 2001).

2.3.4. Atividade anti-hipertensiva

A alta tensão arterial é uma doença caracterizada pela elevação da pressão sanguínea. É uma síndrome metabólica causada pela dieta, principalmente em indivíduos obesos e pode ser uma das complicações desenvolvidas por diabéticos com o tempo (PASUPULETI e ANDERSON, 2008). A enzima conversora de angiotensina (ECA) é uma enzima envolvida na manutenção da tensão vascular, pois converte o dipeptideo histidil-leucina (angiotensina I) em vasoconstritor potente (angiotensina II) que estimula a síntese e liberação de aldosterona, e consequentemente aumenta a pressão arterial através da retenção de sódio nos túbulos renais (**Figura 9**, JOHNSTON, 1994; KWON et al., 2007).

Alguns compostos polifenólicos apresentam atividade anti-hipertensiva através da inibição da ECA (OBOH et al. 2012). Em estudos *in vitro*, Cheplick e colaboradores (2007) mostraram a inibição dessa enzima pelos extratos de framboesa, porém baixa inibição por extratos de morangos (CHEPLICK et al. 2010), possivelmente devido à diferença de composição fenólica entre os frutos. Em estudos com humanos, a quercetina reduziu a pressão sanguínea em hipertensos (EDWARDS et al., 2007). Em estudos com ratos, das antocianinas analisadas, apenas as delfinidinas apresentaram atividade anti-hipertensiva (ANDRIAMBELOSON et al., 1998).

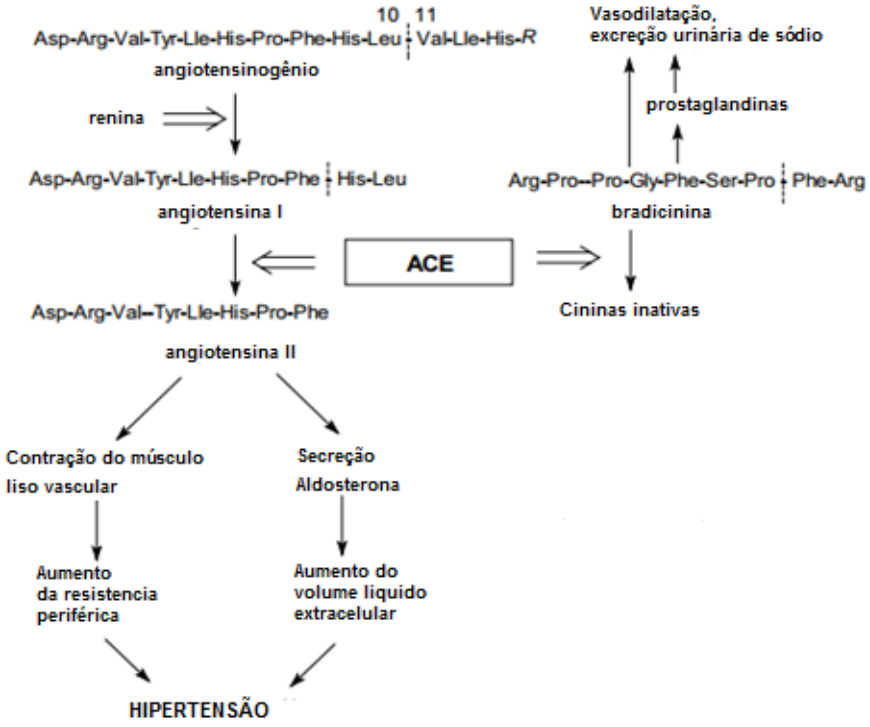


Figura 9. Mecanismo hipertensivo da angiotensina (adaptado de Barbosa-Filho, 2006).

2.4. Secagem

A secagem é uma forma de conservação de alimentos que reduz a umidade dos produtos. Este processo é complexo, pois envolve transferência de calor (convecção, condução ou radiação) e de massa simultaneamente (SARSILMAZ et al., 2000). É aplicada em alimentos com a finalidade de aumentar a vida de prateleira do produto, reduzindo a atividade de água que, por sua vez, irá reduzir a atividade enzimática e microbiana. Além disso, facilita o manuseio, transporte e armazenamento do produto (LESCANO, 2009). A

escolha do tipo de secador irá depender das propriedades e compostos que se quer preservar, buscando um nível de qualidade desejável e um custo que possa ser justificado.

Os métodos mais comuns de secagem de polpa de fruta são: secagem de cilindros rotativos (“drum drying”), por atomização (“spray drying”), secagem a vácuo, liofilização, entre outros.

Neste estudo foi utilizada a secagem por leito de jorro e por atomização, comparando-os com o processo de liofilização.

2.4.1. Liofilização

A liofilização é um processo de secagem em que a água é retirada dos alimentos sem submetê-los a altas temperaturas. A vantagem é que o método mantém intactas as estruturas dos alimentos, permite reidratação mais rápida e completa do produto, o que preserva as características sensoriais e o valor nutritivo, e consequentemente resulta em um produto de alta qualidade (SAGAR e KUMAR, 2010). Entretanto, é um método relativamente caro e lento quando comparado a outros métodos de secagem, como: *spray-drying*, leito de jorro ou secagem em estufa ventilada.

Este processo de secagem consiste de três etapas: congelamento, secagem primária e secagem secundária. Primeiramente, o alimento é completamente congelado e colocado em câmara a vácuo. Em seguida, o produto passa pela secagem primária, isto é, o gradiente de temperatura do produto e o condensador fazem com que ocorra a saída de água do alimento por sublimação (a água passa do estado sólido ao vapor sem passar pelo estado líquido), pois ocorre abaixo do ponto triplo da água (**Figura 10**). E por último, ocorre a secagem secundária ou dessorção da umidade fortemente ligada ao produto (CAMERON, 1997).

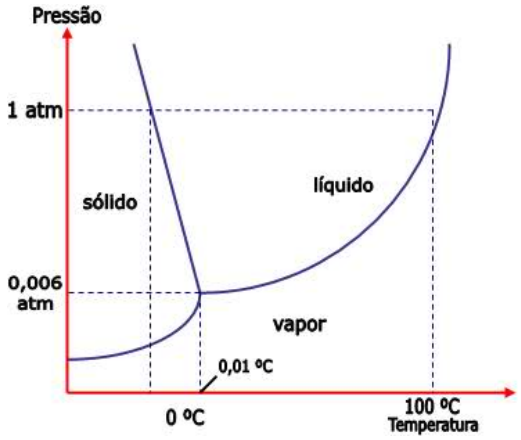


Figura 10. O ponto triplo da água (CAMERON, 1997).

2.4.2. Leito de jorro

O método de leito de jorro foi desenvolvido por Mathur e Gishler (1955) com a finalidade de secar grãos de trigo. Atualmente, tem sido citado como alternativa na secagem de pastas e suspensões termossensíveis na preservação de compostos bioativos, produzindo pós de alta qualidade e baixo custo (BEZERRA et al., 2013; MEDEIROS, 2002). No entanto, a desvantagem deste método é a produção em pequena escala, pois o processo é feito em batelada (COSTA et al., 2006).

Este processo de secagem consiste no contato gás-sólido com boa transferência de calor e massa. Através do orifício central, o gás (ar ambiente) é injetado verticalmente para cima, jorrando o material inerte (**Figura 11**). O atomizador alimenta o leito com a pasta ou suspensão, que reveste o material inerte em uma camada fina. O ar quente em contato com essa camada fina promove a secagem, pois forma um pó devido às colisões interpartículares causadas pelo atrito entre elas. Por fim, o pó é arrastado pela corrente de ar ao ciclone e coletado (KUDRA e MUJUMDAR, 2009; CUI e GRACE, 2008).

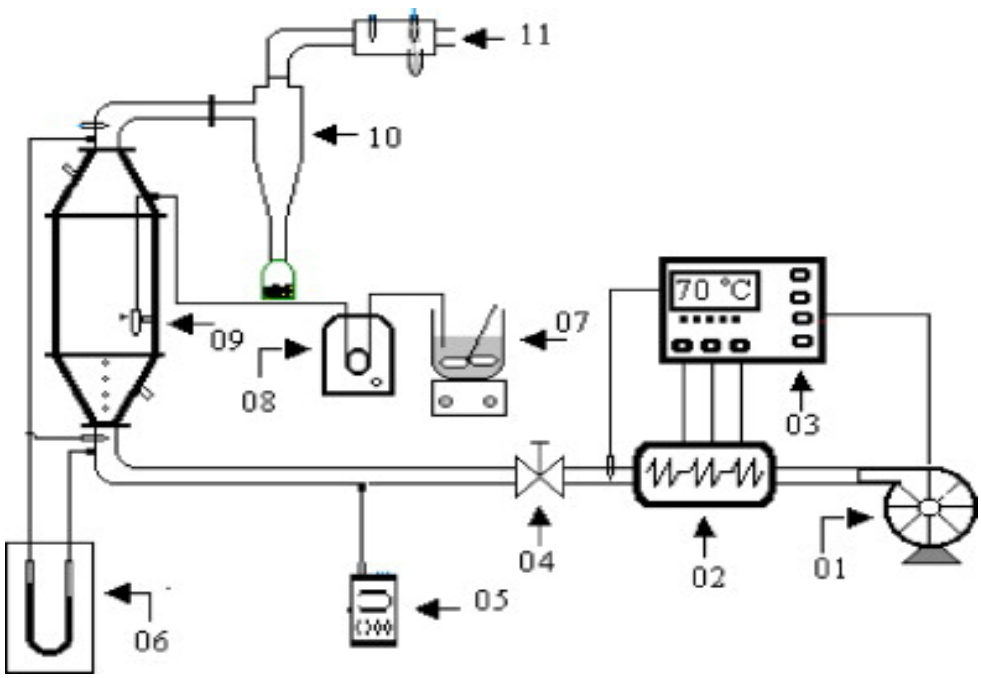


Figura 11. Secador de Leito de jorro. (1) soprador; (2) aquecedor de ar; (3) controlador de corrente elétrica; (4) válvula de regulagem de ar de entrada; (5) medidor do fluxo de ar; (6) manômetro; (7) polpa líquida; (8) bomba peristáltica; (9) leito de jorro; (10) ciclone e (11) psicrômetro (BEZERRA et al, 2013).

2.4.3. Secagem por atomização ou “spray drying”

O método de “spray drying” é uma técnica de secagem utilizada na indústria de alimentos desde 1950. Tem a vantagem de produzir partículas de boa qualidade, além disso é um método econômico, flexível, que possibilita a utilização em grande escala em processo contínuo. Tem sido também utilizado na indústria de aromas (FANG e BHANDARI, 2010; BARROS & STRINGHETA, 2006). Neste processo, utiliza-se o tipo de secador de atomização, isto é, o fluido a ser desidratado é introduzido por aspersão sob pressão, que recebe uma corrente de ar quente sob temperaturas elevadas formando instantaneamente partículas sólidas (**Figura 12) (**RÉ, 1998).

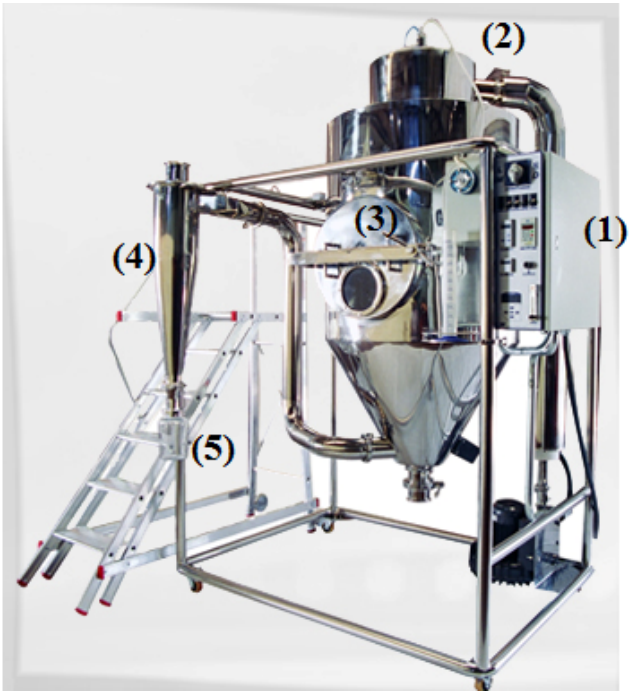


Figura 12. Secador por atomização. (1) controlador (2) alimentação (3) câmara de secagem (4) ciclone (5) pó produzido

A atomização pode ser usada também para microencapsulação de substâncias, líquidas ou sólidas. Este método consiste no envolvimento de uma substância por outra. O objetivo da encapsulação é proteger o material a ser encapsulado de outros ingredientes presentes na formulação e/ou retardar uma alteração que pode resultar em alteração de cor, perda de aroma, mascarar compostos de sabor desagradável, perda do valor nutricional e diminuir a velocidade de evaporação ou de transferência do material encapsulado, além de proteger do meio ambiente (luz, oxigênio, umidade) (RÉ, 1998). Os ingredientes que são normalmente

encapsulados são aromas, vitaminas, minerais, microrganismos probióticos, peptídeos ativos e outras classes de bioativos (FAVARO-TRINDADE et al., 2008).

2.4.4. Agentes carreadores

No processo de atomização de amostras fluídas como sucos de frutas, a utilização de agentes carreadores com alto peso molecular é necessária para promover a secagem. Diversos materiais são utilizados como agentes encapsulantes, dentre eles: goma arábica, ágar, alginato, carboidratos, amido, maltodextrina, carboximetilcelulose, parafina, acetilcelulose, materiais inorgânicos como sulfato de cálcio e silicatos, proteínas do glúten, caseína, gelatina e albumina (JACKSON e LEE, 1991). A escolha do agente encapsulante depende da natureza e da estabilidade do material a ser encapsulado, das características do polímero encapsulador, do processo utilizado na microencapsulação e das características do produto final a ser obtido (FAVARO-TRINDADE et al., 2008).

As maltodextrinas ou malto-oligossacarídeos são considerados produtos da hidrólise parcial do por ácido ou enzimas, e são classificadas pela equivalência em dextrose (DE), que é o percentual do poder redutor da D-glicose pura (dextrose) (**Figura 13**). Quanto menor DE menos higroscópico é o produto. São ligeiramente doces e tem a propriedade de dissolverem facilmente em água (FENNEMA, 2008).

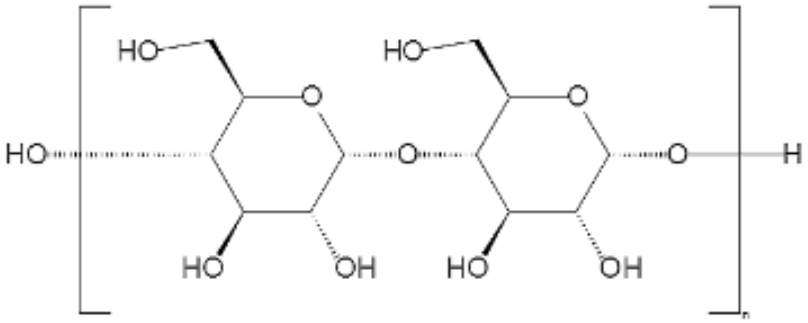


Figura 13. Estrutura química da maltodextrina (adaptado de CARARETO et al., 2010)

A goma arábica (goma acácia) é um exsudado da árvore acácia. Possui uma estrutura química complexa de polissacarídeo-proteína (**Figura 14**). É muito solúvel em água, usada como emulsificante e estabilizante de emulsões e possui baixa viscosidade em altas concentrações. As melhores gomas são encontradas em Sudão e Nigéria (FENNEMA, 2008).

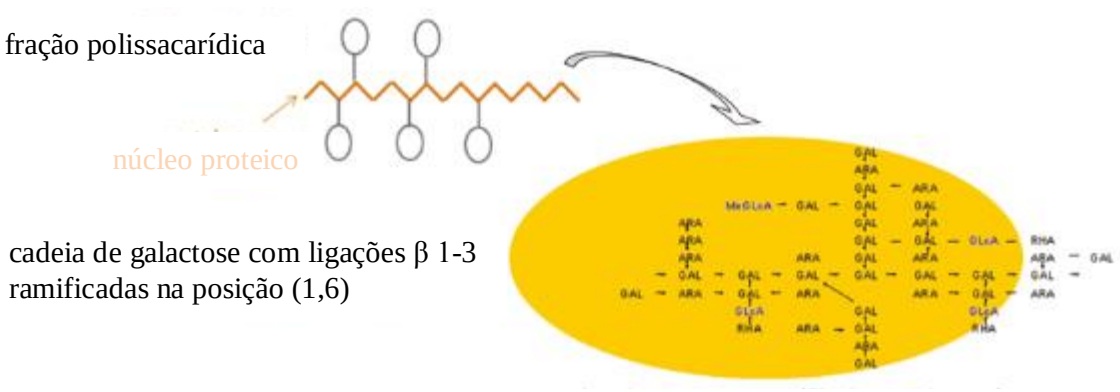


Figura 14. Estrutura química da goma arábica (adaptado de Fincher et al., 1983).

Alguns estudos de microencapsulação por "Spray drying" de vitamina C e compostos fenólicos, como antocianinas e proantocianidinas, obtidos a partir de extratos de frutas, já foram realizados. Angel e colaboradores (2009) utilizaram lactose e maltodextrina na microencapsulação de vitamina C de suco de maracujá por "spray dryng". Valduga e colaboradores (2008) empregaram maltodextrina e goma arábica (em proporção igual) na microencapsulação de antocianinas extraídas a partir de bagaço de uva. Osorio et al. (2010) utilizaram somente maltodextrina na microencapsulação de antocianinas da fruta "*Bactris guineensis*". Proantocianidinas de semente de uva foram produzidas com 40% de goma arábica e 60% de maltodextrina (ZHANG et al., 2007).

A maltodextrina e goma arábica quando utilizadas separadamente, na microencapsulação de suco de camu-camu, apresentaram resultados semelhantes. O pó microencapsulado com maltodextrina apresentou rendimento de 26% e 7% de retenção de vitamina C, numa temperatura de entrada de 150 °C. E o pó com goma arábica, o rendimento obtido foi de 24% e retenção de vitamina C de aproximadamente 6% na mesma temperatura de entrada (DIB TAXI et al., 2003).

Silva e colaboradores (2006) produziram camu-camu liofilizado e compararam a polpa natural com a polpa acrescida de 30% de maltodextrina 20 DE. A adição de maltodextrina aumentou o ponto de congelamento e diminuiu a higroscopicidade da polpa liofilizada. Tonon et al. (2011) compararam maltodextrina (10 DE e 20 DE) e goma arábica, como agentes carreadores para produção de pó de açaí por "spray drying". Os pós produzidos com maltodextrina 10 DE apresentaram menor higroscopicidade que maltodextrina 20 DE e goma arábica. No entanto, aqueles pós apresentaram maior umidade, atividade de água e tamanho de partículas.

Portanto, pode-se verificar que para microencapsulação de extrato de frutas, a maltodextrina e a goma arábica são os agentes encapsulantes mais utilizados. A mistura dos dois compostos também foi testada em diferentes concentrações e DE de maltodextrina (FAZAELI et al., 2012). Os pós de suco de amora preta secos por atomização contendo 6% maltodextrina 6 DE e 2% goma arábica apresentaram melhor rendimento, solubilidade e morfologia da partícula em comparação com outras combinações de mistura.

Estudos indicam que a goma arábica é uma fibra alimentar que pode atuar como prebiótico (PHILLIPS e PHILLIPS, 2011; GLOVER et al., 2009; PHILLIPS et al., 2008). As fibras alimentares auxiliam o trânsito gastrointestinal, pois não são digeríveis. Dessa forma, podem possuir propriedades prebióticas ao estimular o crescimento ou atividade de uma ou mais bactérias no cólon (GIBSON e ROBERFROID, 1995). Comprovou-se em estudo com humanos que a goma arábica promoveu o crescimento de bifidobactérias e lactobacilos no cólon (CHERBUT et al., 2003). Este efeito pode estar associado ao aumento dos produtos da fermentação de prebióticos no cólon, como os ácidos graxos de cadeia curta (SCFas), que são considerados produtos finais da fermentação prebiótica no cólon (PHILLIPS et al., 2008).

2.4.5. Efeito da secagem sobre os compostos fenólicos

Um composto pode polimerizar-se ou hidrolisar-se dependendo da sua característica. Por exemplo, a vitamina C, por um lado, mostra-se mais suscetível a perdas durante a pós-colheita e o armazenamento. Os carotenoides e os compostos fenólicos, por outro lado, são mais estáveis e pode até ocorrer o aumento da quantidade, dependendo das condições de armazenamento (KALT, 2005).

Da Silva et al. (2005) estudaram a degradação de vitamina C do fruto camu-camu, utilizando o secador de bandeja em diferentes temperaturas, e concluíram que há perdas de 22% e 25% de ácido ascórbico a 50 ℃ e 70 ℃, respectivamente. Apesar da secagem a 50 ℃ provocar menor perda da vitamina C, o tempo de secagem foi maior (300 minutos) quando comparado com a secagem a 70 ℃ (180 minutos).

Relatos na literatura mostram que o teor de ácido elágico pode variar de acordo com o processamento e armazenamento. Quando a framboesa foi processada em geleia, houve um aumento de 2,5 vezes no teor de ácido elágico livre. E mais, durante a estocagem da geleia a 20 °C por 6 meses, o teor de ácido elágico continuou a aumentar no primeiro mês e decresceu nos restantes (ZAFRILLA et al., 2001). No entanto, Häkkinen et al. (2000) não observaram

diminuição estatisticamente significativa no teor de ácido elágico livre após processamento e durante o armazenamento por 9 meses de geleia de morango.

Durante o processo de secagem, a temperatura é o fator mais importante para a transformação do composto, seja por perda ou polimerização. Temperaturas elevadas de entrada de ar (160 a 180 °C) no processo de atomização de extrato etanólico de cenouras pretas causaram perdas no teor de antocianinas. No entanto, a utilização de maltodextrina 20-21 DE protegeu o composto durante a secagem e o armazenamento (ERSUS e YURDAGELl, 2007). Ferrari e colaboradores também verificaram a perda de antocianinas em altas temperaturas (140 a 180 °C) durante a atomização de polpa de amora preta com 5 a 25% de maltodextrina 20 DE. Os autores sugeriram como condição ótima, temperatura de 140 °C e 15% de maltodextrina para retenção de antocianinas.

As procianidinas extraídas de semente de uva Cabernet Sauvignon se mantiveram estáveis após a secagem por atomização a 190 °C com 40% de goma arábica e 60% de maltodextrina (ZHANG et al., 2007).

2.5. Compostos fenólicos e probióticos

Os probióticos são definidos como microrganismos vivos que, quando administrados em quantidades adequadas, promovem benefícios à saúde do hospedeiro (FAO/WHO, 2001). Os gêneros mais utilizados como probióticos são os *Lactobacillus* e *Bifidobacterium* (FULLER, 1989). Essas bactérias possuem um número considerável de benefícios à saúde que incluem: regularização da microbiota intestinal, combate a substâncias tóxicas causadoras do câncer e melhoria nas funções imunológicas, podendo também ter um efeito significativo no alívio das doenças alérgicas em crianças (FAO/WHO, 2001). Por esses motivos, têm sido amplamente incorporados a produtos alimentícios, principalmente produtos lácteos (SCHAAFSMA et al., 1998; GROSSO & FAVARO-TRINDADE, 2004; FAVARO-TRINDADE et al., 2007).

Alguns estudos apontam os compostos fenólicos como estimuladores do crescimento de microrganismos. Extratos de romã (contendo elagitaninos) podem estimular o crescimento de *Bifidobacterium* spp e *Lactobacillus* spp e, além disso, podem aumentar a produção urolitinas (metabólitos de elagitaninos) (BIALONSKA et al., 2010). Outro estudo interessante verificou que leite de soja inoculado com a cultura de kefir aumentou o teor de fenólicos solúveis, em virtude da produção de enzimas pelos microrganismos (MCCUE & SHETTY, 2005).

Os compostos fenólicos podem estimular não só os microrganismos probióticos, mas também *Streptococcus thermophilus* CHCC 3534, microrganismo utilizado na produção de iogurte, utilizando baixa concentração de ácido gálico (0,1%) e catequina (0,1%) (KHALIL, 2010).

Em relação à dosagem de compostos fenólicos, baixas concentrações de taninos (0,1 e 0,2 mg/mL) não inibem o crescimento de *L. plantarum* DSM 10492. No entanto, 1 mg/mL inibe seu crescimento (ROZÈS & PERES, 1998). Resultados semelhantes foram obtidos por Cueva et al. (2010) que encontraram a concentração de 1 mg/mL de ácidos fenólicos como inibidora do crescimento de várias espécies de *Lactobacillus*.

Puupponen-Pimia et al. (2002) citaram a necessidade de desenvolver um alimento funcional contendo microrganismos probióticos, carboidratos não digeríveis (prebióticos) e compostos bioativos (fenólicos), a fim de melhorar a saúde intestinal. Hervert-Hernández et al. (2009) notaram que os polifenóis podem exercer duplo efeito positivo, por um lado inibir microrganismos patogênicos e por outro, estimular o crescimento de microrganismos probióticos. Por esse motivo, a ingestão de alimentos ricos em compostos fenólicos pode desempenhar um papel regulador da microbiota do trato intestinal, melhorando a saúde gastrointestinal.

3. OBJETIVOS

Objetivo Geral

O objetivo geral deste projeto foi avaliar e comparar os efeitos de diferentes processos de secagem (em leito de jorro, por atomização e liofilização) sobre os compostos bioativos e potencial funcional da polpa comercial de camu-camu.

Objetivos específicos

1. Estudar o efeito da secagem por leito de jorro em comparação à liofilização de polpa comercial de camu-camu, usando maltodextrina como agente carreador, sobre cor, teores de compostos fenólicos e vitamina C e capacidades antioxidante e antimicrobiana.

2. Estudar o efeito da secagem por atomização em comparação à liofilização de polpa comercial de camu-camu, usando maltodextrina e goma arábica como agentes carreadores, sobre umidade, rendimento, solubilidade, teores de compostos fenólicos e vitamina C, e capacidade antioxidante.

3. Avaliar as perdas e identificar os compostos fenólicos por LC-TOF-MS dos pós liofilizados e secos por atomização. Estudar o potencial funcional associado às propriedades anti-hiperglicêmica, anti-hipertensiva e de proteção celular e rejuvenescimento.

4. Estudar o efeito da adição de pó de cam-camu em leite de soja submetido à fermentação com microorganismos produtores de ácido lático (*Lactobacillus helveticus* e *Lactobacillus plantarum*) sobre o potencial funcional do produto final.

