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STUDY OF THE EFFECT OF BIOACTIVE COMPOUNDS OF BRAZILIAN NATIVE FRUITS OF SOLANACEAE FAMILY IN THE REDUCTION OF OXIDATIVE STRESS AND MODULATION OF GUT MICROBIOTA AND THEIR CONSEQUENCES IN DIABETES

ESTUDO DO EFEITO DOS COMPOSTOS BIOATIVOS DE FRUTAS NATIVAS BRASILEIRAS DA FAMÍLIA SOLANACEAE NA REDUÇÃO DO ESTRESSE OXIDATIVO E NA MODULAÇÃO DA MICROBIOTA INTESTINAL E SUAS CONSEQUÊNCIAS NO DIABETES

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A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno.

“A educação é a arma mais poderosa que você pode usar para mudar o mundo”.

Nelson Mandela

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ABSTRACT

Diabetes mellitus (DM) is characterized by hyperglycemia and micro and macrovascular complications. The increase in the cases of diabetes has been of concern worldwide. Type 1 and type 2 DM are responsible for about 90% of cases. Oxidative stress and low-grade inflammation are the main causes of DM due to failures in glucose regulatory mechanisms (secretion and action of the insulin). Studies suggest that phytochemicals, such as phenolic compounds, for their ability to sequester free radicals, can improve insulin sensitivity, while minimizing the complications of diabetes. On the other hand, short chain fatty acids (SCFA), such as acetate, butyrate and propionate, produced by the intestinal microbiota from the fermentation of prebiotics confer several benefits to the host, such as the reduction of inflammation. In the Brazilian Cerrado there is a great diversity of native fruits that has attracted the attention for its potential as a source of bioactive compounds, besides the sensorial attributes. Most of these fruits are still unknown. Fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendt.) are native fruits from Cerrado belonging to the Solanaceae family. The local population uses fruta-do-lobo starch as a hypoglycemic agent, and this effect is attributed to its high resistant starch content. There are no reports on the composition or popular use of juá-açu. Thus, this study aimed to characterize both fruits as to their physicochemical characteristics and proximate and mineral composition, and to identify their bioactive compounds. From this identification, their biological activities, such as antioxidant capacity and potential biological activity (using the fruta-do-lobo starch), were evaluated. In addition, the changes in the chemical composition during the fruta-do-lobo ripeness were evaluated. Twenty-four phenolic compounds were identified in the three fractions of each fruit (peel, pulp and seed), most of them derived from hydroxycinnamic acid in juá-açu and chlorogenic acid in fruta-do-lobo. Juá-açu pulp presented greater antioxidant capacity and considerable oligosaccharides levels. Regarding prebiotic activity, the fruta-do-lobo starch promoted the growth of probiotic strains tested (*Lactobacillus acidophilus*, *L. caseei* and *Bifidobacterium lactis*), with the production of SCFA. The ripening of the fruta-do-lobo was confirmed by increasing in respiratory activity, among other physicochemical parameters. Changes in the sugar profile were evaluated by HPAEC-PAD, with an increase in glucose, fructose and sucrose and a variation in oligosaccharides. Alkaloids and phenolic compounds were identified by UHPLC-MS/MS. In general, chlorogenic acid was predominant in the green fruit. With ripeness, a predominance of p-coumaroylquinic acid for peel and pulp and 1-o-synapoyl glycoside for seeds was observed. Regarding the alkaloids, there was a reduction in solamargine, with an increase of solasonine and robeneoside B throughout the process. The results of this study provide support for future research aimed at elucidating the physiological and molecular mechanisms involved in the prevention and treatment of diabetes.

Keywords: Resistant starch, phenolic compounds, short chain fatty acid

RESUMO

O diabetes mellitus (DM) é caracterizado por hiperglicemia e complicações micro e macrovasculares, e o crescente aumento no número de casos tem sido motivo de preocupação em todo o mundo. Os DM tipo 1 e tipo 2 são responsáveis por cerca de 90% dos casos. O estresse oxidativo e a inflamação de baixo grau são as principais causas do DM, por provocar falhas nos mecanismos reguladores da glicose (secreção e ação da insulina). Estudos sugerem que fitoquímicos, como os compostos fenólicos, conhecidos por sua capacidade de sequestrar radicais livres, possam melhorar a sensibilidade à insulina, minimizando as complicações do diabetes. Por outro lado, os ácidos graxos de cadeia curta (AGCC) – como acetato, butirato e propionato, produzidos pela microbiota intestinal a partir da fermentação de prebióticos, conferem diversos benefícios ao hospedeiro, como a redução da inflamação. No Cerrado brasileiro há uma grande diversidade de frutos nativos que tem atraído a atenção pelo seu potencial como fonte compostos bioativos, além dos atributos sensoriais. A maioria desses frutos ainda é desconhecida. A fruta-do-lobo (*Solanum lycocarpum* St. Hill) e o juá-açu (*Solanum oocarpum* Sendt.) são frutas nativas do Cerrado pertencentes à família Solanaceae. O amido da fruta-do-lobo é utilizado pela população local como agente hipoglicemiante, sendo esse efeito atribuído ao seu alto teor de amido resistente. Não há relatos sobre a composição nem uso popular do juá-açu. Assim, o objetivo desse estudo foi caracterizar ambas as frutas quanto às suas características físico-químicas e composição centesimal e mineral, além de identificar seus compostos bioativos. A partir dessa identificação, foram avaliadas suas atividades biológicas, como capacidade antioxidante e potencial atividade biológica (utilizando o amido da fruta-do-lobo). Ademais, foram avaliadas as alterações na composição química durante o amadurecimento da fruta-do-lobo. Foram identificados 24 compostos fenólicos nas três frações de cada fruta (casca, polpa e semente), sendo a maioria derivados do ácido hidroxicinâmico no juá-açu e do ácido clorogênico na fruta-do-lobo. A polpa do juá-açu apresentou a maior capacidade antioxidante e também quantidade significativa de oligossacarídeos. Com relação a atividade prebiótica, o amido da fruta-do-lobo promoveu o crescimento das cepas probióticas testadas (*Lactobacillus acidophilus, L. caseei* e *Bifidobacterium lactis*), com produção de AGCC. O amadurecimento da fruta-do-lobo foi confirmado pelo aumento da atividade respiratória, dentre outros parâmetros físico-químicos. Alterações do perfil de açúcares foram avaliadas por HPAEC-PAD, sendo observado um aumento na glicose, frutose e sacarose e uma variação nos oligossacarídeos durante o amadurecimento. Alcalóides e compostos fenólicos foram identificados por UPLC-MS/MS. Em geral, houve uma predominância de ácido clorogênico na fruta verde. Com o amadurecimento, observou-se um predomínio do ácido p-coumaroilquínico para casca e polpa e o 1-o-sinapoil-glicosídeo para sementes. Com relação aos alcaloides, houve redução na solamargina, com aumento de solasonina e robeneosídeo B ao longo do processo. Os resultados desse estudo fornecem subsídios a pesquisas futuras que visem elucidar os mecanismos fisiológicos e moleculares envolvidos na prevenção e no tratamento do diabetes.

Palavras – chave: Amido resistente, compostos fenólicos, ácidos graxos de cadeia curta

SUMÁRIO

INTRODUÇÃO GERAL

A chamada transição nutricional, na qual o crescimento econômico e a globalização do comércio levaram a mudanças drásticas nos padrões alimentares, levou a um ganho de peso excessivo na população em geral, o qual foi acompanhado por um aumento na incidência de diabetes mellitus tipo 2 (Ley, Hamdy, Mohan & Hu, 2014).

O diabetes mellitus (DM) representa um conjunto de doenças autoimunes, metabólicas e genéticas, caracterizado por hiperglicemia e complicações micro e macrovasculares (Ighodaro, 2018; Egan & Dineen, 2019). A hiperglicemia, quando não controlada, leva ao surgimento de diversas comorbidades, que se manifestam a longo prazo, como retinopatia diabética, nefropatia diabética, neuropatia diabética, infarto do miocárdio e aterosclerose (Matough et al. 2012).

O crescente aumento no número casos dessa doença tem sido causa de preocupação a nível global, sendo uma das principais prioridades da Saúde Pública (Forouhi & Wareham, 2019). De acordo com a Federação Internacional de Diabetes (IDF, 2019), estima-se que há 425 milhões de adultos portadores de diabetes em todo o mundo. Apenas no Brasil, há mais de 13 milhões de pessoas vivendo com diabetes, o que representa 6,9% da população (Sociedade Brasileira de Diabetes, 2019). Ainda de acordo com o IDF, 12% dos gastos globais com saúde são com a doença, o que correponde a US$ 727 bilhões.

O diabetes é a sétima principal causa de mortes registradas em todo o mundo e, apenas em 2016, foi responsável por 1,6 milhão de mortes (Organização Pan-Americana de Saúde – OPAS BRASIL, 2019). Portanto, investir na prevenção e manejo efetivo tornou-se necessário para combater essa epidemia global.

Muito progresso tem sido feito na compreensão dos fatores de risco dietéticos. Enquanto dietas caracterizadas pelo alto consumo de carne vermelha e processada, bebidas açucaradas e carboidratos refinados estão associadas com uma maior inciência de casos; um maior consumo de frutas e legumes, produtos lácteos (como iogurte); produtos integrais e alimentos com alto teor de gorduras poli-insaturadas, fibras e compostos bioativos em geral apresentam menor risco de desenvolvimento dessa doença (Ley et al., 2014).

Estudos demonstram que o DM está associado a um aumento da formação de radicais livres e à uma diminuição do potencial antioxidante, caracterizando um estado de estresse oxidativo (Ighodaro, 2018). O estresse oxidativo está associado com a falha dos principais mecanismos reguladores da glicose (secreção de insulina e ação da insulina) no diabetes, bem como com as complicações dessa doença (Asmat et al., 2016).

O organismo deve controlar as concentrações de ambos, pró-oxidantes e antioxidantes, para que haja um equilíbrio homeostático e as funções biológicas sejam preservadas. Estudos sugerem que os fitoquímicos, como os compostos fenólicos, conhecidos por sua capacidade de sequestrar radicais, possam melhorar a sensibilidade à insulina e, portanto, minimizar as complicações do diabete (Bacanli et al., 2019).

Nos biomas do Brasil há uma enorme diversidade de frutos nativos, geralmente não explorados comercialmente, que tem atraído a atenção pelo seu potencial como fonte de compostos bioativos (Neri-Numa et al., 2014). A maior parte dessas frutas apresenta qualidade sensorial excepcional, despertando o interesse do mercado pelo apelo exótico e nutricional.

Estudos em modelo animal têm demonstrado o benefício de compostos extraídos de frutas nativas brasileiras na melhora dos parâmetros bioquímicos associados ao diabetes, como por exemplo, antocianinas, extraídas da casca de jabuticaba (Lenquiste et al., 2012; Dragano et al., 2013; Plaza et al., 2016) e do açaí (Udani et al., 2011; Poulose et al., 2012).

Outro composto que vem sendo estudado devido ao seu efeito hipoglicemiante é o amido da fruta-do-lobo (*Solanum lycocarpum* St. Hill) (Oliveira et al., 2003; Farina et al., 2010). A fruta-do-lobo é nativa do Cerrado e seu amido é utilizado na medicina popular para o controle da glicemia, dos níveis de colesterol sanguíneos e do peso, sendo suas propriedades atribuídas ao seu alto teor de amido resistente (Dall'agnol & Von Poser, 2000).

Diferente dos fitoquímicos supracitados, o mecanismo de ação do amido resistente possivelmente se dá pela produção de ácidos graxos de cadeia curta (AGCC), como acetato, butirato e propionato. Esses AGCC modulam a microbiota

intestinal, promovendo o crescimento de micro-organismos benéficos à saúde, como *Bifidobacterium* e *Lactobacillus* (Sharma & Tripath, 2019).

Embora o efeito hipoglicemiante de algumas espécies tenha sido comprovado através da ação de flavonoides, sesquiterpenos glicosídicos, lignanas, derivados de β-glucano, muitas plantas ainda são consumidas de forma indiscriminada e apresentam risco de serem tóxicas e causar danos à saúde (Malviya et al.,2010; Tundis et al.,2010; Patel et al.,2012). Portanto, a elucidação de moléculas, bem como os estudos de aplicação tecnológica e farmacêutica para formulação de produtos com apelo natural e/ou funcional que possam atuar na promoção da saúde são necessários.

Considerando o potencial das frutas brasileiras, é de grande interesse a caracterização de sua composição química, além do estudo de suas propriedades biológicas. O conhecimento dessas frutas desperta o interesse da população, gerando um novo mercado consumidor, o que poderia beneficiar pequenos produtores e agroindústrias. Ademais, demonstrar a importância dessas espécies pode contribuir para a preservação e reconstituição dos biomas nas quais elas estão inseridas, trazendo benefícios direto para o meio ambiente e gerando renda de forma sustentável.

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OBJETIVOS

Objetivo geral

O objetivo desse estudo foi identificar os compostos bioativos de frutas nativas da família Solanaceae, bem como avaliar sua capacidade antioxidante e potencial prebiótico, visando fornecer subsídios a pesquisas futuras que visem elucidar os mecanismos fisiológicos e moleculares na prevenção e tratamento do diabetes.

Objetivos específicos

- Caracterizar duas frutas da família Solanaceae, fruta-do-lobo (*Solanum lycocarpum* St. Hill) e juá-açu (*Solanum oocarpum* Sendt.), quanto à suas características físico-químicas, composição centesimal e teor de fibras e minerais;
- Identificar e quantificar os mono-, di- e oligossacarídeos;
- Identificar os compostos antioxidantes;
- Avaliar a capacidade antioxidante *in vitro* utilizando o método Capacidade de Absorção do Radical Oxigênio (ORAC);
- Quantificar o teor de amido resistente presente no amido da fruta-do-lobo;
- Avaliar a potencial atividade prebiótica *in vitro* do amido da fruta-do-lobo frente a culturas probióticas dos gêneros *Bifidobacterium* e *Lactobacillus.*
- Avaliar as alterações no perfil de carboidratos, compostos fenólicos e alcaloides durante o amadurecimento da fruta-do-lobo.

REVISÃO BIBLIOGRÁFICA

Diabetes

O diabetes mellitus (DM) representa um conjunto de doenças autoimunes, metabólicas e genéticas, caracterizado por hiperglicemia e complicações micro e macrovasculares (Ighodaro, 2018; Egan & Dineen, 2019). Embora as complicações microvasculares na retina, nos rins ou nos nervos periféricos causadas pela hiperglicemia sejam característicos do diabetes, eles não podem ser usados para defini-lo, pois demoram para se manifestar (Forouhi & Wareham, 2019). Entretanto, as complicações macrovasculares do diabetes, como infarto do miocárdio, acidente vascular cerebral e doença arterial periférica, ocorrem mais frequentemente, uma vez que estas estão frequentemente presentes no estado pré-diabético. Por esse motivo, tem sido sugerido que o diabetes deve ser definido como "aterosclerose prematura com hiperglicemia associada" (Forouhi & Wareham, 2019).

A classificação e diagnóstico do diabetes têm sido objeto de discussão ao longo de décadas. De acordo com a Associação Americana de Diabetes (ADA, 2018), pode ser classificado em:

- **Diabetes tipo 1:** ocorre devido à destruição auto-imune das células β, geralmente levando à deficiência absoluta de insulina;
- **Diabetes tipo 2:** ocorre devido a uma perda progressiva da secreção de insulina pelas células β, frequentemente associado à resistência à insulina;
- **Diabetes gestacional:** é diagnosticado no segundo ou terceiro trimestre de gestação em pacientes que não apresentavam diabetes evidente antes da gestação;
- **Tipos específicos de diabetes devido a outras causas:** por exemplo, síndrome monogênica do diabetes (como *diabetes neonatal e maturity onset diabetes of the young*) - *MODY*), doenças do pâncreas exócrino (como fibrose cística e pancreatite), diabetes induzido por produtos químicos ou drogas (como o uso de glicocorticoides, no tratamento de HIV/AIDS ou após o transplante de órgãos).

O DM tipo 1 e 2 são responsáveis pela maioria dos casos, e apenas o DM tipo 2, também chamado de diabetes não insulinodependente, corresponde a 90% dos casos (Sociedade Brasileira de Diabetes, 2019).

Diabetes mellitus tipo 2

Enquando no DM tipo 1 as células β pancreáticas são destruídas, geralmente por mecanismos inflamatórios autoimunes, levando a ausência de produção e secreção de insulina, o DM tipo 2 está associado à disfunção das células β e a vários graus de resistência à insulina (Forouhi & Wareham, 2019).

Por ser pouco sintomático, o DM tipo 2, na maioria das vezes, permanece por muitos anos sem diagnóstico e sem tratamento, o que favorece a ocorrência de suas complicações no coração e no cérebro (Sociedade Brasileira de Diabetes, 2019). Há um longo período de pré-detecção (3 a 7 anos) durante o qual os níveis de glicose estão elevados, mas muitas vezes não são diagnosticados clinicamente (Forouhi & Wareham, 2019). Estima-se que metade das pessoas com diabetes desconheçam sua doença, embora essa proporção varie de acordo com a região e as oportunidades de rastreamento para o diagnóstico (IDF, 2019).

De acordo com Egan & Dinneen (2019), o diagnóstico de diabetes pode ser estabelecido a partir de qualquer um dos seguintes critérios:

- Concentração de glicose plasmática ≥ 11,1 mmol/L na presença de sintomas clássicos de hiperglicemia, incluindo polidipsia, poliúria e perda de peso (considerado um marcador de deficiência de insulina);
- Glicemia de jejum (mínimo de 8 horas) ≥ 7,0 mmol/L
- Glicose plasmática ≥ 11,1 mmol/L no teste oral de tolerância à glicose (TOTG) (2 horas após a administração de 75 g de glicose anidra dissolvida em água);
- Hemoglobina glicada (HbA1c) ≥ 48 mmol/mol (≥ 6,5 %).

A prevalência de DM tipo 2 é menor nas áreas rurais dos países em desenvolvimento e, geralmente, intermediária em países desenvolvidos. É observado uma maior prevalência em certos grupos étnicos, particularmente aqueles que adotaram padrões de estilo de vida ocidental, e está intimamente relacionado com uma alta prevalência de obesidade, como nos Estados Unidos, por exemplo (Forouhi & Wareham, 2019). Adicionalmente, estudos recentes têm demonstrado que alterações na microbiota intestinal estão associadas com diversas doenças, incluindo o diabetes (Gholizadeh et al., 2019).

Ainda do ponto de vista epidemiológico, ocorre com maior frequência em homens e vem sendo diagnosticada cada vez mais frequentemente em pessoas jovens, embora a prevalência aumenta acentuadamente com a idade em ambos os sexos (Forouhi & Wareham, 2019; SBD, 2019).

Enquanto os principais fatores de risco etiológico são idade, obesidade, história familiar, etnia, inatividade física e dieta, os principais defeitos fisiopatológicos que levam ao surgimento do DM tipo 2 são a resistência à insulina e/ou falha na secreção desse hormônio (Forouhi & Wareham, 2019).

Fisiopatologia do diabetes

Resumidamente, em condições fisiológicas normais, quando há um aumento nos níveis de glicose sanguínea, as células β pancreáticas aumentam o processo de secreção de insulina e, consequentemente, aumenta a taxa de captação dessa molécula. O processo de oxidação é iniciado pela hexoquinase, que fosforila a glicose transformando-a em glicose-6-fosfato (Ighodaro, 2018). A glicose-6-fosfato segue pela via glicolítica, até a formação de ATP (Figura 1).

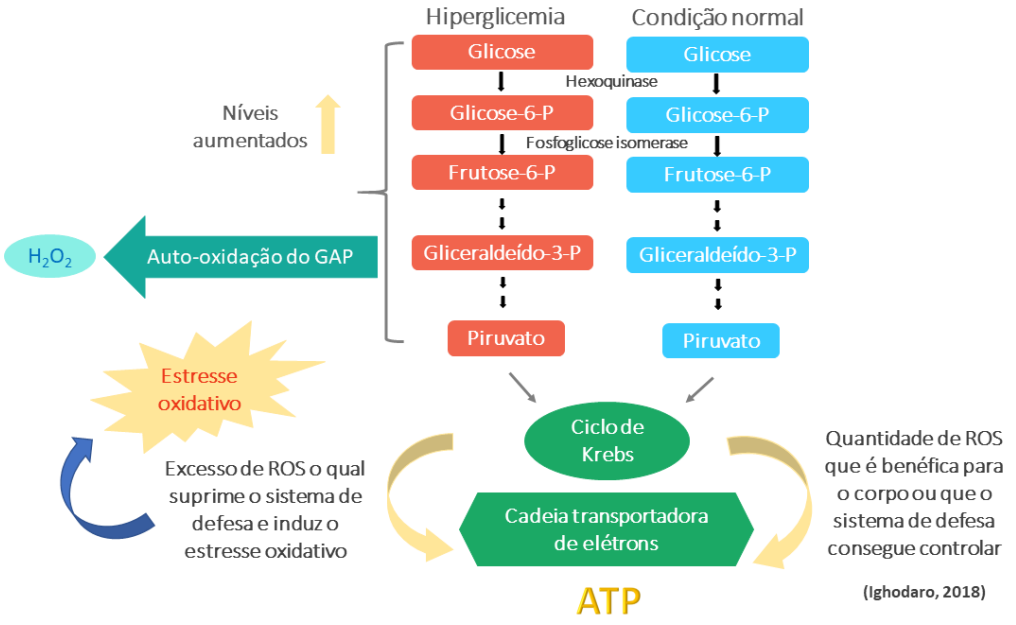

Figura 1. Indução do estresse oxidativo a partir da via glicolítica
Fonte: Adaptado de Ighodaro, 2018.

A insulina, uma vez que atinge a circulação portal, é transportada para os tecidos periféricos, onde se liga ao seu receptor para realizar sua ação de mobilizar glicose nas células dos tecidos insulino-dependentes (Ighodaro, 2018).

Os indivíduos com tolerância à glicose diminuída apresentam hiperglicemia, apesar de apresentarem níveis mais elevados de insulina plasmática, indicando que são resistentes à ação desse hormônio. Entretanto, com a progressão da doença, o nível de insulina diminui, sugerindo que há, também, uma redução em sua secreção em pacientes com DM tipo 2 (Ozougwu et al., 2013).

Tanto a deficiência na produção quanto a resistência à insulina no corpo levam a uma menor absorção tecidual de glicose, que resulta em hipoglicemia intracelular e hiperglicemia extracelular. Esse desequilíbrio resulta em degradação das gorduras (causando cetoacidose diabética) e diminuição da síntese protéica (causando caquexia, polifagia e comprometimento da cicatrização), ambos causados pela hipoglicemia intracelular. Por outro lado, a hiperglicemia extracelular pode resultar em coma hiperglicêmico (Asmat et al., 2016).

Os principais mecanismos reguladores da glicose no diabetes são a secreção e ação da insulina, ambos controlados pela via molecular conhecida como sinalização de insulina (Ighodaro, 2018). Essa via pode ser modulada por espécies reativas de oxigênio e nitrogênio (ROS e RNS, respectivamente) (Asmat et al., 2016).

As espécies reativas são produzidas durante a oxidação da glicose, em resposta à insulina. Por por outro lado, em situações de desequilíbrio, ROS e RNS têm regulação negativa na sinalização da insulina, contribuindo para o quadro de resistência à insulina, que é um fator de risco para o DM tipo 2. Por isso, falhas nesses mecanismos reguladores têm sido associadas ao estresse oxidativo (Ighodaro, 2018).

Além do estresse oxidativo, a inflamação de baixo grau também é um fator fisiopatológico de relevância na progressão do DM tipo 2 (Sharma & Tripathi, 2019). De acordo com esses autores, a redução da diversidade da microbiota intestinal leva ao aumento do número de bactérias patogênicas e inflamação intestinal, contribuindo para a resistância a insulina.

Estresse oxidativo

O corpo humano é continuamente exposto a diferentes tipos de agentes que resultam na produção de espécies reativas, que em baixas concentrações são benéficas ao organismo. O próprio corpo possui sistemas antioxidantes endógenos, além dos antioxidantes exógenos obtidos pela dieta, que são capazes de neutralizar essas espécies e manter a homeostase do corpo (Asmat et al. 2016). Entretanto, quando há qualquer desequilíbrio entre a relação pró- e antioxidantes, favorecendo o aumento de substâncias pró-oxidantes, ocorre o chamado "estresse oxidativo" (Sies, 1985). Em geral, há uma formação excessiva e/ou remoção insuficiente das espécies reativas, que provocam danos às membranas e biomoléculas vitais, como DNA, proteínas e lipídios, com consequente perda de função celular (Asmat et al., 2016).

O estresse oxidativo tem sido amplamente associado à incidência de DM, sendo considerado um elemento-chave no desenvolvimento e progressão da doença e suas complicações associadas (Giacco & Brownlee, 2010; Rains & Jain, 2011; Alipour et al., 2012). Em condições hiperglicêmicas crônicas, a produção de ROS se torna exacerbada, de forma que os sistemas de defesa antioxidantes não sejam capazes de neutralizá-las, o que intensifica ainda mais o estresse oxidativo (Ighodaro, 2018).

