

**UNIVERSIDADE DE SÃO PAULO**  
**FACULDADE DE CIÊNCIAS FARMACÊUTICAS**  
Programa de Pós-Graduação em Ciência dos Alimentos  
Área de Bromatologia

Caracterização e biodisponibilidade de derivados de ácido elágico da jaboticaba  
(*Myrciaria jaboticaba*)

Marcela Roquim Alezandro

Tese para obtenção do grau de

**DOUTOR**

Orientador:

Prof. Dr. Maria Inés Genovese

São Paulo

2013

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Comissão Julgadora

da

Tese para obtenção do grau de Doutor

Prof. Dr. Maria Inés Genovese

orientador/presidente

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1o. examinador

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4o. examinador

São Paulo, \_\_\_\_\_ de \_\_\_\_\_.

**Dedicatória**

À minha família, meu exemplo,  
minha força e meu refúgio.

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“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar. Mas o mar seria menor se lhe faltasse uma gota”

Madre Teresa de Calcutá

## RESUMO

ALEZANDRO, M. R. **Caracterização e biodisponibilidade de derivados de ácido elágico da jabuticaba (*Myrciaria jaboticaba*)**. 2013. 162 f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas – Universidade de São Paulo, São Paulo, 2013.

O ácido elágico é um composto fenólico presente em algumas frutas e sementes. As maiores fontes da dieta humana são as frutas conhecidas como *berries*, a romã e as nozes. Dentre as frutas nativas brasileira, a jabuticaba apresenta teores de ácido elágico comparáveis aos das *berries*. Além disso, a jabuticaba representa uma boa fonte de flavonoides e destaca-se pelo sabor apreciado e pelo grande número de frutos que oferece a cada floração. Dessa forma, os objetivos deste trabalho foram: caracterizar duas espécies de jabuticaba, Sabará e Paulista (*Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg), em diferentes estádios de maturação, assim como as frações polpa, casca e semente, quanto ao teor e perfil de flavonoides, ácido elágico livre e total, elagitaninos, proantocianidinas e capacidade antioxidante *in vitro*. Ainda, avaliar o efeito da administração de extrato bruto e/ou frações fenólicas da jabuticaba Sabará sobre o status antioxidante e perfil bioquímico de ratos Wistar diabéticos induzidos por estreptozotocina. As frações fenólicas de jabuticaba também foram testados em modelo de prevenção de obesidade e diabetes tipo 2 induzidas por dieta hiperlipídica em camundongos C57 Black 6. Também foi avaliado o efeito de extrato bruto e frações fenólicas da jabuticaba em culturas celulares de hepatócitos FAO, macrófagos J774.1 e músculo L6. A biodisponibilidade de derivados do ácido elágico também foi estudada, tanto em modelo *in vitro* de fermentação quanto *in vivo* em ratos Wistar. Os resultados demonstraram que existem diferenças nos teores de compostos bioativos entre as espécies, e entre os estádios de maturação. A variedade Sabará destacou-se em relação à capacidade antioxidante, teor de proantocianidinas e ácido elágico total, e por ser mais cultivada e consumida pela população, foi escolhida para continuar os estudos *in vivo*. Em culturas celulares, o tratamento com os extratos de jabuticaba foi capaz de inibir a produção de óxido nítrico em macrófagos e hepatócitos, e aumentou a captação de glicose em células musculares. Os animais diabéticos tratados com a jabuticaba apresentaram alterações do perfil lipídico plasmático, com reversão dos altos teores de colesterol total e triacilglicerídeos. Outros efeitos como a redução da peroxidação lipídica e aumento da capacidade antioxidante plasmática também foram observados. No modelo de prevenção de obesidade e diabetes tipo 2, o tratamento com os extratos fenólicos da jabuticaba melhorou a sensibilidade à insulina e a tolerância à glicose, mesmo diante do consumo de dieta hiperlipídica e incremento ponderal dos animais. O estudo da biodisponibilidade mostrou que os derivados do ácido elágico são metabolizados especialmente pela microbiota intestinal e seus derivados foram detectados no plasma, cólon, fígado, rins, músculo e cérebro dos animais. Estes resultados demonstraram que a jabuticaba pode ser considerada uma excelente fonte de compostos bioativos e o seu consumo pode ser associado à prevenção de alterações metabólicas causadas pelo diabetes e obesidade, como a dislipidemia e a resistência à insulina.

**PALAVRAS-CHAVE:** ácido elágico, atividade biológica, biodisponibilidade, capacidade antioxidante, compostos bioativos, elagitaninos, jabuticaba.

## ABSTRACT

ALEZANDRO, M. R. **Characterization and bioavailability of ellagic acid derivatives from jaboticaba (*Myrciaria jaboticaba*)**. 2013. 162 f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas – Universidade de São Paulo, São Paulo, 2013.

Ellagic acid is a phenolic compound present in several fruits and nuts. Walnuts and berries are some known sources. Among the Brazilian native fruits, jaboticaba shows ellagic acid content comparable to that of berries. In addition, jaboticaba is a good source of flavonoids and stands out due to its appreciated flavor and the large number of fruits produced in each flowering. In this way, this work aimed to characterize two species of jaboticaba, Sabará and Paulista (*Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg), in different ripening stages, as well as pulp, skin and seeds, in relation to the content and composition of flavonoids, free and total ellagic acid, ellagitannins, proanthocyanidins and *in vitro* antioxidant capacity. Besides, evaluate the effect of raw extract and solid-phase purified phenolic fractions from Sabará jaboticaba administration on antioxidant status and biochemical profile in streptozotocin-induced diabetic Wistar rats. The jaboticaba phenolic fractions were also tested on high-fat-diet-induced obesity and type 2 diabetes in C57BL/6 mice. The effect of phenolic fractions on glucose transport in L6 muscle cells and nitric oxide production in FAO hepatocytes and J774 macrophages were also assessed. The bioavailability of polyphenols from jaboticaba were studied using *in vitro* and *in vivo* methods. The results indicated that the phenolic compounds contents are different between the two species, and among the different ripening stages. Sabará species presented the highest amounts of proanthocyanidins and total ellagic acid, and the highest antioxidant capacity. For being the most cultivated and consumed, this species was chosen for the *in vivo* studies. In cell cultures, the jaboticaba extracts inhibited the nitric oxide production in macrophages and hepatocytes, and increased glucose uptake in L6 muscle cells. In streptozotocin induced diabetic animals, treatment with jaboticaba led to improvement in lipid profile, reducing the levels of total cholesterol and triacylglycerol. Reduction in lipid peroxidation and increase in antioxidant capacity were also observed. In high-fat-diet induced diabetic mice, the phenolic fractions improved insulin sensitivity and glucose tolerance, even when mice become obese. The bioavailability study revealed that the ellagic acid derivatives and other jaboticaba polyphenols were metabolized, especially by the colonic microbiota, and their metabolites were detected in plasma, colon, kidneys, liver, brain, muscle, and stomach. These results demonstrated that jaboticaba may be considered an excellent source of bioactive compounds, and its consumption can be related to reduced risk of metabolic disorders caused by diabetes and obesity, such as dyslipidemia and insulin resistance.

**KEYWORDS:** ellagic acid, biological activity, bioavailability, antioxidant capacity, bioactive compounds, ellagitannins, jaboticaba.

## 1. INTRODUÇÃO

O Brasil é o terceiro maior produtor de frutas do mundo, atrás apenas da China e da Índia. De acordo com o Instituto Brasileiro de Geografia e Estatística (IBGE), em 2010 foram produzidas mais de 40 bilhões de toneladas das 22 espécies de frutas, o que gerou uma receita de US\$ 20,6 bilhões. As cinco frutas de maior produção neste período foram laranja, banana, uva, mamão e abacaxi, representando 70% de toda produção do setor frutícola. A receita é 16,47% a mais que o arrecadado em 2009. A área cultivada superou a marca dos três milhões de hectares, um acréscimo de apenas 1,56% em relação ao ano anterior (IBGE, 2010). Entre as frutas nativas brasileiras que apresentam maior dinâmica da produção, comercialização e inserção nos mercados nacional e internacional estão o açaí (*Euterpe oleracea* Mart) e o cupuaçu (*Theobroma grandiflorum*) (NOGUEIRA; SANTANA, 2009). Ainda, as condições climáticas brasileiras favorecem uma grande diversidade de espécies frutíferas tropicais nativas, como a jaboticaba, que cresce principalmente na mata pluvial e submatas de altitude, e destaca-se entre as oito mil espécies de plantas nativas da Mata Atlântica. Ocorre desde Mato Grosso do Sul e Minas Gerais até o Rio Grande do Sul, mas é mais cultivada no sudeste brasileiro e São Paulo é o estado com maior produção (MATTOS, 1983).

A jaboticaba é uma fruta de cor roxo-escura ou negra, segundo a variedade da planta, e polpa suculenta, mole e esbranquiçada envolvendo de uma a quatro sementes. As suas qualidades incluem o sabor apreciado e a abundância de frutos que oferece a cada floração. É uma fruteira comum em pomares caseiros ou de pequenas plantações, e seu cultivo vem aumentando gradualmente. Segundo Donadio (2000), a Ceagesp (Companhia de Entrepósitos e Armazéns Gerais de São Paulo) comercializou cerca de 900000 kg de jaboticaba em 1980, e em 1998 este valor subiu para mais de 4000000 kg. É pertencente à família Myrtaceae e a principal espécie de jaboticabeira é a *Myrciaria jaboticaba* (Vell.) Berg, conhecida como

Sabar. Mas outras espcies, como a *Myrciaria cauliflora* (Mart.) O. Berg, ou jabuticaba Paulista, tambm so encontradas no estado de So Paulo. A florao ocorre geralmente duas vezes por ano, entre julho e agosto e entre novembro e dezembro, e os frutos maduros podem ser obtidos em agosto, setembro e janeiro.

Entre as frutas nativas brasileiras, a jabuticaba  uma das mais apropriadas para o consumo *in natura*, bem como para aproveitamento industrial, sendo utilizada na fabricao de sucos, geleias, licores, vinhos, sorvetes e tambm como ingrediente de cosmticos (DONADIO, 2000; LIMA et al., 2008). Destaca-se ainda pelos elevados teores de cido elgico, comparveis aos das *berries*, e tambm por ser rica em flavonoides, principalmente as antocianinas presentes de forma abundante na casca (ABE et al., 2012; LEITE et al., 2011).

### **1.1. Compostos bioativos de alimentos**

A dieta habitual garante o fornecimento de macronutrientes e micronutrientes necessrios para o crescimento e desenvolvimento. Alguns desses nutrientes so considerados essenciais e devem ser obtidos a partir do consumo regular de alimentos fontes, uma vez que so fundamentais para a manuteno do funcionamento adequado do organismo e promoo da sade.  o caso dos aminocidos lisina e metionina, e os cidos graxos mega 3, cido eicosapentaenoico (EPA) e cido docosaexanico (DHA) (WHO, 2003).

Recentemente, outros componentes dos alimentos ganharam importncia devido s evidncias de que o seu consumo estaria relacionado a efeitos benficos  sade. So os chamados compostos bioativos, sendo nutrientes ou no nutrientes que apresentam papel metablico ou fisiolgico importante para a manuteno das funes normais do organismo (BRASIL, 2002). O interesse de pesquisadores e profissionais da sade sobre os compostos bioativos teve incio com a observao de dados epidemiolgicos de baixa incidncia de

doenças cardiovasculares e câncer em populações mediterrâneas e asiáticas. Buscou-se então a explicação para tal fato junto aos hábitos alimentares e estilo de vida desses povos, e foi constatado que, por exemplo, a dieta dos países mediterrâneos é baseada no alto consumo de frutas e hortaliças, oleaginosas, peixes, vinho e azeite de oliva. Todos esses alimentos consumidos moderadamente e de forma crônica fornecem compostos bioativos que auxiliam na redução do risco de doenças crônicas (ORDOVAS et al., 2007).

A comprovação da eficiência e a garantia da segurança da ingestão desses compostos bioativos exigem a realização de inúmeros estudos envolvendo não apenas ensaios *in vitro*, mas também experimentos com animais e pesquisas clínicas em humanos. Por essa razão, alguns compostos têm sido investigados cientificamente, mas ainda não apresentam evidências suficientes que garantam sua aprovação pela Agência Nacional de Vigilância Sanitária (Anvisa). Outros, entretanto, já estão registrados junto à agência como substância bioativa com alegação de propriedades funcionais e ou de saúde. Entre eles, estão carotenoides, fibras alimentares, fitoesteróis, proteína da soja e probióticos. Esses compostos podem ser adicionados à matriz alimentar como um ingrediente, mas de forma geral ocorrem naturalmente nos alimentos, como os frutooligossacarídeos presentes no yacon (*Polymnia sonchifolia*) e o licopeno do tomate e seus subprodutos, como molhos e *ketchup* (BRASIL, 1999).

Entre as diferentes classes de compostos bioativos, destacam-se os compostos fenólicos. Estes são substâncias produzidas a partir do metabolismo das plantas, o qual é dividido em primário e secundário para fins didáticos. De fato, o metabolismo deve ser visto de forma integral, uma vez que os compostos orgânicos, como açúcares, aminoácidos e ácidos graxos, produzidos pela atividade metabólica primária apresentam distribuição universal e são vitais para a planta. No entanto, algumas dessas substâncias são utilizadas como precursores para a produção dos metabólitos secundários, como a eritrose 4-fosfato, proveniente da rota

da pentose fosfato, e o ácido fosfoenolpirúvico, resultante da glicólise (MANACH et al., 2004).

Os compostos fenólicos estão amplamente distribuídos no reino vegetal e diversos fatores podem influenciar a sua biossíntese pelas plantas, afetando diretamente o conteúdo desses compostos no material vegetal. São eles: sazonalidade, índice pluviométrico, radiação ultravioleta, ritmo circadiano, temperatura, altitude, ataque de patógenos, presença de herbívoros, composição do solo e atmosférica. Nesse sentido, evidências apontam que a taxa de produção de metabólitos secundários é aumentada à medida que a planta é submetida a condições mais adversas durante o seu desenvolvimento. Deve ser enfatizado ainda, que há um controle genético, especialmente sobre o perfil qualitativo dos compostos sintetizados (GOBBO-NETO; LOPES, 2007).

As principais fontes de compostos fenólicos para a população brasileira são a laranja, rica em naringenina, a alface, o tomate e a cebola, como fontes importantes de quercetina (ARABBI et al., 2004). Outros alimentos também são ricos em fenólicos e contribuem para a ingestão dietética da população, como as uvas e o vinho tinto, chá verde e café (PIMENTEL et al., 2005).

A presença do grupo fenol é uma característica comum a todos os compostos fenólicos. Entretanto, apresentam estruturas e funções diversas e dessa forma, podem ser divididos em três classes: flavonoides, ácidos fenólicos e taninos. Os flavonoides são divididos em oito subclasses, as quais compreendem flavona, chalcona, flavonol, flavanona, flavan-3-ol, flavanonol, antocianidinas, isoflavonas (COOK; SAMMAN, 1996), com 15 átomos de carbono em seu esqueleto básico (C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub>) (TAPAS et al., 2008). Os ácidos fenólicos contêm um grupo carboxílico e sete átomos de carbono (C<sub>6</sub>-C<sub>1</sub>) e são subdivididos em ácidos hidroxibenzoicos e os hidroxicinâmicos (D'ARCHIVIO et al., 2007). Alguns fenólicos não se apresentam na forma livre em tecidos vegetais, mas na forma de polímeros,

como os taninos. Estes são divididos em hidrolisáveis e condensados, de acordo com a sua estrutura química (HELDT, 1997).

O ácido elágico é um composto fenólico presente em algumas frutas e castanhas, tais como: morango (*Fragaria ananassa*), framboesa (*Rubus fruticosus*), romã (*Punica granatum*) e nozes (*Juglans regia*) (DANIEL et al., 1989). Pode ocorrer na forma livre, glicosilada ou ligado como elagitaninos, esterificado com glicose (BATE-SMITH, 1972). Os elagitaninos são compostos fenólicos solúveis em água de alto peso molecular e com capacidade de precipitação de proteínas e alcaloides (SANTOS-BUELGA; SCALBERT, 2000). São ésteres do ácido hexahidroxi-difênico e um poli-ol, geralmente glicose e ácido quínico (HASLAM, 1989). Quando expostos a ácidos ou bases, a porção éster é hidrolisada e o ácido hexahidroxi-difênico se rearranja espontaneamente originando o ácido elágico, substância insolúvel em água. Essa reação é a base para a detecção e quantificação indireta de elagitaninos (CLIFFORD; SCALBERT, 2000).

Segundo Hakkinen et al. (1999), o ácido elágico representa mais de 50% do teor total de fenólicos presentes em morangos e framboesas. O teor de ácido elágico livre varia bastante quando comparamos framboesa (0,6 mg/100 g b.u.), morango (1,8 mg/100 g b.u.) e amora-preta (8,8 mg/100 g b.u.) (AMAKURA et al., 2000). Porém, sabe-se que os teores de ácido elágico livre são geralmente baixos, embora quantidades substanciais possam ser detectadas após hidrólise ácida dos extratos, como resultado da quebra dos elagitaninos (BEATTIE et al., 2005).

O morango representa a principal fonte de derivados de ácido elágico na dieta brasileira (HAKKINEN et al., 2000). Pinto et al. (2008) analisaram sete cultivares de morango (produzidas no mesmo local e sob as mesmas condições) comercializadas no Brasil quanto ao teor de ácido elágico livre e total. O teor de ácido elágico livre variou de 0,6-2,6 (média de 1,6) mg/100 g (b.u.) e esses valores são similares ao encontrado em outro trabalho

para morango (1,8 mg/100 g b.u.) e framboesa (0,58 mg/100 g b.u.) (AMAKURA et al., 2000). O conteúdo de ácido elágico total variou de 17-47 mg/100 g (b.u.), o que está de acordo com resultados anteriores para morango (4-46 mg/100 g b.u.) (MAAS et al., 1991).

As fontes alimentares de ácido elágico são escassas, consistindo principalmente de sementes e de frutas vermelhas tais como framboesa, morango e amora, cujo consumo no Brasil ainda é restrito já que são frutas de países frios. Nesse contexto, a jabuticaba se apresenta como uma fonte promissora desse composto em nossa dieta.

## **1.2. Atividade biológica dos compostos fenólicos**

Os mecanismos pelos quais as doenças crônicas se desenvolvem, geralmente incluem alterações oxidativas de moléculas consideradas críticas, o que engloba proteínas, carboidratos, ácidos nucleicos, além das substâncias envolvidas na modulação da expressão gênica e em respostas inflamatórias (KAWANISHI et al., 2002; LAGUERRE et al., 2007). Adicionalmente, evidências científicas indicam que antioxidantes exógenos, obtidos a partir dos alimentos, são fundamentais para a resposta do organismo ao estresse oxidativo (SAURA-CALIXTO; GOÑI, 2009; VASCO et al., 2008).

No organismo humano, ocorrem diversos processos em células aeróbicas, como a respiração e outras reações oxidativas, as quais são responsáveis pela formação de radicais livres e espécies reativas de oxigênio. Estes, por sua vez, podem causar danos ao organismo e contribuir para o desenvolvimento de processos inflamatórios, doenças cardiovasculares e tumores malignos (SIKORA et al., 2008). Contra esses danos, os tecidos apresentam um eficiente sistema de defesa que compreende componentes enzimáticos (catalase, glutatona peroxidase, superóxido dismutase) e substâncias de caráter hidrossolúvel, como o ácido ascórbico, ou lipossolúvel, como o tocoferol (McLEAN et al., 2005).

A ingestão regular e em longo prazo de compostos fenólicos contribui para a defesa do organismo e está relacionada à redução do risco de doenças crônicas. Estudos epidemiológicos sugerem que uma dieta rica em vegetais tem efeito protetivo contra vários tipos de câncer (HOLLMAN; KATAN, 1999), o que estaria relacionado ao potencial antioxidante e ação anti-inflamatória destes compostos (HARBORNE; WILLIAMS, 2000).

Há um interesse particular na determinação dos teores de ácido elágico presente em frutos devido a crescentes evidências de seus efeitos quimiopreventivos. Estudos com animais utilizando carcinógenos químicos têm demonstrado que a administração do ácido elágico por meio da dieta inibe o desenvolvimento de cânceres de esôfago, fígado e pulmão, dependendo do tipo de composto utilizado. A aplicação tópica de ácido elágico também demonstrou diminuir a incidência de câncer de pele induzido quimicamente, em camundongos (HANNUM, 2004).

Estudos apontam os benefícios associados à ingestão de alimentos ricos em ácido elágico. Algumas atividades biológicas importantes foram demonstradas: atividade antiproliferativa e indução de apoptose em cultura de células carcinogênicas do epitélio cervical (NARAYANAN et al., 1999); prevenção de câncer do trato gastrointestinal atribuída ao acúmulo seletivo de ácido elágico em células epiteliais de rato (WHITLEY et al., 2003); atividade antimicrobiana seletiva em microrganismos patogênicos para o homem (PUUPPONEN-PIMIA et al., 2005); redução da incidência de morte por problemas cardíacos (ANDERSON et al., 2001). Além dessas propriedades o ácido elágico e os elagitaninos são alvo de muitas pesquisas por demonstrar alta atividade antioxidante *in vitro* (MEYER et al., 1998).

Em ratos, o ácido elágico administrado pela dieta inibiu o desenvolvimento de câncer de esôfago induzido por N-nitrosometilbenzilamina (NMBA) em 25 a 50% e as lesões neoplásicas e pré-neoplásicas foram reduzidas (MANDAL; STONER, 1990; DANIEL;

STONER, 1991). Estes pesquisadores observaram que a inibição do tumor ocorria somente quando o ácido elágico era administrado continuamente antes, durante e após a dose de NMBA. Em estudo realizado por Kresty et al. (1998), a administração de framboesas liofilizadas a ratos tratados previamente com NMBA inibiu eventos carcinogênicos de iniciação e pós-iniciação como evidenciado pela diminuição na incidência e multiplicidade de tumores, inibição da formação de adutos de DNA, redução dos índices proliferativos e inibição da formação de lesões pré-neoplásicas.