4. METODOLOGIA, RESULTADOS E DISCUSSÃO

A parte experimental, os resultados e a discussão foram divididos em quatro partes, de acordo com os objetivos específicos, e se encontram na forma de artigos já publicados e/ou submetidos à publicação, enumerados a seguir:

1. Impact of spouted bed drying on bioactive compounds, antimicrobial and antioxidant activities of commercial frozen pulp of camu-camu (*Myrciaria dubia* Mc. Vaugh). Alice Fujita, Kátia Borges, Roberta Correia, Bernadette Dora Gombossy de Melo Franco, Maria Inés Genovese (publicado na Food Research International 54, 495-500, 2013). Fator de impacto 2,818

2. Effect of inlet air temperature and concentration of carrier agents on bioactive compounds and antioxidant capacity of spray-dried powders of camu-camu (*Myrciaria dubia* Mc. Vaugh). Fujita, Alice; Souza, Volnei Brito; Daza, Luis Daniel; Granato, Daniel; Favaro-Trindade, Carmen Silvia; Genovese, Maria Inés (submetido ao Brazilian Journal of Pharmaceutical Sciences). Fator de impacto 0,302

3. Evaluation of phenolic-linked bioactives of camu-camu (*Myrciaria dubia* Mc. Vaugh) for antihyperglycemia, antihypertension, antimicrobial properties and cellular rejuvenation. Alice, Fujita; Dipayan, Sarkar; Shibiao, Wu; Edward, Kennelly; Kalidas, Shetty; Maria Inés Genovese (aceito na Food Research International). Fator de impacto 2,818

4. Improving anti-hyperglycemic functionality of camu-camu (*Myrciaria dubia* Mc. Vaugh) powder by fermenting with *Lactobacillus helveticus* and *Lactobacillus plantarum*. Fujita, Alice; Sarkar, Dipayan; Shetty, Kalidas; Genovese, Maria Inés (a ser submetido a Journal of Food Quality). Fator de impacto 0,838

4.1 Impact of spouted bed drying on bioactive compounds, antimicrobial and antioxidant activities of commercial frozen pulp of camu-camu (*Myrciaria dubia* Mc. Vaugh)

Abstract

Camu-camu (*Myrciaria dubia* Mc. Vaugh) has promising perspectives for agro industrial purposes mainly due to the functional potential. This work deals with the impact of spouted bed drying on bioactive compounds, antioxidant and antimicrobial activities, having the fresh and freeze-dried fruit pulp as references. Commercial camu-camu pulp was spouted bed dried at selected temperatures with Maltodextrin concentrations (carrier agent). The fruit powders were compared in relation to color, *in vitro* antioxidant capacity, total phenolics, ascorbic acid and proanthocyanidins contents. The spouted bed drying of the pulp led to significant losses, in the order ascorbic acid (45 - 64%) > total phenolics (33 - 42%) > proanthocyanidins (16 - 18%) and freeze-drying better preserved antioxidant capacity (74 to 87%) compared to spouted bed drying (29 to 78%). Freeze-dried powder was classified as active, and spouted bed dried powders as partially active, against *S. aureus* ATCC 2913. Despite of losses caused by drying, camu-camu powders still represent excellent sources of bioactive compounds with great potential for use as new bioactive ingredients.

Keywords: phenolics; spouted bed drying; freeze-drying; antioxidant capacity, antimicrobial activity; camu-camu.

1. INTRODUCTION

Camu-camu (*Myrciaria dubia* Mc. Vaugh) is an Amazonian fruit with high vitamin C and polyphenols contents, such as flavonoids and ellagitannins, besides strong *in vitro* and *in vivo* antioxidant capacity (Inoue *et al*., 2008)). This fruit is mainly found at the Brazilian Northern Region and is consumed locally, but it has promising perspectives for agro industrial purposes. Nowadays, the production of camu-camu derivatives, such as frozen pulp, extract and juice, is small (around 20 tons/years; average cost of 4 to 10 dollars per kilograms, Inpa 2011) and mostly destined to Japan, United States of America and the European Union. However, the Brazilian internal market remains unexplored, in part because the rest of the country has little access to the fruit, as well as little knowledge about fruit properties.

Camu-camu, in particular, is highly perishable, which makes the transportation chain more complex and expensive. Dehydration is one of the most important technological processes applied to fruits. Besides extending food shelf life, it is also an interesting way to concentrate bioactives naturally present in food (Bennett et al., 2011; Vinson et al., 2005). Due to its versatility and many practical applications, dried fruits are widely consumed all over the world. The spouted bed drying is considered as a very flexible drying technique, which has the advantage of its lower cost and also the possibility of using lower temperatures when compared to conventional spray driers (Bezerra et al., 2013).

Despite its many advantages, the drying process itself can cause relevant impacts on the bioactive compounds and other food components. Temperature and adjuvant concentration are among the many relevant production parameters which can be assessed in order to evaluate the drying impact on food. On the other hand, freeze drying is a cost drying technique which is known by its little effect on food components. Its low impact is justified by the fact the food remains at a temperature below the freezing-point during the process of sublimation, which tends to better preserve food quality. Despite that, the expensive equipment, high energy costs and long drying times are disadvantages which disable this technique to be applied as a routine basis procedure (Ratti, 2001). However, it can be considered as a drying control process in order to compare and evaluate the possible drying impact on food products.

Therefore, the aim of this work was to evaluate the impact of spouted bed drying on selected bioactive compounds and antioxidant and antimicrobial properties of camu-camu fruit pulp, comparing to freeze dried and fresh samples. Besides this, the bioactive value of camu-camu dried products is also assessed and discussed. Overall, this article approaches the

development of functional ingredients derived from camu-camu fruit, in order to generate potential new fruit ingredients to be added in other food systems.

2. MATERIAL AND METHODS

2.1 Materials. Frozen commercial pulp of camu-camu (*Myrciaria dubia* Mc. Vaugh) was purchased from Cupuama do Amazonas Com. Ind. Exp. Ltda. (Manaus, AM, Brazil).

2.2 Physicochemical characterization. The defrosted pulp was evaluated in regard to its moisture content and titrable acidity (TA) according to AOAC (2005). Total sugars (TS) were determined using the method of Dubois *et al.* (1956), pH with potentiometer (Hanna pH21) and soluble solids (SS,°Brix) with refractometer (Reichert r^2mini).

2.3 Spouted bed drying. The drying process was performed in a stainless steel spouted bed dryer of laboratory size with high density polyethylene (HDPE) inert particles. The angle of the conical base is 60^o, the height is 13 cm and the diameter of the inlet orifice is 3 cm. The cylindrical column is 72 cm high and has a diameter of 18 cm. Drying was performed with 2.5 kg of inert material, 1.8 m/s speed at different temperatures (60, 80, 95 and 110 °C) and different concentration of maltodextrin MOR-REX® 1910 (0%, 3% and 6%) ($9 \leq DE \leq 12$) (Corn Products, Brazil).

2.4 Freeze-drying. Two kilograms of frozen pulp were lyophilized in a Pironi 501 freeze-drier (Thermo Electron Corporation, New York, USA) at -80°C and 100 mTorr for 120 hours.

2.5 Color measurement. The color (L*, a*, and b* values) of camu-camu powders was measured using a reflectance spectrophotometer (Color Quest XE, HunterLab, Fairfax, VA, USA), calibrated by the use of a standard white tile (top of the scale)/light trap (bottom of the scale) included with the instrument. C* (chroma) and h* (hue angle) were calculated by using values of a* and b* coordinates according to the following equations: $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h^* = \tan^{-1} (b^*/a^*)$.

2.6 Ascorbic acid content. Ascorbic acid was extracted with meta-phosphoric acid (3% w/v) and analyzed by reversed-phase HPLC in a HP Agilent 1100 series (Hewlett-Packard, Palo Alto, CA, USA) with autosampler and quaternary pump coupled to a diode array detector as previously reported (GENOVESE *et al.*, 2008). The column used was a 3.9 × 300 mm column 10 µm, 125Å, Waters, µBondapak^{TM} C18 and elution (flow rate of 0.8 mL/min) was performed in isocratic condition with 2 mmol/L potassium chloride buffer (pH 2.5), monitored at 245 nm. Total ascorbic acid was estimated after reduction of dehydroascorbic

acid (DHA) with 10 mM dithiotreitol. Results were expressed as mg ascorbic acid equivalents (AA)/g sample in dry weight (DW).

2.7 Sample extraction for total proanthocyanidin and antioxidant capacity assays. The powders obtained (1 g) were extracted 3 times in a solvent mixture (100 mL the first time, 50 mL the next two times) comprising methanol/water (70:30, v/v) using a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern), at moderate speed for 1 min, while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman N^{o} 1) and it was stored at -18 °C until analysis. All extractions were done in duplicate, and the subsequent assays were run in triplicate.

2.7.1 Folin-Ciocalteu reducing capacity. The analysis was performed according to Singleton *et al.* (1999) with some modifications. A 0.25 mL aliquot of the extract obtained above was mixed with 0.25 mL of the Folin-Ciocalteu reagent and 2 mL of distillated water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture placed at 37 °C in a water bath for 30 min. The absorbance was measured at 750 nm using a Microplate Spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA). Gallic acid was used as the reference standard, and the results were expressed as mg gallic acid equivalents (GAE)/g DW.

2.7.2 Total phenolics. Total phenolics (GAE/g) were calculated subtracting the value of Folin–Ciocalteu reducing capacity due to ascorbic acid, using a standard curve.

2.7.3 FRAP ferric reducing power. The analysis was performed according to Benzie and Strain (1996). The absorbance was measured at 593 nm using a Microplate Spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA), using an aqueous solution of Trolox (0.25 mg/mL), at different concentrations (0.1; 0.2; 0.4; 0.6 and 0.8 mM), as control. The ferric reducing power was expressed as mmols Trolox equivalents/g DW.

2.7.4 DPPH$^{\bullet}$ scavenging activity. DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity was assessed according to Brand-Williams *et al.* (1995), with some modifications. Briefly, a 50 μL aliquot of the extract previously diluted and 250 μL of a methanolic solution of DPPH$^{\bullet}$ (0.5 mM) were shaken and after 25 minutes at 25 ºC the absorbance was measured at 517 nm using the Microplate Spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA), using a methanolic solution of Trolox, at different concentrations (20, 40, 60, 80 and 100 μM), as control. The DPPH$^{\bullet}$ scavenging activity was expressed as μmol Trolox equivalents/g DW.

2.7.5 Total proanthocyanidin content. It was determined according to (Porter, Hrstich e Chan, 1985)) using a reagent comprising 154 mg of $FeSO_4 \cdot 7\ H_2O$ per liter of n-butanol:hydrochloric acid (3:2) was prepared. A total of 250 µL of each sample extract and 2.5 mL of the described reagent was incubated at 90 ºC for 15 min. The blank consisted of 2.5 mL of the reagent and 250 µL of methanol:acetic acid (99.5:0.5). The absorbance was measured at 540 nm using a model U1100 UV/Visible spectrophotometer (Hitachi, Japan). The results were expressed as mg of quebracho tannin/g DW.

2.8 Antimicrobial assay and determination of Minimum Inhibitory Concentration (MIC). The extracts were tested for activity against: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 8739, *Enterobacter aerogenes* ATCC 13048, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 14028 and *Salmonella enteritidis* ATCC 13076. According to CLSI (CLSI, 2009) the cultures were grown in Trypton Soya Agar (Oxoid, Basingstoke, UK) for 18-24 h at 37 °C, and the colonies were suspended in sterile saline solution (0.85%) to reach a turbidity correspondent to 0.5 of the McFarland scale (10^8 UFC mL^{-1}). The suspensions (0.1 mL) were applied to the surface of Muller-Hinton agar plates (Oxoid, Basingstoke, UK) and sterile discs of 13 mm in diameter were placed on the plates. The discs were filled with 100 µL of extracts, and the plates were incubated at 37 °C for 24 hours, when the diameters of the inhibition zones were measured using a calipter rule. Results were evaluated according to the following scale: <16 mm, inactive; 17 – 19 mm, partially active; 20 – 25 mm, active; >25 mm, very active.

For determination of the MIC, the microdilution method was used (CLSI, 2010). Except for the first row, the wells of sterile 96-well microplates were filled with 50 µL of Muller-Hinton broth (Oxoid, Basingstoke, UK). The wells of the first row of were filled with 100 µL of extracts, homogenized and then 50 µL were serially transferred to the subsequent wells. Finally, 50 µL of the microorganisms solution (10^8 UFC mL^{-1}) were added. The microplates were incubated at 37 °C for 24 h and the MIC, correspondent to the lowest concentration that inhibited the visible growth of the microorganism after 24 h. Ampicillin was used as a positive control. The tests were carried out in two repetition.

2.9 Statistical Analysis. All analyses were run in triplicate and results were expressed as mean ± standard deviation (SD). For statistical analysis, the Statistic software package version 11.0 (StatSoft, Inc., Tulsa, OK) was employed. Differences between means were first analyzed by ANOVA test and then Tukey test ($p < 0.05$). Data were subjected to Pearson correlations.

3. RESULTS AND DISCUSSION

3.1 Camu-camu pulp

The physicochemical characterization of camu-camu pulp is presented in **Table 1**. According to the results, camu-camu pulp can be considered too acidic and non-sweet. Compared to sweet orange (*Citrus sinensis)* and açaí (*Euterpe oleraceae* Mart.), camu-camu pulp presented lower pH and higher acidity (Barros et al., 2012; Tonon et al., 2008). The low pH and high acidity observed for camu-camu fruit is similar to those for lemon. Jabuticaba (*Myrciaria cauliflora* Berg), also a Brazilian native fruit of the same genus of camu-camu has higher pH (around 3.5), titrable acidity (about 0.98 g acid citric/100 g) and SS (around 14.5 °Brix) (Lima et al., 2008). Camu-camu acidity and low sugar content are factors that discourage its use *in natura*, and also justify the search for derived products with higher sensory acceptance potential.

Table 1. Physicochemical characterization of camu-camu frozen pulp.

Components	Mean ± SD
Moisture (%)	92.9 ± 0.1
Total sugars (g glucose/100 g)	1.54 ± 0.01
pH	2.62 ± 0.01
Soluble solids (°Brix)	6.97 ± 0.46
Titrable acidity (g acid citric/100 g)	1.44 ± 0.02

3.2 Color of camu-camu powder

Table 2 shows the effect of the spouted bed drying process over the color of camu-camu powder. Overall, the spouted bed dried powders had lower values of lightness (L*), compared to the lyophilized powder. For samples with higher concentration of maltodextrin (6%), the differences in lightness are not so evident ($p<0.05$). The camu-camu pulp contains soluble solids and sugars (**Table 1**), which can cause a stickiness problem during drying (Fang et al., 2012). The product particles clumping together adversely affect the free-flowing of the fruit material into the drier, which can lead to overheating and browning. Maltodextrin favors flowability and could justify the observed behavior.

Table 2. Color measurement of camu-camu powder produced by spouted bed drying in different temperatures (60, 80, 95 and 110 °C) and maltodextrin addition (0, 3 and 6%).

Maltodextrin concentration		Color parameters[b]		
		L*	C*	h*
0%	Fresh pulp	0.36 ± 0.04^{g}	0.89 ± 0.20^{g}	0.61 ± 0.06^{g}
	Freeze dried	60.45 ± 2.78^{ab}	26.04 ± 0.87^{d}	1.18 ± 0.04^{ab}
	60°C	40.84 ± 1.62^{e}	24.55 ± 1.02^{e}	0.84 ± 0.00^{f}
	80°C	46.40 ± 0.42^{d}	24.53 ± 0.27^{e}	1.02 ± 0.01^{e}
	95°C	37.80 ± 0.18^{ef}	21.79 ± 0.16^{f}	1.06 ± 0.00^{cde}
	110°C	36.60 ± 0.35^{f}	21.26 ± 0.19^{f}	1.06 ± 0.00^{de}
3%	60°C	54.33 ± 0.05^{c}	26.83 ± 0.06^{cd}	0.87 ± 0.00^{f}
	80°C	58.66 ± 0.13^{ab}	27.66 ± 0.11^{c}	1.09 ± 0.00^{cde}
	95°C	54.42 ± 0.66^{c}	29.72 ± 0.45^{b}	1.14 ± 0.00^{abc}
	110°C	47.30 ± 0.15^{d}	27.26 ± 0.41^{c}	1.13 ± 0.00^{bcd}
6%	80°C	57.52 ± 0.01^{bc}	26.92 ± 0.02^{cd}	1.08 ± 0.00^{cde}
	95°C	61.40 ± 0.57^{a}	28.00 ± 0.05^{c}	1.20 ± 0.01^{ab}
	110°C	59.87 ± 0.23^{ab}	31.97 ± 0.10^{a}	1.21 ± 0.00^{a}

Values are expressed as means ± SD (n=3). a,b,c,d. means in the same column followed by different superscripts are significantly different ($p<0.05$). [b] L*, lightness (+100=white, -100=black); C*, chroma (color intensity); h*, angle hue.

There were wide variation of C* and h* values regardless the increase in temperature or maltodextrin concentration. Similar observations were made by Tonon et al. (2008) for C* values when investigating açai juice powder produced by spray drying.

3.3 Total phenolics, ascorbic acid and proanthocyanidins content

Total phenolics, ascorbic acid and proanthocyanidins contents of camu-camu fresh and dried pulps, expressed in dry weight (DW), are presented in **Table 3**. Camu-camu is known as having the highest ascorbic acid and total phenolics contents among tropical and exotic fruits (Abe et al., 2012; Akter et al., 2011; Rufino et al., 2010; Genovese et al., 2008; Reynertson et al., 2008). However, drying of the pulp led to significant losses, in the order ascorbic acid>total phenolics>proanthocyanidins. Although these were more extensive for spouted bed, freeze drying also caused losses of around 18% to both ascorbic acid and total phenolics.

Vitamin C is known for the thermolability and a decrease of 45 to 64% in the Vitamin C content in camu-camu was observed during spouted bed drying. The decrease was higher at higher drying temperature, although this temperature-dependence was not observed at 60 and 80 °C in presence of maltodextrin.

Total phenolics and proanthocyanidins losses were 33-42 and 15.5-18.4%, respectively, and not statistically affected by the increase of drying temperature, probably due to the proportional decrease in the drying time. Similar behavior was observed by Vega-Gálvez et al. (2012) when studying apple slices dried by hot air process at 40, 60 and 80 °C. They reported higher total phenolics content at 40 and no statistical difference between their levels at 60 and 80 °C.

When maltodextrin was used in spouted bed drying, total phenolics losses were 22-40 (3% MD) and 18-27% (6%MD), a range similar to that observed without carrier, taking the dilution effect into account.

Table 3. Total Phenolics, ascorbic acid and proanthocyanidins contents of camu-camu powder produced by spouted bed drying in different temperatures (60, 80, 95 and 110 °C) and maltodextrin addition (0, 3 and 6%).

Maltodextrin concentration		Ascorbic acid (AAE mg/g DW)	Total Phenolics (GAE mg/g DW)	Proanthocyanidins (QTE mg/g DW)
0%	Fresh pulp	150.3 ± 0.2^{a}	81.6 ± 6.5^{a}	72.2 ± 2.0^{a}
	Freeze dried	123.0 ± 0.1^{b}	67.1 ± 6.4^{ab}	71.9 ± 2.2^{a}
	60°C	82.7 ± 4.2^{c}	54.7 ± 3.9^{bc}	61.0 ± 3.9^{b}
	80°C	74.7 ± 2.0^{d}	46.9 ± 1.0^{cde}	58.9 ± 1.8^{b}
	95°C	65.2 ± 5.0^{e}	54.3 ± 5.8^{bc}	59.8 ± 1.1^{b}
	110°C	54.2 ± 1.0^{gh}	51.8 ± 1.0^{bcd}	59.5 ± 2.5^{b}
3%	60°C	66.1 ± 2.0^{e}	39.4 ± 1.0^{cde}	49.9 ± 1.1^{c}
	80°C	63.9 ± 3.3^{e}	45.0 ± 3.2^{cde}	48.1 ± 1.3^{cd}
	95°C	55.7 ± 1.5^{gh}	39.5 ± 0.8^{cde}	49.0 ± 0.4^{c}
	110°C	46.7 ± 1.0^{i}	34.5 ± 0.8^{de}	44.6 ± 1.5^{de}
6%	60°C	60.0 ± 3.5^{f}	32.2 ± 3.3^{e}	40.8 ± 3.0^{ef}
	80°C	57.0 ± 2.2^{fg}	32.2 ± 2.0^{e}	40.3 ± 0.5^{f}
	95°C	53.4 ± 1.0^{h}	34.4 ± 0.8^{de}	42.5 ± 0.9^{ef}
	110°C	29.8 ± 1.5^{j}	36.2 ± 0.2^{de}	41.2 ± 1.0^{ef}

Values are expressed as means ± SD (n=3). a,b,c,d. means in the same column followed by different superscripts are significantly different ($p<0.05$).

The contents of proanthocyanidins were not affected by freeze drying. Also, there was a gradual preservation of the proanthocyanidins contents by spouted bed drying with addition of maltodextrin, of about 88-98% and 100%, when 3% and 6% MD were added, respectively. Proanthocyanidins are a large class of polyphenols varying in size from monomer to polymers of 20 or more units. Fruits normally present a range of proanthocyanidins of varying molecular size. The effects of spouted bed drying could be related to an increase in polymerization degree and consequent loss of extractability.

3.4 Antioxidant capacity

The antioxidant capacity of camu-camu powders, measured by three different methods, is shown in **Figure 1A** (Folin-Ciocalteu reducing capacity), **Figure 1B (**FRAP ferric reducing power) and **Figure 1C (**DPPH scavenging activity). Gonçalves et al. (2010) compared the antioxidant activity of several Brazilian tropical fruits and camu-camu showed the highest activity, which was around 10 times higher than that for tucumã and uxi. Antioxidant activity is directly proportional to total phenolic and ascorbic acid contents (Genovese et al., 2008), and, consequently, it was affected by drying processes.

Lyophilization caused a slight decrease in FC and DPPH (13-14%) and a 26% reduction in FRAP (**Figure 1**). Spouted bed drying resulted in temperature-independent decreases in antioxidant capacity. Folin-Ciocalteu reducing (46-67%) and DPPH scavenging capacities (61-71%) reductions were greater than those for the FRAP (22%-46%). This behavior could be explained by differences in losses of ascorbic acid, proanthocyanidins and total phenolics among the processes (**Table 3**).

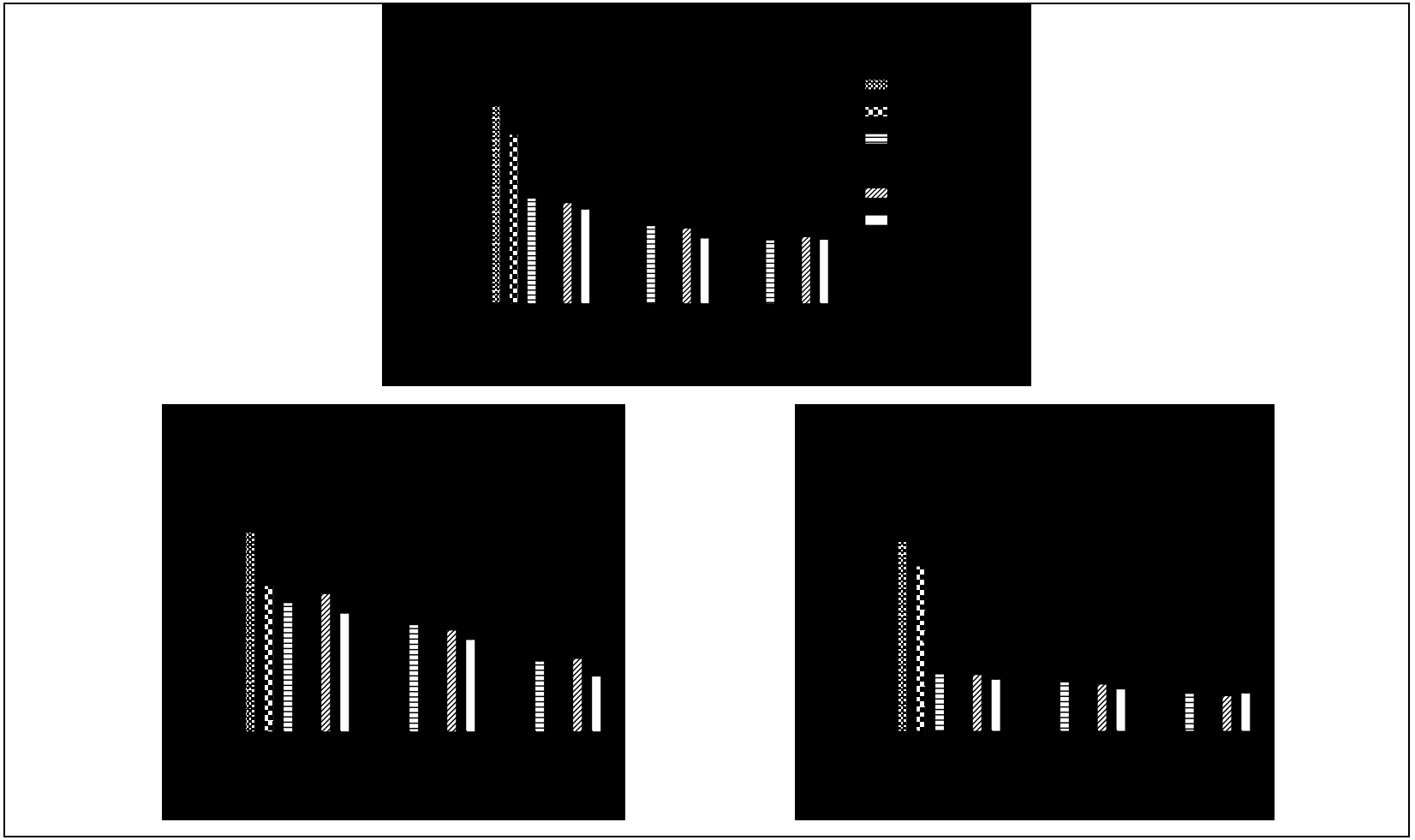


Figure 1. Effect of spouted bed drying of camu-camu pulp in (A) Folin-Ciocalteu reducing capacity; (B) Ferric reducing antioxidant power; (C) DPPH• scavenging capacity

A good correlation between Folin-Ciocalteu reducing and DPPH scavenging capacities was found. The same occurred for Folin-Ciocalteu and FRAP ($r = 0.97$ and $r = 0.90$, respectively, at $p<0.05$). The lower correlation between FRAP and AA values ($r = 0.74$) suggests that the losses of ascorbic acid during drying affected more significantly the Folin-Ciocalteu reducing and DPPH scavenging capacities ($r = 0.99$). DPPH capacity ($r = 0.94$) was also more significantly affected by TP reduction than FRAP ($r = 0.87$). Similar coefficient values were observed by Correia *et al.* (2012) for acerola, jambolan, pitanga and caja-umbu residue powders. Chirinos et al. (2010) and Myoda et al. (2010) also, found a linear relationship between TP and DPPH for camu-camu pulp, and for both seed and peel, respectively. Although Luximon-Ramma *et al.* (2003) found a poor correlation between AA and FRAP, this could be explained by the much lower vitamin C levels in the fruits analyzed by the authors.

3.5 Antimicrobial activity

Among the tested microorganisms, only *Staphylococcus aureus* ATCC 29213 was inhibited by the crude extracts of camu-camu powders (**Table 4**). The extracts of freeze dried powder and spouted bed powders were classified as active and partially active, respectively. Similar results were found by Myoda et al. (2010) with methanolic extracts of seed and peel of camu-camu against *S. aureus* at 5 mg/mL. Others extracts such as those obtained from pericarp of *P. granatum* also presented activity against *S. aureus* (Pradeep et al., 2008).

The MIC value for freeze-dried and spouted bed powders obtained at 60°C without carrier was 0.08 $mg.mL^{-1}$. The MIC values were much higher for powders obtained at higher temperatures and with maltodextrin, ranging between 0.16 $mg.mL^{-1}$ and 0.31 $mg.mL^{-1}$ (**Table 4**). The higher the maltodextrin concentration and inlet drying temperature, the lower the inhibition. In this way, the extract of camu-camu powder produced at 6% MD and from 80 to 100 °C was less effective than the extract produced at 3% MD in the same temperature conditions. Despite loss of inhibitory activity caused by drying, these extracts were more active against *S. aureus* than ampicillin (MIC 0.26 $mg.mL^{-1}$).