Cascatas de eventos moleculares em diferentes vias do metabolismo da glicose e dos lipídeos estão envolvidas na indução do estresse oxidativo. Por exemplo, o ânion superóxido ($O2^{\cdot}$) é produzido pela via glicolítica durante a oxidação da glicose (Figura 1). Em condições fisiológicas normais, os mecanismos de defesa conseguem neutralizar as espécies reativas que são produzidas. Entretanto, quando há excesso de glicose no sangue (hiperglicemia), há uma produção excessiva desse radical ($O2^{\cdot}$), que se sobrepõem aos sistemas antioxidantes do corpo, induzindo o estresse oxidativo, que consequentemente, causa danos ao DNA, bem como em outras biomoléculas (Ighodaro, 2018).

Os radicais livres são formados desproporcionalmente no diabetes pela oxidação da glicose, dentre outros fatores (Alipour et al., 2012). Como consequência do dano ao DNA, uma enzima de reparo é ativada e leva a um aumento nos níveis de gliceraldeído-3-fosfato, que é auto-oxidado, produzindo peróxido de hidrogênio (H_2O_2)

(Figura 2). Todos esses fatores contribuem para um aumento do estresse oxidativo (Ighodaro, 2018).

Em síntese, o papel do estresse oxidativo na patogênese do diabetes se dá pela alteração nos sistemas enzimáticos, peroxidação lipídica, metabolismo da glutationa prejudicada e diminuição dos níveis de vitamina C (Ayepola, Brooks & Oguntibeju, 2014). Portanto, lipídios, proteínas, danos no DNA, glutationa, catalase e superóxido dismutase são considerados biomarcadores do estresse oxidativo no diabetes (Asmat et al., 2016).

O estresse oxidativo, por sua vez, desempenha um papel importante no desenvolvimento de complicações vasculares no diabetes, particularmente no DM tipo 2 (Asmat et al., 2016). A hiperglicemia não controlada está relacionada com diversos danos que acometem o paciente diabético a longo prazo, como disfunção e falência de diferentes órgãos, especialmente os olhos (retinopatia diabética), rins (nefropatia diabética), nervos (neuropatia diabética), coração (infarto do miocárdio) e vasos sanguíneos (aterosclerose) (Matough et al. 2012).

Considerando o ônus que o diabetes e suas comorbidades impõe às economias, além do alto índice de mortalidade prematura (Forouhi & Wareham, 2019), faz-se necessário a busca de terapias adjuvantes que possam auxiliar no seu tratamento. Além da terapia antioxidante, tem sido casa vez mais bem documentado a importância da modulação da microbiota intestinal (Priyadarshini et al., 2016; Colantonio et al., 2019; Sharma & Tripathi, 2019). Os ácidos graxos de cadeia curta (AGCC) produzidos a partir da metabolização dos compostos prebióticos têm sido associados a uma melhorara dos sintomas do DM tipos 1 e 2.

Antioxidantes

Os radicais livres são espécies de curta duração, altamente instáveis e reativos, que contém um ou mais elétrons não pareados. Embora sejam necessários para diversos processos fisiológicos, como na transcrição de genes e ativação de diversas vias de sinalização celular, o excesso de radicais livres induz danos às células, resultando na oxidação de componentes celulares e moléculas (Asmat et al., 2016).

Em contrapartida, os antioxidantes são substâncias cuja disponibilidade, mesmo em concentrações mínimas, retarda ou inibe a oxidação de um substrato (Halliwell, 1990). Em nível molecular, podem atuar de diversas maneiras: inibindo a formação e/ou capturando as ROS e RNS, inibindo enzimas oxidativas, ou ainda reduzindo os danos causados por essas espécies reativas (Borosky et al.2015). São divididos em endógenos, quando sintetizados pelo próprio corpo, e exógenos, quando ingeridos pela dieta.

Os mecanismos de defesa antioxidante endógenos incluem ambas as vias enzimáticas (superóxido dismutase - SOD, catalase - CAT, glutationa peroxidase - GPx, e glutationa redutase - GRx, e não enzimáticas (vitaminas A, C e E e glutationa - GSH) e têm como função contrabalançar as ROS produzidas nas células (Matough et al. 2012). Por exemplo, a CAT converte o peróxido de hidrogênio (H_2O_2) em água e oxigênio, neutralizando-o. Quando há deficiência de catalase, a célula β, que contém grande quantidade de mitocôndrias, produz ROS em excesso, caracterizando estresse oxidativo. Consequentemente, leva à disfunção das células β e, em última instância, ao diabetes (Ighodaro, 2018).

Além dos mecanismos de defesa endógenos, estudos sugerem que dietas contendo antioxidantes e agentes anti-inflamatórios também podem diminuir o estresse oxidativo (Wichansawakun & Buttar, 2019). Um estudo recente mostrou que os antioxidantes dietéticos foram inversamente associados ao risco de câncer do cólon, estômago e endométrio, possivelmente devido à sua capacidade de reduzir os danos ao DNA causados por ROS e RNS (Parohan et al., 2019).

Adicionalmente, dois estudos epidemiológicos foram realizados para investigar a associação entre os antioxidantes dietéticos e o DM tipo 2. Enquanto o estudo de coorte que acompanhou mulheres francesas durante 15 anos mostrou uma associação inversa significativa entre a ingestão de antioxidantes e DM tipo 2 (Mancini et al., 2018), um estudo prospectivo (5 anos) com indivíduos japoneses de ambos os sexos não encontrou associação entre as medidas de antioxidantes dietéticos e o diabetes (Kashino et al., 2019). Um fator importante a se observar é que no estudo de Mancini et al. (2018) 23 % dos antioxidantes vieram de frutas e 19 % de vegetais, sendo esses as principais fontes de fitoquímicos da dieta. Por outro lado, a principal fonte de antioxidantes da população japonesa estudada foi o chá verde (60%).

De maneira geral, as plantas possuem diferentes compostos bioativos, que apresentam estruturas distintas e, assim, oferecem diversas atividades biológicas, como atividade antioxidante (Figura 2) (Chandrasekara & Shahidi, 2012).

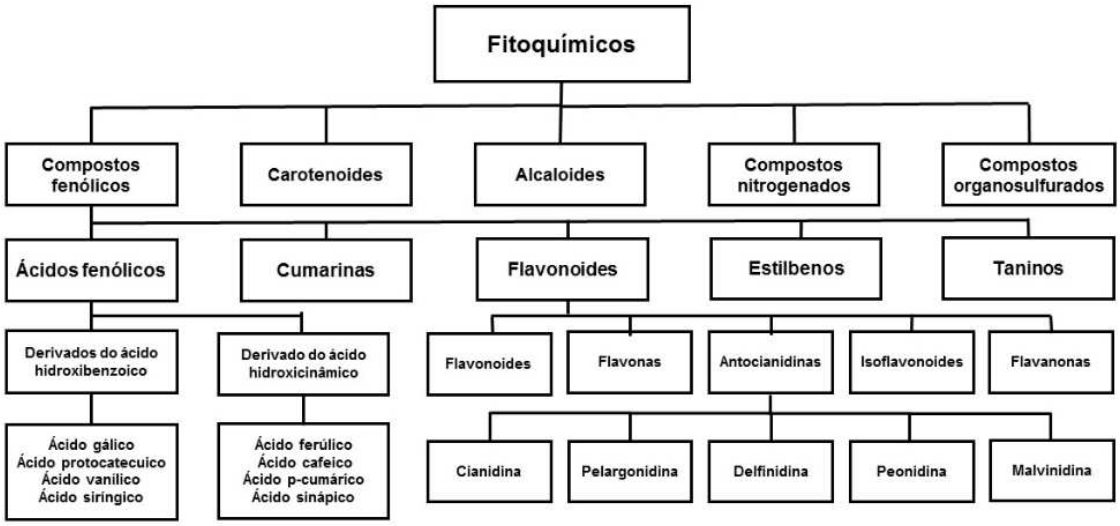

Figura 2. Principais fitoquímicos presentes na dieta

Fonte: Adaptado de Liu, 2004.

Apesar dos benefícios dos antioxidantes, vale ressaltar que alguns desses compostos exibem atividade pró-oxidante sob certas condições (Shahidi & Ambigaipalan, 2015). Portanto, sugere-se que uma combinação de antioxidantes com diferentes modos de ação aumenta a eficácia e minimiza a toxicidade (Lee e Lee, 2006). Ademais, os efeitos aditivos e sinérgicos dos fitoquímicos da dieta obtidos de frutas e vegetais são mais eficientes para reverter o estresse oxidativo do que os suplementos dietéticos (Liu, 2004). Os compostos antioxidantes que ocorrem naturalmente são flavonoides e ácidos fenólicos, lignanas, terpenos, tocoferóis, fosfolipídios e ácidos orgânicos polifuncionais, sendo os compostos fenólicos os mais abundantes (Shahidi & Ambigaipalan, 2015).

Compostos fenólicos

Os compostos fenólicos são produtos do metabolismo secundário das plantas superiores, formados durante condições de estresse (infecções por patógenos, ferimentos, radiações UV, dentre outros) e são essenciais para seu desenvolvimento (Liu, 2004).

Os flavonoides e os ácidos fenólicos derivados do ácido hidroxicinâmico são os principais compostos fenólicos presentes nas frutas e vegetais e exibem uma ampla gama de propriedades fisiológicas, tais como efeitos antialérgicos, antiaterogênicos, anti-inflamatórios, antimicrobianos, antioxidantes, antitrombóticos, cardioprotetores e vasodilatadores (Shahidi & Ambigaipalan, 2015).

Os flavonoides compreendem cerca de dois terços de todos os compostos fenólicos presentes na dieta (Robbins, 2003). Apresentam em comum o esqueleto carbônico composto por $C_6C_3C_6$ (Figura 3), variando apenas sua estrutura em torno do anel de oxigênio heterocíclico (Shahidi & Naczk, 2004). Em geral, a capacidade antioxidante dos flavonoides depende do potencial quelante de metais (dependente do arranjo de hidroxilas e do grupo carbonila ao redor da molécula), da presença de hidrogênio/elétrons capazes de reduzir os radicais livres, e da capacidade do flavonóide para deslocar o elétron desemparelhado levando à formação de um radical fenoxila estável (Shahidi & Ambigaipalan, 2015). De acordo com Shahidi & Ambigaipalan (2015), tanto o mecanismo preventivo quanto o mecanismo de quebra de cadeia são considerados responsáveis pela alta capacidade antioxidante dos flavonoides.

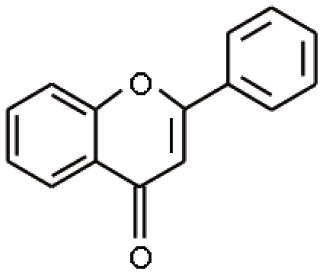

Figura 3. Estrutura química dos flavonoides

Com relação aos ácidos fenólicos, o principal mecanismo se dá pela eliminação de radicais livres via doação de átomos de hidrogênio, embora também possa acontecer a doação de elétrons e a inibição do oxigênio singlete (Shahidi & Wanasundara, 1992). O ácido fenólico mais conhecido é o ácido clorogênico, formado a partir da esterificação dos ácidos cafeico e quínico (Figura 4) (Shahidi & Ambigaipalan, 2015).

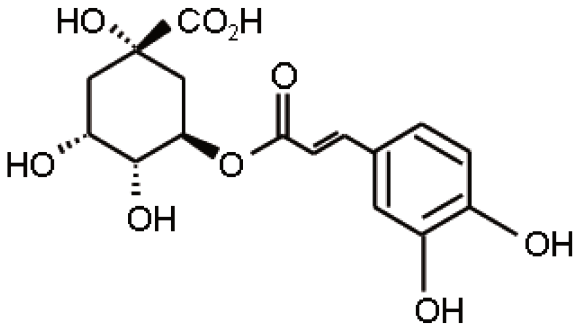

Figura 4: Ácido clorogênico

Prebióticos

Prebiótico, de acordo com a nova definição, é um substrato que é usado seletivamente por micro-organismos hospedeiros conferindo benefícios à saúde (Gibson et al., 2017). Inulina, oligossacarídeos, frutooligossacarídeos (FOS), galactooligossacarídeos (GOS), lactulose e amido resistente são alguns exemplos de polissacarídeos não-digeríves reconhecidos como prebióticos (Colantonio et al., 2019).

Oligossacarídeos não-digeríveis

Os oligossacarídeos não-digeríveis são carboidratos funcionais que consistem em 3 a 10 unidades monossacarídicas, de forma linear ou ramificada, conectadas por ligações α- e/ou β-glicosídicas (Zhao et al., 2017).

O uso de oligossacarídeos melhora o equilíbrio da microbiota intestinal e pode reduzir o risco de doenças relacionadas ao estilo de vida, como doenças cardiovasculares, câncer, obesidade e diabetes tipo 2, que estão relacionados à obesidade (Mussatto & Mancilha, 2007).

Os FOS não são hidrolisados pelas enzimas do corpo humano e, dessa forma, passam por meio do trato digestivo sem serem metabolizados, exercendo funções semelhantes à fibra alimentar e, portanto, são frequentemente utilizados em dietas específicas para distúrbios específicos induzidos pelas síndromes metabólicas (Zhao et al., 2017).

Sugere-se que esses compostos influenciem na absorção de macronutrientes, especialmente de carboidratos, retardando o esvaziamento gástrico e/ou diminuindo o tempo de trânsito no intestino delgado, além de uma possível gliconeogênese mediada por ácidos graxos de cadeia curta, especialmente o propionato (Zhao et al., 2017).

Amido resistente

Amido resistente é a quantidade total de amido, bem como os produtos de sua degradação, que resistem à digestão no intestino delgado e atinge o cólon, sendo depois fermentado pela microbiota intestinal (Zaman & Sarbini, 2015). Como resultado dessa fermentação, tem-se a formação dos AGCC, que são absorvidos pelo hospedeiro e, posteriormente, utilizado em vários processos metabólicos, exercendo diversos efeitos benéficos à saúde (Schwiertz, 2016). Isso faz do amido resistente um potencial agente para a prevenção de doenças associadas à resistência à insulina, como o DM tipo 2 (Higgins, 2004).

De acordo com Colantonio et al. (2019), há evidências suficientes de que os prebióticos, particularmente o amido resistente, dextrina resistente e inulina enriquecida com oligofrutose, podem melhorar os biomarcadores metabólicos e inflamatórios relacionados ao DM tipo 2 em mulheres maiores de 18 anos de idade.

O possível mecanismo de ação se dá pelos receptores de AGCC, FFA2 (para acetato e propionato) e FFA3 (para butirato), encontrados nas ilhotas pancreáticas (Tang et al., 2015). Enquanto o FFA3 modula a secreção de insulina estimulada pela glicose, o FFA2 contribui para a regulação da massa de célula β (Priyadarshini et al., 2016).

Frutas brasileiras como fonte de compostos bioativos

Estudos epidemiológicos evidenciam a importância de nutrientes individuais bem como de padrões alimentares para prevenção e manejo de diversas doenças crônicas não-transmissíveis, incluindo o diabetes, os quais tipicamente apresentam alto consumo de cereais integrais, frutas e vegetais em geral, e nozes;

também consumo moderado de álcool e baixo consumo de grãos refinados, carnes vermelhas ou processadas e bebidas açucaradas (Ley et al., 2014).

A maioria dessas recomendações tem sido desenvolvidas e implementadas em países desenvolvidos (Evert et al, 2014). Considerando que, para se garantir uma adesão a um padrão alimentar, os indivíduos precisam ter flexibilidade nas escolhas alimentares sem comprometer a qualidade global, é necessário um maior desenvolvimento de diretrizes específicas para cada região, visando a acessibilidade aos alimentos (Ley et al., 2014).

Nesse sentido, uma alternativa viável no Brasil seria incentivar o consumo de suas frutas nativas. As frutas estão entre as mais importantes fontes de fibras, vitaminas, minerais e os compostos bioativos, sendo fundamentais para um desenvolvimento e funcionamento adequados do organismo (Boeing et al., 2012). Estes compostos variam extensamente em estrutura química, e, consequentemente, na função biológica, como a atividade antioxidante, modulação de enzimas e detoxificação, estimulação do sistema imune, modulação do metabolismo hormonal, atividade anti-inflamatória, entre outras (Viera et al.,2006).

Grande parte dos estudos que abordam compostos bioativos e seus efeitos na saúde têm focado em frutas de regiões temperadas, subestimando a importância das frutas tropicais (Neri-Numa et al, 2018). Por exemplo, o consumo de mirtilos, uvas e maçãs foi significativamente associado a um menor risco de diabetes (Salas-Salvadó et al., 2014).

Entretanto, no Brasil há uma variedade imensa de frutas nativas, distribuídas ao longo de seus seis biomas (Floresta Amazônica, Caatinga, Cerrado, Mata Atlântica, Pantanal e Pampa) que, embora ainda pouco conhecidas, apresentam potencial para serem exploradas pelos setores alimentício, farmacêutico e agroindustrial (Neri-Numa et al., 2018).

Frutas da família Solanaceae

Embora a família Solanaceae compreenda algumas das espécies mais consumidas em todo mundo, como *Solanum tuberosum* (batata), *S. lycopersicum* (tomate), *S. melongena* (berinjela) e *Capsicum annuum* (pimenta), suas frutas foram

negligenciadas, sendo pouco estudadas ou exploradas comercialmente (Ghatak et al., 2017).

Algumas espécies dessa família têm sido propostas como novas culturas com alto potencial de desenvolvimento e, apenas nos últimos anos que algumas frutas estão sendo gradualmente domesticadas, como o cubiu (*Solanum sessiliflorum*) e o camapu (*Physalis*).

A família Solanaceae apresenta aproximadamente 98 gêneros e mais de 3000 espécies, sendo quase metade pertencente ao gênero *Solanum*, o mais representativo da família (Lima et al., 2014). Esse gênero apresenta-se como um grupo bem caracterizado, apesar da semelhança morfológica existente entre suas espécies, que podem ser utilizadas tanto para fins alimentícios, quanto para fins terapêuticos (Coutinho, 2009). Apenas no Brasil a família Solanaceae apresenta 31 gêneros e cerca de 500 espécies nativas (Pereira et al., 2016).

Há uma forte presença de glicoalcaloides esteroídicos no gênero *Solanum*, aos quais têm atribuídas as atividades biológicas e toxicológicas de várias espécies (Coutinho, 2009). Os principais glicoalcaloides encontradas nas plantas do gênero Solanum são a solasodina e a solamargina.

Embora os glicoalcaloides sejam compostos tóxicos em certos níveis, eles também apresentam diversos efeitos benéficos e tem sido amplamente estudado devido aos seus efeitos antioxidantes, antidiabéticos, antifúngicos, antibiótico e, principalmente, devido às suas propriedades anticancerígenas (Al Sinani & Eltayeb, 2017).

Além dos glicoalcaloides, compostos fenólicos vem sendo amplamente caracterizados em frutas desse gênero, como rutina identificada em tomatinho-do-mato (*Solanum diploconos*) (Ribeiro et al., 2016).

Dentre as frutas do gênero *Solanum*, destaca-se a fruta-do-lobo (*Solanum lycocarpum* St Hill), à qual têm sido atribuídas as propriedades hipoglicemiantes e hipocolesterolêmicas, além de atividades anti-inflamatória e antiproliferativa, cujas vias metabólicas envolvidas ainda não estão bem definidas (Viera et al., 2003; Rocha et al., 2012; Munari et al., 2014).

Fruta-do-lobo

Típica do Cerrado brasileiro, a fruta-do-lobo é utilizada na preparação de geleias e doces por apresentar aroma agradável, polpa abundante e adocicada (Morais et al., 2013). Na medicina popular é utilizada como sedativo, diurético, antiepiléptico e antiespasmódico, assim como agente hipoglicemiante e hipocolesterolêmico (Nakamura et al., 2008; Vieira et al., 2003; Araujo et al., 2010). Os trabalhos sobre a composição química de metabólitos especiais da fruta-de-lobo ainda são escassos, verificando-se uma predominância na investigação de substâncias da classe dos alcaloides (Nakamura et al., 2008; Munari et al., 2014).

Entretanto, grande parte dos estudos sobre a atividade hipoglicemiante da fruta-do-lobo são conduzidos a partir do amido extraído dessa fruta, que já é vendido na forma de cápsulas em farmácias de manipulação. Dall'agnol e Von Poser (2000) realizaram um estudo comparativo entre as frutas e "polvilho da lobeira", como é chamado comercialmente. Eles verificaram que os glicoalcaloides encontrados na fruta não estavam presentes no polvilho. Esses compostos são solúveis em água e possivelmente foram eliminados no processo de extração do amido. O único grupo de compostos que ocorreu nos frutos e no polvilho foram os polissacarídeos, incluindo pectina, mucilagem e amido, aos quais foram atribuídas as atividades hipoglicêmicas e hipocolesterolêmicas atribuídas ao "polvilho de lobeira".

Outro estudo com ensaio animal mostrou uma redução significativa da glicemia (55%) em ratos diabéticos após o tratamento com essa fruta comparando com o grupo diabético, mas ainda assim foi maior que o controle. Além disso, os animais tratados não apresentaram sinais de hipertrofia renal, ao contrário do grupo controle, e mostraram atenuação das alterações fisiológicas associadas à diabetes (poliúria e polifagia).

Em um outro estudo, Perez et al. (2010) avaliaram a validade da indicação terapêutica tradicional dessa fruta como agente hipoglicêmico. Eles utilizaram a fruta seca e triturada. Os resultados mostraram uma redução da glicemia de 230,5 para 92,4 mg/dl em ratos diabéticos induzidos por aloxano. Dentre outros resultados, eles observaram que os animais diabéticos tiveram uma melhora substancial na manutenção da glicemia e parâmetros sanguíneos e, embora o diabetes seja uma doença muito debilitante, os animais foram capazes de realizar diariamente a sessão

de exercícios, o que demonstra uma melhora da qualidade de vida, com menores efeitos colaterais associados ao uso da maioria dos medicamentos hipoglicemiantes orais.

Estes resultados mostraram que o uso da fruta-do-lobo pode ser um suporte eficaz no tratamento do DM.

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CAPÍTULO I

A comprehensive characterization of *Solanum lycocarpum* St. Hill and *Solanum oocarpum* Sendtn: Chemical composition and antioxidant properties

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Abbreviations

FLPE: fruta-do-lobo peel; FLPU: fruta-do-lobo pulp; FLSE: fruta-do-lobo seed; JPE: juá-açu peel; JPU: juá-açu pulp; JSE: juá-açu seed; PCA: Principal component analysis; FOS: fructo-oligosaccharides; GF2: 1-kestose; GF3: nystose; GF4: 1-fructofuranosylnystose; G3: maltotriose; G4: maltotetraose; G5: maltopentaose; G6: maltohexaose; G7: maltoheptaose; HPAE-PAD: high-performance anion-exchange chromatography with pulsed amperometric detection; FAAS: flame atomic absorption spectrophotometry; VOCs: volatile organic compounds; SPME: Solid Phase Micro

Extraction; GC: Gas Cromatography; LRI: Linear Retention Index; ORAC: Oxygen Radical Absorbance Capacity; UHPLC-MS: Ultrahigh-Performance Liquid Chromatography Tandem Mass Spectrometry; TPC: Total phenolic compounds; AAPH: 2,2′-Azobis(2-methylpropionamidine) dihydrochloride; TE: Trolox equivalent; GAE: Galic acid equivalent; TTA: total titratable acidity; TSS: total soluble solids.

Chemical compounds reported in this article

Methyl butanoate (PubChem CID 12180)

Ethyl butanoate (PubChem CID 7762)

Hexanal (PubChem CID 6184)

3-Hexenal (PubChem CID 643139)

Methyl hexanoate (PubChem CID 7824)

2-Hexenal (PubChem CID 5281168)

1-Hexanol (PubChem CID 8103)

Methyl octanoate (PubChem CID 8091)

Cis-3-Methylcyclohexanol (PubChem CID 229503)

2,4-Hexadienal, (E,E) (PubChem CID 637564)

Hexyl butanoate (PubChem CID 17525)

2-Nonenal, (E) (PubChem CID 5283335)

(E)-4-Oxohex-2-enal (PubChem CID 6365145)

Hexanoic acid (PubChem CID 8892)

Methyl 2-oxohexanoate (PubChem CID 545374)

Heptyl butanoate (PubChem CID 62592)

Ethyl hexanoate (PubChem CID 31265)

Ethyl Octanoate (PubChem CID 7799)

Butyl Octanoate (PubChem CID 11517)

Caffeoylputrescine (PubChem CID 5280559)

3-O-Caffeoylquinic acid (Chlorogenic acid) (PubChem 1794427)

Caffeoyl-O-glucoside (PubChem CID 5281761)

Dihydrocaffeoyl-O-glucoside (PubChem CID 15459188)

p-Coumaroyl-O-glucoside (PubChem CID 14158117)

1-O-Feruloylglucose (PubChem CID 13962927)

1-O-Sinapoyl-glucoside (PubChem CID 5280406)

p-Coumaroylquinic acid (PubChem CID 6441280)

Rutin (PubChem CID 5280805)

Di-O-caffeoylquinic acid (PubChem CID 6474310)

Tri-O-caffeoylquinic acid (PubChem CID 6440783)

Highlights

1 For the first time a complete characterization of fruta-do-lobo and juá-açu was realized.

2 Esters and aldehydes were dominant VOCs of juá-açu and fruta-do-lobo.

3 Oligosaccharides GF2 and GF3 was identified by HPAE-PAD.

4 UPLC-Q-ToF fraction analysis of fruta-do-lobo and juá revealed 24 phenolic compounds.

Abstract

In this study we evaluated the proximate composition of two Solanaceae fruits from Brazilian Cerrado, their mineral content, volatile organic compounds (VOCs), phenolic compounds profile, and antioxidant capacity employing Oxygen Radical Absorbance Capacity (ORAC) assay, for each part of the fruits (pulp, peel and seeds). Our results showed that the pulp has a high moisture content (74.62 - 85.40 g/100 g) and soluble

fiber (1.29 - 2.06 g/100 g) content, and low fat, protein, and ash content. The peel exhibited high levels of carbohydrates and total fibers (6.55 - 11.39 and 12.35 - 13.12 g/100 g, respectively), while the seed presented high content of fat, protein, and insoluble fiber (10.14 - 12.62, 9.14 - 13.24 and 19.84 - 23.15 g/100 g). Potassium is the main mineral found in both fruits. It is the first time that the carbohydrate profile, volatile components, and phenolic compounds of the fruta-do-lobo and juá-açu are reported. 1-Kestose (GF2) and nystose (GF3) were found in both fruits. The main VOCs of juá-açu were esters, while in fruta-do-lobo, aldehydes were the major components. UPLC-Q-ToF fraction analysis of juá-açu and fruta-do-lobo revealed 24 phenolic compounds, most being hydroxycinnamic acids derivatives in juá-açu, and chlorogenic acids in fruta-do-lobo. The antioxidant capacity (ORAC) of the fruits ranged from 1.35 to 11.51 µmol TE/100 mL of extract. These results indicate that *Solanum* genus can be interesting for the Brazilian fruit market, and that it has potential to be exploited for agroindustry for diversification of fruit products.