Diversos estudos também já relataram as propriedades antioxidantes *in vitro* dos elagitaninos e do ácido elágico (MULLEN et al., 2002; SRINIVASAN et al., 2002). Priyadarsini et al. (2002) testaram a atividade antioxidante do ácido elágico através da medida da habilidade de inibição da peroxidação lipídica induzida pela irradiação gama em microsossomos. O ácido elágico mostrou atividade antioxidante através do sequestro de ROS (*reactive oxygen species*) e RNS (*reactive nitrogen species*), tais como radicais hidroxila, peroxila, NO<sub>2</sub> e peroxinitrila, com constantes de velocidade comparáveis aos de antioxidantes conhecidos, tais como as vitaminas C e E. Elagitaninos como sanguina H-6 e lambertianina C (presentes em altas concentrações em framboesa, amora-preta e morango) apresentam alta atividade antioxidante, podendo exercer efeitos protetores ao passarem pelo trato gastrointestinal, onde também podem ser despolimerizados liberando ácido elágico, o qual seria mais facilmente absorvido (BEATTIE et al., 2005). Cao et al. (1998) verificaram que a ingestão de suco de morango fresco (240 g/copo) por mulheres aumentou a capacidade antioxidante do plasma em 14-30% após quatro horas, indicando que os compostos antioxidantes presentes eram absorvidos.

Apesar de mecanismos não esclarecidos e poucos estudos relatados, as hipóteses salientam que a alta capacidade antioxidante de compostos fenólicos, principalmente dos flavonoides, também pode ser efetiva na redução do estresse oxidativo e progressão do

diabetes mellitus (SONG et al., 2005). McDougall e Stewart (2005) destacam as antocianinas como bons inibidores de  $\alpha$ - glicosidase e relatam a importância dos polifenóis do chá verde como potentes inibidores de enzimas proteolíticas envolvidas no desenvolvimento de tumores. De modo similar, estudos mais recentes têm demonstrado que os compostos fenólicos, além de apresentar alta capacidade antioxidante, possuem propriedades terapêuticas, sendo estas antidiabética e antihipertensiva (KWON et al., 2006).

### **1.3. Biodisponibilidade dos compostos fenólicos**

Para que um composto químico possa exercer a sua atividade biológica, deve atingir o alvo fisiológico numa concentração mínima que determine tanto esse efeito biológico quanto o mecanismo de ação (OLIVEIRA; BASTOS, 2011). Na dieta habitual, alguns gramas de compostos fenólicos por dia são ingeridos. No entanto, as concentrações desses compostos no organismo humano são muito baixas - na faixa de micromoles, o que está relacionado à sua limitada absorção e biodisponibilidade (BASTOS et al., 2009).

O conceito de biodisponibilidade foi inicialmente proposto pela *Food and Drug Administration* (FDA) para uso em farmacologia e mais tardiamente, passou a ser empregado também por cientistas da nutrição, ao observarem que a presença do nutriente no alimento não seria suficiente para garantir a sua utilização pelo organismo. Neste sentido, biodisponibilidade refere-se à concentração de um determinado composto ou de seus metabólitos na circulação, órgãos e tecidos em relação ao total ingerido (COZZOLINO, 2012).

A biodisponibilidade dos compostos fenólicos é uma característica extremamente importante, já que esta varia amplamente entre os diversos compostos e não necessariamente os mais abundantes em nossa dieta, ou os que apresentam maior capacidade antioxidante *in*

*vitro*, são os mais biodisponíveis. Um composto com alta atividade antioxidante intrínseca pode, ao ser ingerido através da dieta, ser pobremente absorvido, extensamente metabolizado ou rapidamente eliminado, resultando em baixa atividade biológica. Ainda, os metabólitos produzidos pela atividade digestiva ou hepática podem ser completamente inativos ou apresentar atividade maior que o composto original (MANACH et al., 2004).

Os compostos fenólicos apresentam baixa biodisponibilidade, especialmente quando comparada à de macronutrientes. Sabe-se que o organismo não é capaz de distinguir se as substâncias apresentam efeito benéfico ou potencialmente tóxico, mas somente se são ou não nutrientes. Neste sentido, o organismo reconhece os compostos fenólicos como xenobióticos, limitando sua absorção e estimulando mecanismos de detoxificação que visam controlar a concentração fisiológica, prevenindo possíveis efeitos deletérios. De maneira similar ao que ocorre com os micronutrientes, apenas uma parte dos compostos fenólicos é absorvida e metabolizada. Estima-se que a absorção dos polifenóis esteja entre 1% e 60% do total ingerido (JACOBS; TAPSELL, 2007).

A biodisponibilidade sofre a influência de diversos fatores, como a complexidade da matriz alimentar, a forma química da substância presente naturalmente no alimento e a ingestão concomitante de outros componentes alimentícios, os quais podem atuar como ligantes, facilitar ou dificultar a absorção. Outros aspectos, como a massa e a integridade da mucosa intestinal, o tempo de trânsito intestinal, a taxa de esvaziamento gástrico, o metabolismo e o grau de conjugação, e ligação com as proteínas de transporte no sangue e nos tecidos, correspondem às variações intra e interindividuais e afetam diretamente a biodisponibilidade dos compostos fenólicos, que pode variar de 0 a 100% da dose ingerida (JACOBS; TAPSELL, 2007; FRASETTO et al., 2001).

O primeiro passo após a ingestão de compostos fenólicos presentes na dieta é a liberação dos mesmos de sua matriz. A deglicosilação de flavonoides, a clivagem de

proantocianidinas poliméricas e a hidrólise de ácidos fenólicos esterificados são consideradas pré-requisitos para absorção destes compostos através da barreira intestinal (MANACH; DONAVAN, 2004).

Apenas uma pequena parte de compostos fenólicos ingeridos é absorvida pelo intestino delgado. Este processo ocorre através de difusão passiva e está associada com hidrólise e liberação da aglicona pela ação da lactase floridzina hidrolase (LPH) presente nas microvilosidades das células epiteliais do intestino (DONOVAN et al., 2006). Depois de absorvida, a aglicona sofre metabolização no fígado, formando metabólitos sulfatados, glicurônicos e/ou metilados através da ação respectiva das enzimas de fase II sulfotransferase (SULT), uridina-50-difosfato glicuronosiltransferase (UGT) e catecol-*O*-metiltransferase (COMT) (CROZIER et al., 2010).

Os produtos desta metabolização podem entrar na corrente sanguínea e serem excretados através da urina, ou ainda, pela circulação enterohepática, uma fração considerável pode ser excretada pelo fígado como componente da bile de volta para o intestino (MCBAIN; MACFARLANE, 1997). Uma vez liberados no lúmen intestinal, estes conjugados podem ser hidrolisados por enzimas bacterianas como as  $\beta$ -glicuronidases, sulfatases e glicosidases (CROZIER et al., 2010; SELMA et al., 2009). Os compostos que não são absorvidos no intestino delgado vão diretamente para o intestino grosso, onde são degradados pela microbiota colônica a compostos mais simples, como ácidos fenólicos, e assim serem absorvidos pelo sistema circulatório. Uma vez no intestino grosso, os flavonoides e seus metabólitos podem apresentar benefícios à microbiota colônica por selecionar bactérias probióticas ou inibir a proliferação de células cancerígenas (DEL RIO et al., 2010).

O esquema simplificado das diversas etapas envolvidas na absorção, metabolização e excreção de compostos fenólicos é apresentado na **Figura 1**.



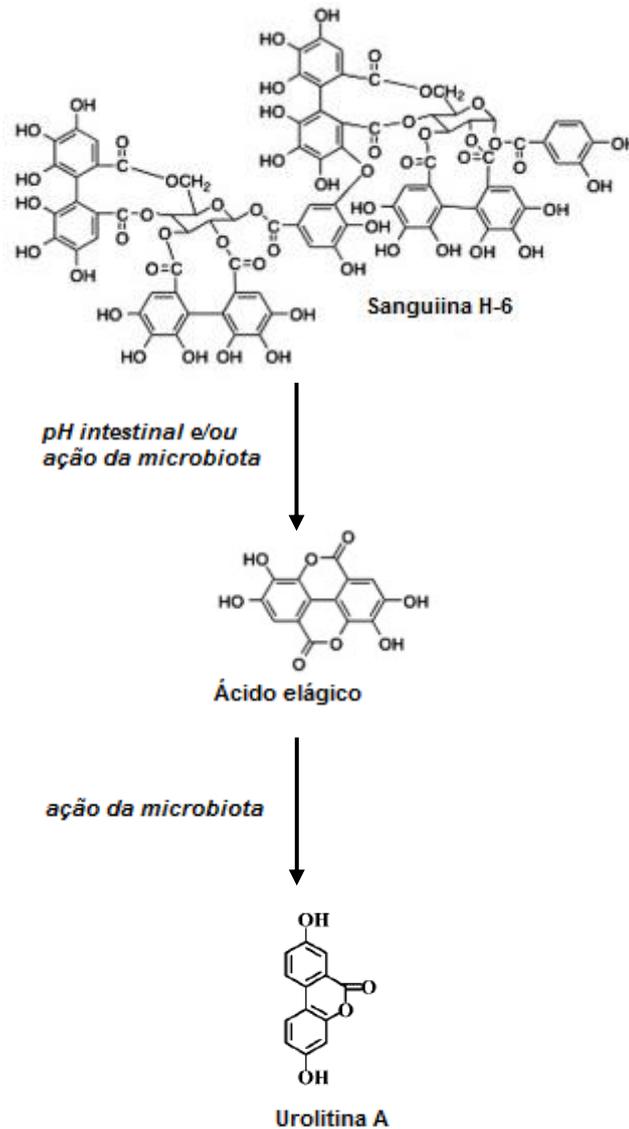
Por muitos anos acreditou-se que a principal função do intestino era apenas de reabsorção de água e sais minerais e uma rota simples de excreção. O intestino humano, considerado um ecossistema microbiano altamente complexo, abriga uma concentração de cerca de mil micro-organismos por grama de fezes, dentre bactérias e fungos, e é um sítio ativo no processo de metabolização. Embora seja um número alto, a diversidade microbiana é limitada em oito classes bacterianas, sendo duas delas mais predominantes, Firmicutes e Bacteroides, que juntos somam 90% da microbiota intestinal, presentes principalmente no cólon (POSSEMIERS et al., 2011).

O perfil qualitativo e quantitativo da formação de metabólitos a partir dos compostos fenólicos é fortemente influenciado pelas variações interindividuais referentes à composição da microbiota, a qual pode ser modificada por fatores genéticos, uso de medicamentos e hábitos alimentares (RECHNER et al., 2004).

Mais recentemente, uma classe de polifenóis tem recebido a atenção da comunidade científica em relação a sua biodisponibilidade, os elagitaninos. Sabe-se que são constituídos por uma ou mais unidades hexahidroxi-difênicas (HHDP) esterificadas a um açúcar, usualmente glicose. Na hidrólise dos elagitaninos que pode ocorrer quimicamente no pH fisiológico, a ligação éster é hidrolisada e os grupos HHDP se rearranjam espontaneamente, formando ácido elágico. Uma série de derivados de ácido elágico existe entre as espécies vegetais e eles são formados através da metilação, glicosilação ou metoxilação dos seus grupos hidroxila. Alguns estudos sugerem que a microbiota colônica também poderia participar da hidrólise dos elagitaninos, formando ácido elágico. A microbiota poderia ainda agir sobre o ácido elágico e após extensivas transformações formar as urolitinas, metabólitos capazes de atingir os tecidos periféricos, como a próstata, e exercer seu papel fisiológico. A metabolização do ácido elágico é iniciada no jejuno e inicialmente é produzida a urolitina D, em seguida a urolitina C e finalmente as urolitinas A e B (GONZALEZ-BARRIO et al., 2012;

LANDETE, 2011).

A via proposta para a metabolização dos elagitaninos pela microbiota do cólon humano está esquematizada na **Figura 2**.



**Figura 2.** Via proposta para a metabolização colônica dos elagitaninos (Adaptado de Gonzalez-Barrio et al., 2012 e Landete, 2011).

Após administração de elagitaninos provenientes de framboesa ou de romã a camundongos (60-600 mg/kg de peso corpóreo, por gavagem), quantidades muito baixas de ácido elágico foram detectadas na urina (0,05% da dose) e pulmões (0,01% da dose). No

entanto, em outro estudo realizado não foi detectada a presença de ácido elágico no sangue ou tecidos dos camundongos alimentados durante uma semana com uma dieta contendo 1% de ácido elágico (~1 mg/kg). Em ratos, após administração oral de ácido elágico, detectou-se o metabólito (~10% da dose) 3,8-dihidróxi-6H-dibenzo[*b,d*]piran-6-ona na urina e fezes, resultado da ação da microbiota (CERDÁ et al., 2005; CERDÁ et al., 2004).

Estudos demonstraram que apenas 3-6% dos elagitaninos consumidos são detectáveis, seja como derivados do ácido elágico ou metabólitos (CERDÁ et al., 2003). Sabe-se que os elagitaninos não são absorvidos como tal e devem ser metabolizados antes da absorção. No entanto, a extensão da metabolização é dependente da habilidade da microbiota em formar metabólitos a partir dos compostos fenólicos. O exemplo mais comum para o efeito da microbiota sobre a formação de metabólitos é em relação ao equol, composto produzido a partir das isoflavonas da soja e que apresenta propriedades estrogênica e antioxidante mais fortes do que o composto original. Porém, apenas 30% a 50% dos indivíduos são capazes de produzir o equol a partir das isoflavonas (YUAN; WANG; LIU, 2007).

## 2. OBJETIVOS

O objetivo geral deste trabalho foi caracterizar os compostos fenólicos do fruto da jabuticaba e avaliar seu potencial biológico na promoção da saúde. Os objetivos específicos são:

- Caracterizar duas variedades de jabuticaba (*Myrciaria cauliflora* e *Myrciaria jaboticaba*) em relação ao conteúdo de fenólicos totais, flavonoides, ácido elágico livre e total, proantocianidinas e capacidade antioxidante *in vitro*, e identificar taninos hidrolisáveis e condensados;
- Verificar o efeito da administração de uma suspensão aquosa de jabuticaba Sabará sobre o perfil bioquímico, capacidade antioxidante do plasma, e a atividade das enzimas antioxidantes catalase, superóxido dismutase e glutathione peroxidase em plasma e tecidos de ratos Wistar diabéticos induzidos por estreptozotocina;
- Determinar o efeito da administração do extrato bruto e extratos de compostos fenólicos obtidos a partir da jabuticaba Sabará sobre o perfil bioquímico, enzimas antioxidantes (catalase, glutathione peroxidase e superóxido dismutase), capacidade antioxidante do plasma, e a atividade dessas enzimas em plasma e tecidos de ratos Wistar caquéticos;
- Verificar o efeito da administração dos extratos de compostos fenólicos obtidos a partir da jabuticaba Sabará sobre o perfil lipídico, metabolismo de glicose, capacidade antioxidante do plasma e ganho de peso de camundongos *C57 Black 6* alimentados com dieta hiperlipídica;

- Verificar o efeito do extrato bruto e extratos de compostos fenólicos obtidos a partir da jabuticaba Sabará em ensaios que mimetizam condições fisiopatológicas de diabetes e inflamação em diferentes tipos de linhagens celulares: transporte de glicose em miócitos (L6), produção de óxido nítrico (NO) em macrófagos (J774.1) e em hepatócitos (FaO);
  
- Verificar a produção de metabólitos a partir dos compostos fenólicos da jabuticaba em modelo *in vitro* de fermentação fecal;
  
- Investigar a biodisponibilidade dos derivados de ácido elágico da jabuticaba em modelo *in vivo* com ratos Wistar, através da identificação de metabólitos em plasma, urina e tecidos.

### 3. RESULTADOS

Os resultados foram divididos em quatro capítulos e foram apresentados na forma de artigos científicos, conforme a seguir:

1. Comparative analysis of chemical and phenolic composition of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg
2. *In vitro* and *in vivo* evaluation of the metabolism of polyphenols from jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg), a Brazilian native fruit
3. Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats

Comparative analysis of chemical and phenolic composition of two species of jaboticaba:

*Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg

Jaboticaba, a rich source of polyphenols among the Brazilian native fruits

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## ABSTRACT AND KEYWORDS PAGE

The two most important commercial species of jaboticaba, Sabará (*Myrciaria jaboticaba*) and Paulista (*Myrciaria cauliflora*), were compared in relation to chemical composition and *in vitro* antioxidant capacity (AC), considering the effect of ripening. Both species presented similar mineral and centesimal composition, and were considered rich sources of Mn (1.8-2.7 mg/100 g DW) and Cu (1.0 mg/100 g DW). Excepting for anthocyanins, phenolic concentrations were much higher in Sabará compared to Paulista. Ellagic acid derivatives (EA) contents varied according to ripening stage and also among the fruit portion. The unripe stage showed the highest contents of proanthocyanidins (PAC) and ellagitannins, and also AC. Ripening led to a decrease of 47% in total EA content, 43% in PAC, 60-77% of AC. Among fractions, seeds showed the highest concentrations of ellagitannins, proanthocyanidins and AC, meanwhile anthocyanins and quercetin derivatives concentrated in the skin. Phenolics strongly inhibit carbohydrate digestive enzymes.

**KEYWORDS:** jaboticaba, ellagitannins, proanthocyanidins, flavonoids, antioxidant capacity.

## 1. INTRODUCTION

Brazil, with its continental dimension, has a very wide diversity of biomes, which results in a huge variety of vegetal species, including some peculiar fruits. Brazilian native fruits such as camu-camu, buriti and maná-cubiu were shown to display expressive amounts of phenolic compounds and consequently high *in vitro* antioxidant capacity (Genovese, Pinto, Gonçalves & Lajolo, 2008). The Atlantic Forest originally extended along the Brazilian coast (92%), and is one of the richest and most varied groups of rainforest in South America (Ribeiro, Metzger, Martensen, Ponzoni & Hirota, 2009). Jaboticaba is a native fruit from the Atlantic Rainforest, whose economic importance has been continuously growing in Brazil due to its attractive and distinctive flavor and potential for the food industry. The plant grows as a large bushy tree and a single tree produces several thousand fruits (Donadio, 2000).

Jaboticaba (*Myrciaria* spp.) is a berry with smooth skin varying from bright green to dark violet, depending on the ripening stage. It is much appreciated due to its sweet and slightly acidic pulp and fruits contain between one and four small seeds (Donadio, 2000). There is an enormous trading potential of this fruit, mainly because of its sensory characteristics, being consumed in the raw form and also used by the food industry to produce jam, juice, liqueur, ice cream and candy. Moreover, jaboticaba presents high levels of minerals (2.8 - 3.8 % DW) and fiber (18-19%) (Lima, Corrêa, Alves, Abreu & Dantas-Barros, 2008). Two anthocyanins, cyanidin 3-glycoside (433 mg/ 100 g DW) and delphinidin 3-glycoside (81 mg/ 100 g DW), were identified in the skin of jaboticaba (Leite, Malta, Riccio, Eberlin, Pastore & Maróstica Júnior, 2011). Gallotannins, such as HHDP-galloyl-glucose, casuariin, pedunculagin, di-HHDP-galloyl-glucose (casuarinin), HHDP-digalloyl-glucose (tellimagrandin I), HHDP-trigalloyl-glucose (tellimagrandin II), and di-HHDP-galloyl-glucose isomer (casuarictin), were detected for the first time in jaboticaba (Wu, Dastmalchi,

Long & Kennelly, 2012). Other compounds, like valoneic acid dilactone, an ellagic acid derivative, and two depsides, 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenyl-acetic acid and methyl 2-[(3,4-dihydroxybenzoyloxy)-4,6-dihydroxyphenyl] acetate (jaboticabin), were also detected and reported earlier in *Myrciaria* species (Reynertson et al., 2006).

Ellagitannins are present in a few berries and nuts, and the main sources of ellagic acid are blackberries, raspberries, pomegranates and walnuts (Clifford & Scalbert, 2000). Recently, Abe, Lajolo & Genovese (2012) evaluated the total ellagic acid content of several fruits commonly consumed in Brazil, and fruits belonging to the Myrtaceae family showed the highest contents of ellagitannins, and jaboticaba the highest among those. Ripening was shown to cause a change in the proportion of the different parts (skin, pulp and seeds), an increase of anthocyanins level, and a reduction of tannins and total ellagic acid contents.

In this way, this work aimed to compare the two most important commercial species of jaboticaba, cultivated in the same geographical region, in relation to the chemical composition, identify and quantify the polyphenols, and measure the *in vitro* antioxidant capacity, considering the ripening stages, as well as the fruit fractions pulp, seed and skin.

## **2. MATERIALS AND METHODS**

### ***2.1 Material***

Ten kilograms of fresh jaboticaba species Sabará (*Myrciaria jaboticaba*), at different ripening stages, and Paulista (*Myrciaria cauliflora*), fully ripened fruit, were obtained from a local producer through the Central Market (Companhia de Entrepósitos e Armazéns Gerais de São Paulo - CEAGESP) in the São Paulo city, Brazil. Fruits of both species were from the same origin, Jaboticabal (SP) at 21° 16' S latitude and 48° 19' W longitude. The fruits were

cleaned and part of the samples were separated into skin, seeds and pulp, freeze-dried and stored at -20 °C until analyses.

Initially, the two species of jaboticaba in the ripe stage were characterized and compared in relation to the polyphenols composition. Afterwards, fruits of the Sabará species, for being the most consumed and commercialized, were separated in five ripening stages according to the size and skin color. Then, additional analysis were carried out in order to characterize the different stages and the effect of ripening on the fruit composition.