Table 4. Antimicrobial activity and minimum inhibitory concentration (MIC) of extracts of camu-camu powders produced by spouted bed drying in different temperatures (60, 80, 95 and 110 °C) and maltodextrin addition (0, 3 and 6%) against *S. aureus* strains.

Maltodextrin concentration		Inhibition (mm)	MIC of extracts ($mg.mL^{-1}$)
0%	Freeze dried	25 ± 1^{a}	0.08^{a}
	60°C	19 ± 1^{b}	0.08^{a}
	80°C	Ne	ne
	95°C	Ne	ne
	110°C	19 ± 1^{bc}	0.16^{b}
3%	60°C	18 ± 1^{bcd}	0.16^{b}
	80°C	18 ± 1^{bcd}	0.16^{b}
	95°C	19 ± 1^{bc}	0.16^{b}
	110°C	Ne	ne
6%	60°C	Ne	ne
	80°C	17 ± 1^{d}	0.31^{d}
	95°C	17 ± 1^{d}	0.31^{d}
	110°C	18 ± 1^{cd}	0.31^{d}
	Ampicillin	Ne	0.26^{c}

Values are expressed as means ± SD (n=3). a,b,c,d. means in the same column followed by different superscripts are significantly different ($p<0.05$). ne = not evaluated.

Phenolic constituents of fruits such as pomegranate were shown to posses significant antimicrobial activity (Pradeep at al., 2008). In this way, the reduction in phenolics concentration caused by drying of camu-camu pulp may have been responsible for the partial loss of inhibitory activity. Kil et al. (2009) suggested that the antimicrobial activity of sorghum may be due to the presence of tannic acid. Saraiva et al. (2012) observed that extract from *Caesalpinia pyramidalis* Tull containing quercetin, catechin, ellagic acid, flavonoids, proanthocyanidins and gallic acid, also presented antistaphylococcal activity. Besides, pure compounds such as flavone, quercetin, naringenin, morin and kaempferol were shown to inhibit *S. aureus* (Rauha et al., 2000). Camu-camu fruit presents high contents of ellagic acid, tannins, cyanidin, quercetin, catechin, kaempferol and rutin (Akter et al., 2011; Gonçalves et al., 2010; Chirinos et al., 2010; Reynertson et al., 2008). Therefore, the detected antimicrobial activity in the extracts was expected.

4. CONCLUSIONS

Drying of camu-camu pulp by spouted bed drying causes a decrease of *in vitro* antioxidant capacity, mainly associated to ascorbic acid and polyphenols degradation. The decrease is more intense for spouted bed drying compared to freeze-drying. Use of maltodextrin may protect the bioactive contents, mainly proanthocyanidins. Despite the observed losses caused by the drying process, camu-camu powders still retain high levels of phenolics, ascorbic acid, proanthocyanidins, antioxidant and antimicrobial activity. Therefore, they represent good sources as antioxidants and antimicrobials and may find several applications in functional food development.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the CNPq and FAPESP for the financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2013.07.025.

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4.2 Effect of inlet air temperature and concentration of carrier agents on bioactive compounds and antioxidant capacity of spray-dried powders of camu-camu (*Myrciaria dubia* Mc. Vaugh)

Abstract

Bioactive compounds, such as phenolics and ascorbic acid, which are abundant in camu-camu (*Myrciaria dubia*), have been shown to display antioxidant activity. This study was aimed to evaluate the effect of inlet air temperature (T) and concentration (C) of maltodextrin and arabic gum in the spray-drying process of two commercial camu-camu pulps on the moisture, solubility, total phenolics (TP), ascorbic acid (AA) and proanthocyanidins (PAC) contents, and *in vitro* antioxidant capacity. Powders with arabic gum resulted in better yield and solubility than maltodextrin. Overall, T had a lower impact on the responses studied than C. Polynomial equations were obtained for AA (R^2=0.993), TP (R^2=0.735) and PAC (R^2=0.946) contents, and also, for antioxidant capacities for three different methods, which showed predictive ($0.867 \leq R^2 \leq 0.963$) in the analyzed range. Additionally, PCA was applied to all results; the powders were separated in three groups using hierarchical cluster analysis, which confirmed freeze-drying as the best way to preserve bioactive compounds. And also, 6% of carrier agents was the best option to develop camu-camu spray-dried powders. The effects and interactions between C and T to produce camu-camu spray-dried powders were modeled, and these equations can be applied to predict and verify the quality of bioactive powders.

Keywords: spray drying; freeze-drying; phenolics; antioxidant capacity; PCA.

1. INTRODUCTION

Diets rich in fruit have been studied for their potential beneficial effects on the risk of cardiovascular disease, diabetes, cancer and strokes.(Crozier, Jaganath e Clifford, 2009) Their high content of phytochemical compounds, mainly polyphenols, has been associated with their health benefits.(Espin, Garcia-Conesa e Tomas-Barberan, 2007) Camu-camu (*Myrciaria dubia* Mc. Vaugh) is an Amazonian fruit with high levels of vitamin C and polyphenols, such as flavonoids and ellagitannins, and with significant *in vitro* and *in vivo* antioxidant capacity.(Inoue *et al.*, 2008; De Souza Schmidt Goncalves, Lajolo e Genovese, 2010; Akter *et al.*, 2011)

Camu-camu fruit has a disadvantage to be highly perishable, which makes the transportation chain more complex and expensive. One alternative to keep the bioactive compounds is dehydration, which is an important method of processing the fruit to extend its shelf life.(Vinson *et al.*, 2005; Bennett *et al.*, 2011) Dried fruits are widely consumed all over the world due to their versatility and many technological applications. The spray-drying method is used in the food industry because of its low cost and wide equipment availability. Microencapsulation by spray-drying has been successfully used not only to increase solubility and production yields, but also to avoid chemical and/or biological degradation.(Gouin, 2004) Previously, the effect of spouted bed-drying on phenolic compounds and bioactivity of camu-camu was assessed.(Fujita *et al.*, 2013)

The aim of this work was to evaluate the influence of carrier agents concentration and inlet air temperature of spray-dried powders of two commercial camu-camu pulps, from Sao Paulo and Amazon States, and to compare them with those of freeze-dried powders and fresh pulp. The parameters evaluated were bioactive compounds contents, such as, ascorbic acid, total phenolics and proanthocyanidins. Finally, antioxidant properties were determined to investigate the potential of spray-dried camu-camu as a source of functional ingredients.

2. MATERIALS AND METHODS

2.1 Materials. Two different commercial pulps were used, one frozen commercial pulp of camu-camu (*Myrciaria dubia* Mc. Vaugh) was purchased from Cupuama do Amazonas Com. Ind. Exp. Ltda. (Manaus, AM, Brazil), and the other pulp was prepared from camu-camu

picked in Registro (São Paulo, Brazil) and immediately frozen. Both materials were defrosted prior to spray-drying.

2.2 Spray-drying. Spray-drying was performed in a pilot scale spray-dryer (Labmaq, SD 5.0, Brazil). The pulp was fed by a peristaltic pump at a fixed rate of 44 mL/min and was spray-dried at different inlet air temperatures (T, 120, 150 and 180 °C) and different carrier agent concentrations (C, 6, 12 and 18%), according to a 3^2 full experimental design (**item 2.5**). Two carrier agents were employed (independent variables): either maltodextrin MOR-REX® 1910 ($9 \leq DE \leq 12$) (Corn Products, Brazil) or arabic gum (Nexira Brazil Com. Ltd., Brazil). The production yield was expressed as ratio of total solids content in the resulting powder to the total solids content in the feed mixture.

2.3 Freeze-drying. Two kilograms of frozen pulp were lyophilized in a Pironi 501 freeze-dryer (Thermo Electron Corporation, New York, USA) at - 80 °C and 100 mTorr for 120 hours.

2.4 Analytical Methods

Moisture (M)

The moisture content of the powder was determined gravimetrically by drying in a vacuum oven at 70 °C until constant weight.(Aoac, 1990)

Solubility (S)

Cold-water solubility of spray-dried samples was determined according to Eastman and Moore.(Eastman e Moore, 1984) A 1% (w/v) powder suspension was agitated for 30 min using a TE-420 shaker (Tecnal, Piracicaba, Brazil) and was then centrifuged at 4000 rpm for 5 min. A 25 mL aliquot of the supernatant was then placed in a porcelain pan and was heated at 105 ºC until constant weight in a drying oven. Solubility was expressed as a percentage g in dry weight (DW) per 100 g^{-1} H_2O).

Ascorbic acid content (AA)

AA was extracted with meta-phosphoric acid (3% w/v) and analyzed by reversed-phase HPLC in a HP Agilent 1100 series (Hewlett-Packard, Palo Alto, CA, USA) equipped with autosampler and quaternary pump coupled to a diode array detector as previously reported.(Genovese *et al.*, 2008) The column µBondapak^{TM} C18 used was a 3.9 × 300 mm i.d., 10 µm pore size, Waters, and elution (flow rate of 0.8 mL/min) was performed in isocratic condition with 2 mmol/L potassium chloride buffer (pH 2.5), monitored at 245 nm. Total ascorbic acid was estimated after reduction of dehydroascorbic acid (DHA) with 10 mM

dithiotreitol. Results were expressed as g ascorbic acid equivalents (AAE) per kg^{-1} sample DW.

Sample extraction for total proanthocyanidin and antioxidant capacity assays

The powders obtained (1 g) were extracted 3 times in a solvent mixture (100 mL the first time, 50 mL the next two times) comprising methanol/water (70:30, v/v) using a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern), at moderate speed for 1 min, while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman N^o 1) and stored at -18 °C until analysis. All extractions were done in duplicate, and the subsequent assays were run in triplicate.

Total proanthocyanidins content (PAC)

Total proanthocyanidins content was determined according to Porter *et al.*(Porter, Hrstich e Chan, 1985) using a reagent comprising 154 mg of $FeSO_4 \cdot 7\ H_2O$ per liter of n-butanol:hydrochloric acid (3:2) was prepared. A total of 250 µL of each sample extract and 2.5 mL of the described reagent was incubated at 90 ºC for 15 min. The blank consisted of 2.5 mL of the reagent and 250 µL of methanol:acetic acid (99.5:0.5). The absorbance was measured at 540 nm using a spectrophotometer (model U1100, Hitachi, Japan). The results were expressed as g of quebracho tannin equivalents (QTE) per kg^{-1} DW.

Folin-Ciocalteu reducing capacity (FC)

The analysis was performed according to Singleton *et al.*(Singleton *et al.*, 1999) with some modifications. A 0.25 mL aliquot of the extract obtained as described above was mixed with 0.25 mL of the Folin-Ciocalteu reagent and 2 mL of distillated water. After 3 min at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added and the mixture was kept in a water bath at 37 °C for 30 min. The absorbance was measured at 750 nm using a microplate spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA). Gallic acid was used as the reference standard, and the results were expressed as g gallic acid equivalents (GAE) per kg^{-1} DW.

Total phenolics (TP)

Total phenolics (GAE kg^{-1} DW) were calculated subtracting the value of Folin–Ciocalteu reducing capacity due to ascorbic acid, using a standard curve.

Ferric reducing antioxidant power (FRAP)

The analysis was performed according to Benzie and Strain.(Benzie e Strain, 1996) Twenty microliters of previously diluted extract and 150 μL of the FRAP reagent were added and then incubated for 4 minutes ate 37 ºC. The absorbance was measured at 593 nm using a microplate spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA), using an aqueous solution of Trolox (0.25 mg mL^{-1}), at different concentrations (0.1; 0.2; 0.4; 0.6 and 0.8 mM), as control. The ferric reducing power was expressed as mol Trolox equivalents kg^{-1} DW.

$DPPH^{\bullet}$ scavenging activity (DPPH)

$DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity was assessed according to Brand-Williams *et al.*(Brand-Williams, Cuvelier e Berset, 1995) with some modifications. Briefly, a 50 μL aliquot of previously diluted extract and 250 μL of a methanolic solution of $DPPH^{\bullet}$ (0.5 mM) were shaken and after 25 minutes at 25 ºC. The absorbance was measured at 517 nm using a microplate spectrophotometer (Benchmark Plus, Biorad, Hercules, CA, USA). A methanolic solution of Trolox, at different concentrations (20, 40, 60, 80 and 100 μM), was used to prepare the calibration curve and thus estimate the antioxidant activity of the extracts. The $DPPH^{\bullet}$ scavenging activity was expressed as mol Trolox equivalents kg^{-1} DW.

2.5. Experimental design. A 3^2 full-factorial design coupled with response surface methodology (RSM) was applied to evaluate the effects of T and C giving a total of 9 trials (**Table 1**). The measured responses were bioactive compounds and antioxidant capacity. All analyses were run in triplicate and were expressed as mean ± standard deviation (SD). Differences between means were first analyzed by one-way ANOVA test and subsequently by the Tukey test ($p<0.05$) and also, data were subjected to Pearson linear correlations.(Granato, De Araújo Calado e Jarvis, 2014) When *p*-value was below 5%, the triplicate results were analyzed using multiple linear regression (Software Statistica 11.0, Stat-Soft Inc., Tulsa, OK, USA). The following polynomial equation was used to model the response variables: $Y = \beta_0 + \beta_1 C + \beta_2 T + \beta_{11} C^2 + \beta_{22} T^2 + \beta_{12} CT$

Where, Y is the response of dependent variable; C (carrier agent concentration) and T (inlet air temperature) are independent variables; β_0 is the mean; β_1 and β_2 are the regression coefficients for linear terms; β_{11} and β_{22} are the quadratic terms and β_{12} is the interaction term, which were calculated based on coded values. The statistical adequacy of the fitted models

was checked by assessing the following parameters: determination coefficient (R^2), adjusted determination coefficient (R^2adj), *p*-value of the model, o-value of each regression coefficient, *p*-value of lack of fit. Moreover, the normality of the residues was also checked by the Anderson-Darling test.

To assess association between the antioxidant activity and phenolic composition with the type of additive used to produce camu-camu powders as well as to understand more comprehensively the effects of the independent variables on the chemical markers and *in vitro* antioxidant power principal component analysis (PCA) and hierarchical cluster analysis (HCA), implemented in the Statistica 11.0 software (Stat-Soft Inc., Tulsa, OK, USA) were used PCA was applied to total contents of AA, TP and PAC, and antioxidant activity measured by Folin, FRAP and DPPH assays. For this purpose, the results obtained for each parameter were adopted as columns and the camu-camu powders as rows and results were autoscaled.(Alezandro *et al.*, 2011) Only eigenvalues higher than 1.0 were used to project the samples on the factor plane (PC 1 *vs* PC2). HCA was performed to assess sample similarities, which were calculated on the basis of the Euclidean metric, while the Ward's hierarchical agglomerative method was used to group the samples.(Zielinski *et al.*, 2014)

3. RESULTS AND DISCUSSION

In the absence of carrier agents, very sticky powders were obtained after spray-dried of camu-camu pulp (preliminary test – data not shown). In order to not obtain a product with this undesirable feature, maltodextrin (MD) and arabic gum (AG) were tested and commercial camu-camu pulps from two local of cultivation were spray-dried using different conditions of inlet air temperature (T;120, 150 and 180 °C) and concentration of carrier agents (C; 6, 12 and 18%). As expected, the higher the drying temperature, the lower the moisture content (M, **Tables 1** and **2**), as evaporation rate is higher when drying temperature is increased. Overall, spray-drying resulted in lower yields (around 25%) and AG showed slightly better yield performances as compared to MD (**Tables 1** and **2**). Dib Taxi et al. (2003) reported similar spray-drying yields for camu-camu juice (26% DW) at 12-32% MD and 120-152 °C. Tonon et al. (2008) on the other hand, observed higher yields (34.4-55.7%) for açai juice using a mini-spray dryer, which, given its dimensions, was expected to provide greater efficiency.

3.1 Solubility (S)

The solubility of camu-camu powders obtained at different concentrations of MD and AG is shown in the **Tables 1** and **2**. Solubility is one of the most important physical properties to be considered when processing foods: the higher the solid content, the more affected the S becomes.(Cano-Chauca *et al.*, 2005) Although the temperature was expected to influence the solubility index, drying temperatures showed no significant effect within the conditions applied in the current study ($p<0.05$).

The solubility of powders ranged between 76.7 and 88% for MD and 84.3 while a mean value of 89.9% was observed for AG. Both carrier agents are highly soluble in water; therefore, as expected, solubility increased with the carrier agent concentration. These data are comparable to those obtained by Ahmed et al. (2010) who found lower S (between 40 and 56%) for spray-dried purple sweet potato. Thus, these results encourage the application of spray-dried powders in food processing, especially when it is necessary to increase product viscosity. On the other hand, the solubility of freeze-dried powders was lower (between 56.5% and 57.1%), which could be explained by the fact that no carrier agent was used in the process.(Cano-Chauca *et al.*, 2005; Caparino *et al.*, 2012)

3.2 AA, TP and PAC contents

AA, TP and PAC contents were determined (**Tables 1** and **2**), and fitted equations were obtained (**Table 3**). The statistical adequacy of these models was checked by calculating the R^2, adjusted R^2, the lack of fit, and also the normality of the residues. In order to assess the process impact, results were expressed on a dry weight (DW) basis, and also, the amount of carrier agents was discounted. Overall, losses for all compounds determined were similar in MD and AG experiments.

Camu-camu has a high AA content and is also rich in TP.(Genovese *et al.*, 2008) AA losses ranged between 53 and 74% and 43 and 74% to MD and AG, respectively ($p<0.05$) (**Tables 1** and **2**). They were not significantly different among the three T investigated, which was expected since the pulp remains a short time (about 10 seconds) in contact with high temperature. This result is in-line with **Figure 1A**, where the T seemed to have no influence in the AA content. The opposite behavior was observed with the effect of C (**Table 3**).

Table 1. Experimental design with coded and real values and moisture content (M), production yield (Y), solubility (S), bioactive compounds (AA, TP and PAC) and antioxidant capacity of spray-dried camu-camu powders using MD.

Tests	Drying conditions		M	Y	S	Bioactive Compounds			Antioxidant Capacity		
	C	T				AA	TP	PAC	FC	FRAP	DPPH
1	6 (-1)	120 (-1)	5.8 ± 0.1^{b}	20.7	76.7 ± 0.9^{b}	52.3 ± 0.5^{c}	43.4 ± 3.8^{b}	47.1 ± 2.6^{d}	57.8 ± 2.4^{bc}	0.78 ± 0.02^{c}	0.29 ± 0.01^{cd}
2	6 (-1)	150 (0)	3.3 ± 0.2^{e}	18.6	81.8 ± 0.2^{ab}	52.4 ± 2.9^{c}	43.8 ± 5.7^{b}	50.8 ± 3.1^{c}	58.4 ± 4.8^{bc}	0.77 ± 0.04^{c}	0.24 ± 0.02^{d}
3	6 (-1)	180 (+1)	3.1 ± 0.4^{e}	21.0	82.7 ± 5.3^{ab}	62.0 ± 0.7^{b}	37.7 ± 3.6^{c}	46.2 ± 2.0^{d}	55.8 ± 3.3^{c}	0.75 ± 0.03^{c}	0.22 ± 0.01^{de}
4	12 (0)	120 (-1)	4.7 ± 0.2^{c}	24.9	80.6 ± 1.1^{ab}	40.6 ± 0.5^{d}	33.9 ± 2.5^{c}	38.2 ± 1.1^{e}	44.9 ± 1.4^{cde}	0.59 ± 0.02^{d}	0.25 ± 0.09^{d}
5	12 (0)	150 (0)	4.0 ± 0.2^{d}	26.2	76.4 ± 3.8^{b}	35.2 ± 0.2^{e}	35.2 ± 3.7^{c}	33.5 ± 1.8^{f}	44.2 ± 3.4^{cde}	0.52 ± 0.05^{de}	0.14 ± 0.02^{ef}
6	12 (0)	180 (+1)	3.0 ± 0.3^{e}	24.3	83.5 ± 2.8^{ab}	39.9 ± 0.5^{d}	26.7 ± 2.1^{d}	30.7 ± 2.7^{f}	37.5 ± 1.2^{de}	0.46 ± 0.05^{de}	0.12 ± 0.03^{f}
7	18 (+1)	120 (-1)	5.0 ± 0.1^{c}	22.1	84.0 ± 0.7^{ab}	27.3 ± 2.9^{f}	29.4 ± 1.2^{d}	30.1 ± 1.0^{f}	35.6 ± 5.4^{de}	0.51 ± 0.03^{de}	0.13 ± 0.01^{f}
8	18 (+1)	150 (0)	4.0 ± 0.3^{d}	24.1	88.0 ± 0.2^{a}	26.7 ± 0.1^{f}	28.2 ± 2.3^{d}	25.8 ± 1.2^{g}	34.3 ± 1.4^{de}	0.44 ± 0.02^{ef}	0.08 ± 0.01^{f}
9	18 (+1)	180 (+1)	3.3 ± 0.1^{e}	23.6	85.1 ± 2.1^{ab}	28.9 ± 0.5^{f}	25.3 ± 2.6^{d}	25.8 ± 1.9^{g}	32.3 ± 1.3^{e}	0.43 ± 0.06^{f}	0.08 ± 0.02^{f}
Control	Fresh pulp		92.5 ± 0.2^{a}			132.2 ± 0.2^{a}	99.9 ± 6.0^{a}	76.5 ± 2.0^{b}	134.5 ± 6.5^{a}	0.96 ± 0.06^{a}	0.76 ± 0.08^{ab}
	Freeze-dried		5.1 ± 0.8^{c}	77.4	56.5 ± 1.3^{c}	128.6 ± 4.7^{a}	97.1 ± 6.9^{a}	83.4 ± 2.1^{a}	132.2 ± 6.5^{a}	0.84 ± 0.01^{b}	0.68 ± 0.03^{b}

C, concentration of carrier agents (%); T, inlet air temperature (°C); M, moisture content (%); Y, yield (%); S, solubility (%); AA, ascorbic acid (g AAE kg^{-1} DW); TP, total phenolics (g GAE kg^{-1} DW); PAC, proanthocyanidins (g QTE kg^{-1} DW); FC, Folin-Ciocalteu reducing capacity; FRAP, ferric reducing antioxidant power; DPPH, DPPH• scavenging capacity, Values are expressed as means ± SD (n=3). a,b,c,d. means in the same column followed by different superscripts are significantly different (p<0.05).

Table 2. Experimental design with coded and real values and moisture content (M), production yield (Y), solubility (S), bioactive compounds (AA, TP and PAC) and antioxidant capacity of spray-dried camu-camu powders using AG.

Tests	Drying conditions		M	Y	S	Bioactive Compounds			Antioxidant Capacity		
	C	T				AA	TP	PAC	FC	FRAP	DPPH
1	6 (-1)	120 (-1)	6.4 ± 1.7^{b}	28.1	84.4 ± 1.5^{b}	137.5 ±2.7^{b}	57.9 ± 2.9^{b}	27.8 ± 1.6^{b}	112.8 ± 3.0^{b}	0.49 ± 0.03^{b}	0.77 ± 0.03^{b}
2	6 (-1)	150 (0)	4.9 ± 0.1^{c}	29.7	84.3 ± 0.4^{b}	134.6 ± 0.4^{b}	46.9 ± 3.0^{bc}	26.8 ± 2.7^{b}	100.0 ± 2.9^{bc}	0.47 ± 0.04^{b}	0.76 ± 0.09^{b}
3	6 (-1)	180 (+1)	4.5 ± 0.3^{c}	24.7	86.6 ± 1.1^{ab}	130.1 ± 6.9^{b}	44.5 ± 7.3^{cd}	24.9 ± 1.6^{b}	95.5 ± 7.4^{c}	0.45 ± 0.02^{b}	0.71 ± 0.08^{bc}
4	12 (0)	120 (-1)	6.4 ± 0.8^{b}	27.5	84.6 ± 2.4^{b}	77.7 ± 2.4^{c}	43.0 ± 2.4^{cd}	18.9 ± 0.7^{c}	74.0 ± 2.4^{d}	0.29 ±0.03^{c}	0.54 ± 0.04^{d}
5	12 (0)	150 (0)	4.5 ± 0.4^{c}	30.3	86.0 ± 1.5^{ab}	73.7 ± 3.3^{c}	41.3 ± 2.2^{cde}	17.1 ± 0.9^{cd}	70.6 ± 2.1^{de}	0.27 ± 0.03^{c}	0.48 ± 0.02^{de}
6	12 (0)	180 (+1)	3.8 ± 0.3^{c}	30.9	86.7 ± 0.9^{ab}	69.8 ± 4.6^{c}	44.7 ± 6.8^{cd}	16.5 ± 1.7^{cde}	72.7 ± 6.4^{d}	0.28 ± 0.03^{c}	0.55 ± 0.03^{d}
7	18 (+1)	120 (-1)	6.1 ± 0.3^{b}	22.5	88.3 ± 1.0^{a}	41.9 ± 3.7^{d}	28.9 ± 4.9^{e}	14.3 ± 1.5^{def}	47.0 ± 4.7^{e}	0.16 ± 0.02^{d}	0.45 ± 0.02^{e}
8	18 (+1)	150 (0)	4.8 ± 0.9^{c}	30.0	89.4 ± 0.8^{a}	39.8 ± 2.7^{de}	33.9 ± 3.9^{de}	13.7 ± 0.6^{ef}	51.9 ± 3.6^{e}	0.15 ± 0.02^{d}	0.42 ± 0.03^{ef}
9	18 (+1)	180 (+1)	3.9 ± 0.4^{c}	ne	89.9 ± 2.1^{a}	35.3 ± 3.2^{e}	32.1 ± 8.0^{de}	13.1 ± 0.9^{f}	47.7 ± 7.7^{e}	0.17 ± 0.03^{d}	0.35 ± 0.03^{f}
Control	Fresh pulp		93.2 ± 0.1^{a}			253.2 ± 3.4^{a}	100.0 ± 7.2^{a}	39.4 ± 0.8^{a}	143.1 ± 7.2^{a}	1.23 ± 0.03^{a}	1.32 ± 0.09^{a}
	Freeze-dried		4.5 ± 0.3^{c}	77.4	57.1 ± 1.3^{c}	248.2 ±1.2^{a}	89.0 ± 1.3^{a}	38.3 ± 0.4^{a}	130.6 ± 1.3^{a}	1.16 ± 0.11^{a}	1.28 ± 0.09^{a}

C, concentration of carrier agents (%); T, inlet air temperature (°C); M, moisture content (%); Y, yield (%); S, solubility (%); AA, ascorbic acid (g AAE kg^{-1} DW); TP, total phenolics (g GAE kg^{-1} DW); PAC, proanthocyanidins (g QTE kg^{-1} DW); FC, Folin-Ciocalteu reducing capacity; FRAP, ferric reducing antioxidant power; DPPH, DPPH• scavenging capacity, ne = not evaluated; Values are expressed as means ± SD (n=3). a,b,c,d. means in the same column followed by different superscripts are significantly different (p<0.05).

Table 3. Regression coefficients and coefficients of determination (R^2 and R adjusted) for bioactive compounds and antioxidant capacity of spray-dried camu-camu powders.