Keywords: Brazilian Cerrado; Fruta-do-lobo; Juá-açu; Oligosaccharides; Phenolic compounds.

1. Introduction

Brazil has a wide variety of native fruits with peculiar sensorial characteristics and high nutritional and economic potential, which have been researched as potential bioactive sources due to the high antioxidant capacity and elevated phenolic levels (Cândido, Silva & Agostini-Costa, 2015).

The Solanaceae family comprises over 3000 species distributed in about 98 genera. The genus *Solanum* is the most representative with almost half of those species (Lima, Santos & Smozinski, 2014). This genus is a well-characterized group, and its species have been employed for both food and therapeutic purposes.

In Brazil, the Solanaceae Family has 31 genera and about 500 natives species (Pereira, Rodrigues & Vegas, 2016). *Solanum lycocarpum* St. Hill, popularly known as fruta-do-lobo, when ripe, exhibits a yellow, sweet and extremely aromatic pulp (Clerici et al., 2011). In popular medicine, it is used to treat diabetes, in obesity control, and to decrease cholesterol level. Studies with experimental models using

animals indicated that the fruta-do-lobo starch can be an effective support in the treatment of diabetes mellitus (Perez et al., 2006; Farina et al., 2010). In addition, preparations from fruta-do-lobo also display antileishmanial, cytotoxic activities, and potential to be used in the treatment of skin cancer (Andrade et al., 2016; Lezama-Dávila et al., 2016; Tiossi et al., 2014). Juá-açu (*Solanum oocarpum* Sendtn.), also a fruit native from Brazil, is a rarer species. In folk medicine, it is used to treat intermittent fevers and lung diseases (Costa, Nunes & Peres, 2010), but little was known about its chemical composition.

Chemically, several members of the Solanum genus have various alkaloids and phenolic compounds with a variety of reported biological activities (Pereira, Rodrigues & Vegas, 2016).

Prebiotics, notably fructo-oligosaccharides (FOS), were the focus of many studies addressing their importance as bioactive compounds with health benefits, for example, bifidogenic activity (Roberfroid, 2007). FOS occur naturally in some vegetables (yacon, Jerusalem artichoke and chicory) and fruits (banana, apple and pear) (L'homme et al., 2001).

In recent years, volatile organic compounds (VOCs), that contribute to the flavor of fruits, have been associated with pharmacological activities such as anti-glycation and anticancer activities. Because of this, VOCs have a potential impact on human health (Dembitsky et al., 2011).

A more detailed chemical characterization of fruits and the quantification of their bioactive components would allow the assessment of their nutritional value, their biological and their technological potential. Thus, the aim of this work was to determinate the proximate composition, mineral content, total titratable acidity (TTA), pH, total soluble solids (TSS), carbohydrate profile, VOCs, phenolic compounds profile, and the antioxidant capacity of two Brazilian native fruits.

2. Materials and methods

2.1 Sample

Fruta-do-lobo (Fig. 1A and 1B) was collected in June 2017 in Carmo do Rio Claro (S 20.555.209; W46.145.379), located in Minas Gerais, and juá-açu (Figure 1C

and 1D) was collected in October 2017 in Campina do Monte Alegre (S 20.555.209; W46.145.379) located in São Paulo, Brazil. Specimens were identified by Dr. Ingrid Koch and Dr. Leandro Giacomini, and a vouchers specimen were deposited in the UNICAMP herbarium (UEC 197248 and 197730, respectively). The fruits that were totally ripe were manually washed. The peel was carefully separated from the pulp using a sharp knife. The seed fraction was composed of seeds without the jelly portion, and the pulp was the portion remaining after removal of the skin and seed fractions. The fractions of each fruit were called as FLPE, FLPU and FLSE (fruta-do-lobo peel, fruta-do-lobo pulp, and fruta-do-lobo seed, respectively), and JPE, JPU and JSE (juá-açu peel, juá-açu pulp, and juá-açu seed, respectively).

2.2 Proximate composition, pH and total soluble solids

Moisture, protein, ash, dietary fiber contents, TSS, and pH values of fruits fractions were determined in triplicate according to official methods (Association of Official Analytical Chemists, 2006). Carbohydrate content and TTA were determined according to the 040/IV and the 016/IV methods (Instituto Adolfo Lutz, 2008) and the lipid content was determined according to Bligh & Dyer (1959). The ratio was obtained by the direct relation of the values of TSS and TTA.

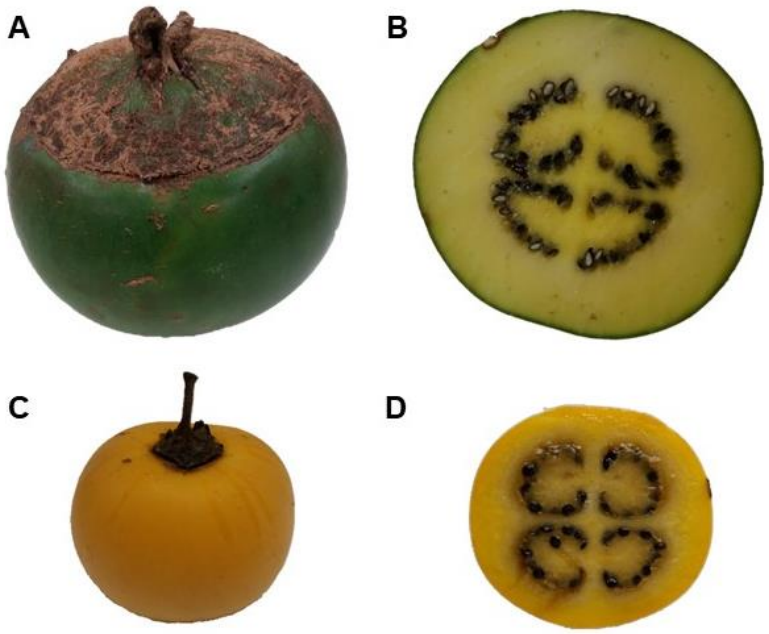

Figure 1: Fruta-do-lobo (*Solanum lycocarpum* St. Hill) (A - B) and juá-açu (*Solanum oocarpum* Sendtn.) (C - D).

2.3 Carbohydrate profile

The carbohydrate profile was performed according to Sancho et al. (2017) with modifications. A high-performance anion-exchange chromatography system coupled to pulsed amperometric detection (HPAEC-PAD) with Chromeleon 7.0 Chromatographic CHM-1, automation software, Dionex (USA) was employed. All analyses were performed in triplicate.

2.3.1 Sample preparation

Freeze-dried pulps of fruta-do-lobo and juá-açu (FLPU and JPU, respectively) were homogenized with deionized water (10 mg/mL) in an ultraturrax (UltraTurrax IKA, T25, Werke, Germany) for 1 minute. The sample was centrifuged (5 ºC, 15 min, 10,000 RPM), the supernatant was removed, diluted in deionized water, and filtered through a 0.20 mm regenerated cellulose membrane filter before analysis.

2.3.2 Mono- and disaccharides

For glucose, fructose, and sucrose, a CarboPac PA-1 (4 x 250 mm) column equipped with a CarboPac PA 1 (4 x 50 mm) guard column was used. The following solutions were used for gradient elution: A (200 mM sodium hydroxide) and B (water). The running was isocratic with 80% (A) and 20 % (B) for 10 min, followed by cleaning with 100 % of A for 5 min and stabilization for 5 min at the same initial status, totalizing 20 min at a flow rate of 1.0 mL/min at 30 ºC. Compounds were quantified using a linear calibration curve of the carbohydrate standards (glucose from Supelco (Bellefont, PA, EUA); sucrose and fructose from Sigma-Aldrich (St. Louis, EUA)). The results are expressed in g/100 g of sample.

2.3.3 Oligosaccharides

For fructo-oligosaccharides and malto-oligosaccharides a CarboPac PA-100 (4 x 250 mm) column equipped with a CarboPac PA 100 (4 x 50 mm) guard column was used. The following solutions were used for gradient elution: A (100 mM sodium hydroxide) and B (500 mM sodium acetate and 100 mM sodium hydroxide). The running was started with 97 % (A) and 3 % (B) for 2 min, followed by 18 min with a linear gradient from 3 to 40 % of B, followed by cleaning with 100% of B for 5 min, and stabilization for 5 min at the same initial status, giving a total time of 30 min at a flow rate of 1.0 mL/min at 30 ºC. Compounds were quantified using a linear calibration curve

of the following carbohydrate standards: 1-kestose (GF2), nystose (GF3), and 1-fructofuranosylnystose (GF4) (Wako Pure Chemical Industries, Osaka, Japan), and maltotriose (G3), maltotetraose (G4), maltopentaose (G5), maltohexaose (G6), and maltoheptaose (G7) (Supelco, Bellefont, PA, USA). The results are expressed in mg/100 g of sample.

2.4 Minerals

Iron, zinc, sodium, calcium, magnesium, potassium, manganese, and copper contents were determined using flame atomic absorption spectrophotometry (FAAS), according to Silva et al. (2017). For the analyses of calcium, iron, magnesium, and zinc, we used a FAAS (AAnalyst 200, PerkinElmer, Waltham, USA) with a deuterium lamp for correction of background radiation, and hollow cathode lamps for determination of iron (248.3 nm), calcium (422.67 nm), magnesium (285.21 nm), manganese (279.48 nm), copper (324.75 nm), and zinc (213.86 nm). Sodium and potassium were determined in FAAS in emission mode in 589.00 nm and 766.5 nm, respectively. We used nitric acid (Sigma-Aldrich, St. Loius, MO, USA) and hydrogen peroxide (Synth, Diadema, Brazil) for the mineralization of samples. After that, each sample was placed into the nebulizer and mixed with air-acetylene flame (2.5/10 L/h) at approximately 2000 °C. For the construction of analytical curves, we used standard solutions of iron, calcium, magnesium, sodium, potassium, manganese, copper and zinc (Sigma-Aldrich), with a concentration of 1,000 mg/L.

2.5 Volatile organic compounds (VOCs)

The fruit pulp was previously homogenized with ultrapure water (1:5/w:v) and grinded to pass through a 32 mesh.

2.5.1 HS-SPME Procedure

Each 5 g of pulp was placed in a 100 mL vial, sealed with a screw-capped top containing a Teflon-lined septum for VOCs headspace microextraction. SPME extractions were performed from the headspace of the samples according to the following conditions: DVB/CAR/PDMS fiber, equilibrium time of 15 min; extraction time of 15 min and extraction temperature of 45 °C. Desorption was performed in the injector of the GC in splitless mode for 10 min at 250 °C. Extractions were performed in triplicate for each fruit.

2.5.2 GC-MS Analysis

The desorbed VOCs were separated in an Agilent 7890A gas chromatograph system (Agilent Technologies) equipped with a GC DB-WAX column (30 m x 0.25 mm x 0.15 µm) and Agilent 5975C inert MSD with Triple-Axis Detector, using helium as a carrier gas. The VOCs were desorbed for 10 min by inserting the SPME fiber into a GC injector (250 °C). The GC oven temperature was programmed to hold at 50 °C for 5 min and then to increase to 200 °C at 5 °C/min, finally holding at 200 °C for 7 min. Column flow rate was 1.0 mL/min. Ion source temperature was 230 °C, and the interface temperature was set at 230 °C. The MS was scanned in the range of 45–650 atomic mass units at 70 eV. Total run time was 42 min. Compounds were identified using NIST 14.0 database and Linear Retention Index (LRI) calculated with a series of n-alkanes (C7-C40).

2.6 Phenolic compounds

2.6.1 Extract preparation

All fractions were freeze-dried (Liotop, Liobras, Brazil) separately for 48 hours, packed (200B Selovac, São Paulo, Brazil) under vacuum in low density polyethylene bags, and frozen at -18 °C until milling, which was carried out in a blender (Colombo AR 2L, Itajobi, Brazil). Extracts were prepared according to Martins et al. (2013) with modifications. The freeze-dried fruit was homogenized with ethanol:water (70:30/v:v) in a homogenizer (Ultra-Turrax T-25, Germany) for 2 min in 100 mg/mL concentration. After that, it was homogenized under constant stirring for 2 hours at room temperature. The extract was centrifuged (5 ºC, 15 min, 10,000 RPM) and concentrated under vacuum until completely dry.

2.6.2 Phenolic compounds profile

Dried extracts were re-suspended in water and samples were prepared diluting 10 times by pipetting 100 µL of solution in 900 µL of water. LC-MS/MS analysis was perform using an Ultrahigh-Performance Liquid Chromatography (UHPLC) (Hewlett Packard, Agilent Technologies 1290 series) coupled to a Q-ToFiFunnel 6550 mass spectrometer using an electrospray ionization (ESI) source with a Poroshell 120 SB-Aq 2.7 µm column (2.1x100 mm, Agilent). Mobile phase A was milli-Q water with 0.1 % of formic acid, and mobile phase B was acetonitrile with 0.1 % of FA. The flow

rate of 0.45 mL/min was used following the linear gradient: 0 - 1 min, 5 % B; 1 - 10 min, 5 % B to 18 % B; 10 -13 min 18% B to 70% B; 13 - 15 min, 70 % B to 100 % B; 15 - 17 min, 100% B and 3 min of post time at 5% B to column re-equilibration. The mass spectrometer voltages and temperatures were set as VCap 3000 V; fragmentor voltage at 150 V; OCT 1RF Vpp at 750 V; gas temperature at 290 ºC; sheath gas temperature at 350 ºC; drying gas at 12 L/min, and the fragmentation were performed using normalized collision energy (NCE) of 30. Mass spectra were acquired in profile negative ion mode and the acquisition range was 100-1200 m/z. Data were treated using Agilent MassHunter Qualitative Analysis B0.7 software, and the data matrix for statistical analysis was generated using Agilent MassHunter Profinder B.06. Compound identification was done using METLIN library and by manual interpretation of MS/MS pattern spectra.

2.6.3 Total phenolic compounds (TPC)

The quantification of phenolic compounds was performed by Folin-Ciocalteau method, which involves the reduction of the reagent by the phenolic compounds of the samples with concomitant formation of a blue complex. The intensity of the color increases linearly at 760 nm (Roesler et al., 2007). Results are expressed in mg of galic acid equivalents (GAE)/100 mL of extract.

2.7 Oxygen Radical Absorbance Capacity (ORAC)

The antioxidant capacity of the samples was performed by ORAC assay according to Ou Hampsch-Woodill, & Prior (2001), adapted by Dávalos, Gómez-Cordovés, & Bartolomé (2004), using a microplate reader (NOVOstar, BMG Labtech®, Offenburg, Germany) with the MARS Data Analysis Software version 1.3 (BMG Labtech®, Offenburg, Germany). Samples and Trolox standards were prepared with 75 mM phosphate buffer (pH 7.4). Each well contained 20 µL of extract or Trolox standard in different dilutions (to reach a linear response) and 120 µL of fluorescein (70 mM). 60 µl of AAPH solution (12 mM) was added to start the reaction, resulting in a final total volume of 200 µL for hydrophilic fraction, while the lipophilic fraction was analysed using 20 µL of extract or Trolox standard, 120 µL of fluorescein (70 mM) and 120 µl of AAPH solution (12 mM). The fluorescence decay was measured every minute for 80 min at 37 °C, with excitation and emission wavelengths of 485 and 528 nm. A blank experiment (fluorescein + AAPH) with buffer instead of the sample or Trolox was

also performed. The results were calculated using the relative area under the curve for samples compared to a Trolox standard curve (65 - 780 μM) prepared under the same experimental conditions. ORAC values were expressed as μM Trolox Equivalents (TE)/100 mL of extract.

2.8 Statistical analysis

Statistical analyses were conducted using R software for Windows, version 3.4.3 (Vienna, Austria). The results were subjected to a two-way ANOVA and differences between means were located using Tukey's multiple comparison test. Significance was determined at $p<0.05$. The normal distribution was checked using a Shapiro- Wilk test. Although some variables violated the homogeneity of variances (Bartlett test), this was compensated by the equal group size. All results are presented as means ± standard deviation for triplicates. The Pearson correlation analysis was performed to evaluate associations between variables, and Principal Component Analysis (PCA) using Pareto scaling was performed to access compound distribution along the samples, both were performed using Metaboanalyst 4.0 software. For PCA analysis, 6 types of sample (FLPE, FLPU, FLSE, JPE, JPU and JSE) were used in triplicate to evaluate the essay repeatability, and the data obtained from the 24 identified phenolic compounds, represented by mz/rt, resulting in a 18 x 24 matrix. Results were considered significant when $p<0.05$.

3 Results and discussion

3.1 Chemical and nutritional characteristics

Table 1 shows the proximate composition, carbohydrate profile, mineral contents, and physicochemical characteristics of fruta-do-lobo and juá-açu. In general, juá-açu exhibited values higher moisture values than fruta-do-lobo ($p<0.05$). Regarding the lipids, the seeds had values about 10 times higher than the other parts for both fruits. The seeds also presented the highest value for protein, and the content of protein of the FLSE was larger than that of the JSE (Table 1).

The carbohydrate content for juá-açu were within the range found for other fruits from the Solanaceae family, such as cubiu-pequeno (*Solanun sessiliflorum* Dunal) (5.28 g/100 g) (Berto et al., 2015). However, the carbohydrate content of fruta-

do-lobo is almost two times higher than that from juá-açu for all fractions (peel, pulp and seeds) ($p<0.05$). Carbohydrates were composed mostly of simple sugars, from which sucrose was the most abundant for both fruits (Table 1). Sucrose was also the main sugar present in chilto pulp (*Solanum betaceum*) (3.23 g/100 g of power) (Orqueda et al., 2017). It was not possible to correlate the sum of the mono- and disaccharides quantified by HPLC with the results of total sugars by the Fehling method. Because it is based on visual assessment, the Fehling method is subjective and is used only for reducing sugars, while HPLC analysis provides a high degree of precision and accuracy (BeMiller, 2010). Therefore, this difference can be explained by the presence of other reducing substances in the sample, such as phenolic compounds and VOCs.

The amounts of GF2, GF3, and G3 are shown in Table 1. GF2 and GF3 were 2.38 and 5.62 times higher in JPU when compared with FLPU. G3 was observed only in juá-açu, but in a small amount (0.66 mg/100 g). The others fructo- and malto-oligosacharides were not identified. There are still few studies on oligosaccharides in fruits. L'homme et al. (2001) found mostly GF2 in dry matter of apple, pear and bananas (16.1 - 44.5, 23.6 - 170.4, and 423.3 - 602.0 mg/100 g, respectively). GF3 was found only in plum (4.7 mg/100 g DM). Corroborant with these authors, our results show that fresh fruits could contribute partly to the daily fructan consumption.

The moisture and protein content of fruta-do-lobo is comparable to that found by Roesler et al. (2007). The most divergent values with respect to the proximate composition were for lipid contents of the FLSE. While Roesler et al. (2007) found 3.73 g/100 g for fruta-do-lobo seeds, we found 12.62 g/100 g for the same fraction in our study. The highest values found in our study can be explained by the method we used. Both in the Soxhlet method and in the Bligh and Dyer method, the fat is quantified by gravimetry. However, in the Soxhlet method, it is possible to determine fatty acids and phospholipids, whereas in the Bligh & Dyer method all classes of lipids are extracted (Min & Ellefson, 2010).

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Table 1. Proximate composition, mineral contents and physicochemical characteristics of fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.) peel, pulp and seeds[1]

		Fruta-do-lobo			Juá-açu		
		Peel	Pulp	Seed	Peel	Pulp	Seed
Proximate composition	Moisture (g/100 g)	66.03 ± 0.22^{d}	74.62 ± 0.11^{c}	40.79 ± 0.06^{f}	77.16± 0.17^{b}	85.40± 0.14^{a}	54.37 ± 1.3^{e}
	Carbohydrate (g/100 g)	11.39 ± 0.33^{a}	10.97 ± 0.57^{a}	9.91 ± 0.23^{b}	6.55 ± 0.17^{c}	5.95 ± 0.04^{c}	5.51 ± 0.06^{d}
	Fat (g/100 g)	1.22 ± 0.05^{c}	0.86 ± 0.22^{c}	12.62 ± 0.41^{a}	1.06 ± 0.22^{c}	0.88 ± 0.14^{c}	10.14 ± 0.50^{b}
	Protein (g/100 g)	2.36 ± 0.01^{c}	1.37 ± 0.07^{e}	13.24 ± 0.31^{a}	2.25 ± 0.04^{d}	2.05 ± 0.04^{d}	9.14 ± 0.37^{b}
	Ash (g/100 g)	1.32 ± 0.04^{b}	0.92 ± 0.01^{c}	2.18 ± 0.01^{a}	0.80 ± 0.06^{d}	0.57 ± 0.01^{e}	0.94 ± 0.03^{c}
	Total fiber (g/100 g)	13.12 ± 0.67^{c}	4.54 ± 0.43^{d}	27.25 ± 2.2^{a}	12.35 ± 0.21^{c}	2.67 ± 0.09^{d}	23.39 ± 0.70^{b}
	Soluble fiber[2] (g/100 g)	3.42	2.48	4.10	7.11	1.29	3.55
	Insoluble fiber (g/100 g)	9.70 ± 0.25^{c}	2.06 ± 0.07^{e}	23.15 ± 0.70^{a}	5.24 ± 0.73^{d}	1.38 ± 0.25^{e}	19.84 ± 0.23^{b}
Sugars	Glucose (g/100 g)	-	0.36 ± 0.005^{b}	-	-	2.12 ± 0.11^{a}	-
	Fructose (g/100 g)	-	0.39 ± 0.006^{b}	-	-	1.45 ± 0.07^{a}	-
	Sucrose (g/100 g)	-	5.26 ± 0.08^{a}	-	-	2.88 ± 0.1^{b}	-
Oligosacharide	GF2 (mg/100 g)	-	26.81 ± 0.5^{b}	-	-	63.80±0.66^{a}	-
	GF3 (mg/100 g)	-	66.69 ± 0.7^{b}	-	-	374.91±1.7^{a}	-
	G3 (mg/100 g)	-	n.d.	-	-	0.66 ± 0.36	-
Minerals	Iron (mg/100 g)	<LOQ	<LOQ	1.97 ± 0.05^{b}	<LOQ	<LOQ	3.44 ± 0.02^{a}
	Zinc (mg/100 g)	<LOQ	<LOQ	2.03 ± 0.02^{a}	<LOQ	<LOQ	0.90 ± 0.01^{b}
	Sodium (mg/100 g)	1.97 ± 0.15^{b}	<LOQ	3.27 ± 0.45^{a}	<LOQ	<LOQ	<LOQ
	Calcium (mg/100 g)	17.80 ± 1.57^{a}	<LOQ	17.07 ± 1.36^{a}	8.00 ± 0.79^{c}	<LOQ	13.13 ± 0.45^{b}
	Magnesium (mg/100 g)	17.57 ± 2.00^{c}	8.43 ± 0.59^{c}	258.50±17.49^{a}	19.93 ± 2.40^{c}	15.47 ± 0.15^{c}	106.97±3.01^{b}
	Potassium (mg/100 g)	510.10±13.66^{a}	396.17±13.72^{b}	445.33±35.62^{b}	455.73±9.96^{b}	300.73±9.96^{c}	172.93±8.11^{d}
	Manganese (mg/100 g)	<LOQ	<LOQ	0.87 ± 0.06^{b}	<LOQ	<LOQ	2.13 ± 0.15^{a}
	Copper (mg/100 g)	0.30 ± 0.00^{c}	0.33 ± 0.06^{c}	2.17 ± 0.06^{a}	<LOQ	<LOQ	1.60 ± 0.00^{b}
Physicochemical characteristics	pH	-	4.87 ± 0.04^{a}	-	-	3.61 ± 0.06^{b}	-
	Total soluble solids (ºBrix)	-	24.0 ± 0.00^{a}	-	-	12.5 ± 0.00^{b}	-
	Total titratable acidity (% citric acid)	-	0.79 ± 0.00^{b}	-	-	1.69 ± 0.02^{a}	-
	Ratio (TSS/TA)	-	30.38	-	-	7.40	-

[1]Values expressed as mean ± SD (n = 3) of 100g of fresh fruit. Values in the same line followed by different letters are significantly different by ANOVA test ($p < 0.05$). [2]Soluble fiber are calculated by difference between total fiber and insoluble fiber. n.d. not detected. -: not determined. Limit of Quantification (LOQ). LOQ Iron = 0.46 mg/100 g; LOQ Zinc = 0.29 mg/100 g; LOQ Calcium = 3.38 mg/100 g; LOQ Magnesium = 0.08 mg/100 g; LOQ Copper = 0.17 mg/100 g; LOQ Manganese = 0.21 mg/100 g; LOQ Sodium= 0.33 mg/100 g; LOQ Potassium = 0.42 mg/100 g.