**2.2 Chemicals.** The 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>), (+)-catechin, and the Folin-Ciocalteu reagents were purchased from Sigma Co (St. Louis, MO). Polyamide SC6 columns were obtained from Macherey-Nagel GmbH and Co. (Düren, Germany). The hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from Aldrich (Milwaukee, WI). Ellagic acid and quercetin were purchased from Sigma Chemical Co. (St Louis, MO, USA). The anthocyanidins cyanidin and delphinidin and the respective 3-glucosides, as well as procyanidin B2 were obtained from Extrasynthèse (Genay, France). All chemicals and solvents used were analytical grade.

**2.3 Physicochemical analysis.** The pH determination was carried out by direct reading with a digital potentiometer, model HM-26S, TOA Instruments, according to Association of Official Analytical Chemists (AOAC, 2005). The titratable acidity (TA) was determined by titration with 0.2 N NaOH and expressed in percentage of citric acid (AOAC, 2005). The content of soluble solids (SS) was determined with a digital refractometer, model ATAGO N1, with temperature compensation, expressed in °Brix.

**2.4 Centesimal composition.** The moisture, ash, protein, lipid and fibers contents were determined according to AOAC (2005). The moisture was measured by freeze-drying under vacuum, using Dura-Top MP, Bulk Tray Dryer, FST Systems®) during 96 hours. The ash content was determined in muffle at 600 °C. The protein and lipid contents were assessed by Kjeldahl and Soxhlet methods, respectively. Determination of total, soluble and insoluble dietary fiber was carried out by enzymatic-gravimetric method (AOAC, 2005). The total carbohydrates content was calculated by difference.

**2.5 Mineral composition.** The mineral composition was assessed by determination of sodium, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, zinc, iron, manganese, copper and selenium. The quantification of sodium and potassium was carried out by *flame-emission* spectrophotometry, and others minerals by atomic absorption spectrophotometry (AOAC, 2005).

**2.6 Sugar content.** The amount of glucose, fructose and sucrose were assessed by HPLC coupled to a refractometer detector with a Sugar-Pak column (Waters, Milford, USA). The samples were extracted in 80% ethanol and water. An aliquot was heated at 70 °C in a water bath and centrifuged. The supernatant was taken and the solvent was evaporated by heating at 40 °C under nitrogen flow. The solid phase was resuspended in water, filtered and analyzed. Identification was based on the retention time, and quantitation on external calibration.

**2.7 Sample extraction for antioxidant capacity assays.** Freeze-dried powders (1 g) were extracted three times in a solvent mixture (100 mL the first time, 50 mL the next two times) comprising methanol/water (70:30, v/v) or methanol/water/acetic acid (70:30:0.5, v/v/v) (for samples containing anthocyanins), using a Brinkmann homogenizer (Polytron-Kinematica

GmbH, Kriens-Luzern, Sweden). The homogenate was filtered under reduced pressure through filter paper (Whatman N° 1) and it was stored at  $-20\text{ }^{\circ}\text{C}$  until analysis (Genovese et al., 2008). All extractions and subsequent assays were performed in triplicate.

**2.7.1 Folin-Ciocalteu reducing capacity.** The antioxidant capacity was assessed using the Folin-Ciocalteu reagent, according to Singleton, Orthofer & Lamuela-Raventós (1999), with some modifications. Results were expressed as mg of catechin equivalents (CE)/100 g of sample dry weight (DW).

**2.7.2 DPPH radical-scavenging ability.** The antioxidant capacity was determined by the DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging method according to Brand-Williams, Cuvelier & Berset (1995), with some modifications. Results were expressed as  $\mu\text{mols}$  Trolox equivalents (TE)/100 g of sample DW.

**2.7.3 FRAP assay.** The ferric reducing antioxidant power (FRAP) was determined using the method of Benzie and Strain (1996). Results were expressed as mmols Trolox equivalents (TE)/100 g of sample DW.

**2.8  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity.** Extracts were obtained by solid phase extraction, according to Arabbi, Genovese and Lajolo (2004). The  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory assays were carried out as described by Gonçalves, Lajolo and Genovese (2010).

## ***2.9 Proanthocyanidins (PAC)***

**2.9.1 Quantification of proanthocyanidins by vanillin assay.** The content of proanthocyanidin was measured by vanillin/HCl assay (Burns, 1971). The extracts were prepared using acidified methanol (methanol:acetic acid 99:1 v/v). Absorbances were measured at 500 nm. Results were expressed as mg catechin equivalents (CE)/ 100 g of sample DW.

**2.9.2 Quantification of proanthocyanidins by Butanol-HCl method.** The content of proanthocyanidins was determined according to Porter, Hrstich and Chan (1986). Results were expressed as mg quebracho tannin equivalents (QTE)/ 100 g of sample DW.

**2.9.3 Quantification of proanthocyanidins by 4-dimethylaminocinnamaldehyde (DMAC) assay.** Total proanthocyanidins in jaboticaba samples were quantified using DMAC method, according to Prior et al. (2010). The extracts were prepared using acetone/water/acetic acid (AWA) (70:29.5:0.5 v/v/v). Total amount was calculated using commercially available procyanidin B2 dimer as a standard, and the calibration curve was in the range of 5-50 µg/mL.

## ***2.10 HPLC-DAD Analysis of Anthocyanins***

**2.10.1 Sample Preparation.** The freeze-dried powders were extracted by methanol/water/acetic acid (70:30:0.5, v/v) as described previously (item 2.7). The extracts were filtered using a 0.22 µm PTFE filter unit [poly(tetrafluoroethylene), Millipore Ltd., Bedford, MA] and analyzed by HPLC. The extractions were run in triplicate.

**2.10.2 Identification and quantification of anthocyanins.** Anthocyanin separation and determination was performed according to Wu and Prior (2005) with a Develosil C18 (5 µm,

25 cm x 4.6 mm id) column (Phenomenex, Torrance, CA, USA) and Agilent 1100 system equipped with an ultraviolet (UV) detector and the software ChemStation (Palo Alto, USA). Identification was based on the spectra and retention time, and quantification was based on external calibration. The anthocyanins standards were purchased from Extrasynthèse (Lyon, France). Results were expressed as mg/100 g of sample DW.

### ***2.11 HPLC-DAD Analysis of Flavonoids and Ellagic Acid***

**2.11.1 Extraction procedure for flavonoids and free ellagic acid analysis.** Extraction was performed according to Arabbi, Genovese and Lajolo (2004) with some modifications. The sum of eluted free ellagic acid and ellagic acid glycosides was considered as ‘free ellagic acid’.

**2.11.2 Total ellagic acid content.** Total ellagic acid was determined after extraction and acid hydrolysis according to Pinto, Lajolo and Genovese (2008). An aliquot (2 mL) of the raw extracts in 80% acetone was dried under nitrogen, 2 N trifluoroacetic acid were added, and the hydrolysis was performed at 120 °C for 90 min. The hydrolyzed samples were evaporated under nitrogen, redissolved in methanol and filtered for HPLC analysis.

**2.11.3 Identification and quantification of flavonoids and free ellagic acid.** Identification and quantification of flavonoids and phenolic acids were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector controlled by the Chemstation software. The column used was 250 × 4.6 mm, i.d., 5 µm, Prodigy ODS3 reversed-phase C18 (Phenomenex, Torrance, CA, USA) and elution solvents were (A) water/tetrahydrofuran/trifluoroacetic acid (98:2:0.1, v/v/v) and (B) acetonitrile. Solvent gradient elution was carried out according to Pinto, Lajolo

and Genovese (2008). Samples were injected in duplicate. For quercetin derivatives, results were expressed as milligrams of aglycone, and ellagic acid derivatives were expressed as mg of the respective standard. Results were expressed per 100 g of sample DW.

## ***2.12 Ellagitannins and proanthocyanidins profile***

**2.12.1 Sample preparation for HPLC and UPLC analysis.** Freeze-dried powders were extracted with AWA (70:30:0.5, v/v) by soaking over night at 37 °C, followed by centrifugation for 5 min at 3500 × g. The solid residue was reextracted with the same solvent by mixing (vortex) for 2 min and ultrasonic agitation at 37 °C for 10 min. After centrifugation, the supernatants were filtered through Whatman N°1 filter paper and concentrated to remove acetone on a rotary evaporator (Rotavapor RE 120; Büchi, Flawil, Sweden). Samples were subjected to semi-purification using a 3-g Sephadex LH-20 SPE cartridge, which were conditioned by using 50% aqueous acetone, methanol and water. The loaded samples were washed with 30% aqueous methanol and eluted with AWA (70:30:0.5, v/v). The samples were evaporated to dryness and made up to 2 mL with AWA (70:30:0.5, v/v). Each sample was extracted in triplicate.

**2.12.2 Instrumentation and chromatographic conditions for ellagitannins and proanthocyanidins identification.** Ellagitannins were identified according to Gasperotti, Masuero, Vrhovsek, Guella and Mattivi (2010). Separation was carried out with a Waters Acquity UPLC system equipped with a UV-Vis Waters PDA (Waters Corp., Milford, MA) and mass spectrometer with an electrospray ionization system (ESI) and MassLynx Software 4.1 (Waters Corp.). The column was a 150 mm x 2.1 mm i.d., 1.7 µm, end-capped reversed-phase Acquity™ UPLC BEH C18 (Waters). The solvents were (A) 1% formic acid in water and (B) acetonitrila. UPLC-MS analysis was performed in negative mode under the following

conditions: capillary voltage 3 kV, source temperature 100 °C, desolvation temperature 350 °C, desolvation gas flow (N<sub>2</sub>) 650 L/h. The m/z range was 50-2000 Da. Ellagic acid and ellagitannins were identified using UV detection at 260 nm. Results were expressed as mg of ellagic acid equivalents (EAE)/100 g sample DW.

Proanthocyanidins characterization was performed according to Robbins et al. (2009). Samples were analyzed using a HPLC Agilent 1260 Infinity Series system equipped with fluorescence detector. Solvents were (A) acetonitrile/acetic acid (98:2, v/v) and (B) methanol/water/acetic acid (95:3:2, v/v/v). Fluorescence detection was conducted with an excitation wavelength of 230 nm and an emission wavelength of 321 nm. Normal phase separations were performed using Develosil Diol 100 Å (250 x 4.6 mm, 5 µm particle size) purchased from Phenomenex (Torrance, CA, USA). Results were expressed as mg epicatechin equivalent per 100 g of sample DW.

**2.13 Statistical Analysis.** All analyses were run in triplicate and results were expressed as mean ± standard deviation (SD). Initially, the results were checked for homogeneity of variances by using the Levene test, and one-way ANOVA and the least significant difference Fisher test was used to compare the means within group. *P*-values below 0.05 were regarded as significant. All statistical analysis were performed by using the Statistica software package version 11.0 (StatSoft, Inc., Tulsa, OK).

### 3. RESULTS AND DISCUSSION

#### *3.1 Comparison between the ripe fruits of two species of jaboticaba*

Among the Brazilian native fruits largely studied in the recent years, the most popular is by far jaboticaba, whose production and commercialization have raised following the increasing acceptance by the Brazilian population (Abe et al., 2012). Nowadays, Paulista and Sabará are the only two species of commercial importance in the agronomic context, since the others grow as a domestic crop (Lima et al., 2008).

Here, fruits of the two species, grown in a commercial plantation under the same conditions (soil, climate and temperature) were collected and compared in relation to chemical and mineral composition (**Table 1**).

The amount of protein (around 1.0%), fiber (17.9 – 19.3%) and carbohydrates (76.5 – 78.2%) was similar, presenting less than 8% of variation. Lipid content, however, was 27% higher in the Paulista species (0.55%), and ashes were higher in the Sabará species (2.9%).

Protein and lipid contents are in accordance with previous reports (Lima et al., 2008), but higher contents of fiber and carbohydrates were found by the other authors, which is 30% and 12% higher than those values obtained in this work, respectively.

**Table 1.** Chemical and mineral composition of two species of jaboticaba, Paulista (*Myrciaria cauliflora*) and Sabará (*Myrciaria jaboticaba*), in the ripe stage.

| <i>Composition (mg/100 g DW)</i> | <i>Paulista</i>          | <i>Sabará</i>            |
|----------------------------------|--------------------------|--------------------------|
| Protein                          | 1.02 ± 0.01 <sup>a</sup> | 0.94 ± 0.02 <sup>a</sup> |
| Lipid                            | 0.55 ± 0.01 <sup>a</sup> | 0.40 ± 0.01 <sup>b</sup> |
| Ashes                            | 2.30 ± 0.08 <sup>b</sup> | 2.90 ± 0.10 <sup>a</sup> |
| <i>Fibers</i>                    | 17.9 ± 1.0 <sup>a</sup>  | 19.3 ± 0.9 <sup>a</sup>  |
| Soluble                          | 1.80 ± 0.15 <sup>a</sup> | 2.30 ± 0.20 <sup>a</sup> |
| Insoluble                        | 16.1 ± 0.7 <sup>a</sup>  | 17.0 ± 0.8 <sup>a</sup>  |
| Carbohydrates*                   | 78.2 <sup>a</sup>        | 76.5 <sup>a</sup>        |
| <b>Mineral (mg/100 g DW)</b>     |                          |                          |
| Nitrogen (N)                     | 800                      | 660                      |
| Phosphorus (P)                   | 110                      | 100                      |
| Potassium (K)                    | 1320                     | 1000                     |
| Calcium (Ca)                     | 20                       | 20                       |
| Magnesium (Mg)                   | 120                      | 100                      |
| Sulfur (S)                       | 80                       | 70                       |
| Boron (B)                        | 0.7                      | 0.8                      |
| Zinc (Zn)                        | 2                        | 2.9                      |
| Iron (Fe)                        | 2.9                      | 2.7                      |
| Manganese (Mn)                   | 1.8                      | 2.7                      |
| Copper (Cu)                      | 1                        | 1                        |
| Sodium (Na)                      | 0.76                     | 1.03                     |
| Selenium (Se)                    | < LQ                     | < LQ                     |

\*calculated by difference; Means in the same line with common letters are not significantly different ( $p < 0,05$ ). LQ: Limit of quantification for Se = 0.002 mg/kg

The mineral composition of the two species of jaboticaba (Paulista and Sabará) was assessed by determination of nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, zinc, iron, manganese, copper, sodium and selenium (**Table 1**). The two species presented similar contents of minerals. Nitrogenium and potassium contents were higher in the Paulista species, but Sabará showed the highest concentrations of zinc, manganese and

sodium. Among the minerals analyzed, zinc, iron and manganese were the elements in highest levels. The contents of phosphorus, iron, copper and calcium were similar between the samples, and differences were less than 10%. The elements with the greatest discrepancy between the two species were manganese and sodium. Environmental conditions during plant development, such as seasonality, soil type, rainfall, ultraviolet radiation and temperature can influence the mineral composition of fruits and vegetables (Siegler, 1998). Since the both species were grown under the same conditions and collected at the same time, the differences observed between Paulista and Sabará can be attributed to the genetic factors.

Lima, Corrêa, Dantas-Barros, Nelson and Amorim (2011) analyzed the mineral profile of whole fruits of the same species Paulista and Sabará, and potassium was the main element detected. Fruits are usually rich in potassium, which is concentrated especially in the skin. Potassium is an element with high motility in plants, due to its low affinity to chelation, which explains the high concentration in plant tissues. The presence of magnesium (100-120 mg/100 g DW) in jaboticaba fruits is due to the accumulation of this element in the skin, which suggests an association with the concentration of anthocyanins, since magnesium has been related to changes in the color of these pigments. The high concentration of phosphorus, in turn, may be associated with phytin in the seeds, a compound which has a function as a phosphorus reservoir for germination (Goodwin & Mercer, 1983).

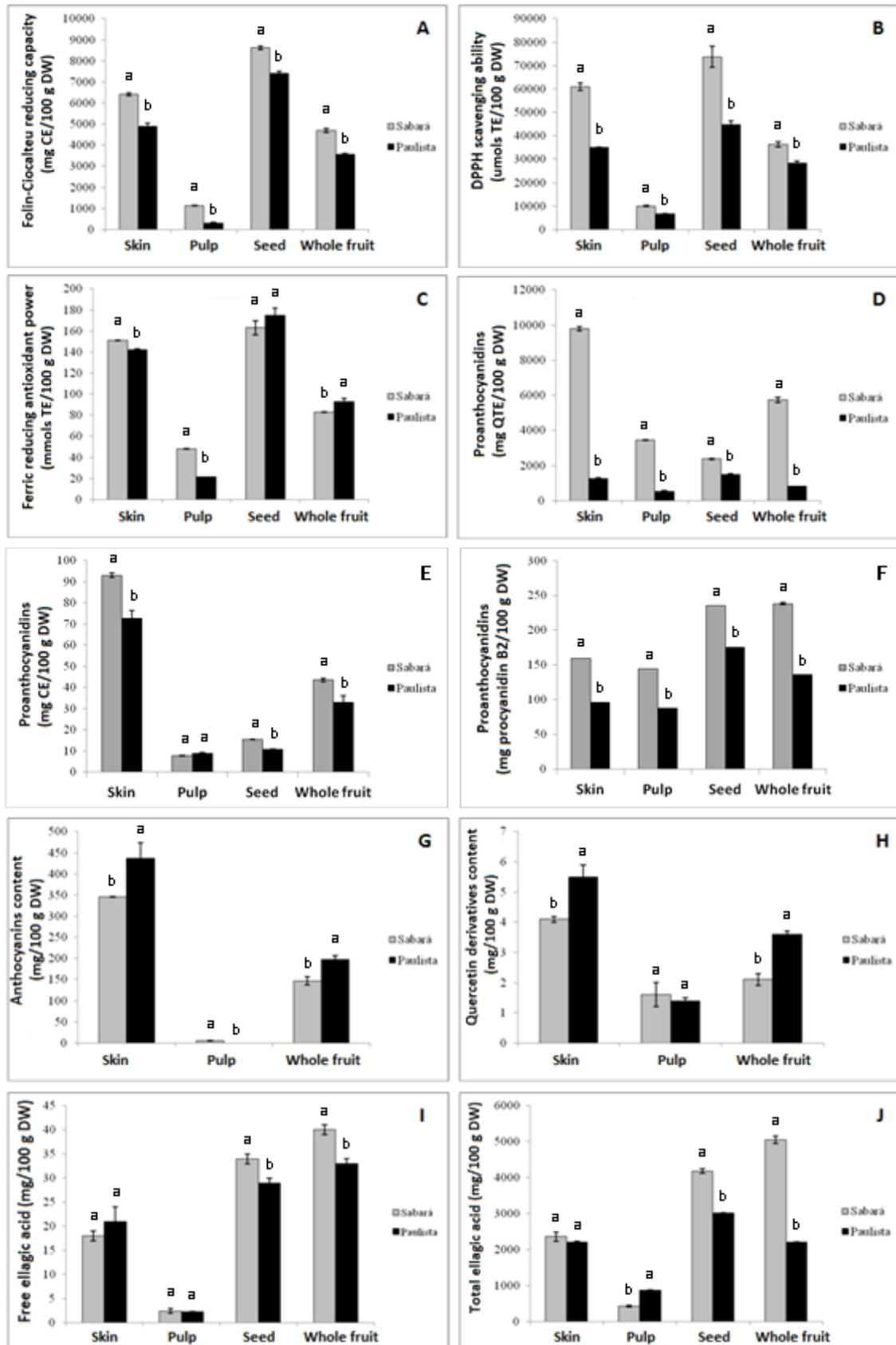
The results presented here show that a serving of 100 g of jaboticaba, approximately 15 units, would provide from 10 to 15% of the recommended daily intake (RDI) of copper, manganese and potassium. Considering the FDA's (Food and Drug Administration's) definition, jaboticaba is a "good source" of these minerals, since one serving contains at least 10% of the RDI.

The two species of jaboticaba in the ripe stage, as well as its fractions skin, pulp and seed were also evaluated in relation to the *in vitro* antioxidant capacity and the phenolic

content and composition (**Figure 1**). Sabará was the species that presented the highest antioxidant capacity, approximately 20% higher than Paulista. Seeds were the most important fraction contributing to the antioxidant properties of jaboticaba, followed by skin and pulp, regardless of the method used (Folin-Ciocalteu reducing capacity, DPPH scavenging ability or Ferric reducing antioxidant power).

Pulp showed the lowest total phenolic content and consequently the lowest antioxidant capacity, which can be associated with several chemical and enzymatic modifications of certain compounds during the ripening process. These alterations include hydrolysis of glycosides by glycosidases, oxidation of phenols by phenoloxidases and polymerization of free phenols. Moreover, the accumulation of soluble phenolics is higher in external plant tissues, such as the skin, compared with the internal parts, like pulp (Robards, Prenzler, Tucker, Swatsitang & Glover, 1999).

Here, the condensed tannins were evaluated by three different methods: the vanillin method (Burns, 1971), the acidified butanol (Porter, Hrstich & Chan, 1986) and the DMAC assay (Prior et al., 2010). The two first are colorimetric methods, and can suffer interferences from sample components, such as anthocyanins, which are measured in the same wavelength used for proanthocyanidins, leading to overestimated results. In this way, the DMAC method developed recently has been considered more appropriate, since proanthocyanidins are quantified at 640 nm. The mechanism of action of the DMAC reagent has not yet been completely understood, although it seems to react with compounds having free hydroxyl groups in a *meta* position and a single bond at the position 2,3 on the C ring (Prior et al., 2010).



**Figure 1.** Comparison between Sabará and Paulista jaboticabas about: (A) Folin Ciocalteu reducing ability, (B) DPPH scavenging ability, (C) Ferric reducing capacity (FRAP), Proanthocyanidin content assessed by (D) Butanol-HCl assay, (E) Vanillin assay, (F) DMAC assay, (G) Anthocyanidin content, (H) Quercetin derivatives content, (I) Free ellagic acid content, (J) Total ellagic acid content. Different letters (a, b) represent significant differences ( $p < 0.05$ ) between the two species Paulista and Sabará.