Param.	Bioactive Compounds								Antioxidant Capacity									
	AA		TP				PAC		FC				FRAP				DPPH	
	AG	*p*	AG	*p*	MD	*p*	AG	*p*	AG	*p*	MD	*P*	AG	*p*	MD	*p*	AG	*p*
Mean	82.25	<0.001	41.48	<0.001	33.73	<0.001	19.24	<0.001	66.81	<0.001	44.54	<0.001	0.30	<0.001	0.58	<0.001	0.50	<0.001
C	-47.31	<0.001	-9.07	<0.001	-6.97	<0.001	-6.40	<0.001	-28.28	<0.001	-11.65	<0.001	-0.15	<0.001	-0.15	<0.001	-0.18	<0.001
T	-3.66	<0.001		ns	-2.84	<0.001	-1.07	0.001	- 2.80	0.01	- 2.12	0.004		ns	-0.04	<0.001		ns
C^2	-6.08	<0.001		ns	-1.36	<0.001	-1.30	<0.001	- 2.30	0.014	- 1.75	0.006	-0.02	<0.001	-0.04	<0.001	- 0.03	0.018
T^2		ns		ns	1.50	<0.001		ns		ns	ns	Ns		ns	Ns	ns		ns
CxT		ns	4.13	0.003	ns			ns	4.21	0.0020	ns	Ns		ns	Ns	ns		ns
R^2	0.9934		0.735		0.875		0.946		0.961		0.940		0.963		0.951		0.867	
R^2adj	0.9929		0.691		0.846		0.937		0.955		0.926		0.956		0.929		0.846	
p (lack of fit)	0.623		0.1459		0.428		0.861		0.136		0.309		0.539		0.282		0.342	
p (residues)	0.057		0.448		0.055		0.869		0.542		0.086		0.007		0.630		0.117	

AA, ascorbic acid (g AAE kg^{-1} DW); TP, total phenolics (g GAE kg^{-1} DW); PAC, proanthocyanidins (g QTE kg^{-1} DW); FC, Folin-Ciocalteu reducing capacity (g GAE kg^{-1} DW); FRAP, Ferric reducing antioxidant power (mol Trolox kg^{-1} DW); DPPH• scavenging capacity (mol Trolox kg^{-1} DW); AG, arabic gum; MD, maltodextrin; C, carrier agent concentration (coded values), T, inlet air temperature (coded values); ns, nonsignificant (p<0.05).

Other fact observed was that the AA content was more abundant in the São Paulo pulp, probably because the Amazonian pulp had degradation during transportation. São Paulo pulp had higher AA content than that reported by Fracassetti et al.(Fracassetti *et al.*, 2013) who found a content of 35.1 g AA kg^{-1} DW in spray-dried camu-camu powder processed with 10% MD at 185 °C. However, São Paulo fresh pulp content of AA was similar to the values obtained by Chirinos et al. (2010) and Rufino et al. (2010).

The levels of TP decreased from about 56% to 75% and from 38% to 65% in the MD and AG experiments, respectively, and were not affected by the T ($p < 0.05$). Fitted mathematic equations could be proposed for TP contents for both carrier agents (**Table 3**). The equations showed that the greater the concentration of carrier agents, the lower phenolic contents. However, the interaction of T and C had positive influence to AG. In addition, T had no influence when camu-camu was spray-dried with AG, and a negative effect was observed for camu-camu spray-dried with MD (**Figure 1B**). TP contents were lower than those obtained by Genovese et al. (2008) Chirinos et al. (2010) and Rufino et al. (2010). Such variation might be explained because phenolic compounds are secondary metabolites which overproduce in response to stress growing conditions.

PAC content was the least affected by the spray-drying process (losses between 25 and 64% for both MD and AG, $p<0.05$). The PAC was observed more thermo stable than other bioactive compounds (Fujita *et al.*, 2013). The polynomial equation was deemed predictive only to AG (**Table 3**), which showed similar behavior for AA, more influence of C than T in the response (**Figure 1C**).

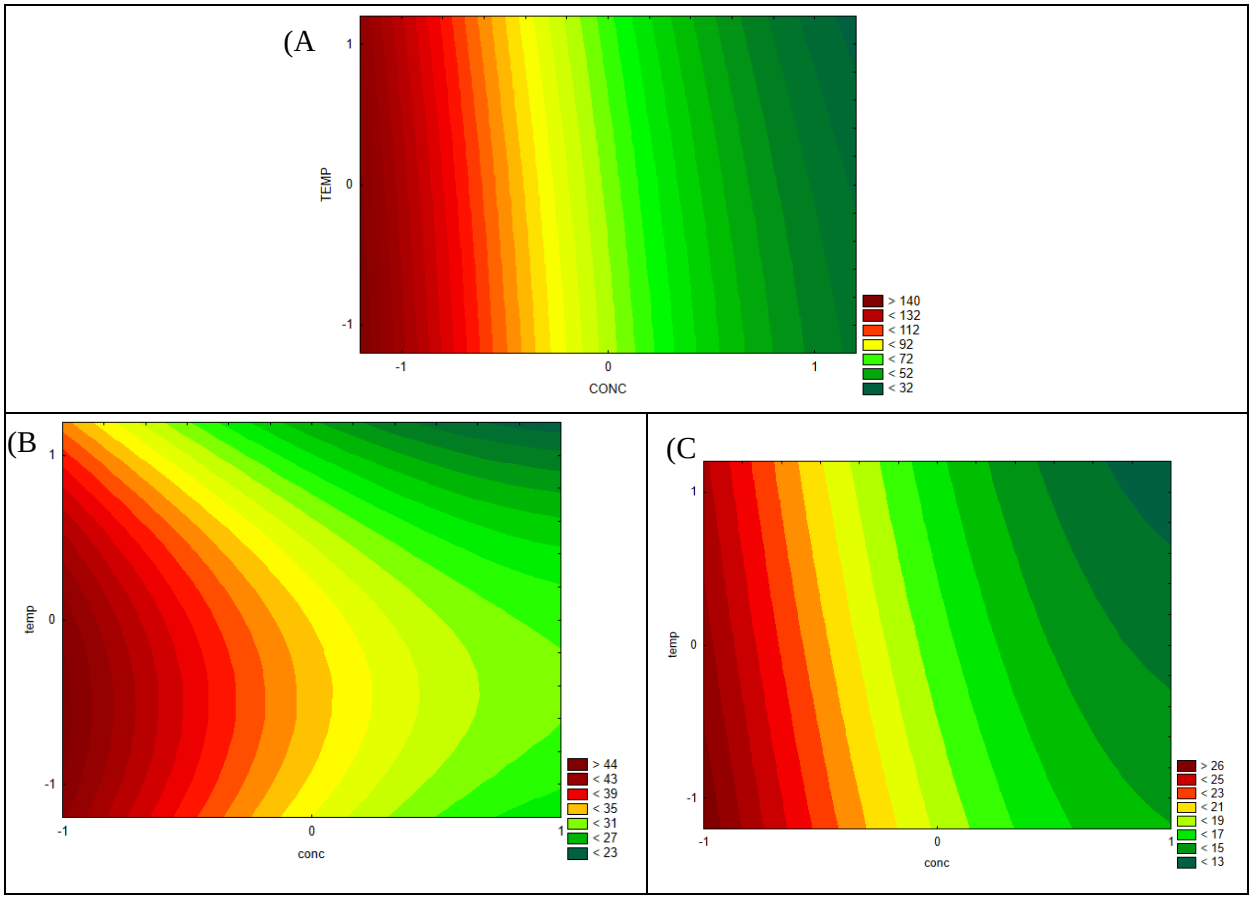

Figure 1. Contour plots for (A) AA, (B) TP and (C) PAC contents of camu-camu spray-dried powders, carrier agents concentration versus inlet air temperature.

3.3 Antioxidant activity

One way to evaluate the potential benefits of bioactive compounds is by measuring their antioxidant activity. Camu-camu powders were tested for antioxidant activity through three methods: FC, FRAP and DPPH (**Tables 1** and **2,** respectively). The greater the TP and AA contents, the higher the antioxidant activity (Pearson's correlation coefficients were around 0.98, **Table 4**). In this context, camu-camu stands out as powerful antioxidant since it is richer in these compounds as compared to many other fruits. For example, in a study carried out by Gonçalves et al. (2010) to compare the antioxidant activity of several fruit pulps, camu-camu showed the highest activity assessed by FC and DPPH methods.

Table 4. Pearson`s correlation coefficient between bioactive compounds and antioxidant capacity for MD and AG powders (P<0.05).

Pearson`s correlation coefficient	Maltodextrin	Arabic gum
AA x FC	0.98	0.97
AA x FRAP	0.99	0.98
AA x DPPH	0.98	0.99
TP x FRAP	0.98	0.98
TP x DPPH	0.99	0.96
PAC x FC	0.96	0.98
PAC x FRAP	0.96	0.96
PAC x DPPH	0.96	0.98

AA, ascorbic acid (g AAE kg^{-1} DW); FC, Folin-Ciocalteu reducing capacity; FRAP, ferric reducing antioxidant power; DPPH, DPPH• scavenging capacity; TP, total phenolics (g GAE kg^{-1} DW); PAC, proanthocyanidins (g QTE kg^{-1} DW).

As expected, freeze-drying process brought about the lowest losses of the *in vitro* antioxidant capacity of camu-camu powders assessed by the three methods (p < 0.05). Except for FRAP, the other two antioxidant capacity values were higher for the spray-dried powders using MD. Losses on the antioxidant activity assessed by DPPH were significant and ranged between around 62-89% and 42-73% for the powders of MD and AG, respectively. Lower losses were observed when antioxidant activity was measured by FC (between about 57-76% for the powders of MD and 21-67% for AG) (**Tables 1** and **2**, p<0.05).

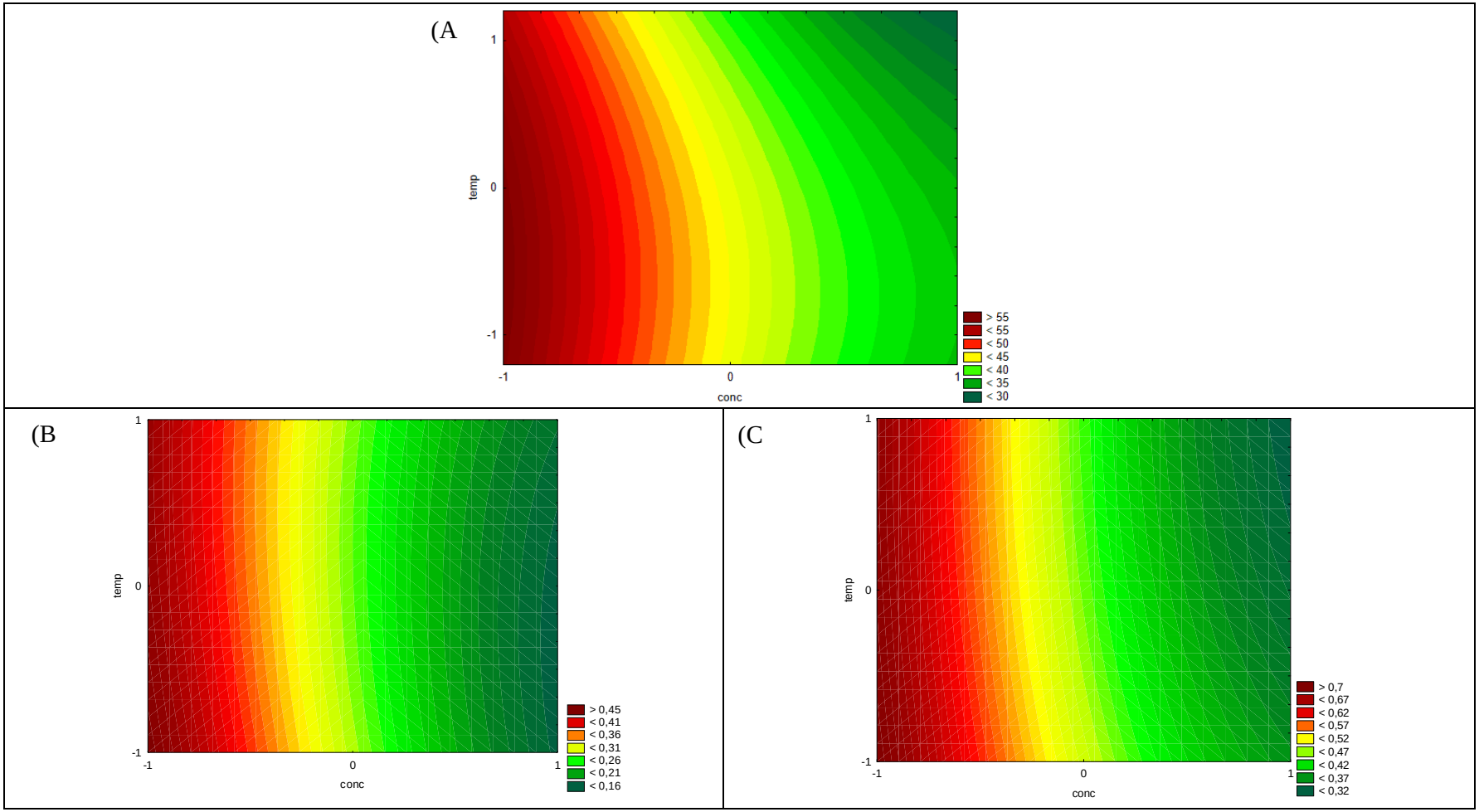

Figure 2. Contour plots for the antioxidant activity by (A) Folin-Ciocalteu reducing capacity for MD; (B) Ferric reducing antioxidant power for AG; (C) DPPH• scavenging capacity for AG, carrier agents concentration versus inlet air temperature.

Polynomial equations were calculated for antioxidant capacity of camu-camu spray-dried powders with MD and AG (**Table 3**). According to those equations, the C affected more significantly the antioxidant capacity than the T, as it can be seen in **Figure 2**. Additionally, the interaction between T and C to AG had positive influence on FC. However, it had negative influence on FRAP for MD addition (**Table 3**). Nevertheless, the model was not predictive for FRAP (arabic gum) once the residues did not follow a normal distribution, meaning the linear model was not efficient in modeling such a response.

Besides of that, the high AA and TP contents in camu-camu powders (**Table 2**) were found to be directly related to their antioxidant capacity, especially for the powders from São Paulo pulp (Pearson's correlation coefficient around 0.99 for all the three methods, **Table 4**). Therefore, the variable that influences bioactive compounds also influences the corresponding antioxidant capacity. Thus, it was expected that T had no significant effect over antioxidant capacity, but the C negatively affected the capacities (**Table 3**). This was also observed by Silva et al. (2014) and Souza et al. (2013) when extracts of jabuticaba peel and bordeaux grape winemaking pomace were spray-dried, respectively.

Moreover, to verify the similarity of all powders in relation to the bioactive compounds and antioxidant activity, HCA was applied and three clusters were suggested. One contained fresh pulp and freeze-dried pulp, other group contained the pulps produced with 6% of carrier agents and the last group contained the camu-camu powders produced with 12 and 18% of carrier agents (**Figure 3**) for both MD and AG. The outputs of multivariate statistical analysis clearly indicate that freeze-drying is the best technological alternative to preserve bioactive compounds. In this sense, the use of 6% of carrier agents, regardless of the temperature used in the manufacturing system, seemed to be the best option to develop camu-camu spray-dried powders with higher contents of bioactive compounds and antioxidant activity.

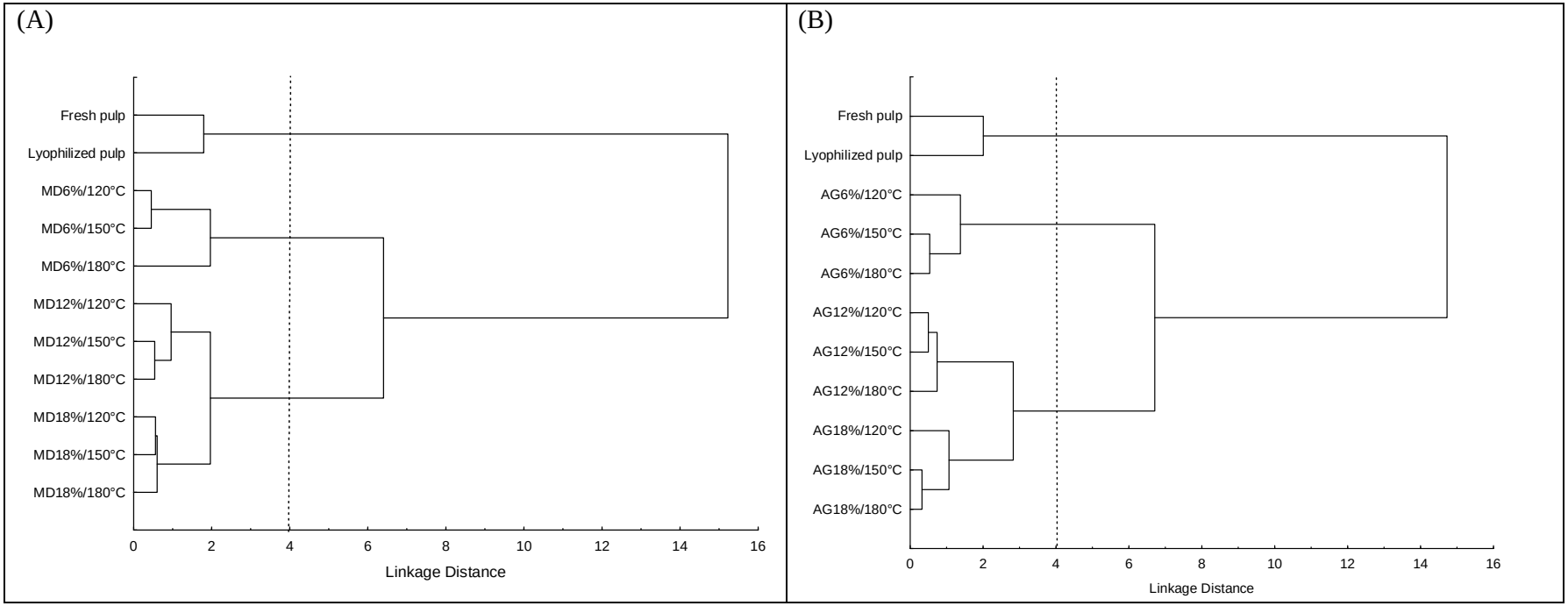

Figure 3. Cluster analysis applied to confirm the similarity among Amazonian pulp using MD (A) and Sao Paulo pulp using AG (B).

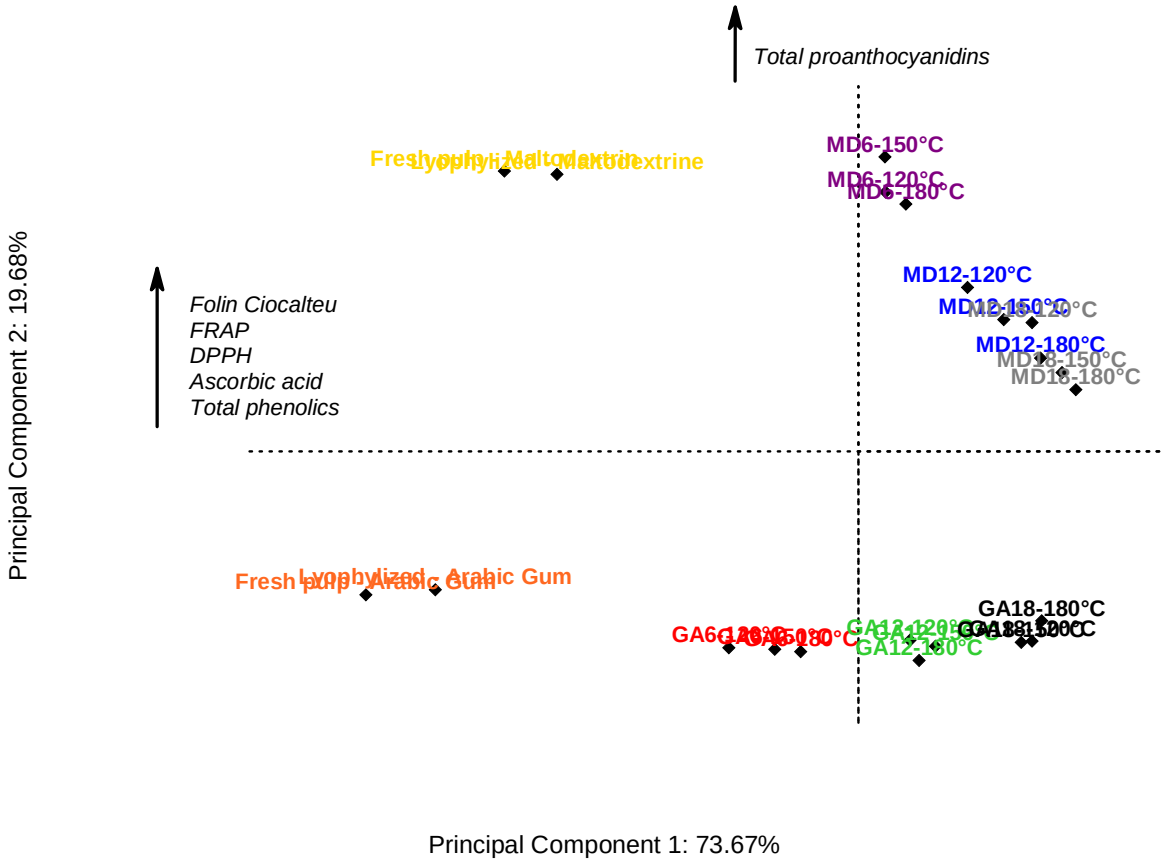

Figure 4. A scatter plot to differentiate samples of camu-camu powders among Amazonian pulp using MD and Sao Paulo pulp using AG.

Additionally, PCA was applied to all results of bioactive compounds and antioxidant capacity for MD and AG (**Figure 4**). The first principal component (PC) explained 73.67% of the variance in experimental data and samples were differentiated on the basis of antioxidant activity (FC, FRAP and DPPH) and content of ascorbic acid, and total phenolic compounds, while the second PC explained another 19.68% of the variance and samples were separated based on proanthocyanidins content. Using this multivariate approach, it is easier to observe differences among samples using all response variables. Besides PCA is not always employed together with RSM, here we demonstrate that their conjoint use may be beneficial to check for distinct and obvious patterns in data when many treatments and responses are assessed.

4. CONCLUSIONS

Camu-camu pulp was spray-dried using different combinations of carrier concentrations and inlet air temperature. Results were subjected to response surface methodology and the effects of these factors could be statistically analyzed. Overall, AG showed slightly better spray-drying yields and solubility than MD. The use of multivariate statistical techniques, namely PCA and HCA, enabled us to conclude that the use of the lowest concentration of carrier agents (6%) in spray-drying resulted in the lowest losses of bioactive compounds and, consequently, the highest antioxidant. Temperature had no significant effect on the bioactive compounds and antioxidant activity. Despite the losses brought about by spray-drying, camu-camu powders preserved high levels of AA, TP and PAC. Given that, the powder could be used as a source of functional ingredients with antioxidant properties.

5. ACKNOWLEDGEMENTS

We would like to thank Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq) for providing a fellowship and CAPES/PNPD for a Post-Doctoral scholarship (D. Granato).

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4.3 Evaluation of phenolic-linked bioactives of camu-camu (*Myrciaria dubia* Mc. Vaugh) for antihyperglycemia, antihypertension, antimicrobial properties and cellular rejuvenation.

Abstract

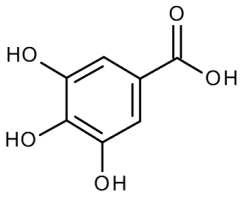
Camu-camu (*Myrciaria dubia* (Kunth) Mc. Vaugh (Myrtaceae)) has shown potential for food and human health applications due to its rich bioactive functional properties linked to high antioxidant activity. The main objective of this study was to identify antioxidant-linked bioactive phenolic profiles in spray-dried and freeze dried camu-camu pulp and to evaluate potential functionality associated with anti-hyperglycemia and anti-hypertension properties using *in vitro* enzyme assay models. Further, antimicrobial properties and planarian-based cellular protection and regeneration were also investigated. Phenolic compounds, such as ellagitannins, ellagic acid, quercetin glycosides, syringic acid and myricetin were detected in camu-camu by LC-TOF-MS. Freeze dried camu-camu powder showed superior phenolic-linked antimicrobial properties and higher hyperglycemia relevant enzyme inhibitory activity compared to the spray dried samples. Besides that, freeze-dried and spray-dried (6% arabic gum at 120 °C) powders were more effective against prokaryotic *Staphylococcus aureus* and showed higher inhibition than ampicillin. Cellular regeneration of eukaryotic planarian model was stimulated with camu-camu powders when compared to control and showed potential of camu camu for redox-linked cellular protection and rejuvenation. Overall camu-camu rich in phenolic bioactive profiles showed superior antidiabetic and antimicrobial properties and has potential as part of dietary strategies in the management of early stages of type 2 diabetes and associated complications.

Keywords: Ellagic acid; *Staphylococcus aureaus*; α-Amylase; α-Glucosidase; ACE; Planaria.

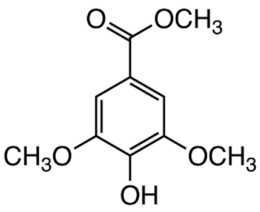
1. INTRODUCTION

Phenolic compounds are secondary metabolites, widely found in fruits, vegetables and grains. These phenolic secondary metabolites have shown significant human health relevant bioactive functionalities (Manach et al., 2005). Some of these phenolic compounds also have antioxidant and anti-inflammatory properties and show potential to counter oxidative stress-induced chronic diseases when consumed as part of the diet. Camu-camu (*Myrciaria dubia* (Kunth) Mc. Vaugh (Myrtaceae)) from Amazon region has high bioactive functionalities, high vitamin C, and rich phenolic profiles, such as flavonoids and ellagitannins (**Figure 1**). The phenolic compounds found in camu-camu are quercetin, cyanidin-3-glucoside, ellagic acid and ellagitannins (Genovese et al., 2008; Gonçalves et al., 2010; Chirinos et al., 2010; Rufino et al., 2010; Fracassetti et al., 2015; Azevedo et al., 2014). This fruit has also shown high antioxidant activity and can be utilized against pathogenic bacteria responsible for human infectious diseases (Fujita et al., 2013, Gonçalves et al., 2010; Inoue et al., 2008). With such a high phenolic bioactive profile and antioxidative potential, camu camu can be incorporated with other functional ingredients and foods for diet based management of oxidative stress linked non-communicable chronic diseases (NCDs), such as type 2 diabetes.

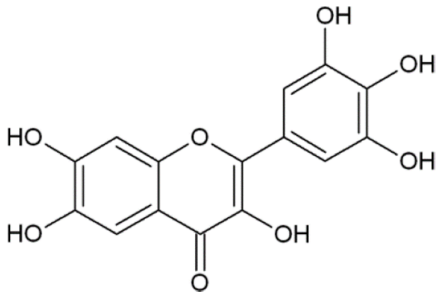
Prevalence of NCDs, including type 2 diabetes and associated cardiovascular diseases are imposing major health care challenges in different countries and across different communities. Diabetes mellitus, especially type 2 diabetes has become an epidemic disease in different parts of the world, causing huge social and economic impacts with rising health care costs. By the year 2030, the population with diabetes cases are estimated to reach over 500 million (Wild et al., 2004), 7^{th} leading cause of death (WHO, 2015). Type 2 diabetes epidemic is a result of genetic and non-genetic factors and can be linked to unhealthy sedentary lifestyles and excess nutrition from diets comprised of refined carbohydrates and fat that cause metabolic breakdown through chronic oxygen malfunction and concurrently induce metabolic syndrome (Oviedo & Beane, 2009). To prevent such health effects it is important to counter with bioactives from plant-based foods with high antioxidant potential that offer cost effective and safe dietary strategies to manage microvascular and macrovascular complications in humans (eukaryote). Further, they have potential to act as antimicrobials to protect against harmful bacteria (prokaryote) under diabetic and immunity breakdown.