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Regarding total dietary fibers, FLSE and JPE present the higher content ~25 %, while FLPU and JPU present the lower values of ~3%. The higher concentration of soluble fiber was found in JPE, followed by FPU and JPU (Table 1). Fruit is a rich source of soluble/viscous fiber: The benefits of fiber are associated with fecal bulking, viscosity, and fermentation. Also, the high fiber values found in eggplant (*Solanum melongena* L.) suggest that their consumption may contribute to a reduction of hyperglycemia, hypertension and oxidative stress in individuals with type 2 diabetes (Gürbüz et al., 2018). These results suggest that dietary fibers of solanum fruits may influence the physiological processes of intestine, playing an important role in the prevention of chronic diseases.

We analyzed the presence of eight minerals in each fraction of each fruit (peel, pulp, and seed). Potassium was the main mineral found in the three fractions of

both fruits. Were observed high levels of potassium for FLPE and JPE, followed by pulps of both fruits (Table 1). In fact, potassium is the most abundant mineral in fruits and high values can be found in banana (376 mg/100 g), passion fruit (338 mg/100 g) and cupuaçu (331 mg/100 g), and other fruits (Lima et al., 2011). Regarding magnesium, the contents found in FLSE were twice higher than JSE while values for pulp and peel did not differ among fruits ($p<0.05$).

For our knowledge, is the first time that minerals assessment was in juá-açu. However, Clerici et al. (2011) characterize the mineral contents in pulp of fruta-do-lobo. These authors found low levels for Ca, Mn, Fe, Na and Zn (14.7, 0.095, 0.30, 0.66 and 0.179 mg/100 g, respectively). Although these minerals being below the quantification limit of the assay in our study, the values of K and Cu was 1.6 and 2.6 times higher. Values of Mg was similar.

The values of Fe and Mn found for the JSE are close to the values reported for the same fraction of the cubiu-pequeno (3.75 and 2.16, respectively) (Berto et al., 2015). However, the Mg values found by the same authors in the cubiu-pequeno pulp are 6.3 times larger than in the JPE. The consumption of 100g of fresh JPU would provide approximately 3.86% and 4.83% of the daily intake of magnesium for male and female adults, while FLSE and JPSE would provide 61.54 and 25.47%, respectively, of the daily intake of magnesium for male (NIH - National Institutes of Health, 2018). According to Resolution nº. 27 (BRASIL, 1998), a food can be considered a source of a mineral when it has at least 15 % of the amount recommended by RDI per 100 g of sample. Even though seed is non-edible fractions, they might be exploited as sources of this mineral for food enrichment, adding value to the product.

The recommended dietary allowances of copper is 0.9 mg/d for adults (NIH, 2018). Therefore, FLPU and FLPE can be considered a source of copper, provide 36.67 and 33.33 % of daily intake, respectively.

The chemical characteristics related to flavor represent important quality attributes for the commercialization and use of the fruit in elaborating industrial products (Inada et al., 2015). Regarding chemical analyses, JPU presented a lower pH value than FLPU, which were similar to those previously reported (4.2) (Roesler et al., 2011). TSS of FLPU were almost twice as large as JPU ($p<0.05$), and it was not possible to correlate this result with the sugar content. The high values of TSS can be

explained for the presence of others compounds in fruits, such as other carbohydrates, organic acids, vitamins, minerals and others. These compounds may have interfered in the samples, causing mistakes in the °Brix determination (Harrill, 1998). The ratio (TSS /TTA) indicates the degree of equilibrium between the sugar and organic acids contents of the fruit, being directly related to the quality of the flavor attribute and, therefore, an important parameter to determine the ripeness and palatability of the fruits. The ratio of FLPU and JPU were 30.38 and 7.40. These data corroborate with the lower pH value found for JPU and the higher carbohydrate value of FLPU.

3.2 Volatile organic compounds

Esters, aldehydes, alcohols, terpenes and their derivatives, are responsible by characteristic flavor in fruits and vegetables (Bicas et al., 2011). As shown in Table 2, esters were found to be the dominant VOCs of juá-açu fruit while fruta-do-lobo mainly contains aldehydes, followed by esters and alcohols.

It is the first time that volatile components from fruta-do-lobo and juá-açu are reported. For juá-açu, ethyl butanoate and ethyl octanoate are the most abundant compounds. Similar results were found for lulo fruit (*Solanum quitoense* Lam.), which was the only fruit of this genus that had the volatile compounds characterized, and ethyl butanoate was also one of the key aroma compounds from this fruit (Forero et al., 2015). Ethyl butanoate is responsible for the fruity and sweet flavor and was previously reported as one of the most common esters present in passion fruits (Janzantti & Monteiro, 2014). On the other hand, ethyl octanoate possesses a waxy flavor and together with ethyl butanoate are responsible for 64% of volatile constitutes of juá-açu (Table2). For fruta-do-lobo, the most abundant component was hexanal, which contribute to the grass flavor, and with ethyl butanoate correspond to 61% of the volatile constituents (Bicas et al., 2011). Despite the low concentration of other components, they all are important to compose the fruit flavor and aroma.

3.4 Phenolic compounds and antioxidant capacity

Twenty-four phenolic compounds belonging to fruta-do-lobo, juá-açu and their respective fractions were separated by UPLC-Q-ToF. Among them, 22 substances were identified considering their exact masses and fragmentation patterns, being the major compounds: tri-O-caffeoylquinic acid, rutin, kaempferol-diglucoside and sinapoyl-O-frutofuranosylglucose (Table 3).

Table 2. Volatile organic compounds identified in fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.) using headspace sampler with Db-wax polar column

	Compounds	LRIexp[1]	LRIref[2]	Área (%)	Odor description
Fruta-do-lobo	Methyl butanoate	974.23	-	4.224	fruit, sweet
	Ethyl butanoate	1028.1	-	12.426	apple
	Hexanal	1077.6	1078	48.732	grass
	3-hexenal	1132.5	1132	3.759	leaf, green
	Methyl hexanoate	1176	1187	1.094	fruit, fresh, sweet
	2-hexenal	1212.8	1212	5.568	apple, green
	Cyclohexanol,4-(1,1-dimethylethyl) -	1218.5	-	0.549	-
	1-hexanol	1355.6	1353	1.038	flower, green
	Methyl octanoate	1385.6	1392	0.693	orange
	cis-3-methylcyclohexanol	1395.5	-	0.706	-
	2,4-hexadienal, (E,E)	1398.4	-	1.379	-
	Hexyl butanoate	1415.6	-	1.893	apple peel
	2-nonenal, (E) -	1537.5	1536	0.693	cucumber, fat, green
	(E)-4-oxohex-2-enal	1596.2	-	0.595	-

[1] LRIexp: Linear retention index experimental; [2] LRIref: Linear retention index of reference. -: References were not found.

Table 2. Volatile organic compounds identified in fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.) using headspace sampler with Db-wax polar column. Continuação…

	Compounds	LRIexp[1]	LRIref[2]	Área (%)	Odor description
Fruta-do-lobo	Hexanoic acid	1634.3	-	0.786	Sweat
	Oxime, methoxy-phenyl -	1871	-	0.969	-
	Methyl 2-oxohexanoate	1963.6	-	0.523	-
Juá-açu	Ethyl butanoate	1036/1048	-	31.285	apple
	Hexanal	1079	1078	7.373	grass
	Heptyl butanoate	1211	-	2.613	-
	Ethyl hexanoate	1233	1232	5.351	apple peel, fruit
	Methyl octanoate	1390	1392	6.891	orange
	Hexyl butanoate	1417	-	1.512	apple peel
	Ethyl Octanoate	1438	1429	33.677	fruit, fat
	Butyl Octanoate	1618	-	0.808	fruit

[1] LRIexp: Linear retention index experimental; [2] LRIref: Linear retention index of reference. -: References were not found.

Table 3. Mass spectral data and tentative identification of phenolic compounds in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.)[1]

Compound	mz/rt	MS/MS	Fruta-do-lobo (% relative)			Juá-açu (% relative)		
			Peel	Pulp	Seed	Peel	Pulp	Seed
Caffeoylputrescine	249.1249/1.84	249/135	1.7±0.1	0.7±0.1	0.2±0.02	11.5±1	13.0±0.4	0.6±0.4
3-O-Caffeoylquinic acid (Chlorogenic acid)	353.0879/2.19	191/179/135	2.5±0.2	4.4±0.5	0.7±0.1	1.4±0.1	n.d.	n.d.
Caffeoyl-O-glucoside	341.0869/2.47	341/181/161/133	9.7±0.4	9.8±1.6	1.3±0.2	7.1±0.2	9.1±0.4	1.0±0.6
Dihydrocaffeoyl-O-glucoside	343.1033/2.49	343/181/137/109	2.4±0.2	0.4±0.1	0.3±0.1	41.8±1.8	46.1±1.4	7.4±2.9
p-Coumaroyl-O-glucoside	325.0934/2.75	325/145/119	3.3±0.1	n.d.	n.d.	0.9±0.1	n.d.	n.d.
Unknown	593.1718/3.22	593/470/385/205/165	n.d.	n.d.	n.d.	0.7±0.03	1.1±0.03	0.2±0.2
p-Coumaroyl-O-glucoside	325.0927/3.32	325/145	2.8±0.2	2.1±0.7	0.2±0.04	5.1±0.7	1.6±0.2	1.3±0.5
p-Coumaroyl-O-glucoside	325.0927/3.6	325/145/117	6.0±0.4	3.5±0.3	0.8±0.1	10.5±1	8.9±0.3	1.8±0.4
Dihydroferulic acid glucoside	371.0983/3.97	371/279/249/121	1.0±0.1	n.d.	n.d.	n.d.	n.d.	41.9±4.1
p-Coumaroyl-O-frutofuranosylglucose	487.1451/4.31	487/341/265/145/117	3.0±0.2	2.5±0.6	0.2±0.1	n.d.	n.d.	n.d.
5-O-Caffeoylquinic acid (Chlorogenic acid)	353.0876/4.35	191/175/160	15.4±1.1	10.4±1.2	10.3±2	3.8±0.2	2.9±0.04	5.0±0.6
1-O-Feruloylglucose	355.103/4.56	355/297/175/160	2.6±0.1	2.7±0.4	1.0±0.1	3.7±0.3	9.5±0.4	3.1±0.6

[1]Values expressed as mean ± SD (n = 3). Values in the same line followed by different letters are significantly different by ANOVA test ($p < 0.05$). n.d. not detected.

Table 3. Mass spectral data and tentative identification of phenolic compounds in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.)[1] . Continuação…

Compound	mz/rt	MS/MS	Fruta-do-lobo (% relative)			Juá-açu (% relative)		
			Peel	Pulp	Seed	Peel	Pulp	Seed
p-Coumaroyl-O-frutofuranosylglucose	487.1455/4.96	487/307/279/163/145/117 487/265/163/145	7.9±0.3	6.8±0.4	1.2±0.2	n.d.	n.d.	n.d.
1-O-Feruloylglucose	355.103/5.43	-	n.d.	0.1±0.01	n.d.	1.4±0.1	2.0±0.1	n.d.
1-O-sinapoyl-glucoside	385.1136/5.47	385/313/294/242/190/175	1.6±0.3	12.1±1.8	0.3±0.1	8.1±0.02	4.8±0.2	4.2±0.4
p-coumaroylquinic acid	337.0932/5.91	337/191/163	10.0±0.5	18.8±2.0	5.4±1.1	n.d.	n.d.	n.d.
Sinapoyl-O-frutofuranosylglucose	547.1659/6.81	547/265/223/205/190/149	0.8±0.1	12.8±1.2	0.2±0.02	n.d.	n.d.	n.d.
Rutin	609.1458/9.03	609/284	1.0±0.1	n.d.	5.9±1.7	3.8±0.4	0.1±0.01	33.3±2.2
Kaempferol-diglucoside	593.151/10.08	-	1.0±0.1	n.d.	1.2±0.3	n.d.	n.d.	n.d.
Di-O-caffeoylquinic acid	515.1191/10.64	515/353/191/179/173/135	1.0±0.7	4.7±4.1	6.5±1.9	n.d.	n.d.	n.d.
Kaempferol-diglucoside	593.1511/10.77	593/285/255	11.1±0.5	n.d.	3.7±2.6	n.d.	n.d.	n.d.
Di-O-caffeoylquinic acid	515.1191/11.16	515/353/191/179/135 515/353/191/179/173/135	1.2±1.0	2.1±2.0	33.7±3.0	n.d.	n.d.	n.d.
Unknown	503.156/11.88	503/307/247/195/145	13.3±2.3	2.4±1.0	2.7±0.5	n.d.	n.d.	n.d.
Tri-O-caffeoylquinic acid	677.1502/12.18	677/515/353/191/179/173/155/135	0.8±0.9	3.9±3.8	24.4±12.5	0.2±0.2	n.d.	n.d.

[1]Values expressed as mean ± SD (n = 3). Values in the same line followed by different letters are significantly different by ANOVA test ($p < 0.05$). n.d. not detected.

There are few studies regarding the phenolic contents of fruits from the genus *Solanum*. Most works about fruta-do-lobo are centered in its alkaloid contents, and no data is available for jua-açu. In a previous study, Morais et al. (2015) identified caffeic and chlorogenic acids in ethanol, dichlorometane, and ethyl acetate fractions of fruta-do-lobo ripe fruits, while we identified caffeic acid derivatives. On the other hand, Radwan et al. (2015) isolated two flavone glycosides from *Solanum elaeagnifolium*, named 2R,3R-5,7,4´-trihydroxy-dihydroflavon-3-O-α-D-glucopyranosyl-6”-O-β-D-glucopyranoside-6”-p-hydroxybenzoate and kaempferol-3-(6´-coumaroyl glucoside). In the same way, Orqueda et al. (2017) identified 31 constituents of chilto, including caffeoyl, quercetin, caffeoylquinic acid, and rosmarinic acid derivatives.

The PCA analysis (Figure 2) and Pearson correlation (Supplementary material) were carried out to gain an overview of the group similarities and differences among the fruta-do-lobo and juá-açu and to investigate the relationships among antioxidant capacity and phenolic contents. The first and second main components together represent a total explained variance of 73.2 % (Figure 2A). The samples were separated into six groups: FLPE, FLPU, FLSE, JPE, JPU and JSE. Figure 2B shows the variables responsible for sample grouping (red arrow). We observed that seed composition of both fruits are quite similar. However, they have different major compounds, for juá-açu they were rutin and dihydroferulic acid glucoside, whereas for fruta-do-lobo they were di and tri-O-caffeoylquinic acid. These phenolic compounds, especially the chlorogenic acids, are present in all parts of plants and they have many functions including acting in plant defense. Their greater amount in seed may be related to host defense through their antibiotic properties (Hammerschmidt, 2014).

Now, looking at the peel and pulp of each fruit, both have the same compounds but in different abundances (Table 3 – Figure 2). For instance, JPE and JPU have mostly hydroxycinnamic acids derivatives, such as caffeoyl and feruloyl O-glucosides and caffeoyl putrescine, whereas fruta-do-lobo have mostly chlorogenic acids such as 5-O-caffeoylquinic acid and O-p-coumaroylquinic acid. Chlorogenic acid (5-caffeoylquinic acid) is also the most widely distributed phenolic acid ester in eggplant (*Solanum melongena* L.) (Niño-Medina et al., 2017).

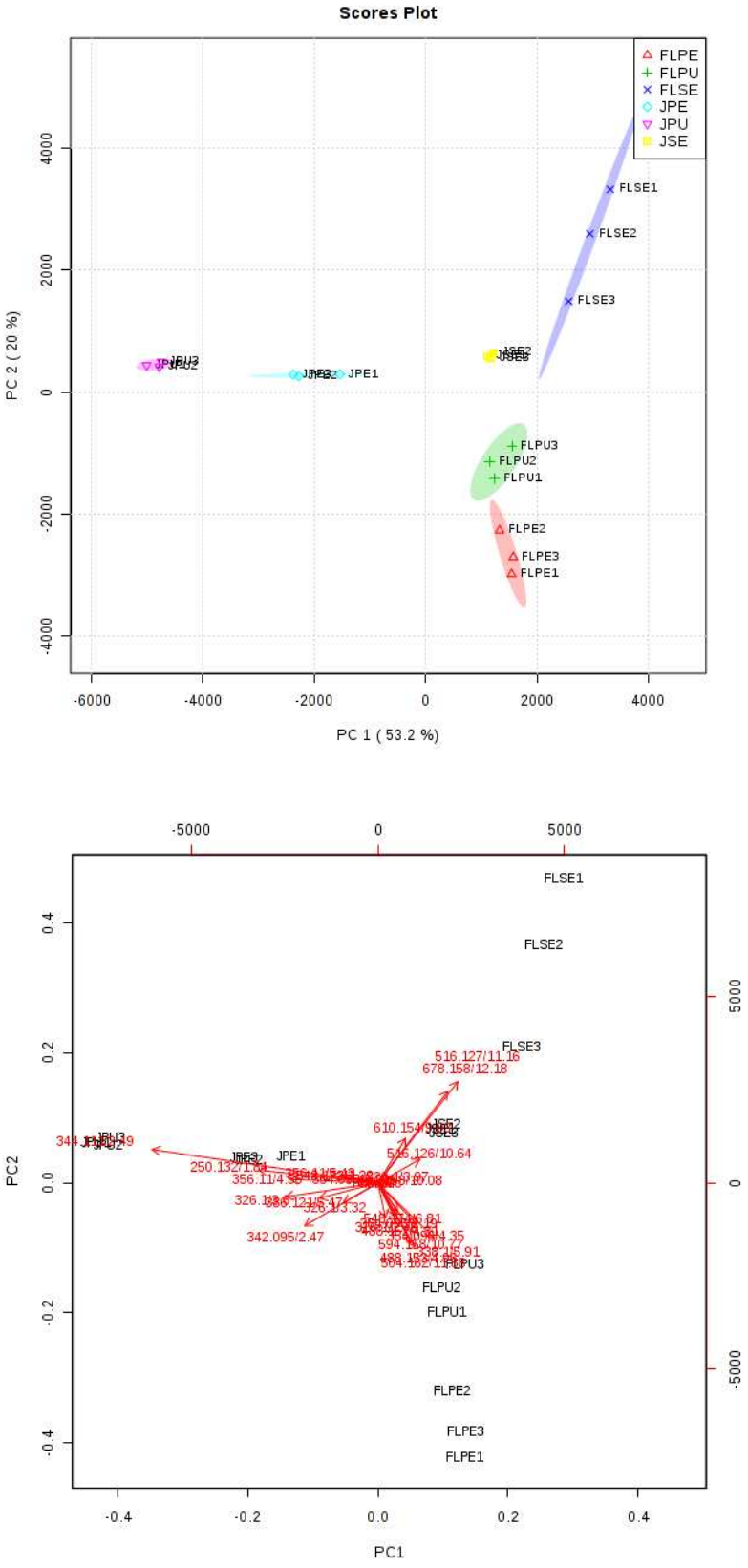
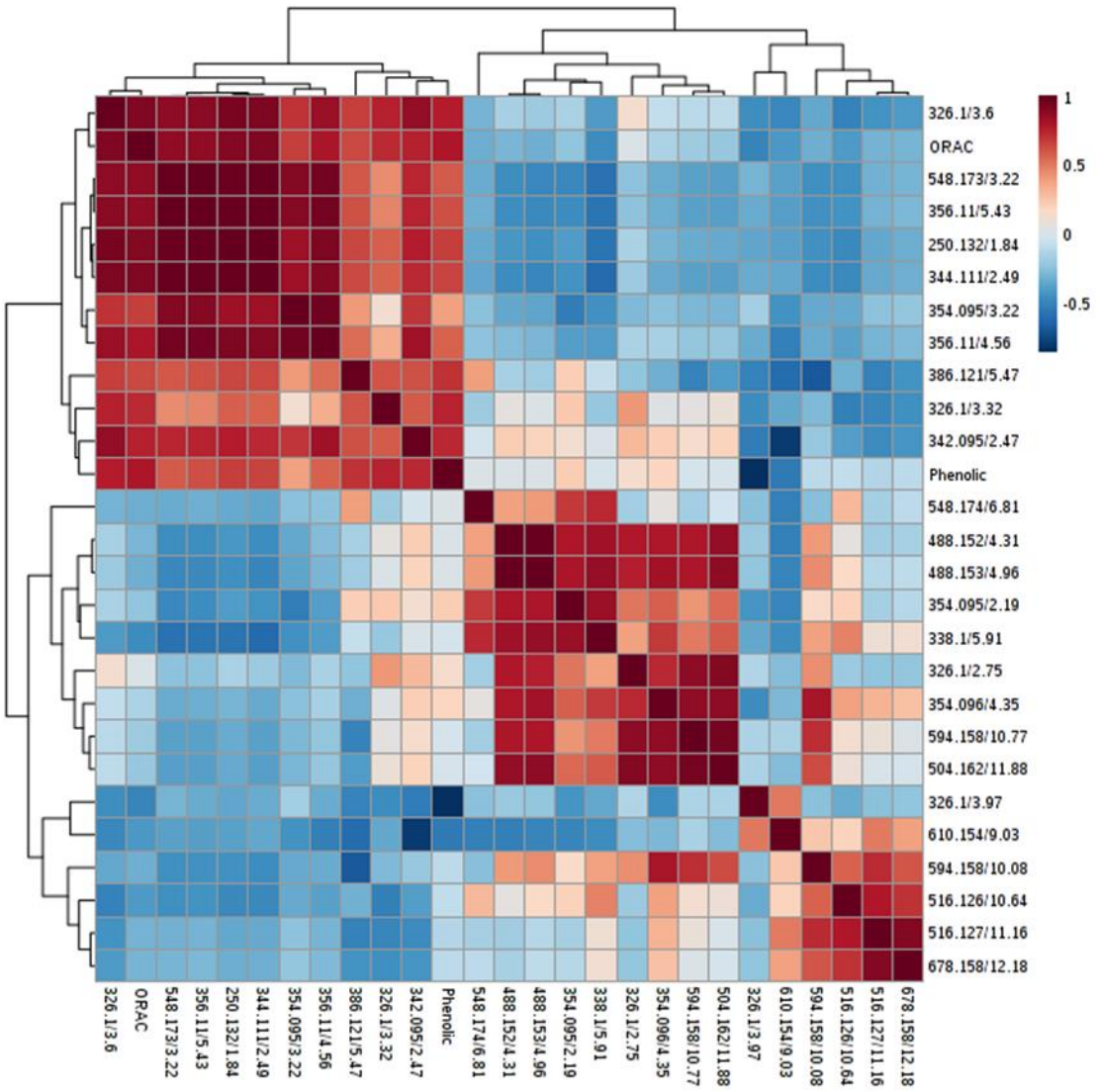


Figure 2. Graphs of scores (A) and loadings (B). Principal Component Analysis for assess phenolic compounds distribution along the fractions (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.). FLPE: fruta-do-lobo peel; FLPU: fruta-do-lobo pulp; FLSE: fruta-do-lobo seed; JPE: juá-açu peel; JPU: juá-açu pulp; JSE: juá-açu seed.



Supplementary material. Interactions between antioxidant tests and phenolic compounds of fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.) by Pearson correlation.

The most common colorimetric method to measure total phenolics is Folin-Ciocalteau (FC), and their results are displayed in Table 4 as total phenolic content (TPC), antioxidant capacity by ORAC, and the total amount of detected phenolic by UHPLC-MS (named as MS phenolic). TPC of fruta-do-lobo and juá-açu extracts ranged from 1.70 to 6.10 mg GAE/mL of each fraction. JPU and JPE have the highest values of phenolic compounds (6.0 and 6.1 mg GAE/mL, respectively), and consequently have the highest antioxidant capacity by ORAC assay (11.51 and 10.53 µmol TE/100 mL of extract, respectively). The same tendency can be observed in MS phenolic (1.84E7). Cândido, Silva & Agostini-Costa et al. (2015) also observed a strong

positive correlation between the total phenolic content and the antioxidant capacity of buriti fruits by the ABTS, DPPH, FRAP and ORAC assays.