Independent of the method used for quantification, the proanthocyanidin content was higher in the Sabará jaboticaba. Among fruit fractions, seeds presented the highest amount, followed by skin and pulp (**Figure 1**). Compared to other fruits, the amounts of proanthocyanidins in strawberry, blueberry and cranberry, considered rich-PAC fruits, are about 10 times higher than that of the ripe jaboticaba. Grapes, in turn, had 20 times more proanthocyanidins, since the peel and seeds are rich in these compounds (Wang, Chung, Song & Chun, 2011). Notwithstanding this, jaboticaba can be considered a good source of proanthocyanidins, especially for the Brazilian population, for whom the availability of blueberries and cranberries is limited.

Flavonoids contents were higher in the Paulista jaboticaba, which presented 25% more anthocyanins and 42% more quercetin derivatives than Sabará. These two classes of compounds were concentrated in the skin. A small amount of quercetin derivatives was detected in the pulp. There was no flavonoids in the seeds (**Figure 1**).

The content of anthocyanins observed in the ripe jaboticaba is higher compared to other fruits of the Myrtaceae family, such as camu-camu and pitanga (Abe et al., 2012). In a previous study, cyanidin 3-glucoside and delphinidin 3-glucoside were identified in the skin of the ripe jaboticaba, and the first one was also detected in small amounts in the pulp (Leite et al., 2011; Lima et al., 2011).

Ellagic acid derivatives were detected in high concentrations in both species (**Figure 1**). The content of free ellagic acid found in the skin, pulp and seeds of both species of jaboticaba was significantly low, varying from 2.2 mg/100 g DW (Paulista pulp) to 34 mg/100 g DW (Sabará seeds). The total ellagic acid content of jaboticaba was much higher (56%) in the Paulista than in Sabará species. Among the fruit fractions, pulp presented the lowest content (420-861 mg/100 g DW) and seeds the highest (3016-4180 mg/100 g DW) (**Figure 1**).

Among several Brazilian native fruits, the highest amounts of ellagic acid derivatives were found in the species of the Myrtaceae family. Jaboticaba, grumixama (*Eugenia brasiliensis*) and cambuci (*Campomanesia phaea*) presented the highest contents of free and total ellagic acid, comparable to fruits such as raspberry and blackberry, known as good sources of these compounds (Abe et al., 2012).

### ***3.2 Changes in the chemical composition during ripening of jaboticaba***

Samples of Sabará jaboticaba were manually classified into five different ripening stages, according to size and skin color. Physicochemical analyses and the sugar content and composition are shown in **Table 2**. There was no significant difference in the moisture content of the different ripening stages and the mean value was of approximately 83%. Fruits showed a continuous increase in the diameter along the ripening process, as expected. Ready-to-consume fruits presented an average diameter of 2.4 cm.

Total soluble solids are highly correlated with soluble sugars and organic acids contents, and the concentration of these compounds is one of the most important variables in fruit quality. For jaboticaba, there was an increase in soluble solids along the ripening, as expected. The increase in pH was accompanied by a decrease in titratable acidity, due to a reduction in the content of organic acids during fruit ripening (**Table 2**).

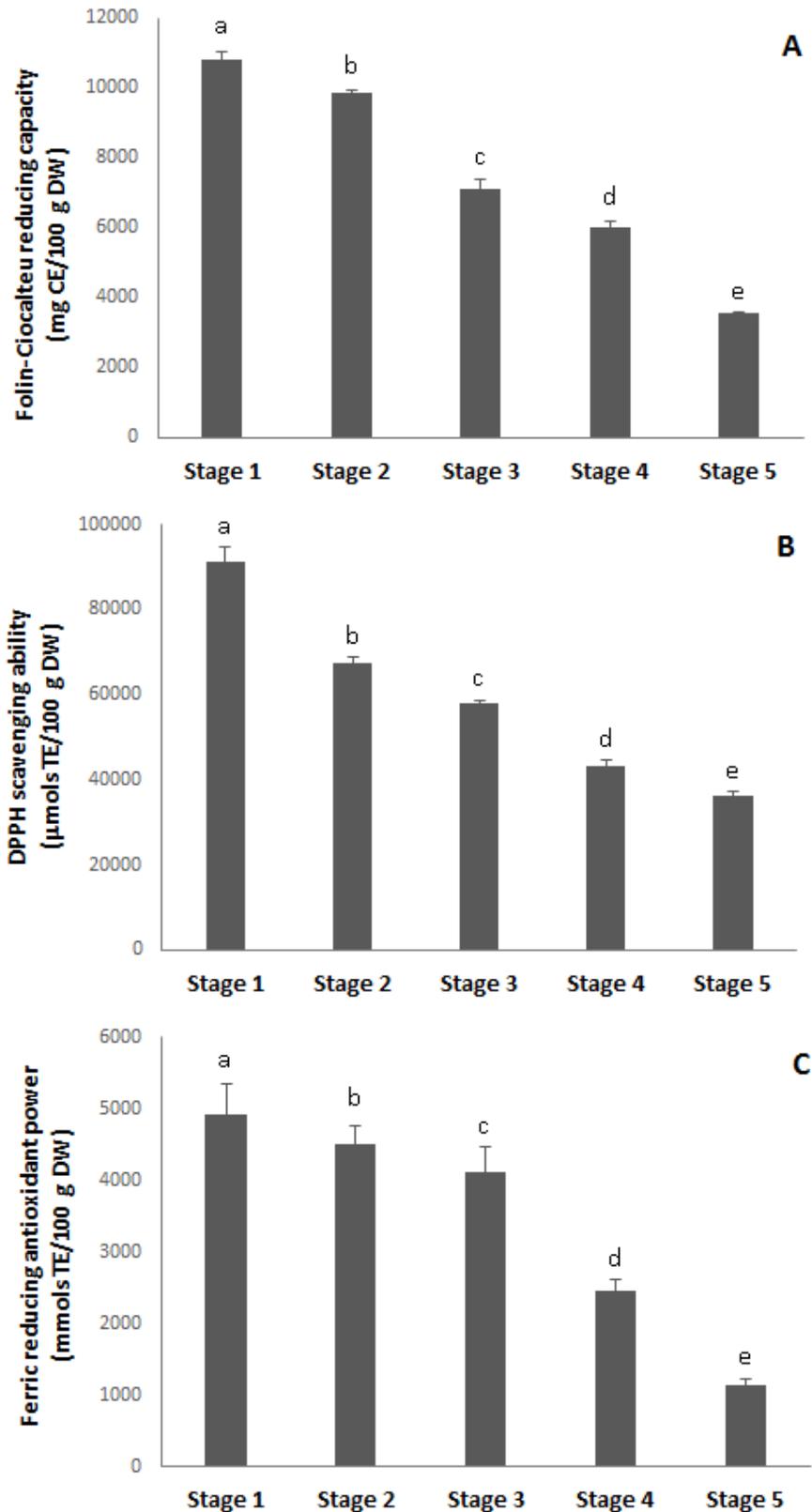
A high amount of sugar was detected in jaboticaba and as expected, the sugar content was higher in the pulp and when ripe. In the ripe fruit, it was detected 3 times more fructose, 10 times more glucose and 18 times more sucrose than in the unripe. A significant sugar content was also observed in the skin, which may be due to the presence of a remaining portion of the pulp adhered to the skin (Lima et al. 2011). Seeds were the portion with the lowest amount of sugar, from 4 to 16 times lower than pulp (**Table 2**).

**Table 2.** Moisture (%), diameter (cm), total soluble solids (°Brix), total titratable acidity (g citric acid/100 g FW), pH, and sugar content (sucrose, glucose and fructose) (g/100 g DW) of Sabará jaboticaba in different ripening stages.

| <i>Physicochemical parameters</i>       |                     |           |           |           |                         |             |             |
|---|---------------------|-----------|-----------|-----------|-------------------------|-------------|-------------|
| Ripening stages                         | Stage 1<br>(unripe) | Stage 2   | Stage 3   | Stage 4   | Stage 5<br>(fully ripe) |             |             |
| Skin colour                             | Green               | Light red | Red       | Dark red  | Dark purple             |             |             |
| Moisture                                | 84                  | 81        | 86        | 83        | 82                      |             |             |
| Diameter                                | 1.6 ± 0.2           | 1.8 ± 0.2 | 1.9 ± 0.2 | 2.0 ± 0.2 | 2.4 ± 0.1               |             |             |
| TSS                                     | 5.6                 | 7.7       | 8.9       | 10.7      | 12.4                    |             |             |
| TTA                                     | 4.73                | 3.52      | 2.10      | 1.74      | 1.39                    |             |             |
| pH                                      | 2.67                | 2.84      | 3.03      | 3.28      | 4.08                    |             |             |
| <i>Sugar content of ripening stages</i> |                     |           |           |           |                         |             |             |
| Sucrose                                 | 0.92                | na        | 6.61      | na        | 16.62                   |             |             |
| Glucose                                 | 1.76                | na        | 9.48      | na        | 16.96                   |             |             |
| Fructose                                | 7.33                | na        | 15.99     | na        | 21.42                   |             |             |
| <i>Sugar content of fruit fractions</i> |                     |           |           |           | <i>Pulp</i>             | <i>Seed</i> | <i>Skin</i> |
| Sucrose                                 | na                  | na        | na        | na        | 11.3                    | 14.5        | 19.1        |
| Glucose                                 | na                  | na        | na        | na        | 18.6                    | 15.9        | 22.9        |
| Fructose                                | na                  | na        | na        | na        | 4.9                     | 1.5         | 1.4         |

na: not analyzed

The antioxidant capacity also varied during ripening, and was assessed by three different methods (**Figure 2**). The Folin-Ciocalteu reducing capacity decreased 67% from the unripe to the ripe fruit. Similarly, there was a reduction of 60% by DPPH scavenging ability and 77% by ferric reducing antioxidant power. A significant positive correlation was found among the three methods used to evaluate antioxidant activity ( $0.90 \leq r \leq 0.95$ ).



**Figure 2.** Antioxidant capacity of jaboticaba assessed by (A) Folin Ciocalteu reducing capacity (mg catechin equivalent/100 g sample DW), (B) DPPH-radical scavenging ability ( $\mu\text{mol}$  Trolox equivalent/100 g sample DW) and (C) Ferric reducing antioxidant power (FRAP) (mmol Trolox equivalent/100 g sample DW) of Sabará jaboticaba in different ripening stages.

Many plant species and compounds isolated from plants have been experimentally studied and used for reducing the postprandial glycemia (Grover, Yavad & Vats, 2002). In order to evaluate the antidiabetic potential of jaboticaba, the *in vitro* assays for inhibitory activity of enzymes involved in carbohydrate metabolism were carried out (**Table 3**). The *in vitro* inhibitory activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase was higher in the ripe fruits, probably due to the highest concentration of anthocyanins in this stage. However, the unripe jaboticaba also exhibited a significant inhibitory activity compared to other fruits, such as tucumã and star fruit (Gonçalves, Lajolo & Genovese, 2010). The phenolic compounds from seeds were the most potent inhibitor of  $\alpha$ -glucosidase, similar to results observed in cambuci and cupuaçu (Gonçalves, Lajolo & Genovese, 2010).

**Table 3.** *In vitro* inhibitory activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase of phenolic fractions obtained from jaboticaba in diferente ripening stages.

| Samples                          | $\alpha$ -amylase                                  |   | $\alpha$ -glucosidase                              |   |
|----------------------------------|--|---|--|---|
|                                  | IC <sub>50</sub><br>(mg sample DW<br>/μL reaction) | IC <sub>50</sub><br>(μg CE <sup>a</sup> /<br>μL reaction) | IC <sub>50</sub><br>(mg sample DW<br>/μL reaction) | IC <sub>50</sub><br>(μg CE <sup>a</sup> /<br>μL reaction) |
| <i>Ripening stages</i>           |  |   |  |   |
| Stage 1                          | 2.9  | 1.2   | 1.7  | 1.3   |
| Stage 2                          | 1.9  | 1.8   | 1.6  | 1.0   |
| Stage 3                          | 1.0  | 0.4   | 1.6  | 0.9   |
| Stage 4                          | 1.3  | 0.6   | 1.8  | 1.6   |
| Stage 5                          | 0.6  | 0.5   | 0.7  | 0.5   |
| <i>Fruit portions of stage 5</i> |  |   |  |   |
| Skin                             | 1.7  | 1.3   | 0.7  | 0.8   |
| Pulp                             | 1.6  | 0.8   | 1.0  | 0.3   |
| Seeds                            | 1.1  | 0.5   | 0.6  | 0.2   |

<sup>a</sup>CE – catechin equivalent

It was previously reported that the most potent inhibitors of  $\alpha$ -glucosidase were myricetin, epigallocatechin gallate, cyanidin, among 16 compounds belonging to six different groups: flavone, flavonol, flavanone, isoflavone, flavan-3-ol and anthocyanidin (Tadera, Minami, Takamatsu, & Matsuoka (2006). Therefore, anthocyanins present in the skin of jaboticaba seem to have an important role in the inhibitory activity of the whole fruit.

Quercetin and anthocyanidin derivatives were the main flavonoids present in jaboticaba, but they were detected only in skin and pulp, and not in seeds (**Table 4**). Ripening caused an anthocyanin accumulation in the skin of the fruit, with a gradual increase in the anthocyanin content of the whole fruit, from 5 to 147 mg/100 g of sample DW, comparing the unripe with the ripe fruit. Regarding quercetin derivatives, they were not detected in the unripe fruit. From the second stage (light red), however, 2 mg/100 g of sample (DW) were found and this value did not change during maturation.

**Table 4.** Flavonoids, free and total ellagic acid content (mg/100 g sample DW) of Sabará jaboticaba in different ripening stages.

| Compounds             | Ripening stages         |                         |                         |                          |                         |
|-----------------------|-------------------------|-------------------------|-------------------------|--------------------------|-------------------------|
|                       | Stage 1                 | Stage 2                 | Stage 3                 | Stage 4                  | Stage 5                 |
| Free ellagic acid     | 19 ± 2 <sup>c</sup>     | 31 ± 3 <sup>b</sup>     | 31 ± 3 <sup>b</sup>     | 39 ± 2 <sup>a</sup>      | 40 ± 1 <sup>a</sup>     |
| Total ellagic acid    | 9566 ± 812 <sup>a</sup> | 8327 ± 129 <sup>b</sup> | 6363 ± 629 <sup>c</sup> | 6810 ± 606 <sup>c</sup>  | 5050 ± 99 <sup>d</sup>  |
| Anthocyanins          | 4.9 ± 0.3 <sup>e</sup>  | 44 ± 1 <sup>d</sup>     | 65 ± 1 <sup>c</sup>     | 74 ± 3 <sup>b</sup>      | 147 ± 10 <sup>a</sup>   |
| <i>Delphinidin</i>    | na                      | na                      | 7.1 ± 0.2 <sup>b</sup>  | na                       | 23.5 ± 0.8 <sup>a</sup> |
| <i>Cyanidin</i>       | na                      | na                      | 58 ± 2 <sup>b</sup>     | na                       | 123 ± 2 <sup>a</sup>    |
| Quercetin derivatives | nd                      | 2.0 ± 0.1 <sup>a</sup>  | 2.1 ± 0.1 <sup>a</sup>  | 2.00 ± 0.05 <sup>a</sup> | 2.1 ± 0.2 <sup>a</sup>  |

nd: not detected; na: not analyzed

Similarly, there was an increase in the anthocyanidins derivatives content during ripening of grapes, while other classes of polyphenols exhibited a pattern of accumulation and subsequent decline, which suggest a degradation or the utilization as a substrate in the biosynthesis of other compounds (Adams, 2006).

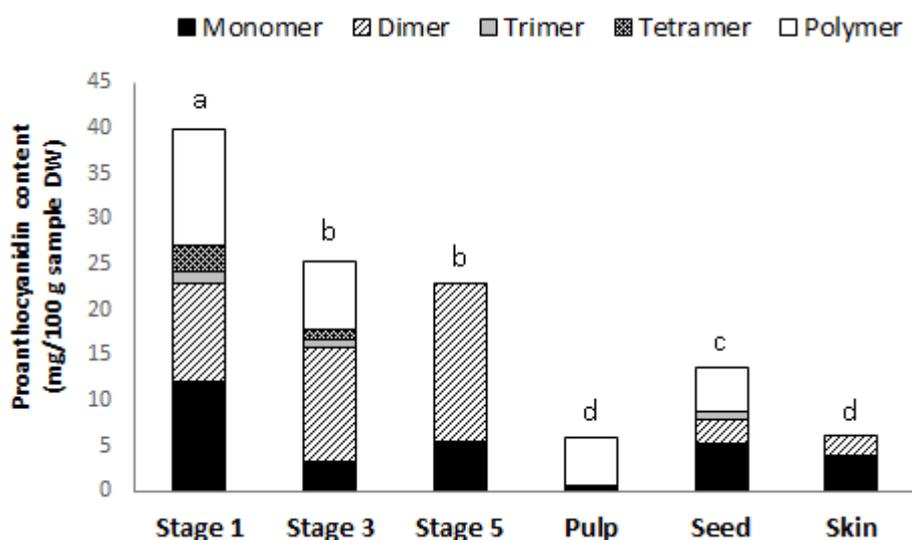
Total ellagic acid decreased as jaboticaba ripened. The unripe fruit presented 9566 mg of total ellagic acid/100 g of sample DW and 5050 mg/100 g DW were detected in the ripe fruit, which indicates a decrease of 50% in the content of total ellagic acid during ripening process (**Table 4**).

The same behavior was observed in strawberry, when five cultivars in three different ripening stages were analyzed in relation to the total ellagic acid content. In the unripe stage, amounts between 8.8 and 17.8 mg/100 g fresh weight (FW) were detected, in the intermediate stage values varied from 4 to 9.4 mg/100 g FW and, from 1.6 to 4.5 mg/100 g FW in the ripe stage. Ripening caused a decrease in the total ellagic acid content in all cultivars analyzed (Williner, Pirovani & Güemes, 2003). Ellagic acid derivatives were the main compounds found in the unripe strawberries, while pelagordin 3-glucoside, an anthocyanin derivative, was the main compound present in the ripe fruits (Kosar, Kafkas, Paydas & Baser, 2004).

Proanthocyanidins contribute significantly to total polyphenols intake in the Western diet, due to their wide distribution among different plant species (Santos-Buelga & Scalbert, 2000). However, there are few data available in the scientific literature on the qualitative and quantitative profile of these compounds in foods, which is mainly due to the difficulty in finding an appropriate method to characterize them.

Generally, tannins present in fruits in the non-polymerized form have tannic properties more accentuated. During ripening, there is a polymerization of tannins and a reduction of astringency (Clifford, 1997), which improves the sensory attributes of fruits.

In jaboticaba, the proanthocyanidins content was approximately 40% higher in the unripe jaboticaba compared to the ripe fruit. Seeds presented the highest amount of proanthocyanidins, 55% higher than pulp and skin. Degree of polymerization changed during ripening, and among ripe fruit fractions. In the pulp and seeds about 90% and 35% of proanthocyanidins were polymers, not detected in the skin (**Figure 3**).



**Figure 3.** Total content and profile (mg/100 g sample DW) of proanthocyanidins identified in jaboticaba in different ripening stages and the fractions pulp, seed and skin of the ripe fruit.

**Table 5** shows the compounds identified as ellagic acid and ellagitannins in jaboticaba at different ripening stages, as well as in the fractions pulp, skin and seed of the ripe fruit. Sanguin H-6 was identified abundantly in jaboticaba, mainly in the unripe fruits (161.5 mg EAE/100 g sample DW) and in seeds (342 mg EAE/100 g sample DW) of the ripe fruit. Sanguin H-6 and lambertianin C are ellagitannins present in high amount in strawberry and raspberry (Mullen et al., 2002). Sanguin H-10, also identified in the samples, has a structure similar to sanguin H-6, but with one less ellagic acid. A previous study detected sanguin H-

10 and sanguin H-3 in *Sanguisorba officinalis*, which belongs to Rosaceae family (Tanaka, Nonaka & Nishioka, 1985).

The ellagitannins composition identified in this work did not reproduce the profile described previously (Reynertson et al., 2006; Wu et al. 2012). Here, 14 compounds were identified as ellagic acid derivatives, while only three were detected in these two anterior studies, along with other eight compounds identified as gallic acid derivatives. However, the fruits of jaboticaba analyzed in the other studies were collected from the Botanical Garden in the United States. We believe that those samples may not be representative of the species, and our fruits were obtained from the main producer of jaboticaba in Brazil.

Quantification and structural characterization of ellagitannins and ellagic acid derivatives in foods, such as the Brazilian native fruits, are starting points for the study of biological effects, since these compounds have been associated with several healthy properties, as antioxidant, antiviral and anticancer (Gasperotti et al., 2010).