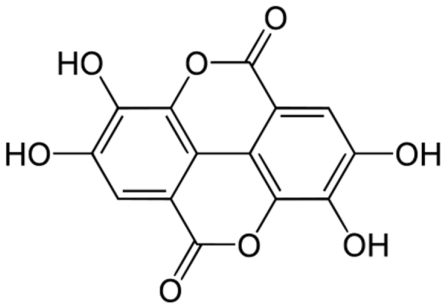
Gallic acid



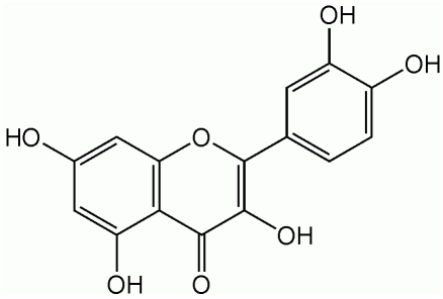
Syringic acid



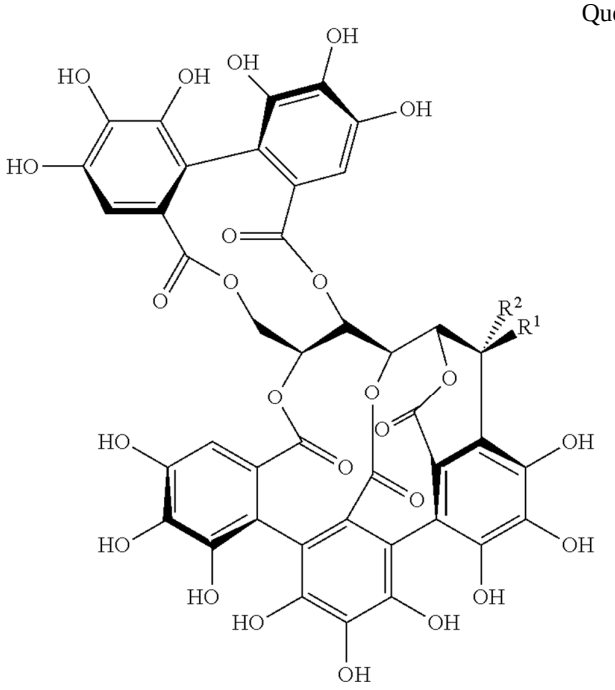
Myricetin



Ellagic acid



Quercetin



1 castalagin R^1 = H, R^2 = OH
2 vescalagin R^1 = OH, R^2 = H

Figure 1. Structures of gallic, syringic and ellagic acids, myricetin, quercetin and ellagitannins (castalagin and vescalagin).

Dietary phenolics with high antioxidant activity have diverse health beneficial properties including antihyperglycemic, antihypertension and antimicrobial. Dietary phenolics from different plant based sources influence glucose metabolism in several ways, such as inhibition of carbohydrate digestion and glucose absorption in the intestine, stimulation of insulin secretion from the pancreatic β-cells, modulation of glucose release from liver, activation of insulin receptors and glucose uptake in the insulin-sensitive tissues, and modulation of hepatic glucose output (Hanhineva et al. 2010; Sarkar & Shetty, 2014). Many *in vitro* studies have been reported indicating that phenolics including flavonoids (anthocyanins, catechins, flavanones, flavonols, flavones and isoflavones), phenolic acids and tannins (proanthocyanidins and ellagitannins) from plant-based foods inhibit starch digesting α-amylase and α-glucosidase enzymes and have potential for management and prevention of early stages type 2 diabetes-linked hyperglycemia and associated cardiovascular complications (Hanhineva et al. 2010).

Based on the above rationale, the primary aim of this study was to evaluate the effect of spray-dried camu-camu pulp comparing to freeze-dried powder on phenolic bioactive profiles identified by LC-TOF-MS, and to study phenolic-linked bioactives functionalities using *in vitro* assay models for antihyperglycemic and antihypertensive properties. Such rapid *in vitro* assay models are health-focused metabolically driven screening of different plant based foods and provide sound biochemical evidence for further validation through clinical and epidemiological studies (Kwon et al., 2007; Ankolekar et al., 2011; Sarkar et al., 2015). These *in vitro* assay models targeted key enzymes (α-amylase, α-glucosidase and angiotensin-I-converting enzyme or ACE) of glucose metabolism and hypertension and are good metabolic indicators to determine the potential role of food bioactives for dietary management of early stage type 2 diabetes-linked hyperglycemia and associated hypertension. The second objective of this study was to investigate camu camu pulp for antimicrobial activities and potential utilization in cellular protection and rejuvenation by determining regeneration of Planaria (*Dugesia* spp.) as a living *in vivo* model. These health-linked functional analyses would help to advance the potential of camu-camu as a functional food ingredient to incorporate in diet based preventative strategies to improve health associated with breakdown towards chronic diseases and infections.

2. MATERIALS AND METHODS

2.1 Materials

Two different commercial pulps of camu-camu (*Myrciaria dubia* Mc. Vaugh): one was purchased from Cupuama do Amazonas Com. Ind. Exp. Ltda. (Manaus, AM, Brazil; latitude 3°8'S and longitude 60°0'W), and another from a commercial plantation located in Registro, SP, Brazil (latitude 24°29'15"S and longitude 47°50'37"W). Both materials were kept frozen (-40 °C for one month) then, defrosted prior to spray-drying.

2.2 Spray-drying

Spray-drying was performed in a pilot scale spray-dryer (Labmaq, SD 5.0, Brazil). The pulp was fed by a peristaltic pump at a fixed rate of 44 mL/min and was spray-dried at different inlet air temperatures (120, 150 and 180 °C) and with different carrier agent concentrations (6, 12 and 18%). Two carrier agents were employed: maltodextrin MOR-REX® 1910 (9≤DE≤12) (Corn Products, Brazil) for Amazonian pulp and gum arabic (Nexira Brazil Com. Ltd., Brazil) for Sao Paulo pulp.

2.3 Freeze-drying

Two kilograms of frozen pulp were lyophilized in a Pironi 501 freeze-drier (Thermo Electron Corporation, New York, USA) at -80°C and 100 mTorr for 120 hours.

2.4 LC-TOF-MS analysis to determine phenolic profiles

For extraction, one gram of each powder (freeze-dried and spray-dried) was homogenized with 70% (v/v) aqueous methanol using a vortex (Vortex Genie2, G-560, Scientific Industries, Bohemia, NY, USA) and then sonicated for 10 min (Sonicator QSonica 5510R-MTH, Ultrasonic Cleaner, Newtown, CT, USA). The extracts were filtered using 25 mm syringe filter (0.45 µm Nylon Membrane). The extracts were characterized with Waters (Milford, MA, USA) Alliance 2695 LC system equipped with a 2695 separation module unit and a 2998 PDA detector using a 100 × 2.0 mm, 2.5 µm Phenomenex Synergi Hydro-RP 100A column with 3 × 4.0 mm Phenomenex Security Guard column (Torrance, CA USA). The mobile phase consisted of solvents (A) 0.1% aqueous formic acid solution and (B) Methyl cyanide (MeCN). Gradient conditions were performed as follow: from 0% to 6% B in 5 min, from 6% to 22% B until 30 min, from 22% B to 30% B until 40 min and from 30% to

40% B to 50 min, followed by a final increase to 95% in 2 min. The flow rate and the injection volume were 0.2 mL/min and 10 µL, respectively.

High-resolution mass spectrometry was performed using an LCT premier XE TOF mass spectrometer (Waters, Milford, MA) equipped with an ESI interface and controlled by MassLynx V4.1 software. Mass spectra were acquired in both positive and negative modes over range *m/z* 100–1000. The capillary voltages were set at 3000V (positive mode) and 2700V (negative mode), respectively, and the cone voltage was 30V. Nitrogen gas was used for both the nebulizer and in desolvation. The desolvation and cone gas flow rates were 600 and 20 L/h, respectively. The desolvation temperature was 400 °C, and the source temperature was 120 °C.

2.5 Extract Preparation

One gram of powdered sample was added to 100 mL of distilled water and mixed using a homogenizer Tissue (Tearor MD 985370-395, Biospec Products, Mexico), at moderate speed for 5 min, while cooled in ice. The extracts were then centrifuged at 8,500 *g* for 30 min and it was stored at 10 °C until analysis. All extractions were done in duplicate, and the subsequent assays were run in triplicate.

2.6 Antimicrobial assay and determination of Minimum Inhibitory Concentration (MIC)

The extracts were tested for activity against: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 8739, *Enterobacter aerogenes* ATCC 13048, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 14028 and *Salmonella enteritidis* ATCC 13076. According to CLSI (CLSI, 2009) the cultures were grown in Trypton Soya Agar (Oxoid, Basingstoke, UK) for 18-24h at 37 °C, and the colonies were suspended in sterile saline solution (0.85%) to reach a turbidity correspondent to 0.5 of the McFarland scale (10^8 UFC mL^{-1}). The suspensions (0.1 mL) were applied to the surface of Muller-Hinton agar plates (Oxoid, Basingstoke, UK) and sterile discs of 13 mm in diameter were placed on the plates. The discs were filled with 100 µL of extracts, and the plates were incubated at 37 °C for 24 hours, when the diameters of the inhibition zones were measured using a calipter rule. Erytromycin (15 µg) and Vancomycin (5 µg) (Oxoid, Basingstoke, UK) were used as a positive controls. Results were evaluated according to the following scale: <16 mm, inactive; 17 – 19 mm, partially active; 20 – 25 mm, active; >25 mm, very active.

For determination of the MIC, the microdilution method was used (CLSI, 2010). Except for the first row, the wells of sterile 96-well microplates were filled with 50 µL of

Muller-Hinton broth (Oxoid, Basingstoke, UK). The wells of the first row of were filled with 100 µL of extracts, homogenized and then 50 µL were serially transferred to the subsequent wells. Finally, 50 µL of the microorganisms solution (10^8 UFC mL^{-1}) were added. The microplates were incubated at 37 °C for 24 h and the MIC, correspondent to the lowest concentration that inhibited the visible growth of the microorganism after 24 h. Ampicillin was used as a positive control. The tests were carried out in two repetitions.

2.7 α-amylase inhibition

The α-amylase inhibitory activity was determined by an assay modified from the *Worthington Enzyme Manual* (Worthington Biochemical Corp. 1993a). A total of 500 µL of each extract and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25 °C for 10 min. After pre incubation, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after the addition of 15 mL of distilled water, and absorbance was measured at 540 nm using a spectrophotometer (Genesys UV-visible, Milton Roy, Inc.). The readings were compared with the controls, containing buffer instead of sample extract. The results were calculated as percent α-amylase inhibition and calculated according to eq 1. Then, expressed as mg of sample per milliliters of reaction time to inhibit 50% of enzyme (IC 50).

$$\% \text{ Inhibition} = [(A540^{control} - A540^{extract}) / A540^{control}] \times 100 \qquad \text{(eq. 1)}$$

2.8 α-glucosidase inhibition

The assay was performed according to the *Worthington Enzyme Manual* (1993b), with some modifications (Mc Cue et al., 2005). Alpha-Glucosidase (1 unit/mL) was assayed by using 50 μL of aqueous camu-camu extracts and 100 μL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution and was incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 10 min. Before and after incubation, absorbance readings were recorded at 405 nm by microplate reader (Spectra Max 190, Molecular Device Co.CA) and compared to a control that had 50 μL of buffer solution in place of the extract. The results

were calculated as percent of α-glucosidase inhibition and calculated according to equation 2. Then, expressed as mg of sample per milliliters of reaction time to IC50.

% inhibition = $[(Abs^{control}5min - Abs^{control}0min) – (Abs^{extract}5min - Abs^{extract}0\ min) / (Abs^{control}5min - Abs^{control}0min)] \times 100$ (eq. 2)

2.9 Angiotensin converting enzyme (ACE) inhibition

ACE inhibition was assayed according to a method modified by Kwon et al. (2006). The substrate hippuryl-histidylleucine (HHL) and ACE-I from rabbit lung (1 unit produces 1.0 μmol of hippuric acid from HHL per minute in 50 mM HEPES and 300mM NaCl at pH 8.3 at 37 °C) were bought (Sigma-Aldrich, St. Louis, MO, USA) and used. Fifty microliters of water soluble supernatant was incubated with 100 μL of 1M NaCl-borate buffer (pH 8.3) containing 2 *milli*units of ACE-I solution at 37 °C for 10 min. After pre-incubation, 100 μL of a 5milliunit substrate (HHL) solution was added to the reaction mixture. Test solutions were incubated at 37 °C for 1 h. The reaction was stopped with 150 μL of 0.5 N HCl. The hippuric acid formed was detected; the spectra were confirmed and quantified by high-performance liquid chromatography (HPLC). Five microliters of the sample was injected using an Agilent ALS 1100 autosampler into an Agilent 1260 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with a DAD1100 diode array detector. The solvents used for the gradient were (1) 10 mM phosphoric acid (pH 2.5) and (2) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for 5 min and then was decreased to 0% for the next 5 min (18 min total run time). The analytical column used was a Nucleosil 100-5 C18, 250 × 4.6 mm i.d., with packing material of 5 μm particle size at a flow rate of 1 mL/min at ambient temperature. During each run, the absorbance was recorded at 228 nm and the related chromatogram was integrated using Agilent Chemstation (Agilent Technologies) enhanced integrator for detection of liberated hippuric acid (A). Pure hippuric acid (purchased from Sigma Chemical Co.) was used to calibrate the standard curve and retention time. The percent inhibition was calculated according to eq. 3:

% Inhibition = $[(\underline{A^{control} – A^{blank}}) – \underline{A^{sample}} / (A^{control} – A^{blank})] \times 100$ (eq. 3)

2.10 Regeneration of black planaria (Dugesia tigrina)

The black planaria were kept and acclimatized for 30 days at room temperature in an aquarium with artificial air pump, fed once a week with fresh liver meat. For the regeneration studies, worms (n = 15) were fed with liver meat with aqueous extracts of different camu-camu powders (10 mg meat dipped into different concentrations of camu camu extracts, 10,

20 and 30 µL of 10 mg/mL for 5 minutes). After one day, worms were cut into cephalic and caudal sections and were collected in two separate fresh water Petri plates at room temperature. Samples were measured every other day for 3 weeks using compound microscope and plotting done in graphical scale. The results were calculated and reported as percentage of regeneration.

2.11 Statistical Analysis

All analyses were run in triplicate and results were expressed as mean ± standard deviation (SD). For statistical analysis, the Statistic software package version 11.0 (StatSoft, Inc., Tulsa, OK) was employed. Differences between means were first analyzed by ANOVA test and then Tukey test ($p< 0.05$). Chemometric data analysis was performed according to Wu et al. (2012), principal component analysis used Markerlynx v4.1 software.

3. RESULTS AND DISCUSSION

3.1 LC-TOF-MS phenolics profile analysis of camu-camu powders

The phenolic composition of freeze-dried and spray-dried camu-camu powders were tentatively identified (**Figure 2)** by their retention times, UV spectra, and exact mass spectra using fragmental ions in both positive and negative modes, and also by comparison with data found in the literature (Fracassetti et al., 2013; Wu et al., 2012) which were shown in **Table 1**. According to **Table 1** and **Figure 2**, it was observed that Amazonian and Sao Paulo pulps have slightly different phenolic compositions. Overall, ellagitannins, such as vescalagin, castalagin, casuarictin, potentillin, and also, ellagic acid, quercetin glycosides, myricetin, myricitin glycosides and syringic acid were detected in both of those fruit extracts. However, cyanidin-3-O-glucoside was detected only in pulp from Sao Paulo, and gallic acid derivatives were detected only in Amazonian pulp (**Figure 2**). The compounds identified are similar to those reported by Fracasseti et al. (2013) and Wu et al. (2012) who analyzed camu-camu and jaboticaba (Plinia cauliflora (Mart.) Kausel, Myrtaceae) fruit, respectively. The reason for not finding anthocyanins in Amazonian pulp could be due to higher proportion of unripe to ripe content in the purchased sample.

Principal component analysis (PCA) was used to determine similarities among powders in groups with same characteristics, providing “visual description” of clustering and differences or outliers groups. PCA confirmed that Sao Paulo pulp, freeze-dried and 6% of

Table 1. Phenolic compounds identified by HPLC-TOF-MS analysis of camu-camu powders.

Number	Compounds	RT (min)	$[M-H]^-$	$[M-H]^+$	λ_{max} (nm)	Formula	Samples detected	Note
1	Vescalagin	3.4	933.0527	935.0923	246	$C_{41}H_{26}O_{26}$	PAM, PSP	Reported Fracassetti et al., 2013
2	Castalagin	5.0	933.0656	935.0919	246	$C_{41}H_{26}O_{26}$	PAM,PSP	Reported Fracassetti et al., 2013
3	Di-HHDP-galloyl-glucose (casuarictin/potentillin)	16.4	935.0833	937.1047	240, 270	$C_{41}H_{28}O_{26}$	PAM,PSP	Reported Fracassetti et al., 2013; Wu et al., 2012
4	Di-HHDP-galloyl-glucose (casuarictin/potentillin)	17.6	935.0717	937.1036	240, 270	$C_{41}H_{28}O_{26}$	PAM	Reported Fracassetti et al., 2013
5	Cyanidin 3-O-glucoside	18.5	447.0938	449.1088	520	$C_{21}H_{21}O_{11}$	PSP	Reported Fracassetti et al., 2013
6	Gallic acid derivative	23.9	569.0947	571.1135	216, 271	$C_{27}H_{38}O_{13}$	PAM	Reported Fracassetti et al., 2013
7	Myricetin 3-O-hexoside	25.7	479.0838	481.0961	264, 358	$C_{21}H_{20}O_{13}$	PAM,PSP	Reported Fracassetti et al., 2013
8	Myricetin 3-O-pentoside	27.1	449.0743	451.0898	258, 356	$C_{20}H_{18}O_{12}$	PAM,PSP	Reported Fracassetti et al., 2013
9	Ellagic acid hexoside	27.7	463.2127	465.1539	255, 362	$C_{20}H_{16}O_{13}$	PAM,PSP	Reported Fracassetti et al., 2013
10	Cyanidin 3-O-glucoside	28.1	447.0567	449.1778	520	$C_{21}H_{21}O_{11}$	PSP	Reported Fracassetti et al., 2013
11	Myricetin 3-O-pentoside	28.8	449.0701	451.0866	258, 356	$C_{20}H_{18}O_{12}$	PAM,PSP	Reported Fracassetti et al., 2013
12	Ellagic acid	29.2	300.9941	303.0154	254, 364	$C_{14}H_6O_8$	PAM,PSP	Reported Fracassetti et al., 2013; Wu et al., 2012
13	Quercetin 3-O-hexoside	30.4	463.0913	465.1049	255, 362	$C_{21}H_{20}O_{12}$	PAM, PSP	Reported Fracassetti et al., 2013
14	Quercetin 3-O-pentoside	31.6	433.0791	435.1058	254,360	$C_{20}H_{18}O_{11}$	PAM,PSP	Reported Fracassetti et al., 2013
15	Gallic acid derivative	33.5	569.0941	571.1154	216, 271	$C_{27}H_{38}O_{13}$	PAM	Reported Fracassetti et al., 2013
16	Syringic	34.5	371.1298	373.1517	249	$C_{17}H_{24}O_9$	PAM,PSP	Reported Wu et al., 2012
17	Myricetin	35.7	317.0293	319.0477	252, 372	$C_{15}H_{10}O_8$	PAM,PSP	Reported Fracassetti et al., 2013
18	Gallic acid derivative	35.9	569.2196	571.2356	216, 271	$C_{27}H_{38}O_{13}$	PAM	Reported Fracassetti et al., 2013
19	Ellagic acid derivative	39.4	719.2197	721.2391	254, 360	$C_{34}H_{40}O_{17}$	PAM,PSP	Reported Fracassetti et al., 2013
20	Ellagic acid derivative	41.3	719.2149	721.2100	254,360	$C_{34}H_{40}O_{17}$	PAM,PSP	Reported Fracassetti et al., 2013
21	Ellagic acid derivative	42.8	719.2187	721.2377	254,360	$C_{34}H_{40}O_{17}$	PAM,PSP	Reported Fracassetti et al., 2013

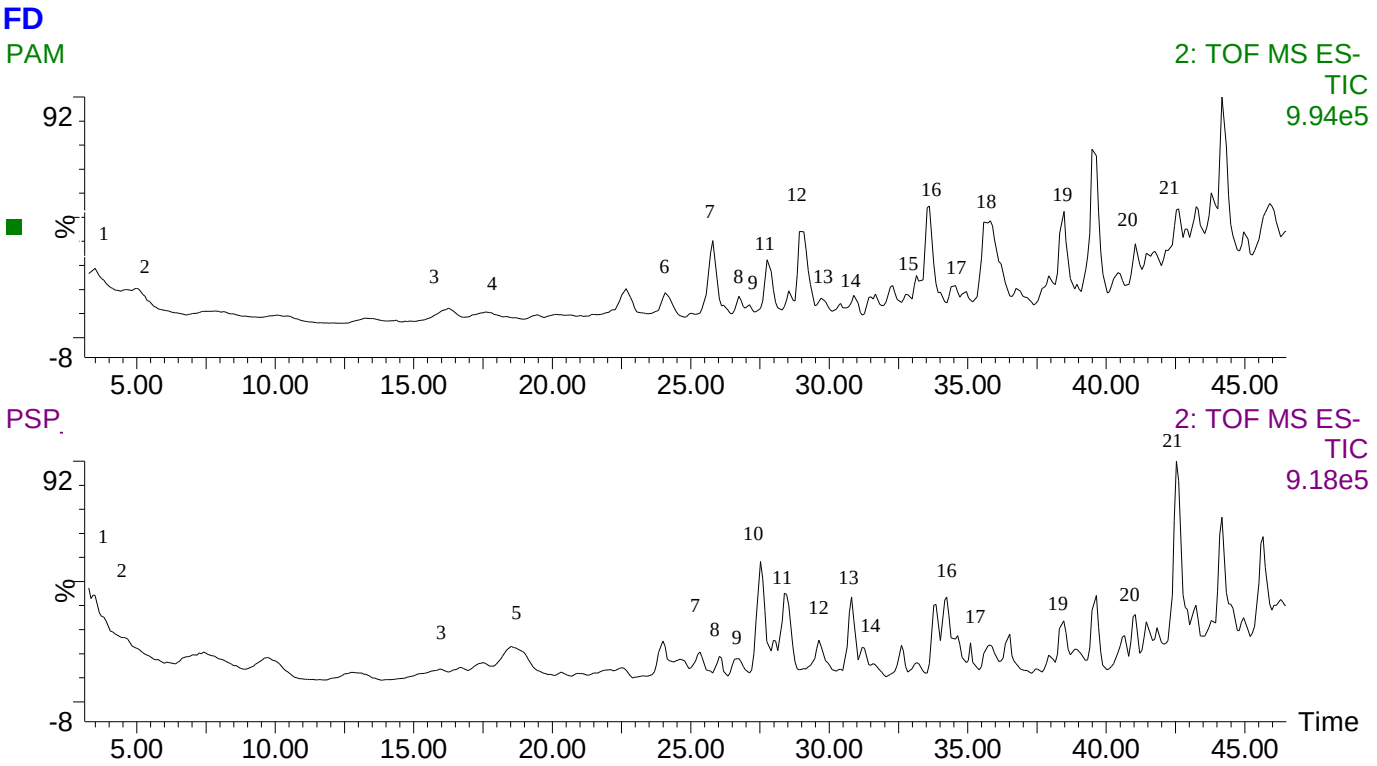


Figure 2. Chromatogram profile of two freeze-dried powders: Amazonian pulp (PAM) and Sao Paulo pulp (PSP). 1, vescalagin; 2, castalagin; 3 and 4, Di-HHDP-galloyl-glucose (casuarictin/potentillin); 5 and 10, cyanidin 3-O-glucoside; 6, 15 and 18, gallic acid derivative; 7, myricetin 3-O-hexoside; 8, myricetin 3-O-pentoside; 9, ellagic acid hexoside; 11, myricetin 3-O-pentoside; 12, ellagic acid; 13, quercetin 3-O-hexoside; 14, quercetin 3-O-pentoside; 16, syringic acid; 17, myricetin; 19, 20 and 21, ellagic acid derivatives.

arabic gum spray-dried powders have high concentration of anthocyanins and Amazonian pulp has high ellagic acid contents (**Figure 3B).**

In order to verify the influence of drying process, it was chosen major phenolic compounds (myricetin, syringic, ellagic acid, casuarictin/potentilin and vescalagin/castalagin) and then determined the relative concentration of those phenolic compounds considering the values in the respective freeze-dried pulps as 100% **(Figure 4A and B**). The results show that Sao Paulo pulp (average of losses were 55% to 87%) was more susceptible to losses and degradation of phenolic compounds than Amazonian pulp (average of losses between 33% and 72%) during spray-drying process. However, such differences could be due to the added adjuvants or microencapsulation of the compounds. Overall, no significant differences were observed between different inlet air temperatures of drying ($p<0.05$). However, addition of Arabic gum showed statistically significant differences between 6% and other concentrations (12 and 18%, $p<0.05$). Such differences could be confirmed in **Figure 3A,** where we applied PCA to LC-TOF-MS data for all powders together and formed three respective groups: one was Amazonian freeze-dried and all their respective spray-dried Amazonian pulp with maltodextrin; another group contained Sao Paulo freeze-dried and 6% of Arabic gum spray-dried powders and the third was spray-dried Sao Paulo pulp with 12 and 18% of Arabic gum powders.

However, when orthogonal partial least squares discriminant analysis (OPLS-DA) was applied separately to each pulp, Amazonian pulp showed two different groups: one was freeze-dried powder and other was all spray-dried powders (data not shown). In contrast, Sao Paulo pulp showed three groups: freeze-dried powder, 6% of Arabic gum and finally, 12% and 18% of Arabic gum together (**Figure 3C**). Overall, this analysis showed Amazonian pulp did not have changes in phenolic composition under the evaluated range of inlet air temperature or concentration of maltodextrin. However, arabic gum concentrations seemed to influence the phenolic composition of camu-camu pulp from Sao Paulo.

The phenolic composition of freeze-dried and spray-dried camu-camu powders were tentatively identified (**Figure 1)** by their retention times, UV spectra, and exact mass spectra; fragmental ions in both positive and negative modes, and also by comparison with data found in the literature (Fracassetti et al., 2013; Wu et al., 2012) which were shown in **Table 1**. According to **Table 1** and **Figure 1**, it was observed that Amazonian and Sao Paulo pulps have slightly different phenolic compositions. Overall, ellagitannins, such as vescalagin, castalagin, casuarictin, potentillin, and also, ellagic acid, quercetin derivatives, myricetin, myricitin derivatives and syringic acid were detected in both of those fruit extracts. However,

cyanidin-3-O-glucoside was detected only in pulp from Sao Paulo, and a gallic acid derivatives were detected only in Amazonian pulp (**Figure 1**). The compounds identified are similar to those reported by Fracasseti et al. (2013) and Wu et al. (2012) who analyzed camu-camu and jaboticaba fruit, respectively. The reason for not finding anthocyanins in Amazonian pulp could be due to higher proportion of unripe parts than ripe in the purchased sample, and also, it might be due to degradation and oxidation during transportation.