Orqueda et al. (2017) found higher TPC in peel (588.8 mg GAE/100 g of powder), following by pulp and seeds (415.2 and 179.4 mg GAE/100 g of powder, respectively). To enable comparison, we converted our results to mg GAE /100 g of each freeze-dried fraction (power). Values ranged from 343.96 to 1225.44 mg GAE/100 g. With the exception of JSE, our results were higher than those reported for by them. Roesler et al. (2007) analyzed the TPC in fruta-do-lobo in both extract (aqueous and ethanolic) and residue. For peel, was found 35.15 and 15.09 g GAE/kg for ethanolic and aqueous extract, respectively). Espin et al. (2016) investigated the phenolic composition and antioxidant capacity of tree tomato fruits (Solanum betaceum Cav) from different cultivars. They found values ranged between 202 and 325 µmol of Trolox equivalents/g of dry pulp for ORAC assays. These authors have been demonstrated the correlation between phenolic compounds and antioxidant capacity. However, is not possible to compare our results with those. The difficulty to compare the results is due to the different assays and extraction methods, ways of expression, and/or type samples considered.

A direct comparison between antioxidant capacity (ORAC) and phenolic composition shows some correlation since a low ORAC value of JSE fraction can be related to their low abundance of phenolic compounds (Table 4). However, for the other fractions that correlations are not so evident, for that, a Pearson correlation was made (Supplementary material) to point out possible interactions between antioxidant tests and phenolic compounds. We were able to observe a positive correlation between the antioxidant activity and the hydroxycinnamic acid mono glucosides, and a negative correlation between the same antioxidant activity and the diglucosides hydroxycinnamic acids. This may occur probably due to an antioxidant activity ‘dilution’ on molar concentration. For example, 1 mg of coumaroyl-O-glucoside (MM 326.3 mmol) with one aromatic hydroxyl group will have a greater antioxidant activity than 1 mg of coumaroyl-O-frutofuranosilglucose (MM 488.2 mmol) with the same hydroxyl group but a greater molar mass.

These results suggest that the phenolic compounds may be one of the main factors responsible for the antioxidant capacity of fruta-do-lobo and juá-açu.

Table 4. Total phenolic compounds, MS phenolic and antioxidant capacity in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendtn.)[1]

	Fruta-do-lobo			Juá-açu		
	Peel	Pulp	Seed	Peel	Pulp	Seed
Total Phenolic Compounds (mg GAE/100 mL of extract)	463.83 ± 24.54b	462.10 ± 26.55b	415.19 ± 22.96b	612.72 ± 43.66a	598.15 ± 38.09a	171.98 ± 25.93c
MS Phenolic[2]	1.1E7 ± 0.2E7c	7E6 ± 1E6d	1.1E7 ± 0.2E7c	1.1E7 ± 0.2E7c	1.84E7 ± 0.05E7a	1.95E6 ± 0.4E6b
ORAC hidro (µmol TE /100 mL of extract)	2.66 ± 0.34d	4.14 ± 0.18c	3.16 ± 0.03d	10.63 ± 0.38b	11.51 ± 0.42a	1.35 ± 0.13e
ORAC lipo (µmol TE/100 mL of extract)	n.d.	n.d.	10.96 ± 1.25a	n.d.	n.d.	5.53 ± 0.56b

[1]Values expressed as mean ± SD (n = 3). Values in the same line followed by different letters are significantly different by ANOVA test ($p < 0.05$). 2MS Phenolic: Total amount of all LC-MS identified phenolics. n.d.: not determined.

4. Conclusions

It was possible to correlate the constituents of both fruits to their antioxidant capacity. The studied fruits had a proximate composition comparable to other fruits of the genus Solanum. In addition to that, the fruits can also contribute partly to the daily fiber and fructan intake, such as GF2 and GF, and can be considered a source of copper and magnesium. With regards to VOCs, aldehydes and esters are responsible by the characteristic flavor of fruits. Apart from the juá-açu seed fraction, the analyzed fractions showed relatively high values of ORAC, which are related to their phenolic compounds. The pulp and peel of juá-açu has the greatest potential for antioxidant capacity. Therefore, these fruits could have an important nutritional and economic value, contributing to the sustainable use of Brazilian fruits, which is of potential interest to the agroindustry, and a possible source of income for the local population. These results provide support for the next steps of this study aiming to elucidate the physiological and molecular mechanisms in the prevention and treatment of comorbidities induced by hyperglycemic and/or hyperlipidic diet, especially regarding the phenolic compounds and oligosaccharides.

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Compliance with Ethical Standards

Conflict of interest: The authors declare that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Publication

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CAPÍTULO II

The potential prebiotic activity of fruta-do-lobo (*Solanum lycocarpum* St. Hill) starch: possible mechanisms involved in glycemic control

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Highlights

1 Fruta-do-lobo starch (FLS) is a good source of resistant starch.

2 For the first time we evaluated the potential prebiotic activity of FLS.

3 *Bifidobacterium* and *Lactobacillus* were able to use FLS as a carbon source.

4 FLS promoted the short chain fatty acids (acetate, propionate and butyrate) production.

Abstract

Fruta-do-lobo (*Solanum lycocarpum* St. Hill) is a native fruit commonly used in Brazilian folk medicine as a hypoglycemic agent. These properties are attributed to their resistant starch content, which has been shown to increases the growth of *Bifidobacterium* and *Lactobacillus* in the gut. In this scenario, this study aimed to investigate the potential prebiotic activity of fruta-do-lobo starch (FLS). FLS showed around 30 % of resistant starch and their prebiotic potential was carried out with five strains *L. acidophilus* (LA3 and LA5), *L. casei* (LC01) and *B. animalis* (BB12) and *B. lactis* (BLC1) in a concentration range of 1.0 to 2.0 % of starch. In preliminary screening, we evaluate, during 48 h the viability of the starch with promoting growth agent. It was observed an increase in the growth of the probiotic strains tested. We also evaluated the microrganisms metabolic activity by assessing the short-chain fatty acid (SCFA) production, using the best starch growth promotion conditions (2% of FLS and strains BLC1, LA5, and LC01). Despite the fact that the growth of these strains was lower when FLS was used as a carbon source when evaluated by optic density, we can observe that microorganisms can grow. The maximum population reached when FLS used was 11 log10 CFU/mL and no differences in populations were observed when the other carbons sources were used. Additionally, FLS promoted SCFA production, particularly acetate. For butyrate, no significant difference was observed ($p < 0.05$) between the FOS and FLS for the three evaluated strains. These data indicate a potential prebiotic activity of FLS and suggest that its supplementation could exert a crucial role in glycemia reduction.

Keywords: Resistant starch, Short-Chain Fatty Acid, Fruta-do-lobo, Prebiotic

1. Introduction

Diabetes is a chronic disease characterized by hyperglycemia that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin that it produces (Tang et al., 2015; World Health Organization

- WHO, 2018). Several classes of drugs have been used to control glycemia, however, most of them are related to unwanted side effects. Therefore, searching for new therapeutic strategies to control glycemia with possible fewer side effects are valuable strategies.

Some studies have been reported that gut microbiota dysbiosis is a factor in a rapid progression of insulin resistance and are directly related to cases of type 2 diabetes, which are responsible for 90% of the almost 500 million total diabetes cases (WHO, 2018). Adjuvant therapy with both prebiotics and probiotics promotes the growth of beneficial bacteria to the host, contributing to a healthy microbiota. This approach has already been shown to be able to reverse metabolic dysfunctions, including tissue inflammation and secretion, and insulin sensitivity (Upadhyaya and Banerjee, 2015; Sharma and Tripath, 2019). So, the microbiota modulation may be a potential therapeutic target for the development of new therapy strategies (Colantonio, Werner and Brown, 2019).

Fruta-do-lobo (*Solanum lycocarpum* St. Hill) is a native fruit commonly used in Brazilian folk medicine as a hypoglycemic agent. Experimental evidence point out that fruta-do-lobo starch (FLS) are involved in the reduction of glycemic index followed by the attenuation of physiological changes associated with diabetes, such as polyuria and polyphagia (Farina et al., 2009). Also, fewer side effects were associated with the use of most oral hypoglycemic drugs (Perez et al., 2006). It means that the resistant starch behavior of the FLS elects them as a novel and promising option to manage diabetes mellitus (Dall'agnol and Von Poser, 2000).

By definition, the resistant starch is the total amount of starch as well as the products of starch degradation which resists digestion in the small intestine, reaching the colon, and then fermented by the gut microbiota (Zaman & Sarbini, 2015). The most prominent metabolites of this fermentation are microbial-derived short chain fatty acids (SCFA) such as acetic, propionic and butyric acids. It is essential to highlight that SCFA is taken up by the host and are subsequently utilized in several metabolic processes as well as exerting beneficial effects on the human body (Schwiertz, 2016). These properties make resistant starch a potential dietary target for the prevention of diseases associated with dyslipidemia and insulin resistance, such as type 2 diabetes, dietary therapies for the treatment of this disease (Higgins, 2004).

In this scenario, we believe that the reduction of glycemic level and the health benefits promoted by ingestion of FLS may be due to the action of SCFA in the host organism. Therefore, this study aimed to investigate the potential enhancement of the prebiotic activity of gut microbiota by FLS intake

2. Material and methods

2.1 Collection and identification of fruit species

Twenty-five kg of fruta-do-lobo (*Solanum lycocarpum* St. Hill) was obtained from Carmo do Rio Claro, Minas Gerais, Brazil, and exsiccate was deposited in the herbarium of the Institute of Biology of UNICAMP (UEC 197248). Fruits were selected taking into consideration the stage of maturation (totally unripe), uniform size, the color of the peel (intense dark green), stalks trapped and absence of injuries caused by insects or mechanical shocks.

2.2 Extraction of starch

The extraction of starch was performed according to Clerici et al. (2011), with modifications. Fruits were washed, peeled, and the seeds were removed. Pulp was cut in small pieces and put into a sodium bisulfite solution (200 mg/L). Subsequently, they were washed for removal of bisulfite excess and ground in a liquefier with distilled water (1:5 v/v) until a homogeneous suspension was formed. The suspension was sieved (22 mesh) and the portion retained was characterized as pomace. The liquid was kept under refrigeration overnight for decanted of starch. Starch was dried at 50 °C for approximately 15 h in convective hot-air dried. For the calculation of the yield of extraction, the relation between the weight of the original raw material and the weight of the final product was established, and the results were expressed in %.

2.3 Characterization of starch

2.3.1 Proximate composition

Moisture, protein, ash, and dietary fiber contents of starch were determined in triplicate according to official methods (Association of Official Analytical Chemists,

2006). Lipid content was determined according to Bligh and Dyer (1959), and carbohydrate content was calculated by difference.

2.3.2 Resistant starch

The resistant starch content was determined according to the methodology proposed by Goñi, Garcia-Diaz, Mañas, and Saura-Calixto (1996). Briefly, a digestion process was performed using pepsin, α-amylase and amyloglucosidase enzymes. After the digestion process, the residue was discarded and the supernatant used to determine the glucose concentration by the glucose oxidase method, using the spectrophotometer with a wavelength of 505 nm. A standard glucose curve was used.

2.4 Irradiation starch

The sterility of FLS was achieved through gamma-irradiation. The packed FLS sample were subjected to a dose of 1.5 kGy using cobalt-60 as source irradiator at room temperature of 25 ± 2 °C. The treatment was performed at Nuclear Energy Center in Agriculture of the University of São Paulo (CENA/USP).

2.5 Evaluation of the potential prebiotic activity

The fruta-do-lobo starch prebiotic potential was carried out with five strains *Lactobacillus acidophilus* (LA5), *Lactobacillus casei* (LC01) and *Bifidobacterium animalis* (BB12) from Christian Hansen (Denmark); *Bifidobacterium lactis* (BLC1) and *Lactobacillus acidophilus* (LA3) from Lyofast Italy). The experiments were done according to Sousa et al. (2015), with modifications.

Before each test, the strains were activated in MRS broth (DeMan-Rogosa-Sharpe) and incubated at 37 °C for overnight. In order to prepare stock cultures, a 1 ml aliquot of the new inoculum was transferred to pre-sterilized microtubes and centrifuged (8000 RPM, 10 min, 4 °C) (Hettich Rotanta 460/460R). The supernatant was discarded and the microorganism maintained in the microtube. Subsequently, 1 mL of sterile freezing medium was added to the microorganism, and each microtube was identified and stored at - 80 ° C for future use as a stock culture.

2.5.1 Starch concentration evaluation

For prebiotic activity, the cryopreserved strain was reactivated in MRS medium and incubated under anaerobic conditions at 37 °C for 48 hours.

The growth promoting activity screening of each strain was performed in microplate assays using MRS with a different carbon source. Each medium was composed of: MRS broth (with glucose), mineral MRS with 2 % lactose (w/v) and mineral MRS with 2 % FOS, which were used as a control, and mineral MRS with starch. The starch concentration used were 1.0, 1.5 and 2.0 % (w/v). All additional carbon source was added in replacement of glucose. Each strain was inoculated with the media mentioned above, considering the initial optical density of 0.6. The microplate was then incubated during 48 h at 37 °C and monitored by measuring absorbance at 655 nm every 30 minutes. Specific growth rates were calculated (by determination of the slope of the trend line, of the viable cell numbers in the log phase of the growth curves) in order to compare the obtained results.

2.5.2 Evaluation of the potential prebiotic activity via viable cell number and metabolic activity

Growth curves and metabolic activity were used to confirm the prebiotic activity potential. The best starch concentration (2 %), as well as three probiotic strains, were selected (BLC1, LC01, and LA5), since the use of the carbon source may differ according to the microorganism. Growth curves were monitored by enumeration of viable cell numbers, as well as by the evaluation of optical density (as described in item 2.5.1). The optical density was measured at 650 nm using a microplate reader (BMG LABTECH, Germany). Bacterial metabolism was assessed by changes in pH and SCFA production. All analyses were performed in duplicate and sampled at 0, 4, 8, 12, 24 and 48h.

2.6 Short-chain fatty acids production

Short-chain fatty acids were identified and quantified according to Zhao, Nyman, and Jönsson (2006) and Silva, Cazarin, Boguz Jr, Augusto and Maróstica Jr (2014) with modifications, as described below:

2.6.1 Preparation of standard solution

Standard solutions were individually prepared and were used for quantification of produced acetic (0.04 – 10.49 mg/mL), propionic (0.04 – 9.93 mg/mL) and butyric acids (0.08 – 9.64 mg/mL). A 2-Ethylbutyric acid solution containing HCl 5

M was used as an internal standard stock solution. All the standard solutions were prepared and immediately used.

2.6.2 Sample preparation

Samples were collected from each Erlenmeyer at different times (0, 4, 8, 12, 24, and 48 h) and immediately were stored at – 18 °C. At the time of analysis, samples were centrifuged (10 min, 10,000 RPM and 5 °C). The supernatant was filtered on 0.22 µm filters. After that, the pH was adjusted to 2–3 by adding acidified water and then kept at room temperature for 10 min during occasional shaking. The internal standard, 2-ethyl butyric acid solution, was spiked into the supernatant at a final concentration of 1 mM and the solution was injected in the GC for analysis.

2.6.3 Short-chain fatty acids (SCFAs) quantification

Chromatographic analysis was carried out using an Agilent 6890N gas chromatograph system equipped with a flame ionization detector (FID) and a capillary column (30 m × 0.25 mm i.d. × 0.25 µm Nukol™, Supelco, Bellefonte, PA, US). The chromatographic conditions were as follows: injector and detector temperatures set at 250 °C, injected volume 1 µL with the split ratio set to 1:10, and the carrier gas was helium at 1.0 mL/min. The initial oven temperature was 100 °C, maintained for 0.5 min, raised to 180 °C at 8 °C/min and held for 1.0 min, then increased to 200 °C at 20 °C/min, and finally held at 200 °C for 5 min totalizing 17.5 min. The SCFAs were identified on chromatograms by their retention times compared with the standard solution.

2.7 Statistical analyses

Statistical analyses were performed using Statistica® and R programs. Analyses of variance (ANOVA) and Tukey's test were used to determine differences between treatments ($p < 0.05$).

3. Results and discussion

3.1 Process yield and characterization of starch

We obtained a yield of 3.7 ± 0.53 % starch, lower than the obtained by Clerici et al. (2011) (4.8 %). Once the same extraction methodology was used, these differences can be explained by the fruit ripeness degree, manipulation, among others.

The proximate composition and resistant starch content of FLS are shown in Table 1. Our results for moisture, lipids, protein, ash, and fiber are lower than the values found by Clerici et al. (2011), 12.15, 0.18, 6.27 0.38, respectively. These variations are common and depend on climatic factors and soil types, for example. However, the content of resistant starch was similar to that found by the same authors (32.32 g/100 g).

Table 1. Proximate composition and resistant starch content of fruta-do-lobo (*Solanum lycocarpum* St. Hill).

Moisture (g/100 g)	Total carbohydrate (g/100 g)	Lipid (g/100 g)	Protein (g/100 g)	Ash (g/100 g)	Fiber (g/100 g)	Resistant starch (g/100 g)
8.66 ± 0.07	90.3	0.24 ± 0.00	0.68 ± 0.02	0.12 ± 0.02	0.00 ± 0.16	29.79 ± 0.61

3.2 Evaluation of the potential prebiotic activity

3.2.1 Choosing of microorganism and starch concentration

All strains were able to consume the FLS among all concentration, and the specific growth rates are shown in Table 2. Our results are smaller when compared with the values found by Sousa et al. (2015), who evaluated the potential prebiotic activity of yacon at different concentrations. This finding can be explained because, once the initial media starch solution was cloudy, we measure the microorganism growth indirectly via starch consumption monitoring, due turbidity decrease. Looking at our results, the higher growth rates were observed for BLC1 when used 2.0 % FLS concentration (0.007 h^{-1}), showing a better ability of this strain in use FLS as a carbon source. For the other strains, there was no difference in the multiplication rate independent of the concentration tested. Therefore, we opted for the highest concentration (2 %). In addition to BLC1, we chose to evaluate two more strains of different species (LA5 and LC01), since the use of the carbon source may differ according to the microorganism.

Table 2. Specific growth rate and maximum growth for the *Lactobacillus* and *Bifidobacterium* strains tested in the different concentrations of fruta-do-lobo-starch (FLS) (1.0 - 2.0 %)

Strain	FLS concentration (%)	Specific growth rate (h^{-1})
LA3	1.0	0.002 ± 0.001
	1.5	0.003 ± 0.000
	2.0	0.003 ± 0.001
LA5	1.0	0.002 ± 0.000
	1.5	0.003 ± 0.001
	2.0	0.004 ± 0.002
LC01	1.0	0.002 ± 0.001
	1.5	0.002 ± 0.000
	2.0	0.003 ± 0.001
BB12	1.0	0.002 ± 0.000
	1.5	0.002 ± 0.000
	2.0	0.002 ± 0.000
BLC1	1.0	0.004 ± 0.002
	1.5	0.003 ± 0.001
	2.0	0.007 ± 0.002

3.2.1 Evaluation of the potential prebiotic activity by determination of viable cell number and metabolic activity

The potential of FLS prebiotic activity was compared with FOS, a non-digestible carbohydrate knows for acting as prebiotic; and lactose, a sugar preferentially and quickly metabolized by lactic acid bacteria. Non-digestible sugars change the composition of gut microbiota by selective increasing of friendly bacteria (Jung, Jeon, and Han, 2015).

First, we evaluate the microorganism growth, and as expected, all strain was able to grow using FLS, MRS, lactose or FOS as the primary carbon source. Figure 1 shows the growth curves using viable cell number, once the presence of starch prevents the direct access or growth rate using OD measurements (Supplementary material 1). Despite no significant growth difference among carbons sources, studies have been shown that when pathogens bacteria are grown together with beneficial bacteria the use of prebiotics used to change their population balance, favoring the beneficial one (Jung et al., 2015). We believe that it may occur because

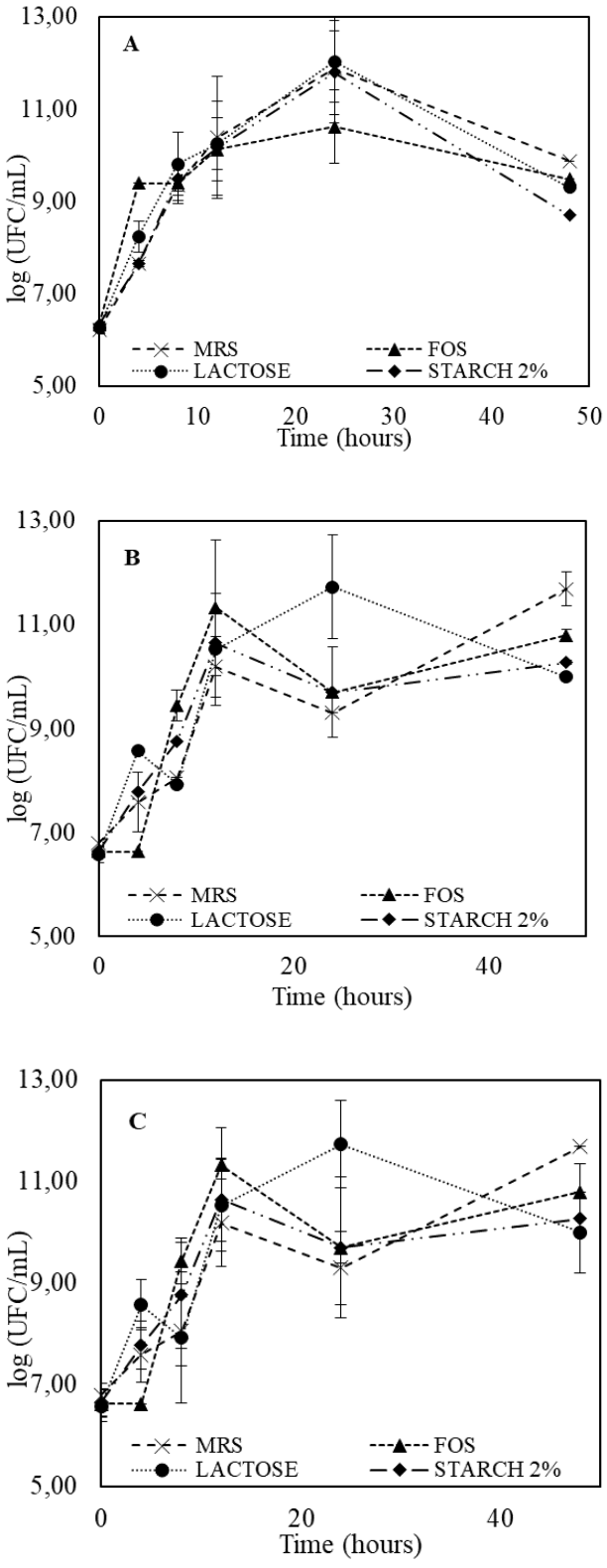
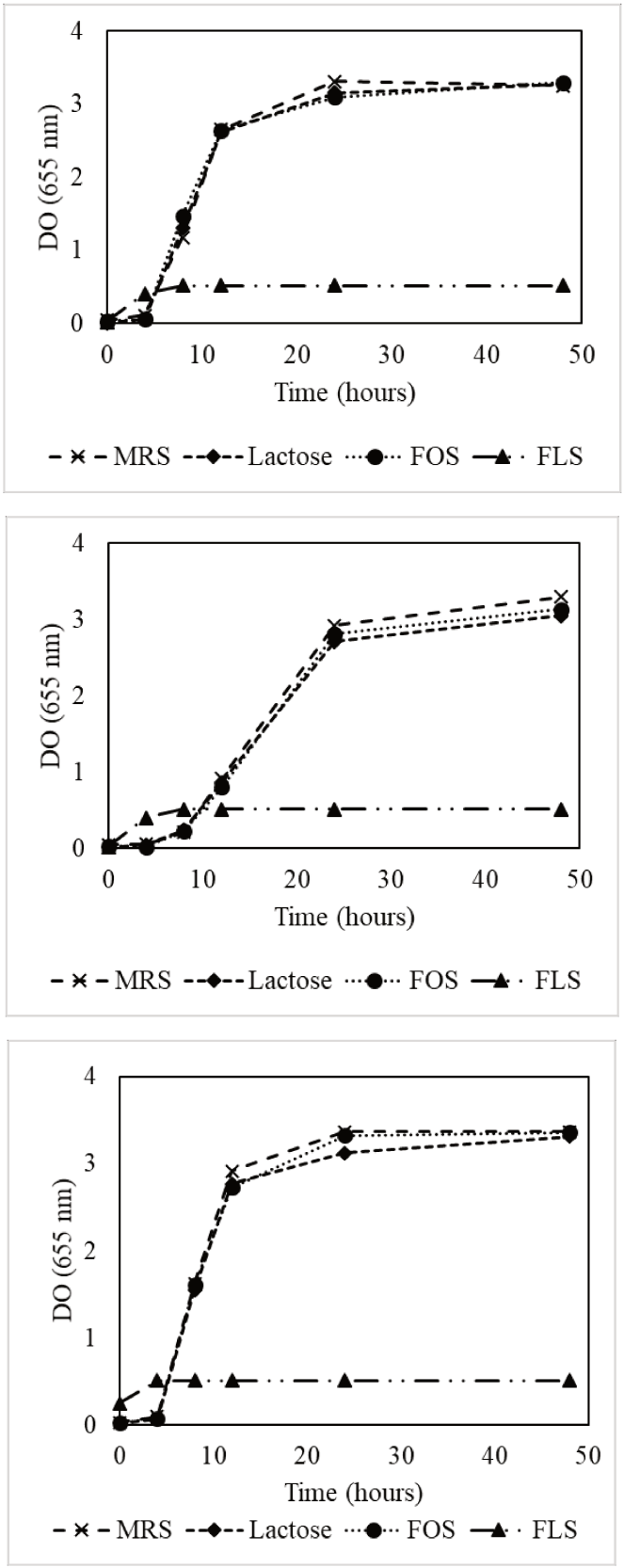


Figure 1. Growth curve for *Bifidobacterium lactis* BCL1 (A), *Lactobacillus casei* LC01 (B) and *Lactobacillus acidophilus* LA5 (C) using different carbon source



Supplementary material 1. Growth curve for strains *Bifidobacterium lactis* BCL1 (A), *Lactobacillus casei* LC01 (B) and *Lactobacillus acidophilus* LA5 (C) in different carbon source to evaluate prebiotic activity. MRS (×); Lactose (♦); FOS (●); FLS (▲)

friendly bacteria such as *Bifidobacterium*, while feeding on prebiotics, lower the pH levels by acids production, making the gut less favorable for pathogens growth (Kolida, Tuohy & Gibson, 2002).