**Table 5.** Ellagitannins profile of jaboticaba during ripening and fractions pulp, seed and skin of the ripe fruit (mg ellagic acid/100 g sample DW).

| Compound                                   | Pulp | Seed  | Skin | Stage 1 | Stage 3 | Stage 5 | Retention time (min) |                     |
|--|------|-------|------|---------|---------|---------|----------------------|---------------------|
| Ellagic acid                               | 9.4  | nd    | 21.2 | 26.0    | 15.4    | 21.9    | 7.7                  | 603; 449; 301       |
| Sanguiin H-10 isomer (1)                   | 1.6  | 12.6  | 19.5 | 61.8    | 24.0    | 9.3     | 3.7                  | 783; 649            |
| Sanguiin H-10 isomer (2)                   | 4.8  | 35.6  | 35.0 | 96.0    | 77.0    | 17.2    | 3.9                  | 783; 649            |
| Sanguiin H-10 isomer (3)                   | 5.4  | 38.6  | 32.3 | 75.8    | 42.0    | 17.9    | 5.0                  | 783; 649            |
| Lambertianin C without ellagic acid moiety | 1.8  | 2.8   | 10.4 | 24.7    | 21.0    | 6.9     | 8.0                  | 1250                |
| Sanguiin H-6 (1)                           | 1.8  | 175.0 | nd   | 84.8    | 37.0    | 25.7    | 3.4                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (2)                           | 6.6  | 145.0 | 3.6  | nd      | 36.0    | 24.5    | 4.4                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (3)                           | nd   | 5.2   | 3.4  | 18.9    | 12.0    | 2.2     | 5.7                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (4)                           | 2.8  | 3.0   | 8.9  | 13.7    | 15.5    | 4.8     | 6.8                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (5)                           | nd   | 4.6   | 1.9  | 9.1     | 4.7     | 1.0     | 7.0                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (6)                           | 2.6  | 4.6   | 8.8  | 19.3    | 15.8    | 4.8     | 7.2                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (7)                           | nd   | 4.6   | 2.2  | 8.0     | 5.6     | 1.0     | 8.0                  | 934; 858; 1235; 469 |
| Sanguiin H-6 (8)                           | nd   | nd    | nd   | 7.7     | 5.5     | 1.2     | 8.6                  | 934; 858; 1235; 469 |
| Unknown ellagitannin                       | 2.2  | 7.5   | 96.4 | nd      | nd      | nd      | 8.8                  | 505; 1011           |

nd : not detected

#### 4. CONCLUSIONS

This work reported, for the first time, a complete and detailed characterization of two commercially important species of jaboticaba, as well as its fractions and ripening stages. The two species of jaboticaba presented a similar centesimal and mineral composition, being important sources of K, Mn and Cu. The Paulista jaboticaba presented the highest contents of quercetin derivatives and anthocyanins. However, the major polyphenols of jaboticaba were the ellagic acid derivatives, and these compounds were detected in higher amounts in the Sabará jaboticaba. Skin was the portion of fruit with the highest content of flavonoids, but the seeds presented the highest amount of ellagic acid derivatives. The unripe fruit showed the highest antioxidant capacity and phenolic contents, and ripening led to a decrease in all these compounds. Ready-to-consume fruits were shown to be very rich sources of polyphenols, especially anthocyanins, and an important source of ellagitannins, comparable to the berries. Phenolic compounds from jaboticaba strongly inhibited the *in vitro* activity of enzymes involved in carbohydrate metabolism. This study showed that jaboticaba is an excellent dietary source of polyphenols and that the most commercially relevant species is also the richest phenolic source. Altogether, these results showed the importance of eating the whole jaboticaba, without discarding the skin or seeds, in order to take advantages of all the compounds present in different portions of the fruit.

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## REFERENCES

- Abe, L. T., Lajolo, F. M., & Genovese, M. I. (2012). Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg). *Journal of the Science of Food and Agriculture*, *92*, 1679-87.
- Adams, D. O. (2006). Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture*, *57*, 249-256.
- AOAC. (2005). *Official Methods of Analysis*. (18<sup>th</sup> ed.). Washington: Association of Official Analytical Chemists.
- Arabbi, P. R., Genovese, M. I., & Lajolo, F. M. (2004). Flavonoids in vegetable foods commonly consumed in Brazil. *Journal of Agricultural and Food Chemistry*, *52*, 1124-1131.
- Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of “Antioxidant Power”: The FRAP Assay. *Analytical Biochemistry*, *239*, 70-76.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, *28*, 25–30.
- Burns, R. E. (1971). Method for estimation of tannin in grain sorghum. *Agronomy Journal*, *63*, 511-512.
- Clifford, M. N. (1997). Astringency. In: TOMÁS-BARBERÁN, F. A.; ROBINS, R. J. *Phytochemistry of fruit and vegetables* (pp. 87-107). Oxford: Clarendon Press.
- Clifford, M. N., & Scalbert, A. (2000). Ellagitannins - nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, *80*, 1118-1125.

Donadio, L. C. (2000). *Jaboticaba (Myrciaria jaboticaba (Vell.) Berg)*. Jaboticabal: FUNEP, 55 pp.

Gasperotti, M., Masuero, D., Vrhovsek, U., Guella, G., & Mattivi, F. (2010). Profiling and accurate quantification of Rubus ellagitannins and ellagic acid conjugates using direct UPLC-Q-TOF HDMS and HPLC-DAD analysis. *Journal of Agricultural and Food Chemistry*, 58, 4602-4616.

Genovese, M. I., Pinto, M. S., Gonçalves, A. E. S. S., & Lajolo, F. M. (2008). Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. *Food Science and Technology International*, 14, 207-214.

Gonçalves, A. E. S. S., Lajolo, F. M., & Genovese, M. I. (2010). Chemical Composition and Antioxidant/Antidiabetic Potential of Brazilian Native Fruits and Commercial Frozen Pulps. *Journal of Agricultural and Food Chemistry*, 58, 4666-4674.

Goodwin, T. W., & Mercer, E. I. (1983). *Introduction to plant biochemistry*. Pergamon: Oxford. 677 p.

Kosar, M., Kafkas, E., Paydas, S., & Baser, K. H. C. (2004). Phenolic composition of strawberry genotypes at different maturation stages. *Journal of Agricultural and Food Chemistry*, 52, 1586-1589.

Leite, A. V., Malta, L. G., Riccio, M. F., Eberlin, M. N., Pastore, G. M., & Maróstica Júnior, M. R. (2011). Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticaba* Vell Berg). *Journal of Agricultural and Food Chemistry*, 59, 2277-2283.

Lima, A. J. B., Corrêa, A. D., Alves, A. P. C., Abreu, C. M. P., & Dantas-Barros, A. M. (2008). Caracterização química do fruto jaboticaba (*Myrciaria cauliflora* Berg) e de suas frações. *Archivos Latinoamericanos de Nutrición*, 58, 416-421.

- Lima, A. J. B., Corrêa, A. D., Dantas-Barros, A. M., Nelson, D. L., & Amorim, A. C. L. (2011). Sugars, organic acids, minerals and lipids in jaboticaba. *Revista Brasileira de Fruticultura*, *33*, 540-550.
- Mullen, W., McGinn, J., Lean, M. E., Maclean, M. R., Gardner, P., Duthie, G. G., Yokota, T., & Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *Journal of Agricultural and Food Chemistry*, *50*, 5191-5196.
- Pinto, M. S., Lajolo, F. M., & Genovese, M. I. (2008). Bioactive compounds and quantification of total ellagic acid in strawberries (*Fragaria x ananassa* Duch.). *Food Chemistry*, *107*, 1629-1635.
- Porter, L. J., Hrstich, L. N., & Chan, B. G. (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, *25*, 223-230.
- Prior, R. L., Fan, E., Ji, H., Howell, A., Nio, C., Payne, M. J., & Reed, J. (2010). Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *Journal of the Science of Food and Agriculture*, *90*, 1473–1478.
- Reynertson, K. A., Wallace, A. M., Adachi, S., Gil, R. R., Yang, H., Basile, M. J., D'Armiento, J., Weinstein, I. B., & Kennelly, E. J. (2006). Bioactive depsides and anthocyanins from jaboticaba (*Myrciaria cauliflora*). *Journal of Natural Product*, *69*, 1228-1230.
- Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F., & Hirota, M. M. (2009). Brazilian Atlantic forest: how much is left and how is the remaining forest distributed? Implications for conservation. *Biological Conservation*, *142*, 1141–1153.
- Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, *66*, 401-436.

Robbins, R. J., Leonczak, J., Johnson, J. C., Li, J., Kwik-Urbe, C., Prior, R. L., & Gu, L. (2009). Method performance and multi-laboratory assessment of a normal phase high pressure liquid chromatography–fluorescence detection method for the quantitation of flavanols and procyanidins in cocoa and chocolate containing samples. *Journal of Chromatography A*, *1216*, 4831-4840.

Santos-Buelga, C., & Scalbert, A. (2000). Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture*, *80*, 1094-1117.

Seigler, D. S. (1998). *Plant secondary metabolism*. Dordrecht: Kluwer Academic. 759p.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178.

Tadera, K., Minami, Y., Takamatsu, K., & Matsuoka, T. (2006). Inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase by flavonoids. *Journal of Nutritional Science and Vitaminology*, *52*, 149-153.

Tanaka, T., Nonaka, G., & Nishioka, I. (1985). Tannins and related compounds. Part 28. Revision of the structures of sanguins H-6, H-2 and H-3, and isolation and characterization of sanguin H-11, a novel tetrameric hydrolysable tannin, and seven related tannins, from *Sanguisorba officinalis*. *Journal of Chemical Research*, 176-177.

Wang, Y.; Chung, S.-J.; Song, W. O., & Chun, O. K. (2011). Estimation of daily proanthocyanidins intake and major food sources in the U.S. Diet. *The Journal of Nutrition*, *141*, 447-452.

Williner, M. R., Pirovani, M.E., & Güemes, D.R. (2003). Ellagic acid content in strawberries of different cultivars and ripening stages. *Journal of the Science and Food and Agriculture*, *83*, 842-845.

Wu, S. B., Dastmalchi, K., Long, C., & Kennelly, E. J. (2012). Metabolite profiling of jaboticaba (*Myrciaria cauliflora*) and other dark-colored fruit juices. *Journal of Agricultural and Food Chemistry*, *60*, 7513–7525.

Wu, X., & Prior, R. L. (2005). Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *Journal of Agricultural and Food Chemistry*, *53*, 2589-2599.

***In vitro* and *in vivo* evaluation of the metabolism of polyphenols from jaboticaba  
(*Myrciaria jaboticaba* (Vell.) Berg), a Brazilian native fruit**

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## ABSTRACT

Ellagic acid derivatives are the main polyphenols found in jaboticaba (*Myrciaria jaboticaba* Vell. Berg), a Brazilian native fruit. Those compounds are known due to their cancer chemopreventive, cardioprotective and antioxidant potential. However, little is known about their metabolites, which are the biologically active compounds indeed. Here, the aim was to investigate the metabolism of different classes of polyphenols, especially ellagitannins and ellagic acid derivatives, from jaboticaba in both *in vitro* and *in vivo* assays. The metabolites formed from the jaboticaba polyphenols were identified in an *in vitro* fermentation model using human feces. In addition, the fate of a wide variety of metabolites was monitored after intragastric administration of jaboticaba extract (15 min – 8 h) in Wistar rats, using an UPLC-MS. The *in vitro* experiment showed that the ellagic acid derivatives were metabolized by the intestinal microbiota and degraded under testing conditions. Two compounds were identified after fermentation with fecal inoculum, *p*-hydroxybenzoic and *p*-hydroxyphenylacetic acids. *In vivo*, thirty eight metabolites were identified in plasma, stomach, liver, kidneys, brain, muscle and colon, and most of them were formed from ellagic acid derivatives.

Keywords : Bioavailability / Ellagitannins / Jaboticaba

## 1. Introduction

Jaboticaba, *Myrciaria jaboticaba* (Vell.) Berg, is a grape-like in appearance and texture and it is by far the best-known and the most consumed Brazilian native fruit. It is from the Atlantic Rainforest and belongs to the Myrtaceae family. There is a continuously growing interest in its production and commercialization, due to the sweet and slightly acidic flavor of the pulp, which encourages its consumption *in natura* and as ingredient in food products, such as jam, juice and liqueur (Donadio, 2000).

Jaboticaba is a rich source of phenolic compounds, which include quercetin derivatives, proanthocyanidins and anthocyanins, that are concentrated in the purple to black skin, when the fruit is ready-to-eat. In addition, recently it was demonstrated that ellagic acid derivatives, such as ellagitannins, are the main compounds found in jaboticaba, being sanguin H-10, sanguin H-6 and lambertianin C the most abundant (Alejandro, Dubé, Desjardins, Lajolo & Genovese, 2013).

Chronic ingestion of phenolic compounds from fruits has been associated with beneficial effects on human health, related to their antioxidant, cancer chemopreventive, anti-inflammatory and cardioprotective properties (Mullen et al., 2002; Priyadarsini, Khopde, Kumar & Mohan, 2002; Youdim, McDonald, Kalt, & Joseph, 2002). However, a chemical compound must be further metabolized to exert its biological activity, being able to achieve the physiological target in a minimum concentration needed to promote the biological effects (Oliveira & Bastos, 2011).

Only a small portion of phenolic compounds ingested is absorbed by the small intestine. This process can occur through passive diffusion and be associated with hydrolysis and release of the aglycone by the action of an enzyme, such as lactase phloridzin hydrolase (LPH), present in the microvilli of intestinal epithelial cells (Donovan, Manach, Faulks, &

Kroon, 2006). Once absorbed, the aglycone is metabolized in the liver, forming sulfated, glucuronidated and/or methylated metabolites by the action of the respective phase II enzymes sulfotransferase (SULT), uridine diphosphate-50-glicuronosiltransferase (UGT) and catechol-O-methyltransferase (COMT) (Crozier, Del Rio, & Clifford, 2010).

The products of this metabolism may entry into the bloodstream and be excreted in the urine, or a considerable fraction can be excreted by the liver into bile followed by entry into the small intestine, through enterohepatic circulation (McBain & Macfarlane, 1997). Once released into the intestinal lumen, these conjugates can be hydrolyzed by bacterial enzymes, such as  $\beta$ -glucuronidases, sulfatases and glycosidases (Crozier, Del Rio, & Clifford, 2010; Selma, Espín, & Thomás Barberán, 2009). Compounds that are not absorbed in the small intestine pass directly to the large intestine, where they are degraded by the colonic microbiota to simpler compounds, such as phenolic acids, and absorbed into the circulatory system (Del Rio, Costa, Lean, & Crozier, 2010).

Several studies have demonstrated that most of the phenolic compounds are metabolized in the colon by the microbiota intestinal before absorption, and this conversion is essential for modulating their physiological actions. It is clear that these metabolites are the main responsible for biological effects observed *in vivo* (Del Rio, Costa, Lean, & Crozier, 2010; Setchell, Brown, & Lydeking-Olsen, 2002; Xu, Harris, Wang, Murphy, & Hendrich, 1995).

However, nothing is known about the metabolites profile formed from the jaboticaba polyphenols, as well as the distribution in different target tissues. These points are important in order to elucidate the biological activity of these compounds. This study aimed to evaluate the bioavailability of jaboticaba polyphenols in an *in vitro* fermentation model using human feces and an *in vivo* assay in Wistar rats.

## 2. Material and methods

**2.1. Chemicals.** Resazurin, tryptone, MOPS, Na<sub>2</sub>EDTA, sodium dithionite, ascorbic acid, ellagic acid, quercetin, (+)-catechin, and the Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St Louis, MO, USA). Ketamine chloride and xylydine chloride were acquired from Bayer (Leverkusen, Germany). The anthocyanidins cyanidin and delphinidin and the respective 3-glucosides, as well as procyanidin B2 were obtained from Extrasynthèse (Genay, France). All chemicals/solvents were of analytical or HPLC grade, according to the requirement, and obtained from Merck (Darmstadt, Germany).

**2.2. Samples.** Fully ripened jaboticaba Sabará (*Myrciaria jaboticaba* Vell. Berg) was kindly provided by a local producer at the São Paulo Central Market (Companhia de Entrepósitos e Armazéns Gerais de São Paulo – CEAGESP, Brazil). Fruits were cleaned and immediately freeze-dried and stored at -20 °C. Then, the dried fruits were ground to a fine powder in a mortar and pestle, using liquid nitrogen to keep sample frozen.

**2.3. Procedure for jaboticaba raw extract preparation.** Extraction was performed according to Arabbi, Genovese and Lajolo (2004) with some modifications. Freeze-dried powder was extracted three times in a solvent mixture comprising methanol/water/acetic acid (70:30:0.5, v/v/v), using a Brinkmann homogenizer (Polytron-Kinematica GmbH, Kriens-Luzern, Sweden), at moderate speed for 1 min, while cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman N<sup>o</sup> 1). The extracts obtained were concentrated until methanol elimination on a rotary evaporator (Rotavapor RE 120; Büchi, Flävil, Sweden) at 40 °C and redissolved in water. The same procedure was used to prepare extracts for the both *in vivo* and *in vitro* experiments, as well as for characterization analyses.

## 2.4. Characterization analyses

2.4.1. *Total phenolics content.* The determination of the total phenolic content was performed using the Folin-Ciocalteu reagent, according to Singleton, Orthofer & Lamuela-Raventós (1999), with some modifications. Catechin was used as the reference standard, and the results were expressed as mg of catechin equivalents (CE)/100 g of sample dry weight (DW).

2.4.2. *Proanthocyanidin content.* Total proanthocyanidins in jaboticaba raw extract were quantified using DMAC (4-dimethylaminocinnamaldehyde) method, according to Prior et al. (2010). Total amount was calculated using commercially available procyanidin B2 dimer as a standard, and the calibration curve was in the range of 5-50 µg/mL.

2.4.3. *Total ellagic acid content.* Total ellagic acid was determined after extraction and acid hydrolysis according to Pinto, Lajolo and Genovese (2008). An aliquot (2 mL) of the raw extracts was dried under nitrogen, 2 N trifluoroacetic acid was added, and the hydrolysis was performed at 120 °C for 90 min. The hydrolyzed samples were evaporated under nitrogen, redissolved in methanol and filtered for HPLC analysis.

2.4.4. *Flavonoids composition.* Identification and quantification of flavonoids, free and total ellagic acid were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector controlled by the Chemstation software. The column used was 250 × 4.6 mm, i.d., 5 µm, Prodigy ODS3 reversed-phase C18 (Phenomenex, Torrance, CA, USA) and elution solvents were (A) water/tetrahydrofuran/trifluoroacetic acid (98:2:0.1, v/v/v) and (B) acetonitrile. Solvent gradient elution was carried out according to Pinto, Lajolo and Genovese (2008). Samples were injected in duplicate. For quercetin derivatives, results were expressed as milligrams of

aglycone. Anthocyanins and ellagic acid derivatives were expressed as mg of the respective standard. Results were expressed per 100 g of sample DW.

## ***2.5. In vitro fermentation experiment***

*2.5.1. Subjects and experiment design.* The *in vitro* fermentation model was based on fecal incubation with conditions designed to simulate events taking place in the human colon, according to Jaganath, Mullen, Lean, Edwards and Crozier (2009). Feces were collected from five subjects, that were required to avoid all alcohol and food rich in polyphenols 48 h before fecal collection. The volunteers were nonsmokers, age between 22 and 27, and had not consumed antibiotics for at least 3 months before the study.

*2.5.2. Fermentation medium.* The fermentation medium was prepared by mixing 2 g of tryptone in 400 mL of distilled water and 100  $\mu$ L of micromineral solution (consisting of 13.2 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 10.0 g of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 1.0 g  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , and distilled water up to 100 mL). This solution was agitated to dissolve the chemicals and then 200 mL of buffer solution (2 g of  $\text{NH}_3 \cdot \text{CO}_3$ , 17.5 g of  $\text{Na}_2 \cdot 2\text{CO}_3$ , and 500 mL of distilled water), 200 mL of macromineral solution (2.85 g of  $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$ , 3.1 g of  $\text{KH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ , 0.3 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 500 mL of distilled water), and 1 mL of 0.1% (w/v) resazurin solution (a redox indicator) were added. This medium was adjusted to pH 7 using HCl, after which it was sterilized at 121 °C for 15 min. This also removed oxygen. Reducing solution (312.5 mg of cysteine hydrochloride, 2 mL of 1 M NaOH, 312.5 mg of sodium sulfide, and 47.5 mL distilled water) was added at 0.5 mL per 10 mL of medium after the solution was purged with oxygen-free nitrogen (OFN) until anaerobic conditions were achieved as indicated by a color change from pale indigo to colorless.

*2.5.3. In vitro fermentation.* Fresh feces from five volunteers were mixed and 6.4 g of fecal sample was homogenized with 20 mL of phosphate buffer to obtain a 32% fecal slurry. Five milliliters of the slurry was added to 44 mL of the prereduced fermentation medium and 1 mL of jaboticaba raw extract (prepared as described in 2.3.). After the substrate was added, the samples were purged with OFN and then, placed in a shaking water bath and incubated at 37 °C for 48 h. Aliquots of the fermented fecal samples (3 mL) were collected after 0, 2, 4, 6, 24, 30, and 48 h and stored immediately at -80°C.

*2.5.4. Extraction of fecal incubates.* Samples of fecal slurry (250 µL) were extracted twice with 500 µL MeOH in 1% formic acid containing 20 mM sodium diethyldithiocarbamate. Samples were centrifuged at 16,000 g for 10 min and supernatants were combined and reduced to dryness under nitrogen flow. Extracts were resuspended in MeOH in 1% formic acid and then analyzed by HPLC-DAD.

*2.5.5. HPLC-DAD analysis.* Samples were analyzed by HPLC-DAD and compounds were identified based on retention time and spectral library. The chromatographic conditions were the same described previously in 2.4.4.

## ***2.6 In vivo bioavailability experiment***

*2.6.1. Animals and experimental design.* The Faculty of Pharmaceutical Sciences/USP Ethical Committee for Animal Research approved all the adopted procedures (Protocol CEUA/FCF/USP no. 355). Fifty male Wistar rats (200 ± 10 g) were obtained from Animal House of Faculty of Pharmaceutical Sciences and Chemistry Institute of University of São Paulo. Animals were kept under standard laboratory conditions of temperature (23 ± 2 °C),

relative humidity ( $50 \pm 5\%$ ), 12 h light-dark cycle. Chow diet and water were provided *ad libitum*. Initially, animals were divided into two groups:

- *Control*: animals receiving water by gavage during 30 days;
- *Jaboticaba*: animals receiving 2.0 g/kg body weight of jaboticaba extract during 30 days;

In the euthanasia day, animals fasted overnight received water or jaboticaba raw extract (2 g/kg body weight) by intragastric administration, according to the group. Then, animals were euthanized after 15, 45 min, 1 h 30 min, 2 h 15 min, 3 h, 4 h, 5 h, 6 h and 8 h after gavage. Animals were anesthetized with ketamine chloride and xylidine chloride. Blood was collected by cardiac puncture into tubes containing EDTA. The plasma was separated by centrifugation at 2,000 g for 10 min at 4 °C. The tissues (liver, kidneys, stomach, gastrocnemius muscle, brain and colon) were removed, weighed and immediately frozen under liquid nitrogen and stored at -80 °C for further biochemical analysis.