Principal component analysis (PCA) was used to determine similarities among powders in groups with same characteristics, providing "visual description" of clustering and differences or outliers groups. PCA confirmed that Sao Paulo pulp, freeze-dried and 6% of arabic gum spray-dried powders have high concentration of anthocyanins and Amazonian pulp has high ellagic acid contents (**Figure 2B).**

In order to verify the influence of drying process, the relative concentration of phenolic compounds were determined considering the values in the respective pulps as 100% **(Figure 3A and B**). The results show that Sao Paulo pulp was more susceptible to losses and degradation of phenolic compounds than Amazonian pulp during spray-drying process. However, such differences could be due to the added adjuvants or microencapsulation of the compounds. Overall, no significant differences were observed between different inlet air temperatures of drying ($p<0.05$). However, addition of Arabic gum showed statistically significant differences between 6% and other concentrations (12 and 18%, $p<0.05$). Such differences could be confirmed in **Figure 2A,** where we applied PCA to LC-TOF-MS data for all powders together and formed three respective groups: one was Amazonian freeze-dried and all their respective spray-dried Amazonian pulp with maltodextrin; another group contained Sao Paulo freeze-dried and 6% of Arabic gum spray-dried powders and the third was spray-dried Sao Paulo pulp with 12 and 18% of Arabic gum powders.

However, when orthogonal partial least squares discriminant analysis (OPLS-DA) was applied separately to each pulp, Amazonian pulp showed two different groups: one was freeze-dried powder and other was all spray-dried powders (data not shown). In contrast, Sao Paulo pulp showed three groups: freeze-dried powder, 6% of Arabic gum and finally, 12% and 18% of Arabic gum together (**Figure 2C**). Overall, this analysis showed Amazonian pulp did not have changes in phenolic composition under the evaluated range of inlet air temperature or concentration of maltodextrin. However, arabic gum concentrations could influence the phenolic composition of camu-camu pulp from Sao Paulo.

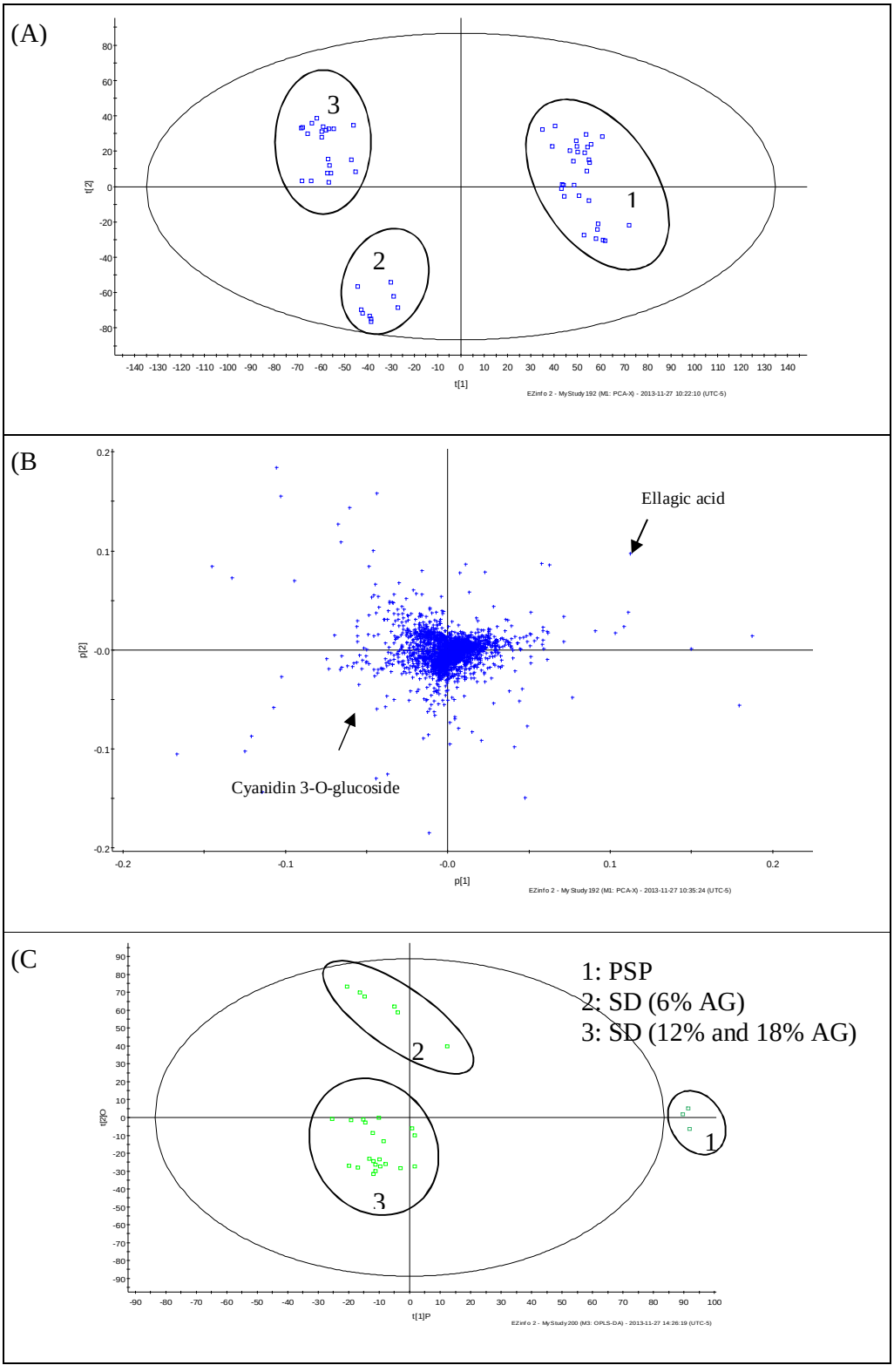


Figure 3. Score (A) and loading plots (B) of freeze-dried and spray-dried powders from Amazonian and Sao Paulo pulps; OPLS_DA plot (C) of freeze-dried and spray-dried powders of camu-camu pulp from Sao Paulo.

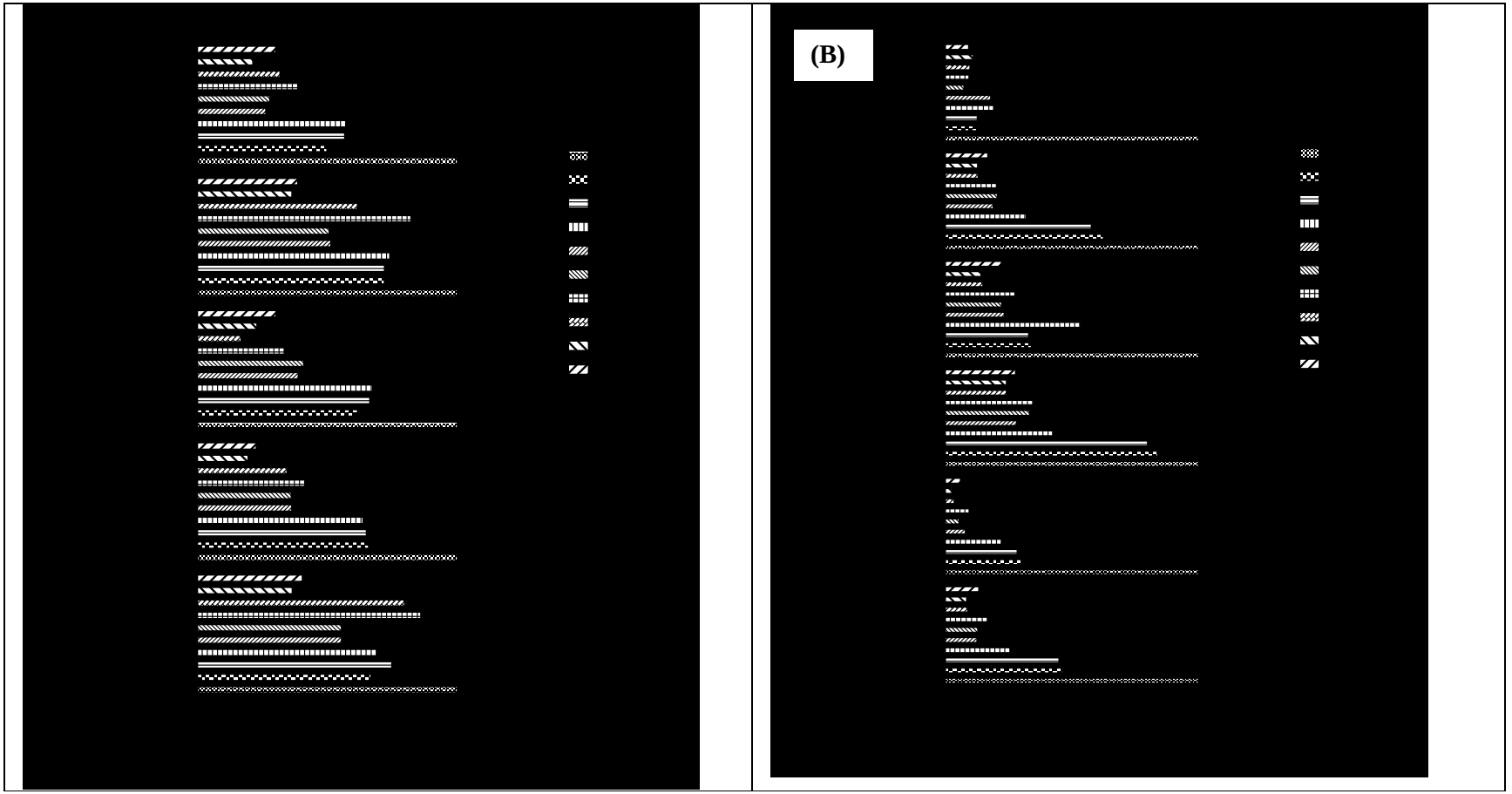


Figure 4. Relative phenolic concentrations of camu-camu powders from Amazonian pulp with maltodextrin (A) and Sao Paulo pulp with arabic gum (B), using the respective freeze-dried powder as a control (100% peak area). Values are expressed as means ± SD (n=3). a,b,c,d means in the same compound followed by different superscripts are significantly different ($p<0.05$).

3.2 α-amylase and α-glucosidase inhibition

It is well-known that α-amylase and α-glucosidase are enzymes directly linked to soluble carbohydrate digestion and associated glucose metabolism, and inhibition of these enzymes helps to reduce postprandial blood glucose levels. Previous *in vitro* studies have shown the potential of phenolic-rich plant-based food extracts for higher inhibition of these enzymes (Hanhineva et al., 2010; Kwon et al., 2006; Gonçalves et al., 2010; Anhê et al, 2013). In this study, potential inhibitory activities of these two early stage digestive enzymes were investigated with aqueous extracts of spray-dried and freeze-dried camu-camu powders.

During carbohydrate digestion, pancreatic α-amylases hydrolyze α1-4 glucosidic linkages and also release oligosaccharides with α-1-6-oligomers (Hanhineva et al., 2010). All camu-camu powders analyzed were less efficient for α–amylase inhibition than acarbose (**Table 2**, IC50 = 3.05 µg/mL). Moreover, spray-dried powders showed less inhibition than freeze-dried powders, and the inhibitory activity decreased when the concentration of carrier agents were increased. Such decrease in α-amylase inhibition might be due to losses during drying processing, microencapsulation of compounds responsible for this enzyme inhibition, therefore difficulty to extract them, it may require more time to release from the structure and also, to act (Fujita et al, 2013).

Table 2. IC50 of α-amylase and α-glucosidase inhibition of extracts of camu-camu powders produced by spray-drying.

Drying conditions		α-amylase (µg.mL^{-1} of reaction)		α-glucosidase (µg.mL^{-1} of reaction)	
		Amazonian	Sao Paulo	Amazonian	Sao Paulo
Acarbose		3.05 ± 0.25^{a}		152 ± 47^{g}	
Freeze-dried		359 ± 105^{b}	299 ± 152^{b}	5.57 ± 1.05^{b}	2.98 ± 1.12^{a}
6%	120 °C	1129 ± 337^{cd}	825 ± 222^{c}	5.13 ± 3.39^{b}	4.28 ± 1.2^{b}
	150 °C	1045 ± 313^{cd}	940 ± 189^{c}	7.03 ± 3.83^{c}	5.78 ± 0.77^{bc}
	180 °C	1098 ± 163^{cd}	937 ± 191^{c}	4.50 ± 3.34^{b}	4.05 ± 0.87^{b}
12%	120 °C	4361 ± 205^{e}	1365 ± 363^{d}	8.92 ± 3.75^{d}	20.74 ± 0.52^{e}
	150 °C	5113 ± 850^{e}	1668 ± 86^{d}	10.83 ± 4.18^{d}	20.18 ± 1.18^{e}
	180 °C	7782 ± 191^{e}	1654 ± 160^{d}	7.86 ± 1.07^{c}	19.06 ± 2.81^{e}
18%	120 °C	ni	11563 ± 242^{f}	9.54 ± 3.52^{d}	26.74 ± 4.54^{f}
	150 °C	ni	11254 ± 174^{f}	8.31 ± 4.15^{d}	22.98 ± 5.11^{ef}
	180 °C	ni	11519 ± 241^{f}	6.58 ± 1.78^{bc}	26.88 ± 1.34^{f}

Values are expressed as means ± SD (n=3). a,b,c,d. followed by different superscripts in the same column indicate significant difference ($p<0.05$). ni = no inhibition in the evaluated concentration.

Further, during the carbohydrate digestion, α-glucosidase hydrolyze terminal α1-4-linked glucose and release glucose in small intestine. Therefore, it is necessary to inhibit this enzyme to decrease absorption of glucose in the small intestine (Hanhineva, 2010). Compared to acarbose (IC50 = 152 µg/mL), all camu-camu powders are highly effective in inhibiting α-glucosidase (**Table 2**). No other fruits analyzed until now, such as strawberry (high inhibition at 25 mg/mL), tea (600 µg/mL), raspberry (200 µg/mL) and pear (500 mg/mL) had same inhibitory potential as camu-camu (50 µg/mL) observed in this study (Pinto et al., 2008, Wang et al., 2012, Cheplick et al., 2007, Barbosa et al., 2013, Sarkar et al. 2015).

For safe and effective management of postprandial hyperglycemia linked to type 2 diabetes, plant-based functional food should have moderate α-amylase and high α-glucosidase inhibitory activities (Pinto et al. 2008). The natural enzyme inhibitors from fruits and vegetables with the above mentioned combination of properties provide health benefits without any side effects such as flatulence, diarrhea and abdominal distention typically caused by drugs (Matsui et al. 2001; Kwon et al. 2006; McDougall et al. 2005; Kotowaroo et al. 2006). This result clearly showed that camu-camu has the potential to be incorporated in functional foods or in dietary strategies for safe and cost-effective management of early stage type 2 diabetes and associated complications.

Different phenolic compounds have shown inhibitory activity against these important enzymes linked to glucose metabolism. McDougall et al. (2005) concluded that soluble tannins are effective in α-amylase inhibition, while anthocyanins have α-glucosidase inhibitory activity. Moreover, You et al. (2012) showed that ellagic acid (IC50 = 2.18 µg/mL) and quercetin (IC50 = 15.2 µg/mL) exhibited α-glucosidase inhibition. In this study, we found that different Pearson's correlation coefficients (**Table 4**, $p<0.05$) for Amazon and Sao Paulo powders. For example, to Amazon powders and α-amylase inhibition had good correlation between all analyzed phenolic compounds, although, Sao Paulo powders did not have good correlation for myricetin (R = 0.52). Those variations may be due to different quantity of those compounds in both powders (**Figure 4**) that could affect inhibition and synergism effects between compounds, differently.

Alpha-glucosidase inhibition had good correlation to ellagitannins and ellagic acid in both powders (Pearson's correlation coefficient around 0.7, **Table 4**). However, syringic acid had more influence to Sao Paulo than Amazon powders (R = 0.83 and 0.57, respectively). Since ellagic acid was the major phenolic compound, found in camu-camu fruit (Fracassetti et al., 2013) it is potentially possible that loss of ellagic acid (**Figure 4**) could be responsible for

the variations in enzyme inhibition with different camu camu powders, although others possibilities pointed out above, were also considered.

3.3 Angiotensin Converting Enzyme-I (ACE) inhibition

ACE inhibition helps manage hypertension, which is an associated risk factor in type 2 diabetes (Pinto et al., 2008). Some studies have mentioned that rich-phenolic extracts may prevent risk of developing high blood pressure (Pan et al., 2010, Edwards et al., 2007). Although no ACE enzyme inhibition was observed in the 10 mg/mL aqueous extracts of camu-camu powders further analysis with more concentrated extracts may be needed to prove the findings.

Previously, Kwon et al. (2006) found that pure compounds of quercetin, ellagic acid and chlorogenic acid did not show any ACE inhibitory activity. Camu-camu has high concentrations of ellagic acid as a major phenolic compound; this could be one reason for not having ACE inhibitory activity in investigated camu-camu extracts.

Table 3. Antimicrobial activity and minimum inhibitory concentrations (MIC) of extracts of camu-camu powders produced by spray-drying against *S. aureus* strains.

Drying conditions		Maltodextrin (Amazonian Pulp)		Arabic gum (Sao Paulo Pulp)	
		Inhibition (mm)	MIC of extracts ($mg.mL^{-1}$)	Inhibition (mm)	MIC of extracts ($mg.mL^{-1}$)
Freeze-dried		25 ± 3^{b}	0.08^{a}	29 ± 0^{b}	0.08^{a}
6%	120 °C	17 ± 2^{cdef}	0.31^{c}	19 ± 1^{c}	0.16^{b}
	150 °C	19 ± 0^{c}	0.31^{c}	19 ± 1^{c}	0.16^{b}
	180 °C	15 ± 0^{f}	0.31^{c}	18 ± 1^{cd}	0.31^{d}
12%	120 °C	17 ± 1^{de}	0.63^{d}	18 ± 1^{cd}	0.31^{d}
	150 °C	16 ± 0^{e}	0.63^{d}	16 ± 1^{de}	0.31^{d}
	180 °C	16 ± 1^{def}	0.63^{d}	17 ± 2^{cde}	0.31^{d}
18%	120 °C	18 ± 2^{cde}	0.63^{d}	16 ± 0^{e}	0.63^{e}
	150 °C	17 ± 1^{de}	0.63^{d}	17 ± 1^{cde}	0.63^{e}
	180 °C	15 ± 0^{f}	0.63^{d}	16 ± 1^{de}	0.63^{e}
Erytromycin (15 µg)		37 ± 1^{a}	ne	37 ± 1^{a}	ne
Vancomycin (5 µg)		21 ± 1^{bc}	ne	21 ± 1^{c}	ne
Ampicillin		ne	0.26^{b}	ne	0.26^{c}

Values are expressed as means ± SD (n=3). a,b,c,d. followed by different superscripts in the same column indicate significant difference ($p<0.05$). ne = not evaluated.

3.4 Antimicrobial activity and Minimum inhibitory concentration (MIC)

Antimicrobial activity is a beneficial trait in plant extracts if they can inhibit bacterial pathogens without affecting beneficial probiotic bacteria. In chronic disease, immunity is always affected and increased susceptibility to bacterial infections and antimicrobial activity can be a common problem. Antimicrobial activity related to phenolic compounds, such as tannic acid, quercetin, catechin, ellagic acid, proanthocyanidins and gallic acid, have been reported earlier (Kil et al., 2009; Rauha et al., 2000; Saraiva et al., 2012). Among all studied pathogenic microorganisms, only growth of *Staphylococcus aureus* ATCC 29213 was inhibited by the crude extracts of camu-camu powders (**Table 3)**. Previous studies reported inhibition against *S. aureus* with extracts of camu-camu and *P. granatum* (Fujita et al., 2013, Myoda et al., 2010; Azevedo et al., 2014; Pradeep et al., 2008; Machado et al., 2002). According to Caillet et al. (2012), cranberry phenolic compounds had significant inhibitory effects on *S. aureus*, Gram-positive bacteria where destabilization of cytoplasmic membrane, permeabilization of plasma membrane, inhibition of extracellular microbial enzymes and metal binding capacity could be reasons for the inhibition. Additionally, Silva et al. (2014) and Souza et al. (2014) reported that Gram-negative bacteria have more complex cell membranes (peptidoglycan, periplasm, and lipopolysaccharide) that making penetration difficult for antimicrobial agents.

As expected, freeze-dried powders were more effective in inhibiting *S. aureus* than spray-dried powders: the higher the carrier agent concentration, the lower the inhibition was found in spouted-bed dried camu-camu pulp (Fujita et al., 2013). For example, the extract of freeze-dried powder was classified as very active in terms of antimicrobial activity, while spray-dried powders were categorized as partially active and inactive (gum arabic and maltodextrin, respectively, **Table 3**),

Besides that, spray-dried extracts showed minimum inhibitory concentration (MIC) values ranging from 0.16 to 0.63 mg/mL, specially, 6% arabic gum at 120 and 150 °C along with freeze-dried extracts (MIC values = 0.08 mg/mL), which were more effective inhibitors than ampicillin (0.26 mg/mL). Therefore, these powders have potential as functional ingredients. This result showed higher inhibition than studies for Bordo grape residues (3.1 to 25 mg/mL, Souza et al., 2014).

Higher phenolic compounds contents showed lower MIC. The major phenolic compounds found in those powders were ellagic acid, quercetin, gallic acid, proanthocyanidins and anthocyanins (**Figure 2, Table 1**), which might be responsible for

antimicrobial activity against *S. aureus* (Saraiva et al., 2012, Rauha et al., 2000, Caillet et al., 2012). In this study, all analyzed phenolic compounds (ellagitannins, ellagic acid, syringic acid and myricetin) from Sao Paulo powders had better coefficient correlation than Amazonian powders (around 0.9 and 0.7, respectively **Table 4**). Therefore, it is reasonable to suggest that the loss and microencapsulation of phenolic compounds, and also, the presence of degradation products during the freeze-drying and spray-drying processes in this study might have reduced the antimicrobial activity of camu-camu powders but they can still be used against prokaryotic harmful bacteria like *S. aureus*.

3.5. Regeneration of Planaria

Planaria animal model is considered a eukaryote regenerative model and was used to determine the potential of camu camu bioactives for cellular protection and rejuvenation (Oviedo & Beane, 2009). The potential of Planaria is based on the fact that entire section of the whole organism can fully regrow from cut sections of the head or lower tail region. Percentage of regeneration was calculated for head and tail section separately (**Figures 5A** and **5B,** respectively). For head regeneration, except camu-camu spray-dried powder with 12% of gum Arabic, all other extracts showed rapid regeneration and re-growth compared to the control (without camu camu extract) (**Figure 5A**). However, for tail regeneration, only freeze-dried camu-camu powder showed superior re-growth (**Figure 5B**). After 10 days of removal of specific cross sections in the head and tail region, the Planaria cells regrew and completed the regeneration process to a fully grown and functioning organism.

This was the first study to verify Planaria regeneration and cellular protection with an aqueous fruit extracts. Further studies are required to more precisely elucidate regeneration and its mechanism. Initial results however suggest that phenolic bioactive compounds (not the level of phenolic bioactive profile, rather composition, stability and bioavailability of phenolic compound might have effect on regeneration) in camu-camu powders might promote eukaryotic cell division and the Planaria model can be utilized as a cellular rejuvenation model. Besides that, oligosaccharides contained in gum arabic could stimulate planaria regeneration. Another possibility is that gum arabic gives better stability of bioactive effects, and consequently, might have stimulatory effects.

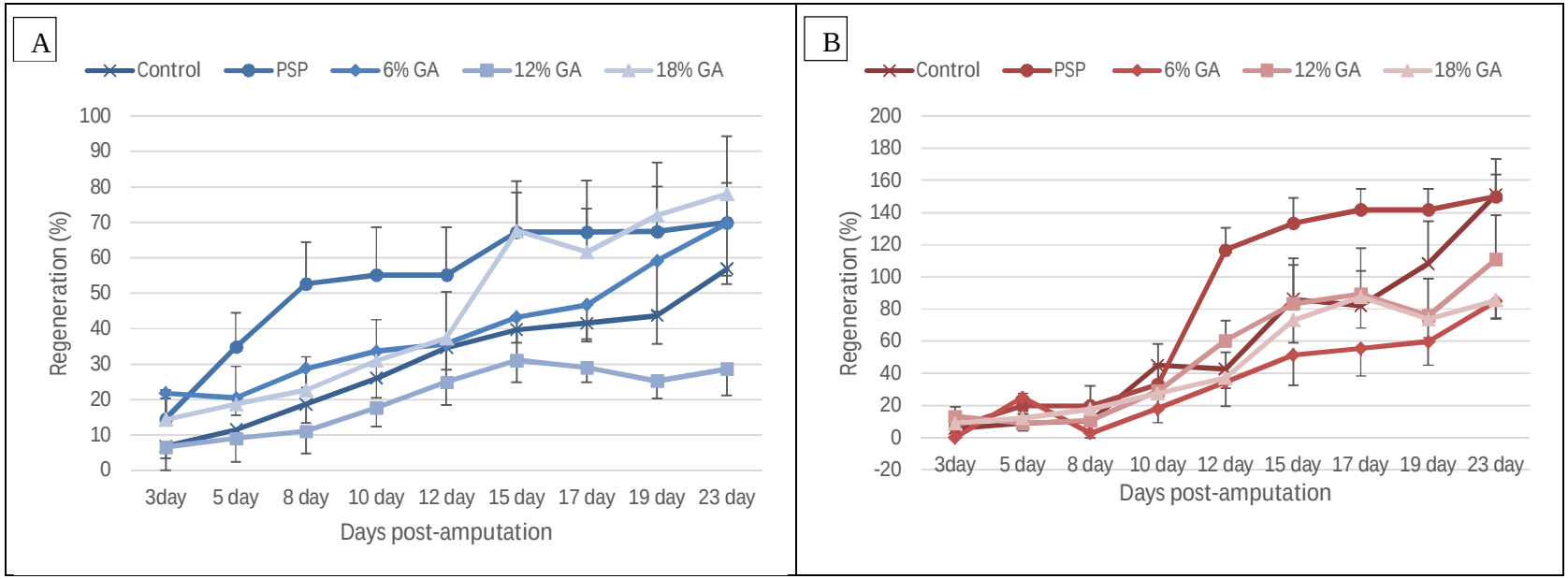


Figure 4. Percentage of planaria (*Dugesia tigrina*) regeneration of (A) head and (B) tail after fed with different camu-camu aqueous extracts (30 µL of 10 mg/mL) and liver.

4. CONCLUSIONS

Two different analyzed pulps of camu camu from commercial sources showed different phenolic compositions and associated health-linked functionalities. Anthocyanidins were found only in Sao Paulo pulp while gallic acid derivatives were predominant in Amazonian pulp. Phenolic compounds such as ellagitannins, ellagic acid, syringic acid and myricetin were major phenolics detected in this study and may be responsible for higher phenolic antioxidant linked functionalities.

In general, inlet air temperature had less effect on bioactive compounds losses than carrier agents concentration during spray-drying processing. Additionally, the lowest concentration of carrier agents (6%) resulted in lower losses of bioactive compounds. Besides that, type of carrier agents (gum arabic and maltodextrin) could influence the results.