We also evaluate the pH reduction during microorganism growth, and they reduce for all conditions confirming the acid production. However, the strains BCL01 and LA5 were able to do it faster than LC01 when using FLS as a carbon source (Figure 2).

The acid production of *Lactobacillus* and *Bifidobacterium* using prebiotics, inhibit the proliferation of harmful bacteria due to increasing in the production of SCFA, IL-10, among others, in addition to modulating lipid metabolism. All of these factors interfere with diabetes (Colantonio et al. 2019; Sharma & Triphanti, 2019).

3.3 Short-chain fatty acids (SCFA) production

SCFA are the primary metabolic products of natural polysaccharides from gut microbiota fermentation in the distal intestine and play an essential role in maintaining the normal function of the colon and in the morphology of their epithelium (Xia, Wang, Yu, Liang, & Kuang, 2019). Thereabout 90–95 % of SCFA are acetate, propionate, and butyrate, with smaller proportions of valerate, hexanoate, isobutyrate, and isovalerate (Wang et al., 2019). Figure 3 shows the final production of the most abundant SCFA produced from FLS and other carbon sources for three probiotic strains previously selected: BLC1, LC01, and LA5. Generally, the proportions of acetate, propionate, and butyrate are approximately 60 %, 25 %, and 15 %, respectively, but such proportions may vary according to the type of fiber (Wang et al., 2019). In our study, there was a predominance of acetate (about 98%) and low production of propionate and butyrate (approximately 1.5 and 0.7%, respectively), regardless of the source of carbon used.

Until now, there are no data on the prebiotic activity about FLS. However, in a systematic review, Colantonio, Werner, and Brown (2019) evaluated the effect of prebiotics and substances with prebiotic properties on the metabolic and inflammatory biomarkers of individuals with type 2 diabetes compared with placebo. Among the 27 analyzed publications, five used resistant starch in doses of 10 to 60 g/day. They found that a supplementing or increased-resistant starch intake improves meal glucose response, blood glucose and lipid levels, and inflammatory biomarkers, improve

endotoxemia and glycemic, oxidative stress, and antioxidant biomarkers among adults with type 2 diabetes.

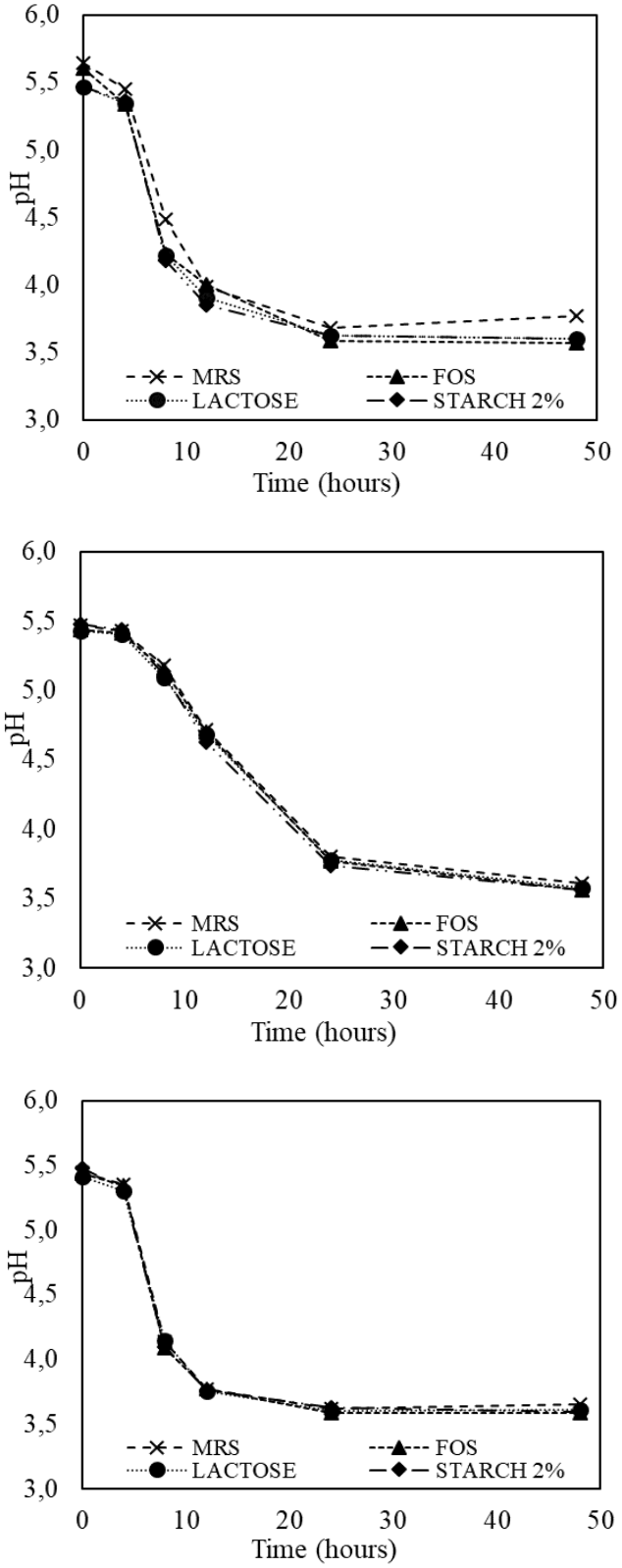


Figure 2. pH curve for *Bifidobacterium lactis* BCL1 (A), *Lactobacillus casei* LC01 (B) and *Lactobacillus acidophilus* LA5 (C) using different carbon sources

According to Lewis and Abreu (2017), the type of SCFA generated in the intestine depends on the type of fiber or substrate consumed as well as the type of bacteria metabolizing, being bacteria of the Firmicutes phylum, such as Lactobacillus, and resistant starch responsible to more production of butyrate. However, as can be seen in the Supplementary material 2, acetic acid was the primary acid produced (4965.99 - 6800.11 mg/L), followed by propionic and butyric acids (62.93 - 87.71 and 38.44 - 46.31 mg/L, respectively). The production of butyrate also depends on the type of resistant starch. For example, Plongbunjong, Graidist, Knudsen, and Wichienchot (2017) reported that resistant starch type 3 produced two times more butyric acid than RS type 2. Lactose was shown to be the best substrate for butyrate production, regardless of the strain used.

At 48 hours, the propionate produced by LC01 was statistically equal ($p < 0.05$) for both FOS and FLS. For the butyrate, there was no significant difference ($p < 0.05$) between the FOS and FLS for the three strains evaluated (Supplementary material).

Regarding the role of SCFA on diabetes, type 1 diabetes is an autoimmune disorder characterized by an absolute deficiency of insulin due to the destruction of the pancreatic β cells mediated by T cells (Sharma & Triphanti, 2019). It also can be related to dysbiosis, since increased permeability leads to intestinal inflammation and reduced/disturbed immune response in the intestinal mucosa (Paun, Yau, & Danska, 2017). SCFA play an essential role in the immune system. While butyric acid inhibits the production of proinflammatory IL-2 and IFN-γ, propionate, and acetate amplify the production of the anti-inflammatory cytokine IL-10 (Khangwal & Shukla, 2019). Butyrate presents beneficial effects on inflammatory conditions because this acid inhibited both in vitro and ex-vivo production of proinflammatory cytokines (TNF-α and CINC-2αβ) and NO by lipopolysaccharide-stimulated neutrophils (Vinolo et al., 2011).

In contrast, type 2 diabetes is a chronic metabolic abnormality with fasting serum hyperglycemia, insulin nonresponsiveness and insulin insufficiency due to reduced insulin secretion from β cells in insulin nonresponsive environment (Sharma & Triphanti, 2019).

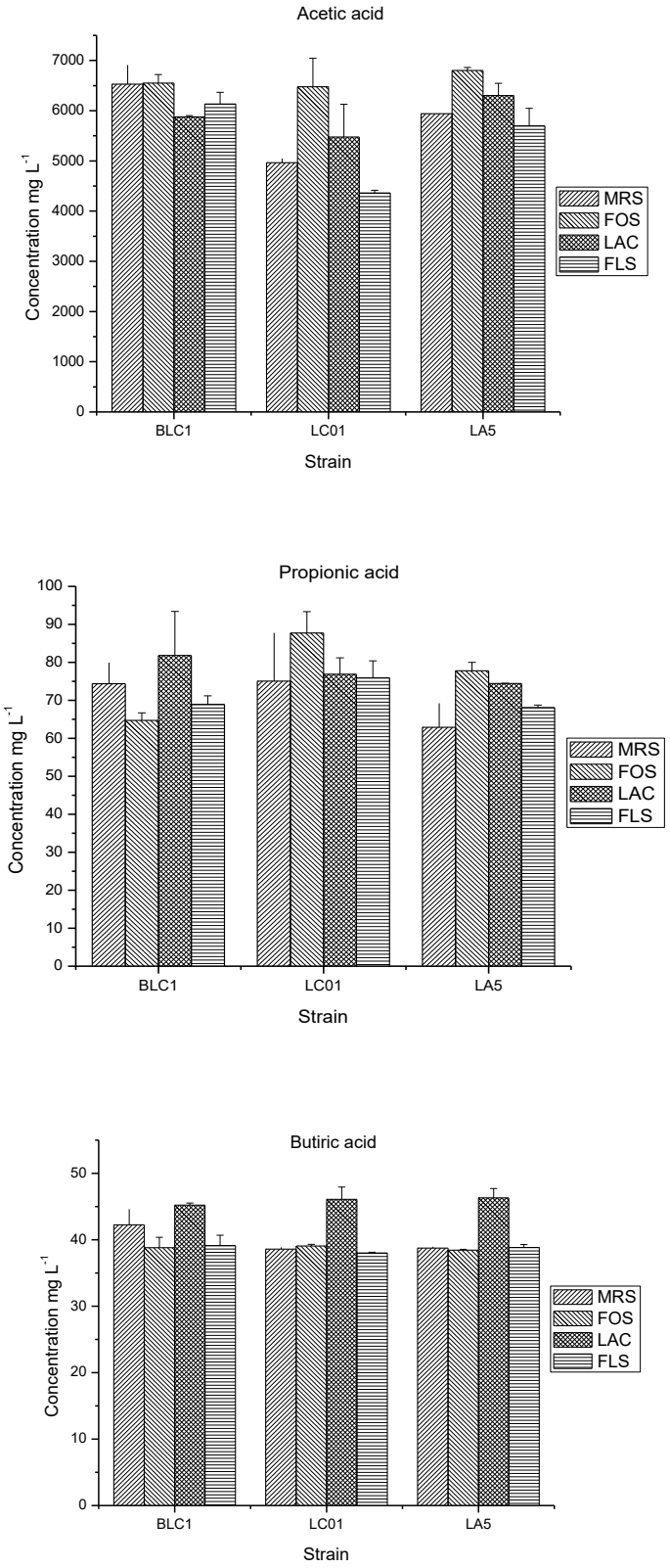


Figure 3. Final production of SCFA (acetic, propionic and butyric) produced from FLS and others carbon sources for probiotic strains for *Bifidobacterium lactis* BCL1, *Lactobacillus casei* LC01 and *Lactobacillus acidophilus* LA5

Supplementary material 2. Short-chain fatty acids concentrations (mg/L) produced by different probiotic strains (BLC1, LC01 and LA5)[1] and different carbon sources (MRS, FOS, LAC and FLS)[2]

Time	Strain	Carbon source (2%)	Acetic acid (mg/L)	Propionic acid (mg/L)	Butyric acid (mg/L)	Total SCFA (mg/L)
T0	BLC1	MRS	2726.52 ± 230.90^{e}	43.69 ± 1.54^{c,d}	34.46 ± 0.19^{c}	2804.67
		FOS	4391.56 ± 382.22^{b}	54.19 ± 2.19^{a,b}	35.69 ± 1.03^{a,b}	4481.44
		LAC	3728.87 ± 35.72^{c}	55.22 ± 0.07^{a,b}	35.99 ± 0.26^{a,b}	3820.07
		FLS	2763.09 ± 139.53^{e}	49.79 ± 2.71^{c,b}	-	2812.87
	LC01	MRS	2503.04 ± 215.39^{e}	43.54 ± 0.80^{c,d}	34.82 ± 0.58^{b,c}	2581.40
		FOS	4429.68 ± 143.02^{b}	54.82 ± 0.02^{a,b}	35.37 ± 0.35^{a,b,c}	4519.87
		LAC	3801.42 ± 97.95^{c}	57.55 ± 4.56^{a}	36.19 ± 0.77^{a}	3895.15
		FLS	2801.28 ± 221.76^{e}	44.50 ± 2.66^{c,d}	-	2845.74
	LA5	MRS	2610.96 ± 73.61^{e}	42.33 ± 0.52^{d}	-	2653.29
		FOS	5064.99 ± 101.54^{a}	59.51 ± 7.93^{a}	36.35 ± 1.40^{a}	5160.85
		LAC	3267.09 ± 13.32^{d}	57.64 ± 3.61^{a}	-	3324.73
		FLS	2669.79 ± 201.55^{e}	46.37 ± 1.50^{c,d}	-	2716.16
T4	BLC1	MRS	3044.52 ± 44.76^{d,e}	51.53 ± 1.11^{d,e}	35.19 ± 0.26^{d,e}	3131.24
		FOS	4809.46 ± 164.88^{a}	62.35 ± 1.06^{b}	36.46 ± 0.57^{b,c}	4908.27
		LAC	4693.23 ± 262.77^{a}	72.80 ± 3.16^{a}	37.70 ± 0.16^{a}	4803.72
		FLS	2329.88 ± 149.36^{f}	51.78 ± 5.39^{d,e}	35.12 ± 1.08^{d,e}	2416.78
	LC01	MRS	2678.77 ± 9.52^{e,f}	48.74 ± 1.46^{e,f}	34.91 ± 0.48^{d,e}	2762.42
		FOS	3661.85 ± 291.55^{c}	55.43 ± 1.40^{c,d}	35.82 ± 0.34^{c,d}	3753.09
		LAC	4096.90 ± 184.24^{b}	59.64 ±1.32^{b,c}	37.43 ± 0.39^{a,b}	4193.97
		FLS	3217.75 ± 177.52^{d}	54.91 ± 6.46^{c,d}	36.31 ± 0.56^{c}	3308.97
	LA5	MRS	2332.73 ± 148.34^{f}	45.69 ± 0.90^{f}	34.64 ± 0.42^{e}	2413.06
		FOS	4662.03 ± 204.50^{a}	61.96 ± 1.57^{b}	36.62 ± 0.75^{a,b,c}	4760.61

Different letters in the same column, for each time interval, means statistical difference. [1]BLC1: *Bifidobacterium lactis*; LC01: *Lactobacillus casei;* LA5: *Lactobacillus acidophilus*. MRS: de Man-Rogosa-Sharpe broth; FOS: Orafti® Oligofructose – P95; LAC: lactose; FLS: Fruta-do-lobo starch.

Supplementary material 2. Short-chain fatty acids concentrations (mg/L) produced by different probiotic strains (BLC1, LC01 and LA5)[1] and different carbon sources (MRS, FOS, LAC and FLS)[2]. Continuação…

Time	Strain	Carbon Source (2 %)	Acetic acid (mg/L)	Propionic acid (mg/L)	Butyric acid (mg/L)	Total SCFA (mg/L)
T8	BLC1	LAC	4013.72 ± 104.97^{b,c}	61.73 ± 0.29^{b}	36.80 ± 0.10^{a,b,c}	4112.24
		FLS	3050.61 ± 429.38^{d,e}	49.06 ± 0.75^{e,f}	34.71 ± 0.71^{d,e}	3134.38
		MRS	3296.24 ± 81.03^{e,f}	72.08 ± 6.07^{b}	36.28 ± 0.29^{d,e}	3404.60
		FOS	5716.98 ± 430.77^{a}	89.22 ± 7.01^{a}	37.62 ± 0.11^{b,c}	5843.82
	LC01	LAC	3625.57 ± 31.42^{d,e}	71.01 ± 7.37^{b,c}	36.95 ± 0.22^{c,d,e}	3733.53
		FLS	3887.49 ± 92.89^{c,d}	65.21 ± 2.83	36.84 ± 0.16^{c,d,e}	3989.54
		MRS	4066.36 ± 364.05^{c,d}	60.30 ± 0.59^{d,e}	36.21 ± 0.41^{d,e}	4162.87
		FOS	4168.11 ± 287.61^{c}	63.16 ± 1.95^{b,c,d}	36.38 ± 0.34^{d,e}	4267.66
	LA5	LAC	4287.93 ± 209.84^{c}	67.08 ± 5.62^{b,c,d}	38.22 ± 0.67^{b}	4393.23
		FLS	3293.53 ± 101.86e,f	58.94 ± 1.78^{d,e}	36.22 ± 0.72^{d,e}	3388.69
		MRS	3086.70 ± 68.05^{f}	52.84 ± 2.13^{e}	35.96 ± 0.66^{e}	3175.50
		FOS	5136.97 ± 125.10^{b}	63.64 ± 3.93^{b,c,d}	37.12 ± 0.24^{c,d}	5237.73
T12	BLC1	LAC	5056.50 ± 177.75^{b}	63.86 ± 1.52^{b,c,d}	40.58 ± 0.86^{a}	5160.95
		FLS	3191.99 ± 302.36^{e,f}	62.34 ± 5.73^{c,d}	36.01 ± 0.76^{e}	3290.34
		MRS	4587.50 ± 376.65^{d,e}	85.74 ± 15.84^{a,b}	37.65 ± 0.21^{c,d,e}	4710.90
		FOS	6624.55 ± 275.29^{a}	75.55 ± 5.03^{a,b,c,d}	38.19 ± 0.17^{b,c,d}	6738.29
	LC01	LAC	5264.73 ± 285.07^{c}	84.40 ± 20.57^{a,b,c}	41.76 ± 1.79^{a}	5390.89
		FLS	4549.24 ± 164.04^{d,e}	70.48 ± 3.58^{b,c,d}	36.79 ± 0.20^{e,f,g}	4656.52
		MRS	3381.19 ± 216.21^{h}	63.06 ± 1.42^{d}	35.61 ± 0.20^{g}	3479.85
		FOS	5821.78 ± 210.86^{b}	69.49 ± 1.31^{b,c,d}	37.11 ± 0.33^{d,e,f}	5928.38
	LA5	LAC	4561.70 ± 124.19^{d,e}	73.64 ± 16.06^{b,c,d}	38.45 ± 0.31^{b,c}	4673.79
		FLS	3664.38 ± 49.49^{g,h}	58.57 ± 5.48^{d}	36.41 ± 0.25^{f,g}	3759.36

Different letters in the same column, for each time interval, means statistical difference. [1]BLC1: *Bifidobacterium lactis*; LC01: *Lactobacillus casei;* LA5: *Lactobacillus acidophilus*. MRS: de Man-Rogosa-Sharpe broth; FOS: Orafti® Oligofructose – P95; LAC: lactose; FLS: Fruta-do-lobo starch.

Supplementary material 2. Short-chain fatty acids concentrations (mg/L) produced by different probiotic strains (BLC1, LC01 and LA5)[1] and different carbon sources (MRS, FOS, LAC and FLS)[2]. Continuação…

Time	Strain	Carbon Source (2 %)	Acetic acid (mg/L)	Propionic acid (mg/L)	Butyric acid (mg/L)	Total SCFA (mg/L)
	LA5	MRS	4398.56 ± 15.63^{e,f}	65.63 ± 4.90^{c,d}	37.22 ± 0.00^{d,e,f}	4501.41
		FOS	4828.09 ± 271.95^{d}	65.42 ± 0.01^{c,d}	36.83 ± 0.10^{e,f}	4930.34
		LAC	4018.89 ± 79.93^{f,g}	64.56 ± 8.84^{d}	39.01 ± 0.43^{b}	4122.46
		FLS	4361.39 ± 105.20^{e,f}	94.21 ± 2.63^{a}	37.82 ± 0.62^{b,c,d,e}	4493.41
T24	BLC1	MRS	5977.74 ± 320.33^{c}	89.77 ± 0.45^{a,b,c}	39.26 ± 0.18^{c}	6106.76
		FOS	6040.37 ± 49.55^{c}	98.76 ± 2.59^{a,b}	38.48 ± 0.34^{c,d,e}	6177.62
		LAC	5876.25 ± 88.56^{c,d}	92.10 ± 13.06^{a,b,c}	45.57 ± 0.02^{b}	6013.91
		FLS	4827.65 ± 206.82^{f,g}	73.82 ± 2.64^{b,c}	37.88 ± 0.02^{d,e,f}	4939.35
	LC01	MRS	4452.67 ± 267.56^{g,h}	75.50 ± 5.42^{b,c}	37.48 ± 0.45^{e,f}	4565.65
		FOS	5844.33 ± 131.50^{c,d}	110.05 ± 33.85^{a}	39.03 ± 0.65^{c,d}	5993.41
		LAC	5425.53 ± 320.14^{d,e}	90.89 ± 28.69^{a,b,c}	44.44 ± 0.03^{b}	5560.85
		FLS	4992.43 ± 401.71^{e,f}	81.90 ± 7.71^{b,c}	38.56 ± 0.94^{c,d,e}	5112.89
	LA5	MRS	4078.00 ± 344.10^{h}	64.94 ± 3.19^{c}	37.17 ± 0.43^{f}	4180.11
		FOS	7403.10 ± 239.58^{a}	87.44 ± 6.85^{a,b,c}	39.28 ± 0.23^{c}	7529.81
		LAC	6881.76 ± 110.78^{b}	82.85 ± 0.14^{a,b,c}	48.27 ± 1.50^{a}	7012.88
		FLS	4800.82 ± 68.11^{f,g}	88.24 ± 3.06^{a,b,c}	38.21 ± 0.05^{c,d,e,f}	4927.26
T48	BLC1	MRS	6527.29 ± 385.84^{a,b}	74.39 ± 5.56^{b,c,d,e}	42.25 ± 2.37^{b}	6643.93
		FOS	6551.34 ± 170.39^{a,b}	64.69 ± 2.00^{d,e}	38.80 ± 1.58^{c}	6654.83
		LAC	5877.72 ± 22.24^{c,d,e}	81.84 ± 11.56^{a,b}	45.23 ± 0.30^{a}	6004.79
		FLS	6131.02 ± 236.65^{b,c,d}	68.94 ± 2.26^{c,d,e}	39.12 ± 1.56^{c}	6239.09
	LC01	MRS	4965.99 ± 82.19^{f,g}	75.09 ± 12.70^{b,c,d,e}	38.59 ± 0.27^{c}	5079.67
		FOS	6476.83 ± 569.28^{a,b,d}	87.71 ± 5.65^{a}	39.06 ± 0.24^{c}	6603.60

Different letters in the same column, for each time interval, means statistical difference. [1]BLC1: *Bifidobacterium lactis*; LC01: *Lactobacillus casei;* LA5: *Lactobacillus acidophilus*. MRS: de Man-Rogosa-Sharpe broth; FOS: Orafti® Oligofructose – P95; LAC: lactose; FLS: Fruta-do-lobo starch.

Supplementary material 2. Short-chain fatty acids concentrations (mg/L) produced by different probiotic strains (BLC1, LC01 and LA5)[1] and different carbon sources (MRS, FOS, LAC and FLS)[2]. Continuação…

Time	Strain	Carbon Source (2 %)	Acetic acid (mg/L)	Propionic acid (mg/L)	Butyric acid (mg/L)	Total SCFA (mg/L)
	LA5	LAC	5477.42 ± 649.78^{e,f}	76.88 ± 4.29^{a,b,c,d}	46.08 ± 1.87^{a}	5600.38
		FLS	4360.03 ± 52.70^{g}	75.89 ± 4.51^{a,b,c,d}	38.00 ± 0.11^{c}	4473.93
		MRS	5940.46 ± 3.04^{b,c,d,e}	62.93 ± 6.35^{e}	38.76 ± 0.21^{c}	6042.15
		FOS	6800.11 ± 58.94^{a}	77.76 ± 2.23^{a,b,c}	38.44 ± 0.11^{c}	6916.31
		LAC	6302.62 ± 242.44^{a,b,c,d}	74.43 ± 0.04^{b,c,d,e}	46.31 ± 1.41^{a}	6423.35
		FLS	5696.99 ± 350.13^{c,e}	68.08 ± 0.66^{c,d,e}	38.86 ± 0.42^{c}	5803.93

Different letters in the same column, for each time interval, means statistical difference. [1]BLC1: *Bifidobacterium lactis*; LC01: *Lactobacillus casei;* LA5: *Lactobacillus acidophilus*. MRS: de Man-Rogosa-Sharpe broth; FOS: Orafti® Oligofructose – P95; LAC: lactose; FLS: Fruta-do-lobo starch.