#### 2.6.2. Processing of biological samples

**Plasma extraction.** Plasma samples were extracted according to the method described by Espín, González-Barrio, Cerdá, López-Bote, Rey and Tomás-Barberán (2007) with some modifications. Aliquots of plasma were mixed with 4% phosphoric acid (1:1 v/v), and then added in a solid phase cartridge Oasis HLB (Waters, Milford, Massachussets, USA), previously conditioned with methanol and 0.2% acetic acid in water. After washing twice with water and 0.2% acetic acid in water, compounds were eluted with acetone/water/acetic acid (70:29.5:0.5 v/v/v), filtered through 0.22 µm PVDF membrane syringe filters (Millipore Ltd., Bedford, MA) and analyzed by UPLC-MS.

**Tissues extraction.** Tissues were extracted according to the method described by Oliveira, Pinto, Sampaio, Yonekura, Catharino and Bastos (2013). The lyophilized samples were mixed with 0.2% formic acid in methanol and 0.3 M sodium dithionite/0.1% (w/v) Na<sub>2</sub>EDTA. The homogenate was centrifuged at 5,000 g for 10 min at 4 °C. The supernatant was collected on a tube containing aqueous ascorbic acid (10 mg/mL). The residue from centrifugation was reextracted with the same extractor solutions, and then, centrifuged under the same conditions cited previously. The combined supernatants were partially vacuum evaporated for 40 min at 40 °C on a CentriVap concentrator (Labconco, Kansas City, USA). The collector tube was washed with 625 mM MOPS buffer (pH 6.8) and this solution was mixed with the concentrated supernatant. The samples were added in a solid phase cartridge Oasis HLB (Waters, Milford, Massachusetts, USA), previously conditioned with methanol and 0.2% formic acid in water. After washing twice with 0.2% acetic acid in water, compounds were eluted with 0.2% formic acid in methanol. The eluate was vacuum evaporated at 40 °C until dryness, reconstituted with water/0.1% formic acid in acetonitrile (94:6 v/v), filtered through 0.22 µm PVDF membrane syringe filters (Millipore Ltd., Bedford, MA) and analyzed by UPLC-MS.

**2.6.3. Metabolites identification.** Samples were analyzed by UPLC-MS and metabolites were identified based on retention time and MS spectra, according to Gasperotti, Masuero, Vrhovsek, Guella and Mattivi (2010). Separation was carried out with a Waters Acquity UPLC system equipped with a UV-Vis Waters PDA (Waters Corp., Milford, MA) and mass spectrometer with an electrospray ionization system (ESI) and MassLynx Software 4.1 (Waters Corp.). The column was a 150 mm x 2.1 mm i.d., 1.7 µm, end-capped reversed-phase Acquity™ UPLC BEH C18 (Waters). The solvents were (A) 1% formic acid in water and (B) acetonitrile. UPLC-MS analysis was performed in negative mode under the following

conditions: capillary voltage 3 kV, source temperature 100 °C, desolvation temperature 350 °C, desolvation gas flow (N<sub>2</sub>) 650 L/h. The m/z range was 50-2000 Da. Compounds were identified using UV detection at 260 nm. Results were expressed as mg of ellagic acid equivalents (EAE)/100 g sample DW.

### 3. Results and discussion

The jaboticaba raw extract showed to be a rich source of phenolic compounds (55.8 mg/mL), which comprehends proanthocyanidins (51 mg/mL) and flavonoids, such as quercetin derivatives (0.02 mg/mL) and anthocyanins (1.5 mg/mL), besides ellagic acid derivatives. Ellagic acid itself and glycosidic combinations were expressed as free ellagic acid (0.4 mg/mL) and the amount obtained after acid hydrolysis corresponded to the total ellagic acid (58 mg/mL) (**Table 1**).

**Table 1.** Content and composition of phenolic compounds found in jaboticaba raw extract, expressed as mg/mL.

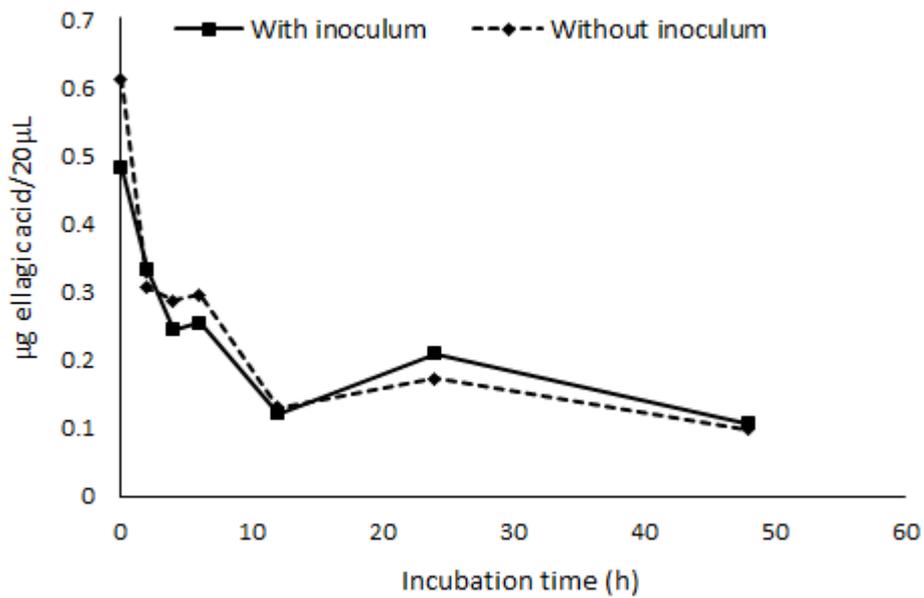
| <b>Polyphenols composition</b> | <b>Contents</b> |
|--------------------------------|-----------------|
| Total phenolics                | 55.80 ± 0.03    |
| Proanthocyanidins              | 50.5 ± 1.0      |
| Flavonoids                     |                 |
| <i>Quercetin derivatives</i>   | 0.021 ± 0.002   |
| <i>Anthocyanins</i>            | 1.5 ± 0.1       |
| Free ellagic acid              | 0.40 ± 0.01     |
| Total ellagic acid             | 58.0 ± 1.5      |

Previously, jaboticaba fruits were largely investigated and it is well-known that ellagic acid derivatives are the main phenolic compounds of jaboticaba. Among the ellagitannins,

sanguiin H-10, sanguiin H-6 and lambertianin C were identified in this species. Cyanidin 3-glucoside and delphinidin 3-glucoside were the anthocyanins detected, especially in the skin of the fruits (Alezandro et al., 2013; Leite, Malta, Riccio, Eberlin, Pastore, Maróstica Júnior, 2011).

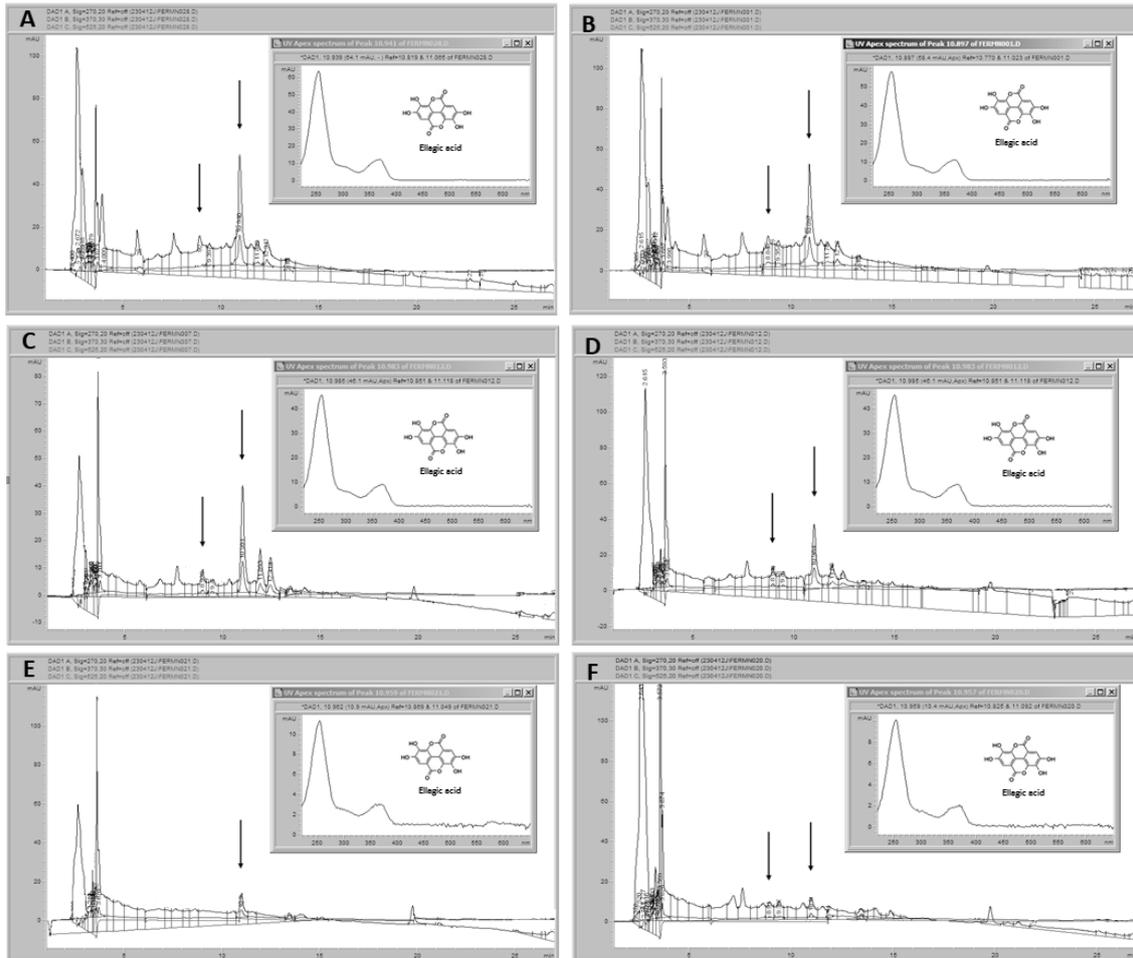
It is known that most polyphenols are metabolized by the colonic microbiota before they can be absorbed, and these transformations are essential in modulating their biological effects. It is clear that these metabolites are the main responsible for the physiological response (Del Rio et al., 2010; Setchell et al., 2002). In order to investigate the bioavailability of phenolic compounds from jaboticaba, an *in vitro* assay was performed using the human fecal fermentation model, aiming for identification of their probable degradation products and metabolites formed by the action of microbiota. In this study, the focus was on the ellagic acid derivatives, as they are the main compounds found in jaboticaba fruits, although other compounds were also identified. The data revealed that incubation led to degradation of ellagic acid derivatives in both fermentation media, with and without feces. This finding indicates that degradation may be related to the conditions inherent to the method, and not only by the action of microbiota (**Figure 1**).

At the beginning, the content of ellagic acid derivatives was higher, approximately 20%, in the sample incubated without fecal inoculum. However, during the fermentation process, degradation was observed in both samples. During the first two hours, there was a reduction of 50% in the amount of ellagic acid derivatives detected in the sample without feces, and 30% in the one with inoculum. After 48 hours, the content of ellagic acid derivatives decreased 84% in the sample without inoculum and 78% in the one with feces (**Figure 1**). The chromatographic profile of the fecal samples over time indicated smaller ellagic acid peaks in the end of fermentation (T = 48 h) compared to those identified in the beginning (T = 0) (**Figure 2**).



**Figure 1.** Fermentation profile of ellagic acid in samples incubated with and without fecal inoculum, indicating the degradation over time.

The same degradation pattern was observed by Serra et al. (2011). There was a higher degradation of procyanidins incubated with the fecal suspension in comparison to the samples without feces. In addition, other authors also verified that the most compounds degraded in the beginning of fermentation process. About 90% of quercetin present in the extracts apparently disappeared during the first 15 minutes of incubation, and only 10% of residues were identifiable after this period (Jaganath et al., 2009).



**Figure 2.** HPLC-DAD analyses of samples with and without fecal inoculum indicating degradation of ellagic acid over time: **(A)** Sample without feces, T = 0; **(B)** Samples with feces, T = 0; **(C)** Sample without feces, T = 2; **(D)** Sample with feces, T = 2; **(E)** Sample without feces, T = 48 h; **(F)** Sample with feces, T = 48 h.

The compound identified as ellagic acid by HPLC-DAD can be from the jaborcaba extract, which corresponds to the free or glycosylated form. It can also be formed during the metabolization of ellagitannins that upon hydrolysis release hexahydroxydiphenic acid, which spontaneously rearranges into ellagic acid. Other polyphenols are often transformed by the colonic microbiota, such as aglycones, which are chemically unstable, and are converted to phenolic acids, as protocatechuic, syringic and vanillic acids (Espín et al., 2007; Selma, Espín, & Tomás-Barberán, 2009). In this way, several compounds were also investigated and

identified in the samples with and without fecal inoculum, which could be formed from different polyphenols from jaboticaba (**Table 2**).

Analysis by HPLC-DAD indicated that many catabolic compounds and intermediate products were found in the samples, being a result of the fission of the C6-C3-C6 skeleton followed by the action of microbiota. Protocatechuic and syringic acid were identified in the samples and may be derived from the anthocyanins metabolism. Protocatechuic acid, however, is a phenolic acid that can be formed from different polyphenols, such as quercetin and ellagic acid. Furthermore, after 2 hours, protocatechuic acid was not detected in the samples, demonstrating that this compound may be transformed in other phenolic acids, such as *p*-hydroxybenzoic acid. This last compound was detected only in the samples incubated with feces, suggesting that the colonic microbiota is involved in the metabolization. Likewise, *p*-hydroxyphenylacetic acid was found only in the samples incubated with fecal inoculum, after 12 hours of incubation. The presence of *p*-hydroxyphenylacetic acid may be associated with the microbial transformations of quercetin or proanthocyanidins, as well.

The colonic microbiota is the most important site of metabolism, especially for hydroxycinnamic acids and flavonoids aglycones released from their conjugated forms by cleavage of their glycosidic or ester bonds (Rechner et al., 2004). Recent studies revealed that those metabolites are biologically more potent than their precursor compounds (Déprez et al., 2000; Keppler & Humpf, 2005; Kim, Jung, Sohng, Han, Kim, & Han, 1998; Larrosa, González-Sarrías, García-Conesa, Tomás-Barberán, Espín, 2006).

**Table 2.** Metabolites detected in the samples incubated with and without fecal inoculum, during 48 hours of fermentation.

| <b>COLLECTION TIME</b> | <b>WITH INOCULUM</b>  | <b>WITHOUT INOCULUM</b> | <b>PRECURSOR COMPOUNDS</b>                           |
|------------------------|---|-------------------------|--|
| T = 0                  | Protocatechuic acid   | Protocatechuic acid     | Ellagic acid<br>Anthocyanin<br>Quercetin             |
|                        | Ellagic acid  | Ellagic acid            | Ellagitannin   |
|                        | Syringic acid   | Syringic acid           | Anthocyanin  |
| T = 2 h                | Protocatechuic acid   | Protocatechuic acid     | Ellagic acid<br>Anthocyanin<br>Quercetin             |
|                        | Ellagic acid  | Ellagic acid            | Ellagitannin   |
|                        | Cinnamic acid   | Cinnamic acid           | -  |
|                        | <i>p</i> -hydroxybenzoic acid                                       | nd                      | Protocatechuic acid<br>Quercetin                     |
| T = 4 h                | Ellagic acid  | Ellagic acid            | Ellagitannin   |
|                        | Cinnamic acid   | Cinnamic acid           | -  |
| T = 6 h                | Ellagic acid  | Ellagic acid            | Ellagitannin   |
|                        | Cinnamic acid   | Cinnamic acid           | -  |
| T = 12 h               | Ellagic acid<br>Cinnamic acid<br><i>p</i> -hydroxyphenylacetic acid | Ellagic acid            | Ellagitannin   |
|                        |   | Cinnamic acid           | -  |
|                        |   | nd                      | Proanthocyanidin<br>Quercetin                        |
| T = 24 h               | Ellagic acid  | Ellagic acid            | Ellagitannin   |
|                        | Cinnamic acid   | Cinnamic acid           | -  |
|                        | <i>p</i> -hydroxyphenylacetic acid                                  | nd                      | Proanthocyanidin<br>Quercetin                        |
| T = 48 h               | Ellagic acid  | Ellagic acid            | Ellagitannin   |
|                        | Cinnamic acid   | Cinnamic acid           | -  |
|                        | <i>p</i> -hydroxyphenylacetic acid                                  | nd                      | Proanthocyanidin<br>Quercetin                        |
|                        | <i>p</i> -hydroxybenzoic acid                                       | nd                      | Protocatechuic acid<br>Proanthocyanidin<br>Quercetin |

nd : not detected

It is known that other tissues are involved in the metabolism of phenolic compounds, besides gut. The hepatic processing, for example, may be responsible for the formation of different metabolites compared to the products obtained after intestinal metabolism. In this way, the bioavailability of jaboticaba polyphenols was also evaluated in an *in vivo* model using Wistar rats. In addition, the UPLC-MS was used to identify the compounds, since the extraction of metabolites from biological fluids and tissues, and their characterization by other spectroscopic means is complicated due to the very low concentrations of these compounds.

The fate of 38 metabolites were investigated in the present study (**Table 3**). Most of them, 20 compounds, are conjugates of ellagic acid and their metabolites, known as urolithins, or conjugated compounds. Besides ellagic acid (**1**), conjugates with methyl ether and glucuronic acid (**2 – 6**) were identified. Ellagic acid methyl ether (**2**,  $m/z^-$  315) and ellagic acid dimethyl ether (**5**,  $m/z^-$  329) were detected only in plasma. Compounds conjugated with methyl ether and glucuronic acid (**3**,  $m/z^-$  329; **4**,  $m/z^-$  329; **6**,  $m/z^-$  329) were more widely distributed, being detected in other tissues, as liver, stomach and colon, besides plasma.

Under the pH conditions of the small intestine, ellagic acid is released from ellagitannins, and the colonic microbiota can also contribute to this transformation. The metabolism of ellagic acid is initiated in the jejuno-ileal portion and urolithin D is the first compound formed, followed by urolithin C and finally, urolithins A and B (González-Barrío et al., 2012; Landete, 2011). Urolithin A (**7**,  $m/z^-$  227) was identified in all the biological samples, unless in the brain. Urolithins B (**8**,  $m/z^-$  211), C (**9**,  $m/z^-$  243) and D (**10**,  $m/z^-$  259) were also detected in all biological samples analyzed, but the amount varied among the different tissues (**Figure 3**).