In comparison to spray drying, freeze-drying led to lower losses of phenolic compounds, which positively correlated with antidiabetic properties such as α-amylase and α-glucosidase inhibitory activity. Overall camu-camu showed low α-amylase and high α-glucosidase inhibition that is potentially ideal for incorporation into dietary strategies for management of early stages of type 2 diabetes and its associated complications. In terms of cellular associated functional studies, freeze-dried and spray-dried (6% arabic gum at 120 °C) powders were more effective against bacterial pathogen *Staphylococcus aureus* than Ampicillin. Further, camu-camu showed cellular regeneration in Planaria model, which showed promise for cellular rejuvenation studies. This study (*in vitro*) suggests that camu camu with its high antioxidant activity and rich phenolic profile potentially provides protection against microvascular complications (associated with type 2 diabetes) as well as against some bacterial borne infections.

5. ACKNOWLEDGEMENTS

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing fellowship during pHD course (Alice Fujita).

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4.4 Improving anti-hyperglycemic functionality of camu-camu (*Myrciaria dubia* Mc. Vaugh) powder by fermenting with *Lactobacillus helveticus* and *Lactobacillus plantarum*

Abstract

Type 2 diabetes is a major chronic disease, which has been rising exponentially around the world. Camu-camu, an Amazonian tropical fruit, has shown anti-diabetic potentials, and the aim of this study was to ferment soymilk added of camu-camu (*Myrciaria dubia* Mc. Vaugh) powders with lactic acid bacteria in order to improving bioactive functionalities of this product. Fermented soymilk-camu-camu mixture was then evaluated for total phenolics contents, antioxidant activity (DPPH) and inhibition of the enzymes responsible for carbohydrates metabolism and hypertension. For comparison, were used two different microorganisms: *Lactobacillus plantarum* NCDO 1193 and *Lactobacillus helveticus* ATCC 12046, besides that, it was also evaluated different camu-camu powders (freeze-dried and spray-dried). Overall, both microorganisms showed similar functions during the fermentation, except to *L. plantarum* dropped pH values faster than *L. helveticus*. Moreover, *L. plantarum* showed faster increase of α-amylase inhibition, at 24 h, than *L. helveticus*, at 72 h of fermentation. Addition of camu-camu powder improved total phenolic contents, retained high antioxidant capacity (around 90% DPPH inibition), increased α-amylase and α-glucosidase inhibition during fermentation. Freeze-dried camu-camu powder has shown better results for bioactive functionalities therefore with fermentation have potential to utilize in dietary management and prevention of early stages type 2 diabetes-linked hyperglycemia and associated cardiovascular complications.

Keywords: total phenolic, antioxidant, α-amylase; α-glucosidase; ACE;

Research Hightlights:

- Soymilk enriched with camu-camu powder increased bioactive functionality after fermentation
- Comparison between *L. plantarum* and *L. helveticus* of camu-camu fermented product were evaluated
- Evaluation of fermentation of spray-dried and freeze-dried camu-camu powders

1. INTRODUCTION

Non-communicable chronic diseases, such as type 2 diabetes is consequence of modern sedentary lifestyle, consumption of diet overloaded with macronutrients and stress. This disease has been exponentially rising, estimated to reach over 592 million cases by year 2035 (WHO, 2014). Therefore, it is necessary to study diet based prevention and management of type 2 diabetes. It has been well known that higher consumption of grains, berries, vegetables and fruits rich in phenolic bioactive profile and antioxidative potential will prevent risk of non-communicable chronic diseases (NCDs) such as type 2 diabetes (Anhê et al., 2013).

One such fruit rich in polyphenol is camu-camu (*Myrciaria dubia* Mc. Vaugh) originally from Amazon. Previous studies showed that this fruit is very high in vitamin C and ellagic acid compared to others fruits (Gonçalves et al., 2010; Inoue et al, 2008), and also has high antioxidant, antimicrobial, antidiabetic properties, specially, α-glucosidase inhibitory activity (Fujita et al., 2015). However, this fruit is highly perishable, which makes transportation and storage expensive. Making camu-camu powder was one alternative to extend shelf-life, and also, to preserve and to concentrate bioactive compounds (Fujita et al. 2013; Fujita et al., 2015).

The next step is to use the powder as an ingredient for functional food. Fermenting fruits with probiotics offer excellent strategy to design functional foods with potential health benefits, such as improved intestinal microflora regulation, enhancing anticarcinogenic and anti-inflammatory activities (FAO/WHO, 2001). In addition, polyphenols in fermented fruits could exert dual functions as antimicrobial against pathogenic microorganisms, while enhancing growth of good bacteria in the gut (Hervert-Hernández et al., 2009). Besides that, gum arabic used as a carrier agent of camu-camu spray-dried has been also cited to have prebiotic properties (Phillips & Phillips, 2011; Glover et al., 2009; Phillips et al. 2008).

With such rationale, the main objective of this study was to investigate the potential of camu-camu powder as an ingredient to incorporate in yogurt to prevent and manage type 2 diabetes. This study was aimed to ferment soymilk enriched with camu-camu powder by probiotic and then, during the fermentation process to evaluate total phenolics contents, DPPH antioxidant activity and *in vitro* anti-diabetic properties such as α-amylase, α-glucosidase and ACE inhibition.

2. MATERIAL AND METHODS

2.1 Materials. Soymilk was purchased from a local supermarket (Hornbachers, Fargo, ND, USA). Freeze-dried and spray-dried of camu-camu pulp (*Myrciaria dubia* Mc. Vaugh) powders from Sao Paulo, Brazil.

2.2 Spray-drying. Spray-drying was performed in a pilot scale spray-dryer (Labmaq, SD 5.0, Brazil). The pulp was fed by a peristaltic pump at a fixed rate of 44 mL/min and was spray-dried at inlet air temperatures (120 °C) and carrier agent concentrations (6, 12 and 18%) of gum arabic (Nexira Brazil Com. Ltd., Brazil).

2.3 Freeze-drying. Two kilograms of frozen pulp were lyophilized in a Pironi 501 freeze-drier (Thermo Electron Corporation, New York, USA) at -80°C and 100 *m*Torr for 120 hours.

2.4 Fermentation with soymilk. Initially, 100 µL of frozen *Lactobacillus helveticus* or *Lactobacillus plantarum* stock were inoculated into 10 mL MRS broth (Difco) and then, inoculated for 18 h at 37 °C. After that, 100 µL of the grown strain was re-inoculated into 10 mL MRS broth for 18 h at 37 °C. One hundred and fifty mL of soymilk were placed into each 250 mL Erlenmeyer flask. Different concentration of camu-camu powders (0.5% and 1.0% w/v) were added and mixed with magnetic stir. Samples were inoculated using 1.5 mL of microorganism (10^6 UFC mL^{-1}). Fermentation was carried out at 37 °C, and 30 mL samples were taken out at 0, 24, 48 and 72 h. The samples were centrifuged at 15000 *g* for 15 min and then used for the assays.

2.5 Enumeration of population of probiotics during fermentation. At 0, 24, 48 and 72 h, CFU/mL was determined by pipetting 100 µL of the sample, serially diluting and plating on MRS medium. The plates were incubated anaerobically in BBL GasPak jars (Becton, Dickinson and Co.) with BD GasPak EZ anaerobe container system sachets (Becton, Dickinson and Co.) at 37 °C for 24 h, and individual colonies were counted. The pH of the samples was also measured at 0, 24, 48 and 72 h.

2.6 Total phenolics assay. All samples were determined by according to Shetty et al. (1995). In brief, 0.5 mL of fermented sample was added to a test tube and mixed with 0.5 mL of 95% ethanol and 5 mL of distilled water. To each sample, 0.5 mL of 50% (vol/vol) Folin-Ciocalteau reagent was added and mixed. The absorbance was read at 725 nm using a spectrophotometer (Genesys UV/Visible, Milton Roy, Inc, Rochester, NY, USA). Results were expressed as mg of gallic acid/ mL of sample.

2.6 Antioxidant activity by DPPH radical inhibition assay. It was determined according to Kwon et al. (2006). A 250 µL aliquot of the sample extract was mixed with 1250 µL of DPPH (60 µM in ethanol). Absorbance was measured at 517 nm using Genesys UV/Visible spectrophotometer. The percentage inhibition was calculated by:

$$\% \text{ Inhibition} = \frac{Abs^{control} - Abs^{extract}}{Abs^{control}} \times 100 \qquad \text{(eq. 1)}$$

2.7 α-amylase inhibition. The α-amylase inhibitory activity was determined by an assay modified from the *Worthington Enzyme Manual* (Worthington Biochemical Corp. 1993a). A total of 500 µL of each extract and 500 µL of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing α-amylase solution (0.5 mg/mL) were incubated at 25 °C for 10 min. After pre incubation, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube at timed intervals. The reaction was stopped with1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted after the addition of 15 mL of distilled water, and absorbance was measured at 540 nm using a Genesys UV-visible spectrophotometer. The readings were compared with the controls, containing buffer instead of sample extract. The results were expressed as percent α-amylase inhibition and calculated according to eq 1.

2.8 α-glucosidase inhibition. The assay was performed according to the *Worthington Enzyme Manual* (1993b), with some modifications (Mc Cue et al., 2005). α-glucosidase (1 unit/mL) was assayed by using 50 μL of aqueous camu-camu extracts and 100 μL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase solution and was incubated in 96-well plates at 25 °C for 10 min. After pre-incubation, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25 °C for 10 min. Before and after incubation, absorbance readings were recorded at 405 nm by microplate reader (Spectra Max 190, Molecular Device Co., CA) and compared to a control that had 50 μL of buffer solution in place of the extract. The results were expressed as percent of α-glucosidase inhibition and calculated according to eq 2.

$$\textbf{\% inhibition} = \frac{(Abs^{control}\,5min - Abs^{control}\,0\ min) - (Abs^{extract}\,5min - Abs^{extract}\,0\ min)}{(Abs^{control}\,5min - Abs^{control}\,0\ min)} \times 100 \qquad \text{(eq. 2)}$$

2.9 Angiotensin converting enzyme (ACE) inhibition. ACE inhibition was assayed according to a method modified by Kwon et al. (2006). The substrate hippuryl-histidylleucine (HHL) and ACE-I from rabbit lung (1 unit produces 1.0 μmol of hippuric acid from HHL per minute in 50 mM HEPES and 300 mM NaCl at pH 8.3 at 37 °C) were used. Fifty microliters of water soluble supernatant of water extracts was incubated with 100 μL of 1M NaCl-borate buffer (pH 8.3) containing 2 *m*units of ACE-I solution at 37 °C for 10 min. After pre-incubation, 100 μL of a 5 *m*units substrate (HHL) solution was added to the reaction mixture. Test solutions were incubated at 37 °C for 1 h. The reaction was stopped with 150 μL of 0.5 N HCl. The hippuric acid formed was detected; the spectra were confirmed and quantified by high-performance liquid chromatography (HPLC). Five microliters of the sample was injected using an Agilent ALS 1100 autosampler into an Agilent 1260 series HPLC (Agilent Technologies, Palo Alto, CA) equipped with a DAD1100 diode array detector. The solvents used for the gradient were (1) 10 mM phosphoric acid (pH 2.5) and (2) 100% methanol. The methanol concentration was increased to 60% for the first 8 min and to 100% for 5 min and then was decreased to 0% for the next 5 min (18 min total run time). The analytical column used was a Nucleosil 100-5 C18, 250 × 4.6 mm i.d., with packing material of 5 μm particle size at a flow rate of 1 mL/min at ambient temperature. During each run, the absorbance was recorded at 228 nm and the related chromatogram was integrated using Agilent Chemstation (Agilent Technologies) enhanced integrator for detection of liberated hippuric acid (A). Pure hippuric acid (purchased from Sigma Chemical Co.) was used to calibrate the standard curve and retention time. The percent inhibition was calculated according to eq 3:

$$\% \text{ Inhibition} = \frac{(A^{control} - A^{blank}) - A^{sample}}{(A^{control} - A^{blank})} \times 100 \qquad \text{(eq. 3)}$$

2.10 Statistical Analysis. All analyses were run in triplicate and results were expressed as mean ± standard deviation (SD). For statistical analysis, the Statistic software package version 11.0 (StatSoft, Inc., Tulsa, OK) was employed. Differences between means were first analyzed by ANOVA test and then Tukey test ($p < 0.05$).

3. RESULTS AND DISCUSSION

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3.1 Determination of pH and viable cells counts

Some studies showed that polyphenols at lower concentration could promote probiotic growth (Bialonska et al., 2010; Curiel et al., 2010). Besides that, low concentration of tannins (0.1 or 0.2 mg/mL) did not inhibit *L. plantarum* DSM 10492 growth however, 1 mg/mL inhibited bacterial growth (Rozés and Peres, 1998). On the other hand, Nualkaekul et al. (2011) showed cranberry and lemon juices could decrease the cells survived due to the acidity. Therefore, 0.5% and 1% of freeze-dried camu-camu powder which is acidic and also rich in polyphenols was added to soymilk and were fermented by *L. plantarum* and *L. helveticus*, separately. After that, pH values and number of microorganisms were counted by colony counts and expressed in log CFU/mL of samples (**Table 1**).

The pH values of fermented camu-camu soymilk mixture by *Lactobacillus plantarum* showed a more rapid drop in pH than those by *L. helveticus* samples (**Table 1**). Yoon et al. (2004) observed the same results and concluded that could be due to *L. plantarum* has ability to produce more lactic acid than other analyzed microorganisms (*L. acidophilus*, *L. casei* and *L. delbrueckii*).

Overall, addition of camu-camu powder decreased pH values because the acidity of the fruit. As a result, higher the amount of camu-camu powder, lower the pH values were observed (**Table 1**, test 3 and 4). Besides that, variations in type of camu-camu powders did not show any statistical differences ($p<0.05$) during fermentation, especially, when 0.5% of camu-camu spray-dried powders were added (**Table 1**, test 3).

The population of microorganisms growth indicated that both microorganisms could grow in soymilk when camu-camu powder was added, however, *L. helveticus* showed better preservation of growth than *L. plantarum* after 72 h of fermentation (**Table 1,** test 1 and 2, $p<0.05$). Both microorganisms are lactic acid bacteria, although *L. helveticus* are well known as protease producer and *L. plantarum* as tannase producer. Therefore, soymilk protein contributed for *L. helveticus* growth. Addition of 0.5% and 1% of camu-camu powder did not show growth difference to *L. plantarum* (Test 1, $p<0.05$). In contrast, *L. helveticus* growth was inhibited when

we compared to the control. Type of microorganisms, presence of phenolics in camu-camu and lactic acid contents could influence these results.

Further, test 3 and test 4, soymilk were fermented adding spray-dried camu-camu powders with different concentration of gum arabic (6%, 12% and 18%) by *L. helveticus*. Gum arabic showed preobiotic properties, therefore could promote or preserve probiotic microorganisms growth (Phillips et al., 2008). Although, addition of 0.5% of camu-camu powders did not show statistical difference (test 3, $p<0.05$), we observed indication of microorganisms preservation when 1% were added in comparison to freeze-dried powder (test 4, $p<0.05$).

3.2 Total phenolics contents

Total phenolic contents were quantified during the fermentation time (0, 24, 48 and 72 h). Firstly, different concentrations of freeze-dried camu-camu powders (0.5% and 1%) were fermented using probiotic microorganisms *L. plantarum* and *L. helveticus* (**Figures 1A and 1B,** respectively). As expected, high phenolic contents were found when camu-camu powder was added. Overall, total phenolic contents did not change during the fermentation. Both microorganisms had similar behavior, which did not show statistical differences at 0, 48 and 72 h. However, at 24 h a decrease of 30% of phenolic contents was observed with both microorganisms. Those results suggested that probably at 24 h had polymerization of phenolics and then at 48 h enzymes produced by fermentation could release the phenolics compounds. Apostodis et al, (2011) and Ankolekar et al, 2011 found same decrease in phenolic content when cranberry and cherry were fermented, respectively. Similarly, McCue et al. (2003) suggested that lignin and lignans suffered polymerization due to high antioxidant activity during early stages of fermentation. Besides that, production of peroxidase, β-glucosidase and laccase were observed after 24 h of fermentation (McCue et al. 2005). Especially *L. plantarum* can produce tanase after 24 h of growth (Rodriguez et al., 2009). Those enzymes can hydrolyze glycosides and break down plant cell walls that facilitate polyphenol extraction, and also, contribute to yield aglucone forms.

Table 1. pH measurement and microrganisms growth in Log CFU/mL of samples by *Lactobacillus plantarum* NCDO 1193 and *Lactobacillus helveticus* ATCC 12046 with camu-camu powders

Test	Microorg.	Conc. of powder	Powder	pH Time (h) 0h	pH Time (h) 24 h	pH Time (h) 48h	pH Time (h) 72h	Log CFU/mL Time (h) 0h	Log CFU/mL Time (h) 24 h	Log CFU/mL Time (h) 48h	Log CFU/mL Time (h) 72 h
		0%	w/o powder	6.16 ± 0.02^{a}	4.15 ± 0.04^{d}	4.03 ± 0.07^{de}	4.03 ± 0.05^{de}	6.30 ± 0.05^{bc}	6.49 ± 0.14^{a}	6.29 ± 0.03^{bc}	5.01 ± 0.08^{d}
1	*L. plantarum*	0.5%	Freeze-dried	5.65 ± 0.07^{b}	3.94 ± 0.04^{ef}	3.82 ± 0.03^{fg}	3.80 ± 0.01^{fg}	6.40 ± 0.12^{ab}	6.48 ± 0.05^{a}	6.17 ± 0.09^{c}	4.92 ± 0.09^{d}
		1%	Freeze-dried	5.26 ± 0.06^{c}	3.85 ± 0.08^{fg}	3.75 ± 0.02^{g}	3.73 ± 0.02^{g}	6.30 ± 0.06^{bc}	6.52 ± 0.02^{a}	6.26 ± 0.07^{c}	4.98 ± 0.10^{d}
		0%	w/o powder	6.42 ± 0.08^{a}	6.23 ± 0.02^{b}	5.86 ± 0.09^{c}	5.21 ± 0.04^{e}	5.82 ± 0.07^{c}	5.28 ± 0.09^{de}	6.49 ± 0.12^{b}	6.78 ± 0.08^{a}
2	*L. helveticus*	0.5%	Freeze-dried	5.83 ± 0.06^{c}	5.43 ± 0.07^{d}	4.02 ± 0.06^{g}	3.92 ± 0.02^{g}	5.80 ± 0.03^{c}	4.95 ± 0.05^{f}	5.10 ± 0.07^{ef}	5.85 ± 0.09^{c}
		1%	Freeze-dried	5.36 ± 0.05^{de}	4.51 ± 0.09^{f}	4.04 ± 0.05^{g}	3.98 ± 0.03^{g}	5.81 ± 0.04^{c}	4.41 ± 0.11^{g}	4.38 ± 0.10^{g}	5.38 ± 0.07^{d}
		0%	w/o powder	6.48 ± 0.06^{a}	6.27 ± 0.01^{ab}	5.94 ± 0.08^{bc}	5.28 ± 0.11^{de}	5.82 ± 0.07^{d}	5.28 ± 0.09^{g}	6.49 ± 0.12^{b}	6.78 ± 0.08^{a}
		0.5%	Freeze-dried	5.91 ± 0.08^{cd}	5.47 ± 0.01^{d}	5.05 ± 0.02^{ef}	4.30 ± 0.09^{g}	5.80 ± 0.03^{de}	4.95 ± 0.05^{h}	5.10 ± 0.07^{gh}	5.85 ± 0.09^{cd}
3	*L. helveticus*	0.5%	6% GA	6.10 ± 0.08^{ab}	5.51 ± 0.02^{d}	5.26 ± 0.06^{de}	5.06 ± 0.06^{ef}	5.80 ± 0.09^{de}	4.38 ± 0.08^{i}	5.59 ± 0.05^{ef}	5.98 ± 0.07^{cd}
		0.5%	12% GA	6.18 ± 0.07^{ab}	5.58 ± 0.02^{cd}	5.03 ± 0.01^{ef}	5.00 ± 0.12^{ef}	5.85 ± 0.03^{cd}	5.52 ± 0.04^{f}	5.84 ± 0.07^{d}	6.06 ± 0.08^{c}
		0.5%	18% GA	6.24 ± 0.07^{ab}	5.46 ± 0.07^{d}	5.02 ± 0.08^{ef}	4.73 ± 0.11^{f}	5.90 ± 0.02^{cd}	5.91 ± 0.03^{cd}	5.89 ± 0.04^{cd}	5.81 ± 0.09^{d}
		0%	w/o powder	6.04 ± 0.01^{a}	6.22 ± 0.03^{a}	5.71 ± 0.02^{b}	5.31 ± 0.03^{de}	5.82 ± 0.07^{c}	5.28 ± 0.09^{de}	6.49 ± 0.12^{b}	6.78 ± 0.08^{a}
		1%	Freeze-dried	5.19 ± 0.06^{e}	4.99 ± 0.05^{f}	3.74 ± 0.04^{ij}	3.74 ± 0.02^{ij}	5.81 ± 0.04^{c}	4.41 ± 0.11^{h}	4.38 ± 0.10^{h}	5.38 ± 0.07^{d}
4	*L. helveticus*	1%	6% GA	5.47 ± 0.01^{cd}	4.50 ± 0.13^{h}	3.74 ± 0.10^{ij}	3.64 ± 0.09^{j}	5.96 ± 0.07^{c}	4.82 ± 0.15^{g}	5.15 ± 0.11^{def}	5.80 ± 0.05^{c}
		1%	12% GA	5.65 ± 0.02^{bc}	5.12 ± 0.11^{ef}	3.88 ± 0.12^{i}	3.61 ± 0.04^{j}	5.98 ± 0.04^{c}	4.92 ± 0.11^{fg}	5.10 ± 0.06^{ef}	5.88 ± 0.09^{c}
		1%	18% GA	5.83 ± 0.01^{b}	5.36 ± 0.06^{de}	4.99 ± 0.03^{f}	4.76 ± 0.06^{g}	5.88 ± 0.09^{c}	5.10 ± 0.06^{ef}	5.14 ± 0.12^{def}	5.80 ± 0.07^{c}

Values are expressed as means ± SD. $^{a-j}$Different letters in each Test denote significant differences for pH and log CFU/mL, distinctly ($p<0.05$).

As previously observed, phenolic contents in samples with spray-dried camu-camu powders were lower than freeze-dried powders due to loss in the drying process, especially, it might be occurred due to microencapsulation with gum arabic and subsequent difficulty in extraction (Fujita et al., 2013, Souza et al., 2014; **Figure 1C and 1D**).

Adding 0.5% of spray-dried camu-camu powders (m/v) with gum arabic (6%, 12% and 18%) did not show any statistical difference ($p<0.05$) during the fermentation (**Figure 1C**). On the other hand, when 1% of powder was added the total phenolics contents were decreased, except in camu-camu powder with 18% of gum arabic at 72 h fermentation (**Figure 1D**). Two probable mechanisms were considered for the increasing of phenolic contents at late stages of fermentation. Gum arabic is polysaccharide, arabino-galactan proteins, the sugars bonded are D-galactose, L-arabinose, L-rhamnose, D-glucuronic acid and 4-O-methyl glucuronic acid (Phillips et al., 2008). Probiotic microorganism released the sugars, which reacted with the reagent Folin-Ciocalteau overestimating the phenolic contents. Another possibility is that gum arabic stimulated the microorganisms to produce enzymes responsible for depolymerization of high molecular weight phenolic compounds. However, further investigations for biochemical changes should be studied in the future.

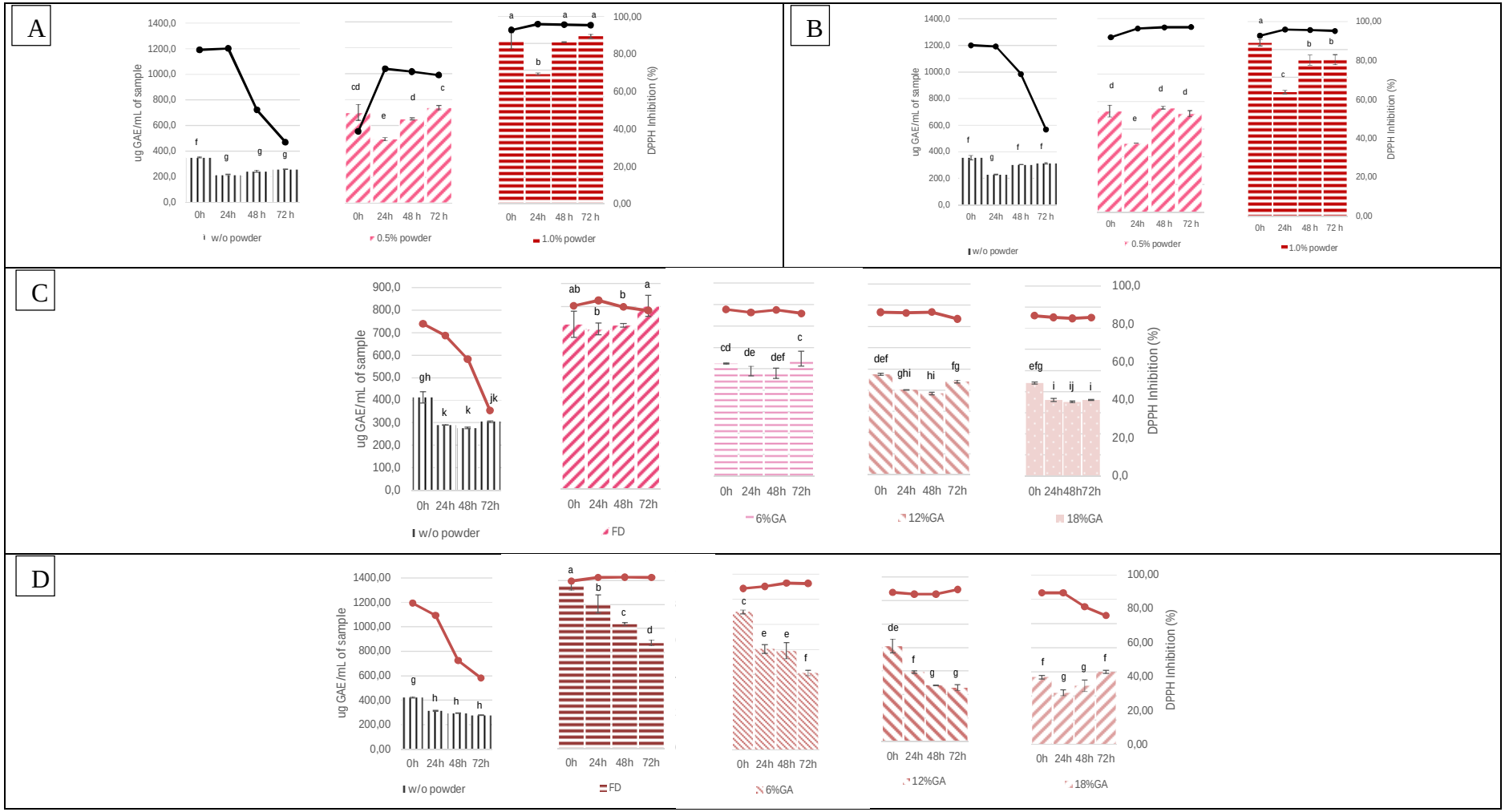

Figure 1. Total phenolics contents (µg GAE/mL of sample) and DPPH inhibition (%) during fermentation by *Lactobacillus plantarum* NCDO 1193 (A) *Lactobacillus helveticus* ATCC 12046 (B) in two concentrations: 0.5% of powder and 1.0% of powder of freeze-dried camu-camu powder. Different type of camu-camu powders, where FD is freeze-dried powder compared to spray-drying powders contained 6%, 12% and 18% of gum arabic in two concentrations: 0.5% (C) and 1.0% (D) by *Lactobacillus helveticus.*

3.3 Antioxidant activity by DPPH inhibition

Antioxidant activity by DPPH inhibition were measured at the same time as a total phenolic and plotted together (**Figure 1**). Although, studies have been shown contribution of antioxidant capacity from soy fermented products (Lin et al., 2006; Hubert et al., 2008), samples without camu-camu powders had decreased antioxidant capacity after 72 hours of fermentation, which were 33.5% and 40.6% for *L. plantarum* and *L. helveticus*, respectively (**Figures 1A and 1B**). In contrast, samples with camu-camu powders showed high antioxidant capacity, around 90% of inhibition of DPPH-linked free radical scavenging even at 72 h (**Figure 1**). Camu-camu has high vitamin C and is rich in phenolics compounds, as a consequence, high antioxidant capacity (Myoda et al., 2010; Fujita et al., 2013). The maintenance of high antioxidant capacity during the fermentation is probably because of conversion of glycosides to aglycone forms by enzymes (Vattem et al, 2003). Besides that, synergism between polyphenols and other compounds should be contribute. Thus, fermentation with camu-camu powders improved the final products, resulting in better prevention of risk of chronic diseases and promoting health.