It is known that two short-chain fatty acid receptors FFA2 (acetate and propionate) and FFA3 (butyrate) have a direct effect on insulin secretion and β cell proliferation. These receptors were found in pancreatic islets (Tang et al., 2015). FFA3 modulate glucose-stimulated insulin secretion, and FFA2 contributes to the regulation of β cell mass (Priyadarshini et al., 2016). According to Tang et al. (2015), under diabetic conditions, elevated acetate acts on FFA2 and FFA3 to inhibit proper glucose-stimulated insulin secretion and acetate is more likely to regulate insulin secretion through these receptors because it reaches sufficiently high plasma concentrations (Tang et al., 2015. These data corroborate our results because acid acetic reached concentrations up to 170 times higher than the other SCFA (Supplementary material).

These data suggest a potential prebiotic activity of FLS. However, a resistant starch, to be fully identified as prebiotic must meet the following criteria: 1) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, 2) able to be fermented and be utilized by gut microbiota and 3) selectively stimulate activity and/or growth of one or a limited number of gut bacteria that contribute to host health and well being (Zaman & Sarbini, 2015).

Although there is scientific evidence that SCFA modifies the microbiota and consequently benefit diabetic patients, such as glycemic reduction, and FLS are promoting the SCFA production further studies are needed to complete evaluated these benefits.

4. Conclusion

Our data indicate that FLS can increase the levels of acetic propionic, and butyric acid via stimulating their production by probiotic strains and, thus, may promoting blood glucose lowering, as already observed in studies in animal models. Besides, FLS promotes the growth of healthy bacteria and reduces intestinal pH due to the production of AGCC. These data help elucidate the mechanisms of action by which the fruta-do-lobo starch acts.

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Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Publication

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CAPÍTULO III

Ripening behavior in fruta-do-lobo (*Solanum lycocarpum* St. Hill): carbohydrate, phenolic and alkaloid profiles

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Abstract

Fruta-do-lobo (*Solanum lycocarpum* - Solanaceae) is used in folk medicine as an anti-inflammatory and hypoglycemic agent due to their alkaloids, phenolic

compounds and resistant starch. This study evaluated the biochemical changes on carbohydrates, phenolic and alkaloids compounds during ripening of this climacteric fruit. It was observed an increase in glucose, fructose and sucrose ($p<0.05$), while oligosaccharides levels, mainly G3, GF3 and G4 varied during this period. Regarding to antioxidant compounds, there was a predominance of chlorogenic acid in all parts of the green fruit. However, throughout the ripening process, this compound reduces its levels and predominate the p-coumaroylquinic acid for peel and pulp and 1-O-sinapoyl-glucoside for seeds. In alkaloids was observed a relative reduction in solamargine along with the ripening and an increase of solasonine and robeneoside b. These data provide support for future research aimed at exploring the application of these compounds with respect to their biological activity.

Keywords: *Solanum lycocarpum*; Brazilian native fruit; Brazilian Cerrado; bioactive compounds; oligosaccharides; antioxidant capacity; phenolic compounds

Chemical compounds reported in this article

Caffeoylputrescine (PubChem CID: 5280559)

3-O-Caffeoylquinic acid (Chlorogenic acid) (PubChem CID: 1794427)

Caffeoyl-O-glucoside (PubChem CID: 5281761)

Dihydrocaffeoyl-O-glucoside (PubChem CID 15459188)

5-O-Caffeoylquinic acid (Chlorogenic acid) (PubChem CID: 5280633)

p-Coumaroyl-O-glucoside (PubChem CID 14158117)

O-Caffeoylquinic acid (Chlorogenic acid) (PubChem CID 1794427)

1-O-Feruloylglucose (PubChem CID 13962927)

p-Coumaroylquinic acid (PubChem CID 6441280)

p-Coumaroyl-O-frutofuranosylglucose

Robeneoside B (PubChem CID 16109798)

Rutin (PubChem CID 5280805)

Solamargine (PubChem CID 73611)

Solasonine (PubChem CID 119247)

Di-O-caffeoylquinic acid (PubChem CID 6474310)

Tri-O-caffeoylquinic acid (PubChem CID 6440783)

1-O-Sinapoyl-glucoside (PubChem CID 5280406)

1. Introduction

Fruta-do-lobo (*Solanum lycocarpum* St. Hill) is a native and climacteric fruit commonly found in the Brazilian Cerrado. The most of studies on fruta-do-lobo are focused on the biological activities of their alkaloids, solasonine and solamargine. Many studies have been demonstrated in vitro and in vivo activity of their alkaloids on treatment of skin and bladder cancer cells, as well as in leishmaniasis treatment (Tiossi et al., 2014; Carvalho et al., 2019; Clementino et al., 2018, respectively). Their starch has also been employed in popular medicine to treat diabetes and decrease cholesterol levels (Farina et al., 2010; Rocha et al., 2012).

Recently, studies have shown a more comprehensive characterization of its constituents, for instance, Pereira and coworkers (Pereira et al., 2018) report the importance of fruta-do-lobo phenolic compounds for their antioxidant capacity. During ripeness, several sensorial changes take place, such as flavor, color and flesh firmness, which make the fruit acceptable for consumption (Choo, 2019). Nonetheless ripeness occurs followed by chemical composition alteration. Some of these changes can be observed by visual analysis, such as physical transformations or by chemical endogenous transformations, such as pigment, organic acids, phenolic compounds and carbohydrates alteration (Chitarra & Chitarra, 2005). All these transformations happen in a synchronized way and are under genetic and environment control.

One of the most well-known processes that occurs during fruit ripeness is the starch hydrolysis, which increase levels of soluble sugars, such as glucose, fructose and sucrose (Mesa et al., 2016). Consequently, specific flavors are developed in conjunction with sweetness increases, and reduction of acidity and astringency.

Despite soluble sugars have their ripeness metabolism well defined, oligosaccharides alteration are still poor understood. Oligosaccharides are present in foods and has been highlighted due to their health benefits, they are consisted of 3–10 sugar residues linked by glycosidic bonds, which can be hydrolysed to their monosaccharide units by acids or specific enzymes. Additionally, the oligosaccharides may be obtained by controlled hydrolysis of carbohydrates with higher degree of polymerization (e.g. starch) or may be synthesized from simple sugars (e.g., sucrose) by transglycosylation reactions (Sancho et al., 2017). As examples of oligosaccharides, we can include malto-oligosaccharides (MOS) and fructo-oligosaccharides (FOS), which can be classified as digestible and non-digestible, according to their physiological properties. While MOS may be found in tubers, roots, and bulbs, FOS occur naturally in vegetables such as yacon, Jerusalem artichoke and chicory, and in fruits such as banana, apple and pear and are not hydrolysed by the human digestive enzymes.

Therefore, the aim of this work is to elucidate the chemical and biochemical transformations behavior for these components (carbohydrates, phenolic compounds, and alkaloids) during the ripeness of fruta-do-lobo, bringing information about the crucial harvest time to improve the yield for those compounds.

2. Materials and Methods

2.1. Obtaining and selection of fruits

Fruta-do-lobo was randomly and manually collected in August 2017 in Carmo do Rio Claro (S20.555.209; W46.145.379), located in Minas Gerais. The fruits (n=60) were selected considering the ripeness stage, size, color of the peel (intense green), stalk fully trapped and structural integrity (absence of injuries). The fruits evaluated had approximately 553.46 ± 84.15 g. The specimen was identified by Dr Ingrid Koch and a voucher was deposited in the UNICAMP herbarium (UEC 197248).

2.2. Monitoring the fruits ripeness

The monitoring ripeness of fruits was performed according Oliveira Junior, Santos, Abreu, Corrêa and Santos (2004), with modifications. The fruits were stored

in Biochemical Oxygen Demand (BOD) incubator (25 °C) for 12 day. Every two days, 10 fruits were selected, washed and processed according to the respective analysis.

2.2.1. Respiration rate

The respiration rate was performed according to Yang, Cao, Su and Jiang (2014). Whole fruits were held in gas-tight jars at 25°C for 30 minutes to gas sampling. CO2 and O2 were measured using a gas analyzer (Mocon, EUA). The respiration rate was calculated according to the Equation 1:

$$P_{CO2} = \frac{\%\mathrm{CO_2}}{100} * \frac{\mathrm{V}_{empty}\,[ml]}{m_{product\,[Kg]}} * \frac{1{,}98\ [\mathrm{mg/mL}]}{\mathrm{t\ [h]}} \quad \text{Eq. 1}$$

where %CO_2 = production of CO_2 by product in %; Vempty = headspace in mL; Consider the difference between the percentage of the total volume that the fruit occupied. mproduct = product mass in the container, in kg; 1,98 [mg/mL] = converter from mLCO_2 to mgCO_2, in the normal temperature and pressure (NTP); t [h] = time the containers remained closed. The results were expressed in mL CO_2/kg.h.

2.2.2. Physicochemical analysis

The fruits were peeled, and the seeds were removed. Total soluble solids (TSS), pH values and titratable total acidity (TTA) were determined in pulp in triplicate according to official methods (Association of Official Analytical Chemists, 2006) and the 016/IV methods (Instituto Adolfo Lutz, 2008), respectively.

2.2.3 Firmness

Firmness was measure according to Bentes and Mercadante (2014), with modifications. Were used a TA-XT Plus texturometer (Stable Micro Systems Ltd., Surrey, UK) equipped with a P/2N needle probe, following the post-test speed, 5 mm/s and penetration depth, 10 mm. The results were expressed in newtons (N).

2.2.4. Instrumental color

Instrumental color was measured in a CR-400 Chroma Meter (Konica Minolta) using the CIE L (brightness ranging from zero (black) to 100 (white)) a* (+ a* indicating tendency for red and -a* tendency for green) b* (+ b* indicating tendency for yellow and -b* tendency for blue) system. It was calibrated in the reflectance mode, using the illuminant D65 and an observation angle of 0°. The peel color was determined at four equidistant points around the equatorial region and the pulp at three points.

2.3. Sample preparation

The fruits were peeled and chopped, and the fractions of the peel, pulp and seeds were separated. These fractions were freeze-dried (Liotop, Liobras, BR) for 48 hours, then were ground in an industrial blender for 1 minute. All samples were stored in a hermetic container, protected from light at - 18 °C until analysis.

2.4. Changes in carbohydrate profile during the ripeness

This analysis was performed according Pereira et al. (2018). Briefly, homogenized freeze-dried fruta-do-lobo pulp with deionized water (10 mg/mL) were centrifuged. Then the supernatant was filter before analysis, which was carried out by high performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). Standards of glucose, fructose, sucrose, FOS (GF2 to GF4) and MOS (G3 to G7) were used for identification and quantification by standard curve calibration. All analyses were performed in triplicate.

2.5. Phenolic compounds profile

The phenolic compounds profile was performed according to Pereira et al. (2018).

2.5.1. Extract preparation

Each freeze-dried fraction of the fruit was separately homogenized (Ultra-Turrax T-25, Germany) with ethanol:water (70:30/v:v) for 2 min. After that, it was homogenized under constant stirring for 2 h at room temperature. The extract was centrifuged (5 °C, 15 min, 10,000 RPM) and concentrated under vacuum until completely dry. The extracts were re-suspended in water until the 50 mg/mL initial concentration.

2.5.2. Phenolic and alkaloid compounds profile

The analysis was performed using an Ultrahigh-Performance Liquid Chromatography (UHPLC) (Hewlett Packard, Agilent Technologies 1290 series) coupled to a Q-ToF iFunnel 6550 mass spectrometer using an electrospray ionization (ESI) source with a Poroshell 120 SB-Aq 2.7 µm column (2.1 × 100 mm, Agilent). Mobile phase A was water, with formic acid 0.1 %; and mobile phase B was acetonitrile with formic acid 0.1 %. The sample were eluted with a flow rate of 0.45

mL/min, using the following gradient: 0-10min, 5 % to 18 % of B; 10-15 min; 18 % to 100 % of B and 15-17 min hold in 100 % B. Sample were analysed in negative ions mode for phenolic compounds and in positive ion mode for alkaloids. The mass spectrometer parameters used were: VCap 3000 V; fragmentor voltage at 100 V; OCT 1RF Vpp at 750 V; Gas Temperature at 290 ºC; Sheath Gas Temperature at 350 ºC; Drying Gas at 12 Lmin-1. Mass spectra were acquired in profile mode and the acquisition range was 50-1200 m/z.

2.6. Antioxidant capacity

The antioxidant capacity of the samples was performed by ORAC assay according to Ou Hampsch-Woodill and Prior (2001), adapted by Dávalos, Gómez-Cordovés and Bartolomé (2004), using a microplate reader (NOVOstar, BMG Labtech®, Offenburg, Germany) with the MARS Data Analysis Software version 1.3 (BMG Labtech®, Offenburg, Germany). Samples and Trolox standards were prepared with 75 mM phosphate buffer (pH 7.4). ORAC values were expressed as µM Trolox Equivalents (TE)/100 mL of extract.

2.7. Statistical analyses

Statistical analyses were conducted using Statistica (StatSoft). The results were subjected to a one-way ANOVA and differences between means were located using Tukey's multiple comparison test. For TSS and TTA was used Regression analysis. The Pearson correlation analysis was performed to evaluate associations between mono- and disaccharides and TSS. Significance was determined at $p < 0.05$.

3. Results and discussion

3.1 Respiration rate

Ripening of fruits are responsible for the flavor of fruits such as sweetness or sourness and textural changes (Choo, 2019). Respiration is one of the main factors related to this process. The stored sugar is converted into energy in the presence of an oxygen substrate, thus leading to senescence (Yang et al., 2014). An increase in the respiration rate of the fruta-do-lobo was observed during the period evaluated (Figure 1). Based on respiration, fruits can be grouped as climacteric and non-climacteric. Ripening in climacteric fruits manifests itself by a rise in respiration, which is responsible for auto-catalytic ethylene production (Pech et al., 2012). According to

Yang et al. (2014), the fruit respiration process involves a series of oxidation-reduction reactions, mediated by respiratory enzymes located on the internal mitochondrial membrane in plant cells. For example, succinic dehydrogenase catalyzes the oxidation of succinate to fumarate, whereas cytochrome C oxidase catalyzes the transfer of electrons from ferrocytochrome C to molecular oxygen (Soto, Fontanesi, Liu, & Barrientos, 2012).

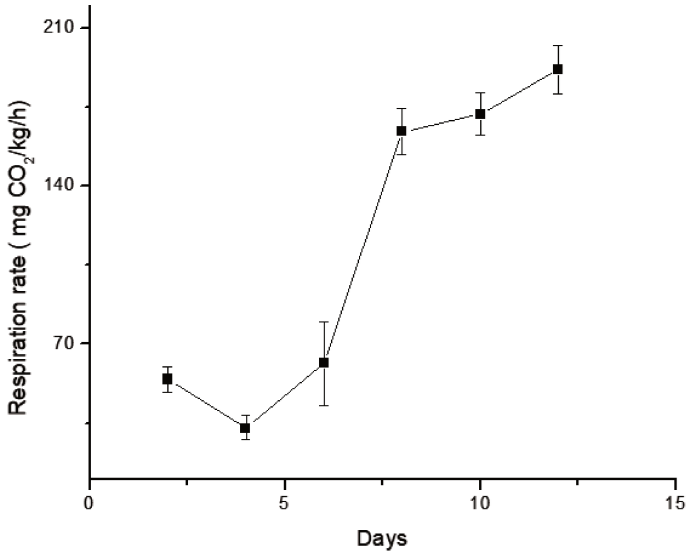


Figure 1: Respiration rate during the ripeness of fruta-do-lobo (*Solanum lycocarpum* St. Hill). Values are the means ± SD of triplicate assays.

3.2 Physicochemical analysis

The ripening of the fruit was also evaluated by physical and chemical changes (pH, TTA, TSS, firmness and instrumental color) and the results are shown in Table 1 and 2. The TTA increased significantly ($p < 0.05$) during ripening and values ranged from 0.18 to 0.82 % citric acid/100 g of pulp, however practically no change was observed in pH. Corrêa, Abreu, Santos and Ribeiro (2000) also followed the fruta-do-lobo physicochemical parameters variations and they did not observe significant alterations in pH and TTA values despite the TTA increases fruta-do-lobo are not an acidic fruit.

Table 1. Changes in pH, TTA, TSS and firmness during the ripening of fruta-do-lobo (*Solanum lycocarpum* St. Hill)[1]

Day	2	4	6	8	10	12
pH	5.23±0.03^{a,b}	5.07±0.02^{a,b}	5.36±0.08^{a}	5.18±0.12^{a,b}	4.87±0.04^{b}	5.05±0.32^{a,b}
TTA[2]	0.20±0.02^{c}	0.28±0.02^{c}	0.18±0.02^{c}	0.58±0.02^{b}	0.80±0.00^{a,b}	0.82±0.27^{a}
TSS[3]	2.50±0.00^{e}	5.00±0.00^{d}	8.33±1.44^{c}	12.50±0.00^{b}	17.50±0.00^{a}	17.50±0.00^{a}
Firmness	155.08±4.45^{a}	141.83±8.68^{a,b}	127.35±14.63^{b}	106.67±18.66^{c}	71.08±13.18^{d}	62.30±13.18^{d}

[1]Values expressed as mean ± SD (n = 3). Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$). [2] TTA: Total titratable acidity are expressed in % citric acid/100 g of pulp. [3] TSS: Total soluble solid.

There was a linear increase in TSS content until day 10 of ripening ($p<0.05$) (Table 1). These increases is expected, and it is according to the results of Oliveira Junior, Santos, Abreu, Corrêa and Santos (2004). They showed that the starch reduction occurred simultaneously with the amylase activity reduction and soluble solids content increase.

Corroborating with the increase of TSS, also, there was a linear reduction in the firmness of the fruit during maturation (Table 1). This is one of the indicators of fruit senescence (Lurie & Crisosto, 2005). The textural changes in fruit during ripening are mainly associated with the dissolution of the middle lamella and the depolymerization of pectins and the loss of sugars from their side chains. These changes are mediated by the action of a broad variety of cell wall degrading enzymes or proteins secreted from the symplast into the cell wall space, generally encoded by ripening-related genes (Mercado, Matas, & Posé, 2018).

Color intensity is associated with the ripening and understanding the fruit pigments changes during this period is important for harvesting these fruits at their correct stage of maturity. This is crucial to fruit market, for processing or for the functional food market where consumers' perceptions of health or nutritional effects of the fruits are critical (Choo, 2019).

Table 2. Changes in instrumental color during the ripeness of fruta-do-lobo (*Solanum lycocarpum* St. Hill)[1]

Peel					
Day	L*	a*	b*	C*	h*
2	39.30 ± 1.19^{c}	-10.80 ± 1.01^{a}	17.56 ± 2.15^{c}	20.60 ± 2.22^{d}	121.52 ± 2.48$^{n.s.}$
4	46.70 ± 1.79^{a}	-14.92 ± 1.16^{b}	26.76 ± 1.74^{a}	30.57 ± 2.05^{a}	118.74 ± 1.75$^{n.s.}$
6	41.24 ± 0.53^{b,c}	-12.24 ± 1.21^{a,b}	20.13 ± 1.11^{b,c}	23.61 ± 1.61^{c,d}	121.39 ± 1.33$^{n.s.}$
8	43.61 ± 1.06^{a,b}	-11.19 ± 1.14^{a}	23.53 ± 0.94^{a,b}	26.13 ± 0.58^{b,c}	115.31 ± 3.15$^{n.s.}$
10	44.29 ± 1.01^{a,b}	-11.25 ± 2.55^{a}	23.89 ± 0.99^{a,b}	26.49 ± 1.46^{a,b,c}	115.09 ± 5.11$^{n.s.}$
12	45.23 ± 3.17^{a}	-13.06 ± 1.24^{a,b}	25.45 ± 2.85^{a}	28.63 ± 2.98^{a,b}	117.27 ± 1.70$^{n.s.}$
Pulp					
Day	L*	a*	b*	C*	h*
2	77.97 ± 2.96^{b,c}	-3.98 ± 3.33$^{n.s}$	27.99 ± 0.83^{b,c}	28.42 ± 1.20^{b,c,d}	97.91 ± 6.37$^{n.s}$
4	82.87 ± 0.47^{a}	-3.50 ± 0.37$^{n.s}$	18.55 ± 3.31^{c}	19.0 ± 2.95^{d}	98.10 ± 2.24$^{n.s}$
6	80.98 ± 1.34^{a,b}	-3.68 ± 1.74$^{n.s}$	26.93 ± 3.54^{b,c}	26.22 ± 2.05^{c,d}	96.71 ± 1.91$^{n.s}$
8	76.29 ± 1.09^{c}	-5.26 ± 0.48$^{n.s}$	37.51 ± 4.25^{a,b}	37.90 ± 4.26^{a,b}	98.12 ± 0.40$^{n.s}$
10	74.54 ± 0.53^{c}	-5.51 ± 0.41$^{n.s}$	41.86 ± 1.55^{a}	42.16 ± 1.54^{a}	97.35 ± 0.29$^{n.s}$
12	75.22 ± 1.06^{c}	-4.61 ± 0.69$^{n.s}$	34.76 ± 8.11^{a,b}	35.08 ± 8.13^{a,b,c}	97.67 ± 0.53$^{n.s}$

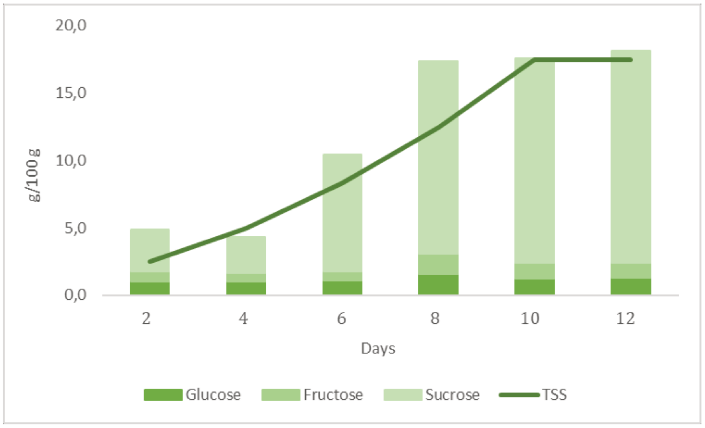
[1]Values expressed as mean ± SD (n = 4 for peel and n = 3 for pulp). Values in the same column followed by different letters are significantly different by ANOVA test ($p < 0.05$).

During the ripeness, changes occurred mainly on the lightness (L) and coordinate b* (Table 2). This result shows that despite the green color persistence the maturation process turns it more yellowish (Supplementary material 1), which is related to the decline in chlorophyll concentration (Chitarra & Chitarra, 2005). The structural degradation of chlorophyll happens due to several factors such as pH change (by release of organic acids) and oxidative systems with phytol or Mg2+ loss (Charoenchongsuk, Ikeda, Itai, Oikawa, & Murayama, 2015). For pulp, the values of luminosity and the coordinate a* decreased, while the values of coordinate b* increased (Table 2), characterizing a yellow-green color (Supplementary material). These structural changes on chlorophyll are also related to endogenous ethylene released from fruit. A chroma (C*) differentiation was observed from day 8. Chroma represented the purity or intensity of the color and its increase indicated that both peel and pulp developed more intense staining with ripeness. The hue angle (h*) combines both a* and b* values and shows that for peel there was a tendency to change the tonality to lighter green, whereas for the pulp the hue values were not significant.

Supplementary material 1. Changes in peel and pulp color of fruta-do-lobo (*Solanum lycocarpum* St. Hill) during the ripeness. A-B: unripe; C-D: totally ripe.

3.3. Carbohydrates profiles

The main sugars present in the fruits are glucose, fructose and sucrose, in different proportions according to the species or cultivars. The sweetening power of each sugar is different, so it is important the individual determination to better characterize the flavor. We monitor changes in glucose, fructose and sucrose levels during maturation, as well as in oligosaccharides and the results are shown in Table 3. During ripeness the soluble sugar concentration increase. These occurs due action of hydrolytic enzymes, such as amylases, which degrade starch. The increases in soluble sugars (glucose, fructose and sucrose) are directly related to TSS, as can be evidenced by Pearson's correlation (0.95, $p<0.05$), i.e. as mono and disaccharide increases also TSS increase (Supplementary material 2).



Supplementary material 2 – Changes in mono- and disaccharides (g/100 g) and TSS (%) levels during the ripeness of fruta-do-lobo (*Solanum lycocarpum* St. Hill) ($p<0.05$)

In most fruits, sucrose is the main translocation sugar that transport itself from leaves to the fruit and its concentration increase, while the concentration of reducing sugars decrease after day 8. The same tendency was also observed for *Solanum peruvianum* and *Solanum habrochaites* and this increase may be at least

partially, related to the activities of soluble invertase and sucrose synthase enzymes (Kortstee, Appeldoorn, Oortwijn & Visser, 2007).