**Table 3.** Polyphenols metabolites detected in Wistar rats after chronic ingestion of jaboticaba raw extract.

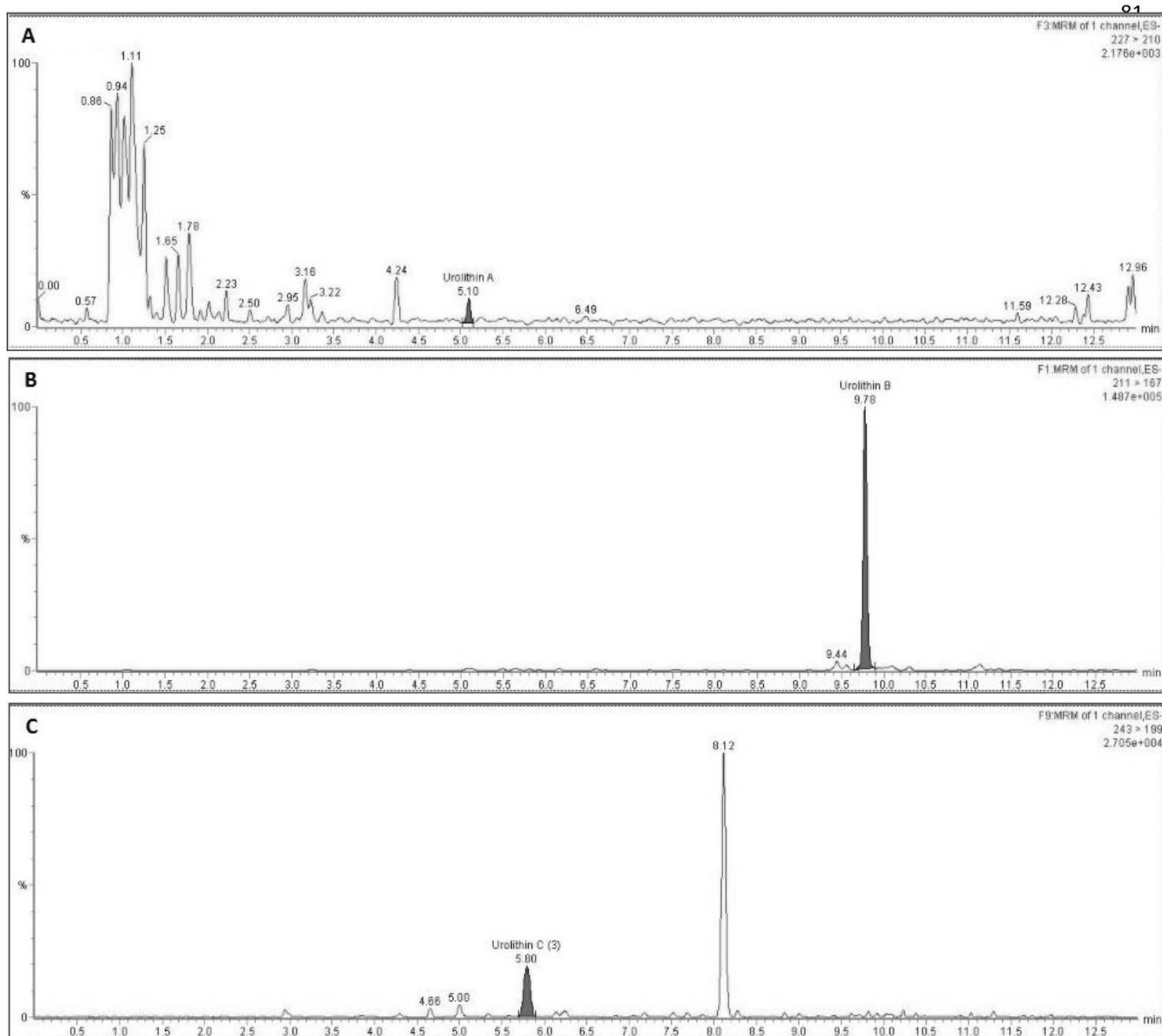
| Phenolic metabolites                    | n° | t <sub>R</sub> (min) | MRM transition | Occurrence          |
|---|----|----------------------|----------------|---------------------|
| <i>ETs derivatives</i>                  |    |                      |                |                     |
| ellagic acid                            | 1  | 4.72                 | 301 / 145      | P, S, K             |
| ellagic acid methyl ether               | 2  | 6.37                 | 315 / 300      | P                   |
| ellagic acid methyl ether glucuronide   | 3  | 9.85                 | 491 / 315      | P, L, S, K, M, C    |
| ellagic acid methyl ether diglucuronide | 4  | 6.79                 | 667 / 491      | P, S                |
| ellagic acid dimethyl ether             | 5  | 6.72                 | 329 / 315      | P                   |
| ellagic acid dimethyl ether glucuronide | 6  | 5.94                 | 505 / 315      | P, L                |
| urolithin A                             | 7  | 5.10                 | 227 / 210      | P, L, S, K, M, C    |
| urolithin B                             | 8  | 9.75                 | 211 / 167      | P, L, S, K, B, M, C |
| urolithin C                             | 9  | 5.80                 | 243 / 199      | P, L, S, K, B, M, C |
| urolithin D                             | 10 | 2.84                 | 259 / 241      | P, L, S, K, B, M, C |
| urolithin A glucuronide                 | 11 | 7.55                 | 403 / 227      | P, L, S, K, C       |
| urolithin A diglucuronide               | 12 | 7.89                 | 579 / 227      | P, L, S             |
| urolithin B glucuronide                 | 13 | 8.80                 | 389 / 211      | P, L, C             |

|  |    |      |           |                     |
|--|----|------|-----------|---------------------|
| urolithin C glucuronide                      | 14 | 4.53 | 419 / 243 | P, L, S, K, C       |
| urolithin C diglucuronide                    | 15 | 3.55 | 595 / 243 | P, L, S, K          |
| urolithin C methyl ether glucuronide         | 16 | 7.65 | 433 / 257 | P, L, S, K, M, C    |
| urolithin C methyl ether glucuronide sulfate | 17 | 8.80 | 513 / 337 | P, L, S             |
| urolithin D glucuronide                      | 18 | 3.29 | 435 / 259 | P, L, S, K          |
| urolithin D methyl ether                     | 19 | 3.38 | 273 / 258 | P, S, K             |
| urolithin D methyl ether glucuronide         | 20 | 6.90 | 449 / 273 | P, L, S, K, C       |
| <i>hydroxyphenylpropionic acids</i>          |    |      |           |                     |
| hydroxyphenylpropionic acid                  | 21 | 6.93 | 165 / 121 | P, L, S, K, B, M, C |
| dihydroxyphenylpropionic acid                | 22 | 5.55 | 181 / 137 | P, L, S, K, B, M, C |
| <i>hydroxyphenylacetic acids</i>             |    |      |           |                     |
| hydroxyphenylacetic acid                     | 23 | 3.50 | 151 / 107 | P, L, S, K, B, M, C |
| dihydroxyphenylacetic acid                   | 24 | 3.62 | 167 / 123 | P, L, S, K, B, M, C |
| <i>hydroxybenzoic acids</i>                  |    |      |           |                     |
| hydroxybenzoic acid                          | 25 | 3.30 | 137 / 93  | P, L, S, K, B, M, C |
| <i>hydroxycinnamic acids</i>                 |    |      |           |                     |

|                                |    |      |           |                     |
|--------------------------------|----|------|-----------|---------------------|
| coumaric acid                  | 26 | 4.72 | 163 / 119 | P, L, S, K, B, M, C |
| caffeic acid                   | 27 | 3.80 | 179 / 135 | P, L, S, K, M, C    |
| ferulic acid                   | 28 | 4.85 | 193 / 134 | P, L, S, K, C       |
| <i>flavan-3-ols</i>            |    |      |           |                     |
| (+)-catechin                   | 29 | 3.73 | 289 / 245 | P, L, S, K, B, C    |
| (-)-epicatechin                | 30 | 3.94 | 289 / 245 | P, L, S, K, B, C    |
| <i>flavonols</i>               |    |      |           |                     |
| quercetin-glucoside            | 31 | 4.74 | 463 / 301 | P, L, S, C          |
| quercetin-glucuronide          | 32 | 5.50 | 477 / 301 | P, L, S, K, C       |
| <i>anthocyanins</i>            |    |      |           |                     |
| delphinidin 3-glucoside        | 33 | 1.59 | 465 / 303 | S                   |
| cyanidin 3-glucoside           | 34 | 1.91 | 449 / 287 | S                   |
| cyanidin diglucuronide         | 35 | 1.89 | 639 / 287 | S                   |
| delphinidin-acetylglucoside    | 36 | 2.96 | 507 / 303 | S                   |
| cyanidin-acetylglucoside       | 37 | 2.28 | 491 / 287 | S                   |
| delphinidin-coumaroylglucoside | 38 | 3.00 | 611 / 303 | S                   |

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**Figure 3.** UPLC-MS analyses of metabolites in plasma: **(A)** Urolithin A (**7**,  $m/z^-$  227); **(B)** Urolithin B (**8**,  $m/z^-$  211); **(C)** Urolithin C (**9**,  $m/z^-$  243).



Urolithins were mainly detected in conjugated forms with methyl ether and glucuronic acids, and rarely with sulfates. Urolithin C methyl ether glucuronide sulfate (**17**,  $m/z^-$  513) was the only sulfated metabolite found in this study. Glucuronides of urolithin A (**11**,  $m/z^-$  403; **12**,  $m/z^-$  579) were identified, as well as of urolithin B (**13**,  $m/z^-$  389), C (**14**,  $m/z^-$  419) and D (**18**,  $m/z^-$  435). Different compounds were formed by different conjugate combinations (**16**,  $m/z^-$  433; **20**,  $m/z^-$  449) and were largely distributed among the biological samples.

Quercetin is one of the most widely distributed phenolic compounds in human diet, including vegetables, fruits, tea, and wine. Quercetin derivatives can be partly absorbed into the body and accumulated in the circulation (Hollman, de Vries, Van Leeuwen, Mengelers, & Katan, 1995; Hollman et al., 1997). In the gut, quercetin aglycone is released from glycosides by the action of bacterial enzymes, as  $\beta$ -glucosidases. Then, the portion that is not absorbed is degraded to simpler products, as phenolic acid (hydroxyphenylpropionic, hydroxyphenylacetic and hydroxybenzoic acids) (Serra, Macià, Romero, Reguant, Ortega, & Motilva, 2012). After absorption, the aglycone is metabolized in the liver, producing metabolites conjugated with methyl, glucuronate and sulfate groups, and this process is mediated by catechol-*O*-methyltransferase (COMT), uridine diphosphate glucuronosyltransferase (UGT) and sulfotransferase (SULT), respectively (Crozier, Del Rio, & Clifford, 2010). However, it was demonstrated that glucuronide conjugates are the main quercetin metabolites (Manach et al., 1998).

In this study, two compounds were identified as result of ingestion of quercetin derivatives present in the raw jaboticaba extract. Quercetin-glucoside (**31**,  $m/z^-$  463) was detected, especially in the stomach, but some animals presented small amount in plasma, liver and colon. Quercetin-glucuronide (**32**,  $m/z^-$  477) was also found in plasma, liver, stomach, kidneys and colon. Hydroxyphenylpropionic (**21**,  $m/z^-$  165), dihydroxyphenylpropionic (**22**,  $m/z^-$  181), hydroxyphenylacetic (**23**,  $m/z^-$  151), dihydroxyphenylacetic (**24**,  $m/z^-$  167) and

hydroxybenzoic acids (**25**,  $m/z^-$  137) were present in different biological tissues, and may be derived from quercetin metabolism.

Jaboticaba showed to be a rich source of proanthocyanidins, which were metabolized by the colon microbiota, releasing catechin (**29**,  $m/z^-$  289) and epicatechin (**30**,  $m/z^-$  289). Then, these monomeric units were converted in hydroxyphenylpropionic acid (**21**, **22**), hydroxyphenylacetic (**23**, **24**), hydroxybenzoic (**25**) and hydroxycinnamic acids (**28**,  $m/z^-$  193). The presence of these compounds in biological fluids and tissues has been related to the procyanidins metabolism (Aura, 2008; Serra, Macià, Romero, Anglés, Morelló, & Motilva, 2011).

Delphinidin 3-glucoside (**33**,  $m/z^-$  465) and cyanidin 3-glucoside (**35**,  $m/z^-$  449) were the anthocyanins previously identified in jaboticaba fruits (Alejandro et al., 2013) and were also detected in the stomach of rats as the intact forms. One glucuronide conjugate of cyanidin (**35**,  $m/z^-$  639) was found, as well. Apart from the conjugated metabolite, three anthocyanins were identified in the stomach: delphinidin-acetylglucoside (**36**,  $m/z^-$  507), cyanidin-acetylglucoside (**37**,  $m/z^-$  491) and delphinidin-coumaroylglucoside (**38**,  $m/z^-$  611).

According to the literature, only 1–2% of anthocyanins were absorbed after ingestion of high amounts (500 mg) of these compounds. However, when a lower quantity (100 mg) is consumed, no products derived from their metabolism were found in biological fluids, as plasma and urine (Clifford, 2000). This fact, along with the high unstability and susceptibility to degradation, can explain why the anthocyanins metabolites were not detected in other tissues, besides stomach.

The bioavailability of polyphenols is generally evaluated by means of acute administration of one or more compounds. In this study, a long-term experiment (30 days) was performed, as the scientific literature related that repeated oral doses of ellagitannins are necessary to make the colonic microbiota able to metabolize ellagic acid derivatives and

produce urolithins (Cerdá, Llorach, Ceron, Espín, & Tomás-Barberán, 2003). Our findings showed that metabolites formed from jaboticaba polyphenols were largely distributed in many biological tissues, but the kind and fate of metabolites were not related with time of euthanasia. Probably, the compounds found in the tissues may have been produced and accumulated during all the supplementation period, and not only on the day of euthanasia.

#### **4. Conclusion**

The dietary polyphenols are effective substrates for the action of the microbiota in human colon and are extensively metabolized, forming simpler phenolic or non-phenolic compounds. The type and amount of compounds generated by metabolism are influenced by the interindividual differences, which here was minimized by the use of a pool of feces. It is known that the formation of metabolites is essential for the absorption and consequently, their presence in target tissues in chemical form and concentration adequate to exert their physiological role. Thirty eight compounds were identified as metabolites formed from different classes of jaboticaba polyphenols and they were widely distributed in many biological tissues and plasma. The data obtained in this study are the first step towards the elucidation of the bioavailability of phenolic compounds from jaboticaba and provide important information about the possible compounds that may be found in the human body after consumption of this fruit.

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### References

Alezandro, M. R., Dubé, P., Desjardins, Y., Lajolo, F. M., & Genovese, M. I. (2013). Comparative analysis of chemical and phenolic composition of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg. *Food Research International*, submitted.

Arabbi, P. R., Genovese, M. I., & Lajolo, F. M. (2004). Flavonoids in vegetable foods commonly consumed in Brazil. *Journal of Agricultural and Food Chemistry*, 52, 1124-1131.

Aura, A. (2008). Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry Reviews*, 7, 407–429.

Cerdá, B., Llorach, R., Ceron, J. J., Espín, J. C., Tomás-Barberán, F. A. (2003). Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. *European Journal of Nutrition*, 42, 18–28.

Clifford, M. N. (2000). Review. Anthocyanins – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, 80, 1063–1072.

Crozier, A., Del Rio, D., & Clifford, M. N. (2010). Bioavailability of dietary flavonoids and phenolic compounds. *Molecular Aspects of Medicine*, 31, 446-467.

Del Rio, D., Costa, L. G., Lean, M. E. J., & Crozier, A. (2010). Polyphenols and health: What compounds are involved? *Nutrition, Metabolism and Cardiovascular Diseases*, *20*, 1-6.

Déprez, S., Brezillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C., & Scalbert, A. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *Journal of Nutrition*, *130*, 2733–2738.

Donadio, L. C. (2000). *Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.* Jaboticabal: FUNEP, 55 pp.

Espín, J. C., González-Barrio, R., Cerdá, B., López-Bote, C., Rey, A. I., & Tomás-Barberán, F. A. (2007). Iberian pig as a model to clarify obscure points in the bioavailability and metabolism of ellagitannins in humans. *Journal of Agricultural and Food Chemistry*, *55*, 10476–10485.

Gasperotti, M., Masuero, D., Vrhovsek, U., Guella, G., & Mattivi, F. (2010). Profiling and accurate quantification of *Rubus* ellagitannins and ellagic acid conjugates using direct UPLC-Q-TOF HDMS and HPLC-DAD analysis. *Journal of Agricultural and Food Chemistry*, *58*, 4602-4616.

González-Barrio, R., Truchado, P., García-Villalba, R., Hervas, G., Frutos, P., Espín, J. C., & Tomás-Barberán, F. A. (2012). Metabolism of oak leaf ellagitannins and urolithin production in beef cattle. *Journal of Agricultural and Food Chemistry*, *60*, 12, 3068-3077.

Hollman, P. C., de Vries, J. H., van Leeuwen, S. D., Mengelers, M. J., Katan, M. B. (1995). Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *The American Journal of Clinical Nutrition*, *62*, 1276–1282.

Hollman, P. C., van Trijp, J. M., Buysman, M. N., van der Gaag, M. S., Mengelers, M. J., de Vries, J. H., et al. (1997). Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters*, *418*, 152–156.

Jaganath, I. B., Mullen, W., Lean, M. E. J., Edwards, C. A., & Crozier, A. (2009). In vitro catabolism of rutin by human fecal bacteria and the antioxidant capacity of its catabolites. *Free Radical Biology and Medicine*, *47*, 1180-1189.

Keppler, K., & Humpf, H. U. (2005). Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. *Bioorganic & Medicinal Chemistry*, *13*, 5195–5205.

Kim, D. H., Jung, E. A., Sohng, I. S., Han, J. A., Kim, T. H., & Han, M. J. (1998). Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. *Archives of Pharmacal Research*, *21*, 17.

Landete, J. M. (2011). Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. *Food Research International*, *44*, 1150-1160.

Larrosa, M.; González-Sarriás, A.; García-Conesa, M. T.; Tomás-Barberán, F. A.; Espín, J. C. Urolithins, ellagic acid-derived metabolites produced by human colonic microflora, exhibit estrogenic and antiestrogenic activities. *J. Agric. Food Chem.* 2006, 54, 1611–1620.

Leite, A. V., Malta, L. G., Riccio, M. F., Eberlin, M. N., Pastore, G. M., & Maróstica Júnior, M. R. (2011). Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticaba* Vell Berg). *Journal of Agricultural and Food Chemistry*, 59, 2277-2283.

Manach, C., Morand, C., Crespy, V., Demigné, C., Texier, O., Régéat, F., et al. (1998). Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. *FEBS Letters*, 426, 331–336.

McBain, A., & Macfarlane, G. (1997). Investigations of bifidobacterial ecology and oligosaccharide metabolism in a three-stage compound continuous culture system. *Scandinavian journal of gastroenterology. Supplement*, 222, 32.

Mullen, W., McGinn, J., Lean, M. E., Maclean, M. R., Gardner, P., Duthie, G. G., Yokota, T., & Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *Journal of Agricultural and Food Chemistry*, 50, 5191-5196.

Oliveira, D. M., Bastos, D. H. M. (2011). Biodisponibilidade de ácidos fenólicos. *Química Nova*, 34, 1051-1056.

Oliveira, D. M., Pinto, C. B., Sampaio, G. R., Yonekura, L., Catharino, R. R., & Bastos, D. H. M. (2013). Method validation for extraction of phenolic acids from plasma, urine and liver and analysis by ultra-performance liquid chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry*, submitted.

Pinto, M. S., Lajolo, F. M., & Genovese, M. I. (2008). Bioactive compounds and quantification of total ellagic acid in strawberries (*Fragaria ananassa* Duch.). *Food Chemistry*, *107*, 1629-1635.

Prior, R. L., Fan, E., Ji, H., Howell, A., Nio, C., Payne, M. J., & Reed, J. (2010). Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. *Journal of the Science of Food and Agriculture*, *90*, 1473–1478.

Priyadarsini, K. I., Khopde, S. M., Kumar, S. S., & Mohan, H. (2002). Free radical studies of ellagic acid, a natural phenolic antioxidant. *Journal of Agricultural and Food Chemistry*, *50*, 2200-2206.

Rechner, A. R., Smith, M. A., Kuhnle, G., Gibson, G. R., Debnam, E. S., Srai, S. K. S., Morre, K. P., & Rice-Evans, C. A. (2004). Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. *Free Radical Biology & Medicine*, *36*, 212-225.

Selma, M. A. V., Espín, J. C., & Tomás-Barberán, F. A. (2009). Interaction between phenolics and gut microbiota: role in human health. *Journal of Agricultural and Food Chemistry*, *57*, 6485-6501.

Serra, A., Macià, A., Romero, M.-P., Anglés, N., Morelló, J.-R., & Motilva, M.-J. (2011). Metabolic pathways of the colonic metabolism of procyanidins (monomers and dimers) and alkaloids. *Food Chemistry*, *126*, 1127–1137.

Serra, A., Macià, A., Romero, M.-P., Reguant, J., Ortega, N., & Motilva, M.-J. (2012). Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. *Food Chemistry*, *130*, 383-393.

Setchell, K. D. R., Brown, N. M., & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol—a clue to the effectiveness of soy and its isoflavones. *The Journal of Nutrition*, *132*, 3577-3584.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178.

Xu, X., Harris, K. S., Wang, H.-J., Murphy, P. A., & Hendrich, S. (1995). Bioavailability of soybean isoflavones depends upon gut microflora in women. *The Journal of Nutrition*, *125*, 2307-2315.

Youdim, K. A., McDonald, J., Kalt, W., & Joseph, J. A. (2002). Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. *The Journal of Nutritional Biochemistry*, *13*, 282-288.

**Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats**

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## ABSTRACT

Jaboticaba (*Myrciaria jaboticaba* Vell. Berg) is a Brazilian Atlantic rainforest fruit of the Myrtaceae family. In this work, the effect of the daily intake (40 days) of jaboticaba (1.0 and 2.0 g DW/kg body weight) on oxidative stress and plasma lipid profile of streptozotocin (STZ)-induced diabetic rats was evaluated. Jaboticaba was shown to be a good source of phenolic compounds, proanthocyanidins and ellagitannins. Daily administration of jaboticaba resulted in ameliorated water consumption and energy intake in STZ-diabetic rats. Plasma total cholesterol levels were reduced in 32% and triacylglycerol decreased 50% when both doses of jaboticaba were administered. This reduction of total cholesterol and triacylglycerol levels seems to be associated to the strong *in vitro* inhibition of pancreatic lipase presented by jaboticaba extracts. Plasma antioxidant capacity of diabetic rats assessed by FRAP assay increased (2 to 2.5 times) after supplementation with both doses of jaboticaba along with a decrease of lipid peroxidation in plasma (22%) and brain (10-17%). Diabetic rats consuming jaboticaba presented higher activity of SOD in the brain, CAT and GPx in kidneys and liver, and GPx in plasma, as compared to the control group. These results suggest that chronic ingestion of jaboticaba may represent a dietary strategy for controlling oxidative stress in pathological conditions.

**KEYWORDS:** *Myrciaria jaboticaba*; oxidative stress; streptozotocin-induced diabetes; lipid profile.

## 1. Introduction

Jaboticaba, *Myrciaria jaboticaba* (Vell.) Berg, is a Brazilian native fruit from the Atlantic Rainforest that belongs to the Myrtaceae family, grape-like in appearance and texture. Its economic importance has been continuously growing in Brazil because of the sweet and slightly acidic flavor of the pulp. Jaboticaba has a huge trading potential, since it can be consumed *in natura* and also used by industry as ingredient to produce cosmetics and food products (Donadio, 2000).