3.4 Anti-diabetic properties by α-amylase, α-glucosidase and ACE inhibition *in vitro* assays

Phenolic-rich extracts could inhibit α-amylase and α-glucosidase, which are key-enzymes directly linked to control postprandial hyperglycemia and help to manage Type 2 diabetes (Hanhineva et al., 2010, Anhê et al, 2013, Sarkar & Shetty, 2014, Barbosa et al. 2013). In this study, inhibition of two enzymes from fermented soymilk samples after 0, 24, 48 and 72 h in two concentration (0.5% and 1%, m/v) and type of camu-camu powders were investigated.

Previously, our results showed that aqueous extract of camu-camu powder has high potential to be incorporated in functional foods to manage early stages of type 2 diabetes related to hyperglycemia due to low α-amylase and high α-glucosidase inhibitory activity (Fujita et al., 2015). The approach of this study was to verify the changes of those enzymes inhibition during fermentation as one alternative for the powder utilization. The analyzed microorganisms had different behavior related to α-amylase inhibition (**Figure 2)**. For *L. plantarum* (**Figure 2A**) the highest enzyme inhibition was for 1% of camu-camu powder after 24 and 48 h of fermentation. In contrast, for *L. helveticus* the highest enzyme inhibition was after 72 h of fermentation with 1% of camu-camu powder (**Figure 2B**).

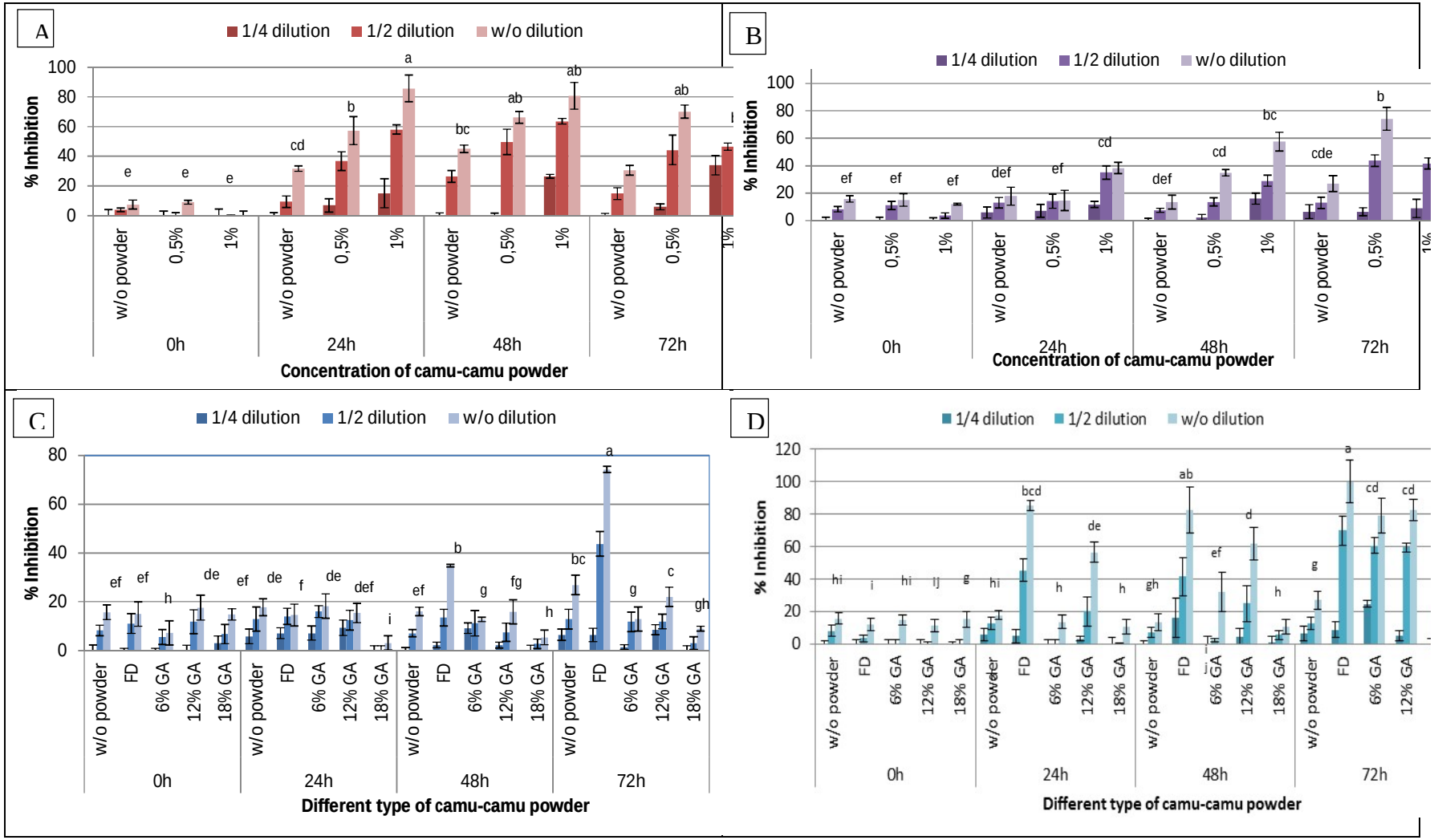

Figure 2. Alfa-amylase inhibition of camu-camu powders after fermentation by *Lactobacillus plantarum* (A) and *Lactobacillus helveticus* (B) in two concentrations: 0.5% of powder and 1.0% of powder of freeze-dried camu-camu powder. Different type of camu-camu powders, where FD is freeze-dried powder compared to spray-drying powders contained 6%, 12% and 18% of gum arabic in two concentrations: 0.5% (C) and 1.0% (D) by *Lactobacillus helveticus*.

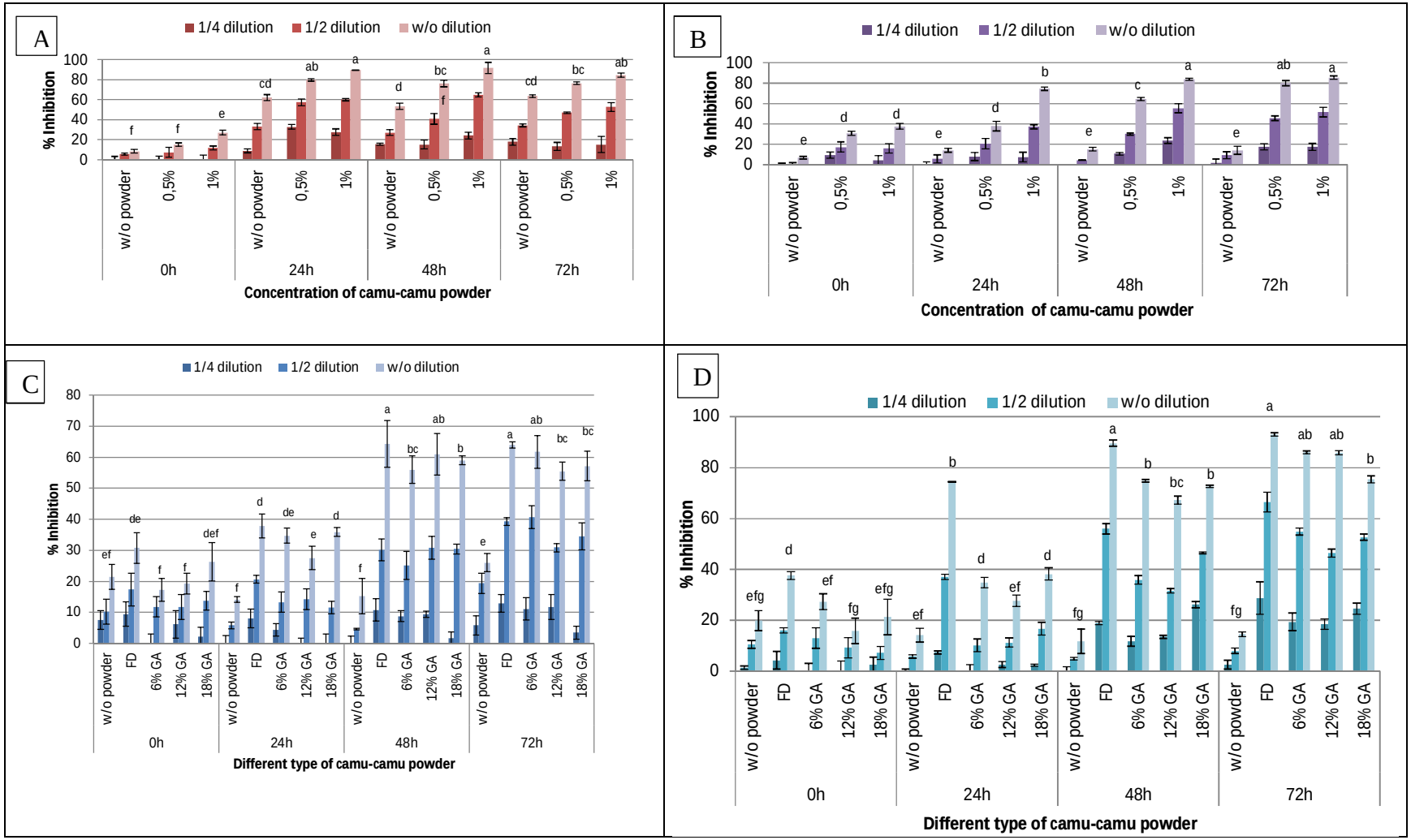

Figure 3. Alfa-glucosidase inhibition of camu-camu powders after fermentation by *Lactobacillus plantarum* (A) and *Lactobacillus helveticus* (B) in two concentrations: 0.5% of powder and 1.0% of powder of freeze-dried camu-camu powder. Different type of camu-camu powders, where FD is freeze-dried powder compared to spray-drying powders contained 6%, 12% and 18% of gum arabic in two concentrations: 0.5% (C) and 1.0% (D) by *Lactobacillus helveticus*.

Comparison of different type of camu-camu powders were performed (**Figures 2C and 2D**). Our results showed that freeze-dried powder were better than others powders. When 0.5% of powder was added, freeze-dried powder increased enzyme inhibition 2 times after 48 h and 4 times after 72 h of fermentation compared to the control (**Figure 2C**). Additionally, 1% of camu-camu powder enhanced enzyme inhibition in an order to freeze-dried > 6% gum arabic = 12% gum arabic = 18% gum arabic > soymilk without camu-camu powder at 72 hours of fermentation (**Figure 2D**, $p<0.05$). Since α-amylase inhibition are related to phenolic contents, the superiority of freeze-dried powder than others powders were expected.

Aqueous extracts of camu-camu powders showed very high α-glucosidase inhibition than acarbose (Fujita et al, 2015). After fermentation, using *L. plantarum* and *L. helveticus* (**Figures 3A and 3B**) similar functions as α-amylase inhibition were observed. The higher amount of camu-camu powders showed higher α-glucosidase inhibition. Moreover, the percentage of α-glucosidase inhibition was remained high during the period of fermentation.

Figures 3C and **3D** showed results of α-glucosidase inhibition after fermentation with soymilk and camu-camu spray-dried powders. Overall, there were no statistical difference among all analyzed camu-camu powders ($p<0.05$). After 48h and 72h of fermentation, the enzyme inhibition increased for all analyzed powders for 0.5% and 1% of addition. Alpha-glucosidase inhibition increased 2 times after 48h of fermentation to freeze-dried powder compared to the control (**Figure 3C**). When 1% of freeze-dried powder was added the enzyme inhibition increased 2 times at 24h of fermentation and reached the highest after 48h of fermentation (**Figure 3D**).

Some phenolic compounds have been cited as responsible for those enzyme inhibitions (Cheplick et al., 2007, Sarkar et al., 2015, Pinto et al., 2008). Especially in camu-camu, the major phenolic compounds are ellagic acid, ellagitannins, anthocyanins and quercetin (Fracassetti et al., 2013; Azevedo et al., 2014; Fujita et al., 2015). Degradation and/or production of those compounds might be the reason of variation of enzyme inhibition. For example, tannins are effective for α-amylase inhibition (McDougall et al. 2005) therefore, production of tannase by fermentation might yield tannins contents. Besides that, anthocyanins, ellagic acid and quercetin exhibit α-glucosidase inhibition (Matsui et al., 2001; You et al., 2012) thus, enhance of aglucones by β-glucosidase might explain the increase of this enzyme inhibition.

Other enzymes associated with type 2 diabetes is ACE, which is an enzyme responsible for hypertension activity (Pinto et al., 2008). Phenolic-rich extracts may prevent risk for developing high blood pressure (Manach et al., 2005; Pan et al., 2010). Quercetin and cyanidin-3-*O*-glucoside showed IC50 = 71 and 174 µM, respectively for ACE inhibition (Balasuriya et al., 2012). Although, ellagic acid did not showed ACE inhibition (Kwon et al., 2006). Isoflavones, specially genistein, have been cited to have anti-hypertension effects *in vivo* models (Montenegro et al., 2009; Edwards et al., 2007).

In this study, high ACE inhibition was found, around 90%, for all samples analyzed (**Table 2**), even in fermentation without camu-camu powders. Therefore, isoflavones could be responsible for the inhibition. Both microorganisms had similar behavior, at 72 h of fermentation were increased enzyme inhibition when 0.5% and 1% of camu-camu powders were added (test 1 and 2). However, there were no statistical differences ($p<0.05$) were observed when 0.5% of different types of camu-camu powders were added (test 3). When 1% of powders were added, spray-dried powders (6% and 12% of gum arabic) showed slightly better inhibition than control (test 4). Since camu-camu powder did not exhibit ACE inhibition (Fujita et al, 2015), those results suggested synergism of compounds present in soymilk and camu-camu powder. Further, specific compounds should be tested for more accurate conclusions.

Table 2. Angiotensin I-converting enzyme (ACE) inhibitory activity of fermented products by *Lactobacillus plantarum* NCDO 1193 and *Lactobacillus helveticus* ATCC 12046 with camu-camu powders

Test	Microorg.	Conc. of powder	Powder	% ACE inhibition Time (h) 0h	24 h	48h	72h
		0%	w/o powder	90.1 ± 0.6^{b}	86.5 ± 0.3^{d}	86.5 ± 0.6^{d}	87.3 ± 0.7^{cd}
1	*L. plantarum*	0.5%	Freeze-dried	88.9 ± 0.3^{bc}	89.2 ± 1.7^{bc}	90.6 ± 1.3^{b}	94.5 ± 0.2^{a}
		1%	Freeze-dried	89.8 ± 0.9^{b}	92.8 ± 0.3^{a}	94.5 ± 0.1^{a}	94.0 ± 1.0^{a}
		0%	w/o powder	89.5 ± 1.3^{cd}	90.2 ± 0.1^{c}	90.2 ± 0.2^{c}	89.4 ± 0.2^{cd}
2	*L. helveticus*	0.5%	Freeze-dried	88.9 ± 1.2^{d}	92.8 ± 0.2^{b}	90.4 ± 0.4^{c}	94.3 ± 0.1^{a}
		1%	Freeze-dried	93.5 ± 1.2^{ab}	90.5 ± 0.9^{c}	93.8 ± 1.5^{ab}	93.7 ± 0.5^{ab}
		0%	w/o powder	79.0 ± 0.8^{ab}	69.3 ± 0.9^{cde}	73.1 ± 0.7^{abcde}	67.2 ± 3.4^{de}
		0.5%	Freeze-dried	79.8 ± 2.3^{a}	77.0 ± 0.3^{abcd}	78.2 ± 1.0^{abc}	74.0 ± 0.5^{abcde}
3	*L. helveticus*	0.5%	6% GA	66.0 ± 8.1^{e}	78.0 ± 0.6^{abc}	75.6 ± 0.6^{abcde}	68.5 ± 1.3^{cde}
		0.5%	12% GA	78.9 ± 1.4^{ab}	76.4 ± 1.3^{abcd}	72.8 ± 0.5^{abcde}	68.5 ± 0.5^{cde}
		0.5%	18% GA	79.4 ± 0.7^{ab}	74.9 ± 0.4^{abcde}	69.7 ± 2.2^{bcde}	68.8 ± 0.5^{cde}
		0%	w/o powder	87.8 ± 0.5^{h}	93.0 ± 0.2^{c}	91.9 ± 0.2^{e}	89.8 ± 0.2^{g}
		1%	Freeze-dried	91.9 ± 1.4^{e}	93.7 ± 1.2^{ab}	94.2 ± 0.5^{a}	92.1 ± 0.4^{de}
4	*L. helveticus*	1%	6% GA	93.2 ± 0.2^{bc}	92.9 ± 0.2^{bc}	90.7 ± 0.1^{f}	94.1 ± 0.3^{a}
		1%	12% GA	92.7 ± 0.1^{cd}	93.9 ± 0.1^{bc}	90.8 ± 0.4^{f}	94.2 ± 0.3^{a}
		1%	18% GA	92.8 ± 0.4^{cd}	93.1 ± 0.2^{bc}	91.7 ± 0.3^{e}	92.3 ± 0.1^{de}

Values are expressed as means ± SD. $^{a-h}$Different superscript letters in a test denote significant differences for ACE inhibition activity ($p<0.05$).

4. CONCLUSION

Fermentation of soymilk enriched with camu-camu powder could promote health benefits with improving total phenolic contents and antioxidant capacity.

Both microorganisms *L. plantarum* and *L. helveticus* showed similar functions during the fermentation, except to α-amylase inhibition. *L. plantarum* showed faster increase in carbohydrate enzyme inhibition at 24 h. On the other hand, for *L. helveticus* the increase occurred at 72 h fermentation.

The higher the amount of camu-camu powder, the higher total phenolic contents was observed and resulted in higher α-amylase and α-glucosidase inhibition. In this study, freeze-dried camu-camu powder showed better results and might have potential to incorporate as functional foods into dietary strategies for management of early stages of type 2 diabetes associated with anti-hyperglycemia and anti-hypertension.

5. ACKNOWLEDGEMENTS

We would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing fellowship.

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5. CONCLUSÕES

Ao comparar os três processos de secagem conclui-se que as perdas de compostos bioativos são menores no processo de liofilização, seguido do leito de jorro e por último, spray-drying. Porém, nos processos de leito de jorro e atomização pode ter ocorrido a microencapsulação dos compostos fenólicos, devido ao uso de agentes carreadores, o que poderia, por um lado, protegê-los da degradação durante a secagem, e por outro lado, levar à diminuição de sua extratabilidade. Foi observado também que a goma arábica pode se liga mais que a maltodextrina aos compostos fenólicos.

Os compostos bioativos mais afetados durante a secagem foram a vitamina C, seguida dos fenólicos totais, e por último as proantocianindinas. O conteúdo destes compostos foi influenciado pela temperatura e adição do agente carreador no processo de leito de jorro. No entanto, no processo de atomização, foi influenciado apenas pela concentração do agente carreador.

A capacidade antioxidante foi correlacionada com os compostos bioativos, principalmente os compostos fenólicos. Portanto, os pós obtidos pelo processo de liofilização apresentaram maior capacidade antioxidante.

Os pós de camu-camu apresentaram atividade antimicrobiana contra *S. aureus*. O halo de inibição foi maior para os pós liofilizados (classificados como ativos), sendo mais eficazes que o antibiótico Vancomicina. Além disso, a concentração do pó liofilizado mínima para inibir o *S. aureus* foi menor que a do antibiótico Ampicilina. Os pós obtidos pelo processo de leito de jorro (0 e 3% de maltodextrina) e pelo processo de atomização (6% de goma arábica a 120 e 150°C) também se mostraram mais efetivos que a Ampicilina.

Os extratos aquosos dos pós de camu-camu demonstraram baixa inibição da α-amilase e alta inibição da α-glicosidase *in vitro*. A inibição de α-glicosidase pelos pós de camu-camu apresentou IC50 menor que a Acarbose. Portanto, os pós têm potencial para incorporação como ingredientes em dietas para o controle do estágio inicial da diabetes tipo 2.

Os pós de camu-camu mostraram potencial de proteção e rejuvenescimento celular no modelo com planárias.

Os compostos fenólicos identificados nos pós obtidos foram elagitaninos, ácido elágico, derivados de quercetina, ácido siringico e miricetina. Além disso, o potencial benéfico à saúde

avaliado como capacidade antioxidante *in vitro*, atividade antimicrobiana contra *S. aureus*, inibição de α-amylase e α-glicosidase *in vitro*, proteção e rejuvenescimento celulares em planárias, foi maior para os pós liofilizados. No entanto, não foi observada a inibição de ECA pelos compostos bioativos do camu-camu.

O pó de camu-camu usado como ingrediente na fermentação probiótica com leite de soja resultou no aumento do potencial bioativo do produto final. Observou-se, principalmente, o aumento da inibição *in vitro* das enzimas α-amilase e α-glicosidase após 72 horas de fermentação. Portanto, podemos concluir que os pós de camu-camu são boas fontes de compostos bioativos, e possuem grande potencial para serem usados como ingredientes em alimentos funcionais.

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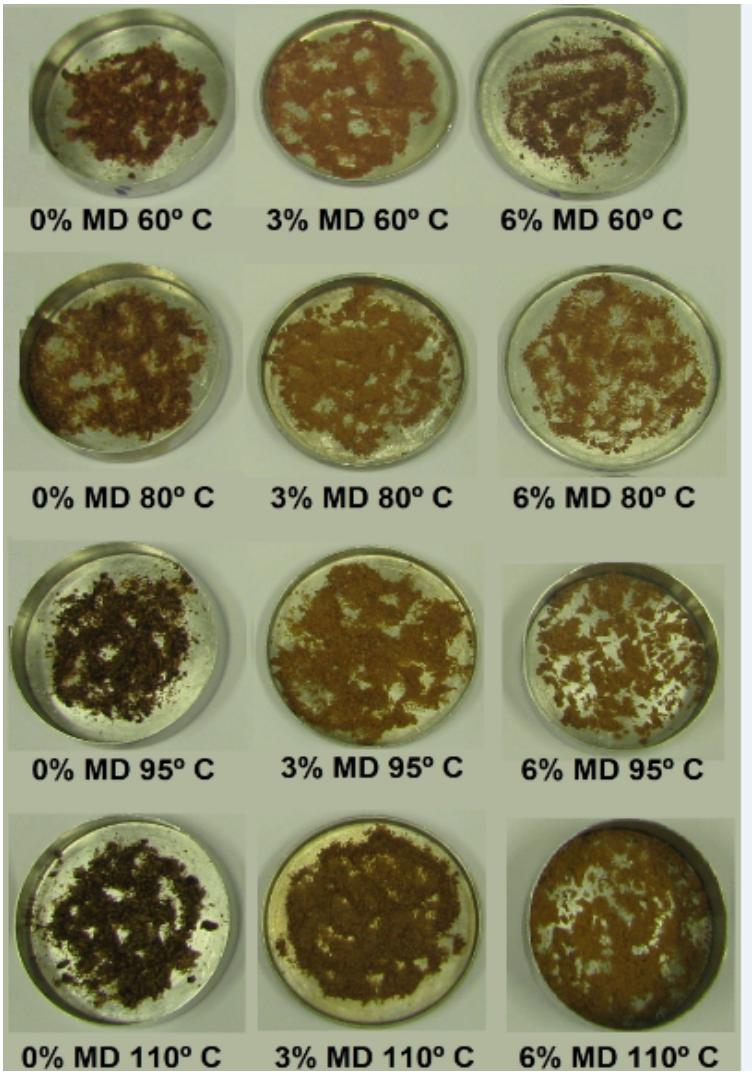
7. ANEXOS

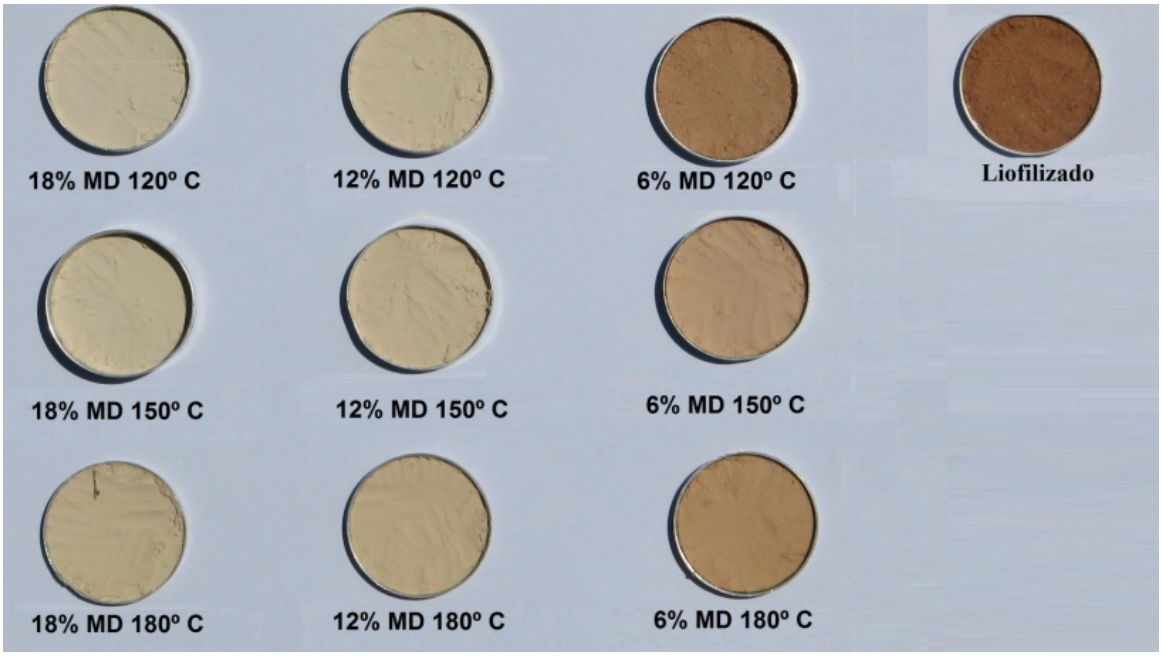
Anexo I
Fotos dos pós obtidos pela secagem em leito de jorro



Anexo II

Fotos dos pós obtidos pela secagem por atomização

A – Polpa do Amazonas com maltodextrina



B – Polpa de São Paulo com goma arábica