In the growth phase, sugars are translocated to the fruit and used partly for the synthesis of starch (energy reserve material), and partly for the synthesis of pectic substances and other cell wall components (structure substances) (Chitarra & Chitarra, 2005). These polysaccharides are formed by the monosaccharides condensation or by their oligosaccharide derivatives, which bond through glycosidic linkages giving rise to compounds of higher molecular mass. During the ripening, an enzymatic depolymerization of polysaccharides occurs, resulting in smaller fragments composed of two to thirteen monosaccharide units, called oligosaccharides (Giese et al., 2011). Until now, there is few information in literature regarding to the oligosaccharide in fruits, which are even more scarce when we considering the maturation process.

The mainly oligosaccharides found in ripe fruta-do-lobo were G3, followed by GF3, GF2 and G4. In our previous study, we found only GF2 and GF3 in fruta-do-lobo ripe (Pereira et al., 2018). It can be observed a decrease ($p<0.05$) in the GF3, G3 and G4 contents with the ripening (Table 3). GF4 and other MOS were found only in a few days and in small amounts, which can be explained by the continuous depolymerization process that occurs during this period.

Differently, GF2 concentrations increase during ripening, reaching the maximum value on day 8 ($p<0.05$). This pattern was reported for L'homme, Peschet, Puigserver, and Biagini (2001) in banana, pear Williams and pear Guyot, respectively, which observed that the levels of fructans increase during maturation, mainly GF2. Later, Agopian, Soares, Purgatto, Cordenunsiand and Lajolo (2008), confirmed this trend in a study with different cultivar of bananas. FOS can be obtained from the transfructosylation of sucrose by the action of β-fructosylfuranosidases (Passos & Park, 2003). It is already known that sucrose is the main substrate for FOS biosynthesis acting both as a signal triggering to the GF2 synthesis and as a substrate to the fructan's synthesis (Agopian, Soares, Purgatto, Cordenunsiand & Lajolo, 2008).

Table 3. Changes in mono-, di- and oligosaccharide levels during the ripeness of fruta-do-lobo (*Solanum lycocarpum* St. Hill)[1]

Carbohydrate		Day					
		2	4	6	8	10	12
Monosaccharide (g/100g)	Glucose	1.00±0.01^{b}	1.05±0.09^{b}	1.11±0.09^{b}	1.56±0.14^{a}	1.26±0.04^{a,b}	1.30±0.06^{a,b}
	Fructose	0.80±0.01^{c}	0.58±0.06^{d}	0.65±0.04^{c,d}	1.51±0.09^{a}	1.13±0.04^{b}	1.11±0.06^{b}
Disaccharide (g/100g)	Sucrose	3.08±0.06^{c}	2.72±0.22^{c}	8.68±0.70^{b}	14.31±0.51^{a}	15.21±1.20^{a}	15.74±0.04^{a}
Oligosaccharide[2] (mg/100g)	GF2	1.56±0.09^{d,e}	1.24±0.11^{e}	2.48±0.04^{c}	14.30±0.26^{a}	6.85±0.23^{b}	2.07±0.12^{c,d}
	GF3	12.13±3.71^{a}	0.05±0.07^{b}	6.45±0.16^{a,b}	5.11±2.06^{b}	3.97±1.13^{b}	4.60±0.45^{b}
	GF4	0.30±0.13	n.d.	0.85±0.04	n.d.	n.d.	n.d.
	G3	6.75±0.13^{a,b,c}	7.76±0.49^{a}	7.34±0.21^{a,b}	6.39±0.27^{b,c}	6.43±0.28^{b,c}	5.89±0.32^{c}
	G4	4.11±0.06^{a}	2.83±0.12^{b}	4.37±0.24^{a}	2.93±0.09^{b}	2.66±0.10^{b}	2.98±0.14^{b}
	G5	0.32±0.02	n.d.	0.12±0.01	n.d.	n.d.	n.d.
	G6	2.73±0.03	1.09±0.08	1.18±0.04	n.d.	n.d.	n.d.
	G7	3.34±0.06	1.69±0.10	1.12±0.05	0.01±0.01	n.d.	n.d.

[1] Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$). [2]GF2: 1-kestose, GF3: nystose, GF4: fructofuranosylnystose, G3: malttriose, G4: maltotetraose, G5: maltopentaose, G6: maltohexaose, G7: maltoheptaose.

In our study, we observed a concomitant increase of sucrose and GF2 until day 8, which can suggest that GF2 production and sucrose concentration may be related. After this period, GF2 levels decrease, while sucrose increase.

Differently, GF2 concentrations increase during ripening, reaching the maximum value on day 8 ($p<0.05$). This pattern was reported for L'homme, Peschet, Puigserver, and Biagini (2001) in banana, pear Williams and pear Guyot, respectively, which observed that the levels of fructans increase during maturation, mainly GF2. Later, Agopian, Soares, Purgatto, Cordenunsiand and Lajolo (2008), confirmed this trend in a study with different cultivar of bananas. FOS can be obtained from the transfructosylation of sucrose by the action of β-fructosylfuranosidases (Passos & Park, 2003). It is already known that sucrose is the main substrate for FOS biosynthesis acting both as a signal triggering to the GF2 synthesis and as a substrate to the fructan's synthesis (Agopian, Soares, Purgatto, Cordenunsiand & Lajolo, 2008). In our study, we observed a concomitant increase of sucrose and GF2 until day 8, which can suggest that GF2 production and sucrose concentration may be related. After this period, GF2 levels decrease, while sucrose increase.

3.4. Changes in phenolic and alkaloid profiles

As shown in Table 4, 21 phenolic compounds were tentatively identified considering their chromatographic and spectrometric characteristics and two compounds were not identified. Phenolic acids detected in fruta-do-lobo are mainly chlorogenic acids. In general, we noticed a large predominance of 5-O-caffeoylquinic acid in all parts of the unripe fruit, corresponding about 80% of all the phenolics detected. Our results corroborate with Clifford (1999). According to these authors, this acid, formed from the esterification of one or more derivatives of the transcinnamic acid with the quinic acid, was the main acid found in fruits, regardless of the part analyzed. With ripening, its relative abundance decreases, but remains as the main phenolic compound present in the seed along with di-o-caffeoylquinic acid. p-Coumaroylquinic acid became predominant for the pulp, while in the peel the formation of two unknown compounds was observed. We also observed an increase in the relative abundance of chlorogenic acid dimers and trimers. This behavior has already been reported in leaves of plants of the same family, which showed that chlorogenic acid may be linked to a greater initial protection of vegetal material against sunlight and to herbivory. During fruits ripening these compounds are being relocated in the plant, as they

Table 4. Mass spectral data and tentative identification of phenolic compounds in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) unripe and ripe[1].

Phenolic compounds		mz/rt	MS/MS	Peel		Pulp		Seed	
				Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
Phenolic compounds	Caffeoylputrescine		-	0.02 ± 0.16^{b}	0.65 ± 0.16^{a}	-	-	-	-
	3-O-Caffeoylquinic acid (Chlorogenic acid)	354.0987/2.38	354/191/179/135	0.22 ± 0.34^{c}	2.70 ± 0.34^{b}	0.21 ± 0.00^{c}	3.33 ± 2.70^{a}	0,19 ± 0.07^{c}	2.13 ± 0.67^{b}
	Caffeoyl-O-glucoside	341.0870/2.83	179/161/133	0.14 ± 0.87^{c}	6.32 ± 0.87^{a}	0.35 ± 0.02^{c}	5.45 ± 4.43^{a,b}	0.12 ± 0.05^{c}	1.21 ± 0.26^{b,c}
	Caffeoyl-O-glucoside isomer	341.0875/3.2	282/221/177	0.02 ± 0.00^{b}	2.21 ± 0.27^{a}	0.03 ± 0.00^{b}	2.07 ± 0.02^{a}	-	-
	Dihydrocaffeoyl-O-glucoside	343.1035/3.5	343/181/137	0.13 ± 0.06^{b}	5.32 ± 1.16^{a}	0.01 ± 0.00^{b}	0.58 ± 0.05^{b}	0.01 ± 0.00^{b}	1.21 ± 1.20^{a}
	Dihydrocaffeoyl-O-glucoside	343.1031/3.8	181/135	0.32 ± 0.17^{b}	1.99 ± 0.22^{a}	-	-	0.03 ± 0.00^{c}	0.74 ± 0.35^{b}
	Caffeoyl-O-glucoside	341.0873/3.9	-	0.02 ± 0.00^{b}	2.09 ± 0.19^{a}	0.04 ± 0.02^{b}	2.11 ± 0.17^{a}	-	-
	5-O-Caffeoylquinic acid (Chlorogenic acid)	353.0873/4.0	353/191	76.97 ± 1.20^{a}	10.20 ± 1.20^{b}	79.34 ± 0.22^{a}	16.80 ± 5.08^{b}	52.82 ± 1.52^{a,b}	9.54 ± 2.74^{b}
	Caffeoyl-O-glucoside	341.0875/4.1	-	0.14 ± 0.05$^{n.s}$	0.46 ± 0.08$^{n.s}$	-	-	-	-

[1] Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

Table 4. Mass spectral data and tentative identification of phenolic compounds in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) unripe and ripe[1]. Continuação…

Phenolic compounds	mz/rt	MS/MS	Peel		Pulp		Seed	
			Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
p-Coumaroyl-O-glucoside	325.0928/4.2	325/279/164/145/119	0.82 ± 0.40^{b}	3.78 ± 0.76^{a}	0.10 ± 0.01^{b}	1.66 ± 0.07^{b}	0.04 ± 0.00^{b}	0.28 ± 0.36^{b}
O-Caffeoylquinic acid (Chlorogenic acid)	353.0870	353/191/192/173/135	-	0.43 ± 0.07$^{n.s.}$	0.89 ± 0.05$^{n.s.}$	1.21 ± 0.28$^{n.s}$	1.22 ± 0.04$^{n.s}$	1.64 ± 0.71$^{n.s}$
1-O-Feruloylglucose	355.1021/5.12	-	0.07 ± 0.03^{b}	0.51 ± 0.05^{a}	0.08 ± 0.01$^{a.b}$	0.49 ± 0.01^{a,b}	0.07 ± 0.00^{b}	0.35 ± 0.24^{a,b}
O-Caffeoylquinic acid (Chlorogenic acid)	353.0873/5.5	353/191	1.81 ± 0.18^{a}	1.17 ± 0.16^{b}	0.61 ± 0.12^{a}	0.78 ± 0.01^{b}	1.09 ± 0.60^{a}	1.13 ± 0.35^{b}
p-Coumaroylquinic acid	337.0924/5.6	337/191	0.12 ± 0.07^{c}	0.63 ± 0.12^{c}	14.11 ± 0.19^{a,b}	25.94 ± 0.93^{a}	8.87 ± 0.43^{b}	17.39 ± 6.57^{a,b}
p-Coumaroyl-O-frutofuranosylglucose	487.1451/5.3	487/307/163/145	-	4.94 ± 0.62^{b}	0.01 ± 0.00^{c}	9.10 ± 0.40^{a}	-	1.64 ± 0.74^{b}
Rutin	609.1430/9.0	609/302/301	2.56 ± 0.89$^{n.s.}$	2.82 ± 0.21$^{n.s.}$	-	-	-	-
Di-O-Caffeoylquinic acid	515.1195/10.4	515/353/191/179	0.06 ± 0.03^{b}	3.63 ± 0.77^{a}	0.03 ± 0.00^{c}	2.07 ± 0.41^{a}	1.17 ± 0.25^{b}	2.02 ± 1.66^{a,b}

[1] Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

Table 4. Mass spectral data and tentative identification of phenolic compounds in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) unripe and ripe[1]. Continuação…

Phenolic compounds		mz/rt	MS/MS	Peel		Pulp		Seed	
				Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
	Di-O-Caffeoylquinic acid	515.1195/10.6	515/353/191/179/135	0.06 ± 0.03^{c}	3.63 ± 0.68^{a,b}	0.72 ± 0.15^{b,c}	5.79 ± 0.40^{a}	0.97 ± 0.03$^{n.s}$	2.13 ± 1.67^{b}
	Unknown	961.4653/12.0	961/915/753	0.02 ± 0.01^{c}	14.25± 4.01^{a}	2.39 ± 0.06^{a}	10.09 ± 0.14^{a}	0.02 ± 0.00^{c}	6.17 ± 6.44^{b}
	Unknown	961.4651/12.3	-	6.81 ± 1.97^{a}	8.34 ± 1.59^{b}	-	-	2.57 ± 0.27^{a}	9.97 ± 4.78^{b}
	Tri-O-Caffeoylquinic acid	677.1505/12.1	677/515/353/179	0.15 ± 0.11^{c}	1.15 ± 1.43^{b}	0.07 ± 0.01^{c}	4.92 ± 2.92^{a}	0.12 ± 0.03^{c}	4.67 ± 4.81^{a}
	1-O-Sinapoyl-glucoside	385.1134/5.6	-	-	-	0.01 ± 0.00^{b}	1.28 ± 0.10^{a}	-	-
Alkaloids	Solasonine	884.5026/11.83	884/866/848	64.46 ± 0.23^{c}	79.24± 1.19^{a}	64.76 ± 0.25^{c}	84.79 ± 1.51^{a}	63.93 ± 0.61^{c}	80.22± 1.67^{a}
	Robeneoside B	868.5073/11.64	868/850	7.84 ± 0.21^{b}	10.71± 1.27^{a}	7.9 ± 0.04^{b}	11.81 ± 0.36^{a}	6.78 ± 0.80^{b}	10.13± 0.74^{a}
	Solamargine	900.4974/11.89	900/882/864	27.70 ± 0.24^{a}	10.06± 0.74^{b}	27.34± 0.21^{a}	3.39 ± 1.99^{c}	29.29± 0.62^{a}	9.65 ± 2.30^{b}

[1] Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

participate in their lignification process (Mondolot, La Fisca, Buatois, Talansier, De Kochko & Campa, 2006). These migrations causing a decrease in fruit phenolic content. Same behavior was observed by Seraglio et al. 2018 in fruits of the Myrtaceae family, who shows that a reduction of the primary metabolism in the ripe fruit, resulting in a lack of substrates that are essential for the biosynthesis of phenolic compounds.

Glycoalkaloids are commonly found in plants of the *Solanum* genus. During the ripennes, we observe a relative reduction in solamargine along the maturation process and an increase of solasonine and robeneoside B (Table 4). According to Al Sinani and Eltayeb (2017), glycoalkaloids of *Solanum* species are found generally in all plant organs, with the highest concentrations occurring in flowers, sprouts, unripe berries, young leaves or shoots, because are parts more metabolically active.

Glycoalkaloids are formad by an aglycone unit with nitrogen incorporated into F ring bound in a carbohydrate side chain attached to 3-OH position (Milner et al., 2011). While solamargine had a chacotriose sugar residue, both solasonine and robeneoside B had a solatriose sugar linked to saponin skeleton.

Solamargine and solasonine are the most common glycoalkaloids of Solanum genus and were previously identified by Munari et al. (2012). These authors also evaluated the possible cytotoxic, genotoxic and antigenotoxic potentials of these compounds in lung fibroblasts and it was found that there was no genotoxic activity. In addition, the different concentrations of extract showed a protective effect against genomic and chromosomal damages induced by methyl methanesulfonate.

3.5. Antioxidant capacity

The antioxidant capacity of fruta-do-lobo as measured by ORAC assay. The highest ORAC hydrophilic values was observed by seeds for both unripe and ripe stage, which was statistically equal ($p < 0.05$) to the values found for peel and unripe pulp (Table 5). For lipophilic fraction, the highest values were found in the fractions of the unripe fruit, except for seed, that showed higher antioxidant capacity after ripening ($p < 0.05$).

Chlorogenic acid seems to have been responsible for the antioxidant capacity of fruta-do-lobo, representing about 85% of the phenolic compounds of seeds and 82 and 80 % of the compounds found on pulp and peel, respectively. The

antioxidant capacity of chlorogenic acid is due to the presence of an ortho-di-hydroxyl group in the aromatic ring of caffeic acid, which would act as a free radical aceptor (De Maria & Moreira, 2004). Agunloye et al. (2019) demonstrated the antioxidant capacity of chlorogenic acid in the stabilization of antioxidant enzymes like catalase and consequent cellular prevention against oxidative damage, exerting antihypertensive and cardioprotective effect.

Tabela 5: Antioxidante capacity in each fraction (peel, pulp and seed) of fruta-do-lobo (*Solanum lycocarpum* St. Hill) unripe and ripe[1]

		$ORAC_H$	$ORAC_L$	$ORAC_T$
		μmol Trolox equivalent (TE)/mg of fraction[2]		
Peel	Unripe	964.072 ± 1.77[a,b]	769.55 ± 1.69[a]	1131.57
	Ripe	914.27 ± 2.05[a,b]	437.06 ± 0.43[b]	1193.43
Pulp	Unripe	1086.61 ± 6.64[a,b]	796.70 ± 2.62[a]	1883.31
	Ripe	581.56 ± 1.17[b]	459.33 ± 1.11[b]	1040.89
Seed	Unripe	1256.75 ± 4.70[a]	680.68 ± 2.63[a,b]	1937.43
	Ripe	1145.89 ± 0.89[a]	810.75 ± 3.02[a]	1280.25

[1]Values expressed as mean ± SD (n = 3). [2]Results are expressed in dry base. Values in the same column followed by different letters are significantly different by ANOVA test ($p < 0.05$).

Our results show that the unripe fruta-do-lobo presents a higher concentration of phenolic compounds and alkaloids. In addition, starch, which is used to glycemia reduction, is extracted from unripe fruit pulp (Clerici et al., 2011). Therefore, it seems feasible to use the peel and seed of this fruit, allowing a greater use.

4. Conclusion

For the first time was evaluated the changes in carbohydrate, phenolic compounds and alkaloids profile during the ripening of fruta-do-lobo, native from Brazilian Cerrado, besides a comprehensive characterization on the physical and chemical parameters involved in this process.

Regarding the changes in the carbohydrate profile, the glucose, fructose and sucrose contents increased with the ripening of the fruits and it was possible to correlate the total soluble solids with the levels of these sugars. Changes in oligosaccharide levels did not follow a trend. While G3, GF3 and G4 contents decreased during the ripening, GF2 levels seems to be related to sucrose contents.

Further studies are needed to give a better understanding of the behavior of oligosaccharides during the ripening of fruits.

Among phenolic compounds, there was a predominance of chlorogenic acids on unripe fruit, which were correlated with their antioxidant capacity. Finally, about the alkaloids of fruta-do-lobo, there was observed a relative reduction in solamargine with an increase of solasonine and robeneoside B along the ripening process.

This study provides practical information about what is the best stage of ripening to achieve of the bioactive compounds according to the health implications. The elucidation of these mechanisms is of great interest and will contribute to the improvement of agroindustry, as well as to the pharmaceutical and chemical industries, which have an interest in these compounds.

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DISCUSSÃO

O Brasil apresenta seis biomas, com características distintas, o que lhe confere uma biodiversidade riquíssima, entretanto subexplorada. Com o avanço do desmatamento em áreas protegidas, faz-se cada vez mais necessário estudos que demonstram a importância da flora brasileira, buscando garantir sua conservação e exploração de forma sustentável.

Por outro lado, tem sido crescente o aumento das doenças crônicas não-transmissíveis, como o diabetes, que tem como principais fatores fisiopatológicos o extresse oxidativo e a inflamação. Ambos quadros provocam falhas nos principais mecanismos que regulam a glicose no organismo, que são a secreção e ação da insulina.

Estudos tem demonstrados que compostos bioativos, como compostos fenólicos e carotenoides, desempenham diversas atividades biológicas, como antioxidante, anti-inflamatória e antibacteriana, sendo, portanto, capazes de minimizar as complicações associadas ao diabetes. Ademais, outros compostos com potencial prebiótico, como o amido resistente, passam intactos pelo intestino delgado e, ao atingirem o cólon, são metabolizados pelas bactérias do intestino, produzindo ácidos graxos de cadeia curta (AGCC). Esses ácidos conferem diversos benefícios ao hospeiro ao modular a microbiota intestinal, sendo, também, capazes de contribuir para uma melhora do quadro de diabetes.

As frutas da família Solanacea estão distribuídas ao longo de todo território brasileiro, e embora sejam neglicenciadas, são ricas em diversas classes de fitoquímicos e poderiam contribuir para a ingestão desses compostos, trazendo benefícios à saúde. Neste sentido, este estudo se debruçou sobre a caracterização química e biológica da fruta-do-lobo e do juá-açu.

Foi observada capacidade antioxidante pronunciada de ambas frutas, sendo maior na polpa e casca do juá-açu, a qual foi correlacionada com os compostos fenólicos identificados. Essa propriedade é importante no combate ao estresse oxidativo, que além de estar envolvido na fisiopatologia, contribui para o desenvolvimento das comorbidades associadas. Também foram encontrados níveis significaticos de FOS na polpa do juá-açu, particularmente de GF3, o qual poderia ser explorado em trabalhos futuros.

Ademais, ao avaliar a potencial atividade prebiótica do amido da fruta-do-lobo, frente a cepas probióticas dos genêros *Lactobacillus* e *Bifidobacterium*, observou que o amido promoveu o crescimento das cepas testadas, assim como o FOS, reconhecido prebiótico, ao qual o amido foi comparado. Também houve produção de AGCC, majoritariamente acetato, que minimiza a inflamação.

Complementando o estudo, após identificar os principais compostos dessas frutas e alguns de seus mecanismos de ação, também foi avaliado as alterações que ocorrem na fruta-do-lobo durante seu amadurecimento. Dessa forma, permitiu-se entender melhor seu metabolismo e, assim, identificar o melhor período de coleta para maximizar a extração de seus compostos de interesse e obter melhor rendimento, seja do amido, dos alcaloides ou dos compostos fenólicos.

CONCLUSÃO

Os dados apresentados nesse estudo contribuem de forma significativa para o avanço no entendimento de quais mecanismos de ação que os compostos bioativos das frutas da família Solanaceae usam para atenuar os efeitos do diabetes no organismo, seja pela capacidade antioxidante ou pela potencial atividade prebiótica. Entretanto, mais estudos são necessários, como modelos experimentais utilizando animais e seres humanos, para que possa reforçar seus efeitos positivos à saúde.

Além disso, conhecer a importância dessas frutas permite o incentivo à preservação de áreas nativas, contribuindo para a preservação do meio ambiente. O bioma Cerrado, por exemplo, vem sendo constantemente degradado para o cultivo de monoculturas e pastagens. A lobeira é uma das árvores que mais são derrubadas por acreditarem se tratar de uma planta venenosa. Entretanto, além dos benefícios dessa planta à saúde humana demontrado nesse estudo, a fruta é o principal alimento do lobo-guará. Portanto, incentivar sua preservação também é importante para a preservação da fauna local.

Por fim, essas informações podem despertar o interesse de pequenos produtores que venham a se interessar pelo cultivo dessas frutas. Os resultados desse estudo, particularmente os dados sobre o amadurecimento da fruta-do-lobo, podem contribuir para futuras iniciativas que visam desenvolver pomares comerciais para essas frutas, a fim de abrir novos mercados.

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ANEXO I
Comprovante de depósito de material biológico no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado - SISGEN

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

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

Comprovante de Cadastro de Acesso

Cadastro nº ADE6A88

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

Número do cadastro:	**ADE6A88**
Usuário:	**Ana Paula Aparecida Pereira**
CPF/CNPJ:	**086.860.436-40**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Solanum lycocarpum
Solanum oocarpum

Título da Atividade:	**Caracterização de Solanum lycocarpum St. Hill e Solanum oocarpum Sendtn: composição química e propriedades antioxidantes**

Equipe

Ana Paula Aparecida Pereira	**UNICAMP**
Célio Fernando Figueiredo Angolini	**Universidade Estadual de Campinas**
Bruno Nicolau Paulino	**Universidade Federal do Amazonas**
Leonardo Borges Chatagnier Lauretti	**Universidade Estadual de Campinas**
Eduardo Adilson Orlando	**Universidade Estadual de Campinas**
Joyce Grazielle Siqueira Silva	**Universidade Estadual de Campinas**
Iramaia Angelica Neri-Numa	**Universidade Estadual de Campinas**
Jane Delane Reis Pimentel Souza	**Universidade Federal do Sergipe**
Juliana Azevedo Lima Pallone	**Universidade Estadual de Campinas**
Marcos Nogueira Eberlin	**Universidade Estadual de Campinas**
Glaucia Maria Pastore	**Universidade Estadual de Campinas**

Data do Cadastro:	**13/09/2018 17:49:39**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **12:03** de **14/09/2018**.

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

ANEXO II

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Title: A comprehensive characterization of Solanum lycocarpum St. Hill and Solanum oocarpum Sendtn: Chemical composition and antioxidant properties

Author: Ana Paula Aparecida Pereira,Célio Fernando Figueiredo Angolini,Bruno Nicolau Paulino,Leonardo Borges Chatagnier Lauretti,Eduardo Adilson Orlando,Joyce Grazielle Siqueira Silva,Iramaia Angelica Neri-Numa,Jane Delane Reis Pimentel Souza et al.

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ANEXO III

Comprovante de submissão de artigo – Food Research International

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Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
View Submission Send E-mail	FOODRES-D-19-01510	Potential prebiotic activity of fruta-do-lobo (Solanum lycocarpum St. Hill) starch: possible mechanisms involved in glycemic control	Apr 23, 2019	May 06, 2019	Under Review

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Action Links		Ripening behavior in fruta-do-lobo (Solanum lycocarpum St. Hill): carbohydrate, phenolic and alkaloid profiles	May 19, 2019	May 19, 2019	Submitted to Journal

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