Jaboticaba fruits are a rich source of polyphenols, such as anthocyanins, which are concentrated in the dark purple to almost black skin, when the fruit is ripe, besides quercetin derivatives and proanthocyanidins. Furthermore, a recent study carried out with two species of jaboticaba proved ellagic acid derivatives, such as ellagitannins, are the main compounds detected in jaboticaba (Alejandro, Dubé, Desjardins, Lajolo & Genovese, 2013). Long-term ingestion of fruits with high levels of flavonoids (anthocyanins) and tannins (ellagitannins) has been associated with positive effects on human health, related to their antioxidant potential and consequent inhibitory activity against lipid peroxidation, reducing the risk of cardiovascular diseases (Mullen et al., 2002; Priyadarsini, Khopde, Kumar & Mohan, 2002; Srinivasan, Vadhanam, Arif, & Gupta, 2002). Despite the unclear mechanisms and few studies reported, several hypotheses indicate the high antioxidant capacity of phenolic compounds, especially flavonoids, may also be effective in reducing oxidative stress (Macedo, Rogero, Guimarães, Granato, Lobato, & Castro, 2013), progression of diabetes mellitus (Song, Wang, Li & Cai, 2005), and hypertension (Kwon, Vattem, & Shetty, 2006).

Streptozotocin-induced diabetes is an animal model used to promote metabolic dysfunctions related to oxidative stress (Raza & John, 2012). Oxidative stress occurs when there is an overproduction of free radicals and the endogenous antioxidants are not enough to

buffer these unstable molecules (Mullarkey, Edelstein, & Brownlee, 1990), and leads to modifications involved in the inflammation process and in the initiation and progression of atherosclerosis (Strobel, Fassett, Marsh & Coombes, 2010), thus being intrinsically linked with non-transmissible chronic diseases (Mullarkey, Edelstein, & Brownlee, 1990).

Studies about the potential beneficial effect on health of jaboticaba are sparse, but research has shown that native fruits are promising sources of bioactive compounds. Herein, the research available in the literature mostly focused on the role of anthocyanins present in the skin of the fruit (Lenquiste, Batista, Dragano, Marineli & Maróstica Jr., 2012; Dragano et al., 2013), and usually ignores the existence and importance of ellagitannins. Based on these considerations, this work aimed to evaluate the effects of chronic administration of whole jaboticaba on the oxidative stress related to streptozotocin-induced diabetes in an animal model using Wistar rats.

## **2. Material and methods**

**2.1. Chemicals.** The hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI, USA). Malondialdehyde, fluorescein, 2,2-azobis(2-methylpropionamide) dihydrochloride (AAPH), xanthine oxidase (from bovine milk), cytochrome C (from horse heart) and xanthine were purchased from Sigma Chemical Co. (St Louis, MO, USA). Glutathione, glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Merck Chemical Co. (Darmstadt, Germany). TPTZ (2, 4, 6-tripyridyl-S-triazine) was purchased from Fluka Chemie AG (Buchs, Switzerland). Ketamine chloride and xyloidine chloride were acquired from Bayer (Leverkusen, Germany). All chemicals/solvents were of analytical or HPLC grade, according to the requirement.

**2.2. Samples.** Fully ripened jaboticaba Sabará (*Myrciaria jaboticaba* Vell. Berg) was obtained from a local producer at the São Paulo Central Market (Companhia de Entrepósitos e Armazéns Gerais de São Paulo – CEAGESP, Brazil). Fruits were cleaned and immediately freeze-dried and stored at -20 °C. Then, the dried fruits were ground to a fine powder in a mortar and pestle, using liquid nitrogen to keep sample frozen. Protein, lipid, fiber and ashes contents of jaboticaba were assessed according to AOAC (2005). Total phenolics (Singleton, Orthofer, & Lamuela-Raventos, 1999), proanthocyanidins (Porter, Hrstich, & Chan, 1986), ellagitannins (Pinto, Lajolo, & Genovese, 2008) and total tannins (Hagerman & Butler, 1978) were also determined. Results were expressed as mg/g fresh weight (FW).

**2.3. Sample extraction for lipase inhibition assay.** Samples were extracted in a 70% aqueous methanol solution for 2 h at room temperature, at a 1:20 (w/v) ratio of sample-to-solvent. After filtration (Whatman N° 1), extracts were kept at -80 °C until analyses (Arabbi, Genovese, & Lajolo, 2004).

**2.4. Measurement of pancreatic lipase activity.** The pancreatic lipase activity was measured using 4-methylumbelliferyl oleate (4-MU oleate) as a substrate, according to Jacks and Kircher (1967) with some modifications (You, Chen, Wang, Jiang, & Lin, 2012). Twenty-five microliters of a sample solution dissolved in water and 50 µL of a 0.1 mM 4-MU solution dissolved in a buffer consisting of 13 mM Tris-HCl, 150 mM NaCl, and 1.3 mM CaCl<sub>2</sub> (pH 8.0) were mixed in the well of a microtiter plate, and 25 µL of the lipase solution (50 U/mL) in the above buffer was then added to start the enzyme reaction. After incubation at 25 °C for 30 min, 0.1 mL of 0.1 M sodium citrate (pH 4.2) was added to stop the reaction. The amount of 4-methylumbelliferone released by lipase was measured with a fluorometrical microplate reader (Fluoroskan Ascent C LabSystems, Inc.) at an excitation wavelength of 355 nm and an

emission wavelength of 460 nm. Results were expressed as IC50 values, considering the amount of sample (mg sample DW/mL reaction) and the content of phenolic compounds (mg catechin equivalents (CE)/mL reaction), determined according to Singleton et al. (1999).

**2.5. Animals and experimental design.** The Faculty of Pharmaceutical Sciences/USP Ethical Committee for Animal Research approved all the adopted procedures (Protocol CEUA/FCF/USP no. 355). Thirty six male rats weighing  $200 \pm 10$  g were obtained from Animal House of Faculty of Pharmaceutical Sciences and Chemistry Institute of University of São Paulo. Animals were kept under standard laboratory conditions of temperature ( $23 \pm 2$  °C), relative humidity ( $50 \pm 5\%$ ), 12 h light-dark cycle. Chow diet and water were provided *ad libitum*. For diabetes induction, overnight fasted rats received intraperitoneal injection (*i.p.*) of STZ (65 mg/kg) in citrate buffer (pH 4.5), followed by an aqueous solution of 10% glucose for eight hours. After three days the glycemia was measured and all rats presented glucose levels upper than 200 mg/dL. The STZ-diabetic animals were divided into three groups of 12 animals, as follows:

- *Control*: animals receiving water by gavage during 40 days;
- *Group1*: animals receiving 1.0 g/kg body weight of jaboticaba powder dispersed in water during 40 days;
- *Group2*: animals receiving 2.0 g/kg body weight of jaboticaba powder dispersed in water during 40 days.

The food and water consumptions were recorded daily. Every three days the animals were weighted and the fast blood glucose was measured every five days in all animals. Results were reported as average energy intake (Kcal/day), water consumption (mL/day), plasma glucose levels (mg/dL) and body weight (g) for the 40 days period.

**2.6 Blood and tissue samples.** After 40 days the animals were anesthetized with ketamine chloride and xylidine chloride. Blood was collected by cardiac puncture into tubes containing EDTA. The plasma was separated by centrifugation at 2,000 g for 10 min at 4 °C. Erythrocytes were washed three times with ice-cold 9 g/L NaCl solution and hemolyzed with distilled water (1:4 v/v). The tissues were exhaustively perfused with sterilized ice-cold 9 g/L NaCl solution through heart puncture until the liver was uniformly pale. Kidneys, brain and liver were removed, weighed and immediately frozen under liquid nitrogen and stored at -80 °C for further biochemical analysis. At the time of analysis, the tissues homogenates were prepared with ice-cold 50 mM phosphate buffer (pH 7.4) (1:4 w/v) and centrifuged at 10,000 g for 10 min at 4 °C.

**2.7 Plasma antioxidant capacity.** Antioxidant capacity of plasma towards peroxy radicals was evaluated by the ORAC method described by Huang, Ou, Hampsch-Woodill, Flanagan and Prior (2002) and the ferric reducing ability of plasma (FRAP) assay was determined according to Benzie and Strain (1996). Both methods were performed on a Synergy H1 Hybrid Multi-Mode microplate reader (BioTek Instruments, Winooski, VT) and the results were expressed in  $\mu\text{mols Trolox equivalents (TE)/mL plasma}$ .

**2.8 Lipid peroxidation levels.** Thiobarbituric acid reactive substances (TBARS) levels in plasma and tissues were measured according to Ohkawa, Ohishi, and Yagi (1979). An aliquot of plasma or tissue homogenate was mixed with 8.1% sodium dodecyl sulphate (SDS), 20% acetic acid, 0.67% thiobarbituric acid and water. The mixture was heated for 1 h at 95 °C and the pink chromogen formed was extracted into 1.4 mL of n-butanol. The absorbance of the organic phase was measured at 532 nm using a Synergy H1 Hybrid Multi-Mode microplate reader (BioTek Instruments, Winooski, VT). Malondialdehyde (MDA) was used as a standard. Results were expressed as  $\eta\text{mol MDA/mL plasma or mg protein}$ .

**2.9 Antioxidant enzymes.** Antioxidant enzymes activities were measured in plasma, and tissues (liver, brain and kidney). Briefly, catalase (CAT) activity was assayed at 25 °C by a method based on the disappearance of 10 mM H<sub>2</sub>O<sub>2</sub>. The decomposition of H<sub>2</sub>O<sub>2</sub> by CAT contained in the samples follows a first-order kinetic and changes in absorbance were measured 60 s after addition of H<sub>2</sub>O<sub>2</sub>, and then at 60 s intervals over 4 min (Hugo & Lester, 1984). Glutathione peroxidase (GPx) catalyses the oxidation of glutathione by terc-butyl hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is converted to the reduced form with a concomitant oxidation of NADPH to NADP<sup>+</sup>, which is reflected as a decrease in the absorbance at 340 nm ( $\epsilon_{340}$  6.22 L/mmol/cm). Changes in absorbance were measured at 60 s intervals over 6 min (Albrecht & William, 1981). Superoxide dismutase (SOD) activity was measured by the decrease in the rate of cytochrome c reduction in a xanthine/xanthine oxidase superoxide-generating system consisting of 10 mM cytochrome c, 100 mM xanthine, 50 mM sodium phosphate buffer (pH 7.8) and the necessary quantity of xanthine oxidase to yield a variation of 0.025 absorbance/min at 550 nm (Gunzler et al., 1984). Enzymatic activities were expressed as units of activity (UA, corresponding to 0.1 absorbance changes for CAT and GPx and to 0.0125 absorbance change for SOD) min<sup>-1</sup>.g<sup>-1</sup>.protein or mg<sup>-1</sup> haemoglobin (Hb). Protein concentration in plasma samples was determined by the method described by Lowry, Rosebrough, Farr and Randall, (1951). Haemoglobin was measured using Drabkin's reagent.

**2.10 Biochemical analysis.** The concentrations of total cholesterol (TC), triacylglycerol (TAG), HDL cholesterol (HDL-c), glucose, urea and creatinine in plasma were determined using commercial kits LABTEST (Lagoa Santa, MG, Brazil). The fast blood glucose was determined by means of the Accu-Check Performa<sup>®</sup> system.

**2.11 Statistical analysis.** Data were presented as mean  $\pm$  SD. Initially, the results were checked for homogeneity of variances by using the Levene test while one-way ANOVA (parametric) or the Welch test (non-parametric) was used to assess differences among the three treatments. The least significant difference Fisher test or Kruskal-Wallis test was used to compare the means within groups. *P*-values below 0.05 were regarded as significant. In order to observe the experimental results simultaneously, nine biomarkers (triacylglycerol, total cholesterol, GPx liver, TBARS brain, TBARS plasma, CAT liver, SOD plasma, SOD brain, ORAC plasma) were submitted to a principal component analysis, adopting the biomarkers as columns and Wistar rats as cases. Analyses were based on linear correlation, variances were computed as Sum of Squares/(n-1) and a scatter plot that contained the variables and rats was built adopting the factor-plane (1 x 2) (Granato, Katayama, & Castro, 2012). Statistical standardization was performed to obtain relativized data to which the multivariate technique was applied. The standardization of the variables was performed using the Equation (1):

$$Z_{ij} = \frac{X_{ij} - \bar{X}_j}{s_j}$$

Where *Z* is the standardized value for each value of the response,  $X_{ij}$  represents the original value for the object (*i*) of measured attribute (*j*),  $\bar{X}_j$  is the mean value of variable *j*, and  $s_j$  is the standard deviation for the attribute. All statistical analyses were performed with Statistica v. 11 software (Statsoft Inc., Tulsa, OK, USA).

### 3. Results

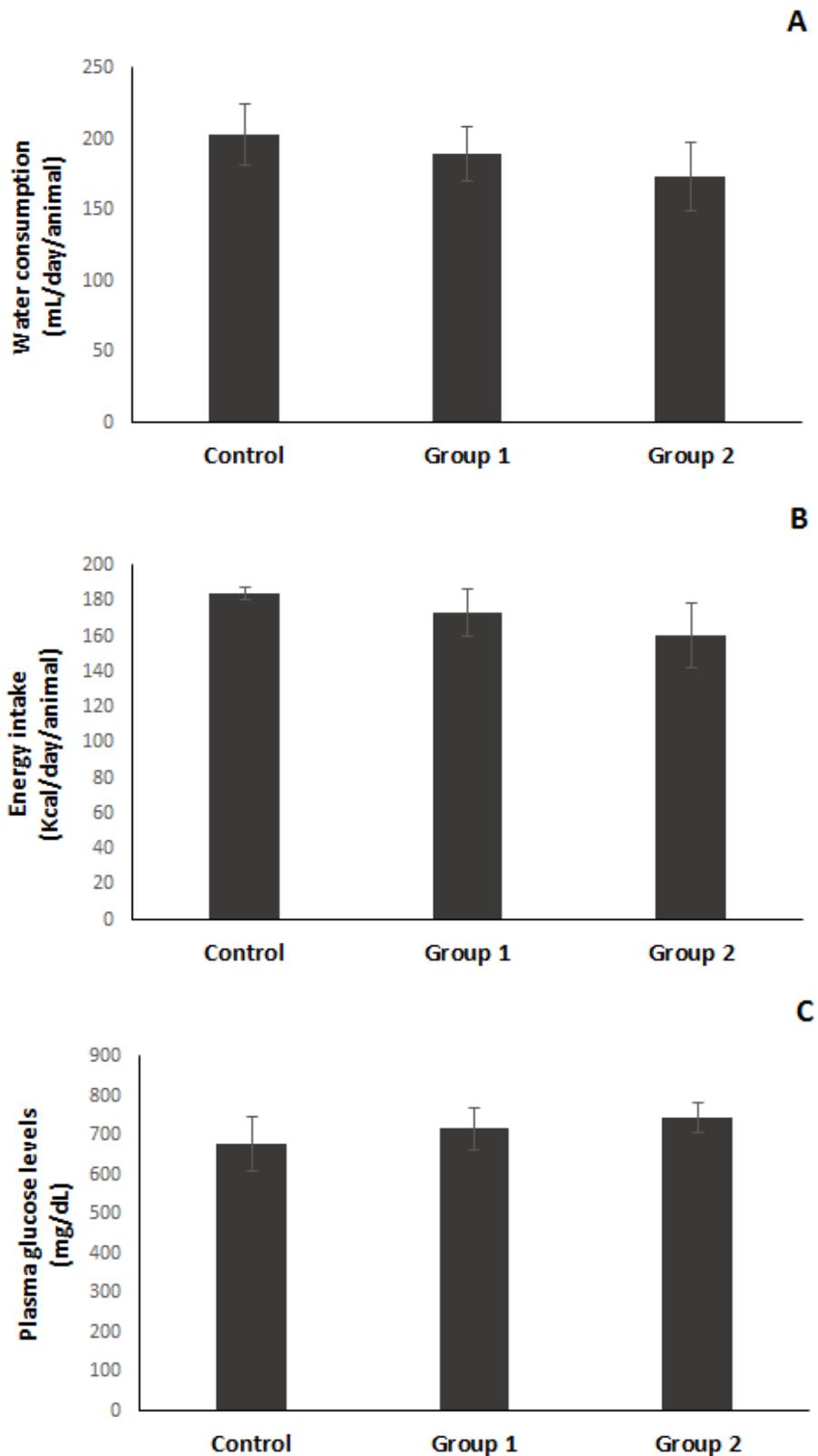
Jaboticaba was shown to be a good source of phenolic compounds, which included proanthocyanidins (10.4 mg/g FW), ellagitannins (9.1 mg/g FW) and total tannins (7.3 mg/g FW). Besides being rich in polyphenols, jaboticaba provides fibers (34.7 mg/g FW) and ashes (5.2 mg/g FW), important nutrients in maintaining health (**Table 1**).

Besides, jaboticaba raw extract showed a strong inhibitory activity against pancreatic lipase ( $IC_{50} = 1.08$  mg sample/mL reaction) and its polyphenols largely contributed to this effect ( $IC_{50} = 0.06$  mg CE/mL) (**Table 1**).

**Table 1.** Chemical composition (mg/g FW) and *in vitro* inhibitory activity of pancreatic lipase (IC<sub>50</sub>) of jaboticaba berry.

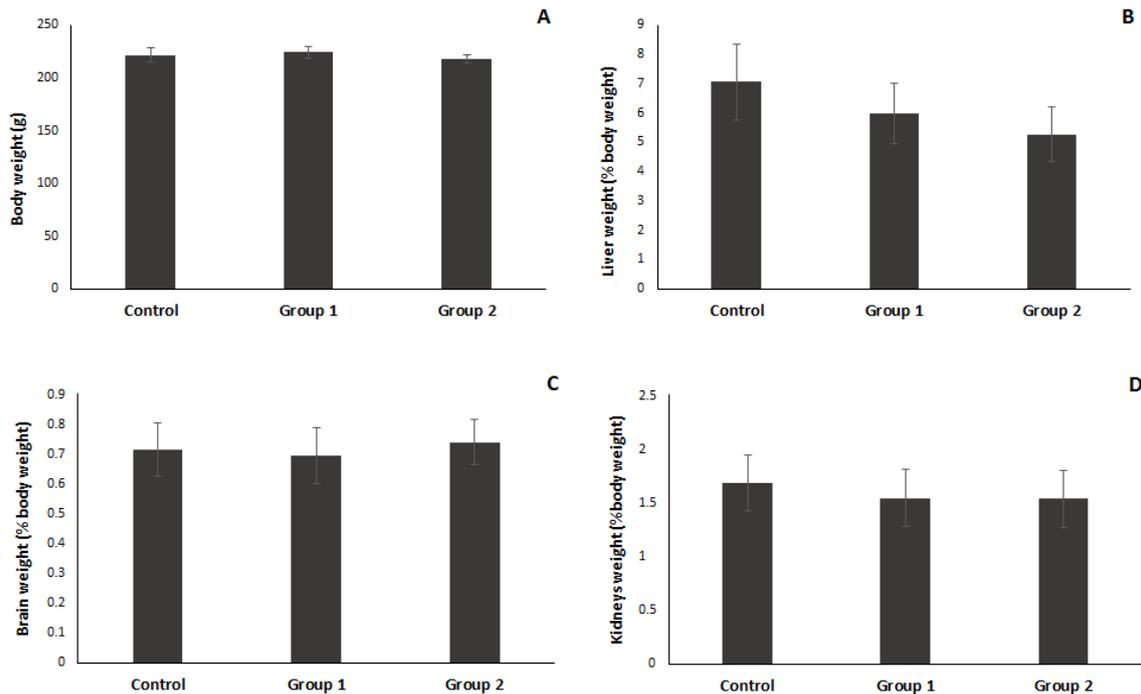
| <i>Chemical composition</i>  |                      |                    |                    |                    |                                       |                   |                  |                            |
|--|----------------------|--------------------|--------------------|--------------------|---------------------------------------|-------------------|------------------|----------------------------|
| <i>Nutritional composition</i>   |                      |                    |                    |                    | <i>Polyphenols composition</i>        |                   |                  |                            |
| Carbohydrate <sup>a</sup>  | Protein <sup>a</sup> | Lipid <sup>a</sup> | Ashes <sup>a</sup> | Fiber <sup>a</sup> | Total phenolic <sup>b</sup>           | PACs <sup>c</sup> | ETs <sup>d</sup> | Total tannins <sup>e</sup> |
| 138 ± 5  | 1.7 ± 0.1            | 0.72 ± 0.02        | 5.2 ± 0.2          | 35 ± 2             | 6.4 ± 0.1                             | 10.4 ± 0.3        | 9.1 ± 0.2        | 7.26 ± 0.05                |
| <i>Inhibitory activity of pancreatic lipase</i>  |                      |                    |                    |                    |                                       |                   |                  |                            |
| IC <sub>50</sub> (mg sample DW /mL reaction)   |                      |                    |                    |                    | IC <sub>50</sub> (mg CE/ mL reaction) |                   |                  |                            |
| 1.08   |                      |                    |                    |                    | 0.06                                  |                   |                  |                            |
| Contents expressed as mg/g FW <sup>a</sup> ; mg catechin equivalent/ g FW <sup>b</sup> ; mg quebracho tannin/g FW <sup>c</sup> ; mg ellagic acid/g FW <sup>d</sup> ; mg tannic acid/g FW |                      |                    |                    |                    |                                       |                   |                  |                            |

Due to this unique chemical composition and the antihyperlipidemic potential, we believed that jaboticaba would be powerful against oxidative stress associated to diabetes. Here, it was demonstrated that the daily administration of jaboticaba was effective for controlling oxidative stress and hyperlipidemia in STZ-diabetic rats. Polyphagia and polydipsia (**Figure 1**), common symptoms of diabetic animals, were also assuaged when the diabetic rats received both doses of jaboticaba. The mean energy intake was significantly reduced ( $p < 0.01$ ) in 6%, after administration of the lowest dose, and 13% for the highest dose. Similarly, significant ( $p < 0.01$ ) reductions of 8% and 14% in water consumption were observed after treatment with 1 and 2 g DW (dry weight)/kg of jaboticaba, respectively. However, an increase ( $p = 0.03$ ) in blood glucose levels was detected after administration of the highest dose of jaboticaba (**Figure 1**), not physiologically relevant due to the already elevated glycemia of the animals ( $> 680$  mg/dL).



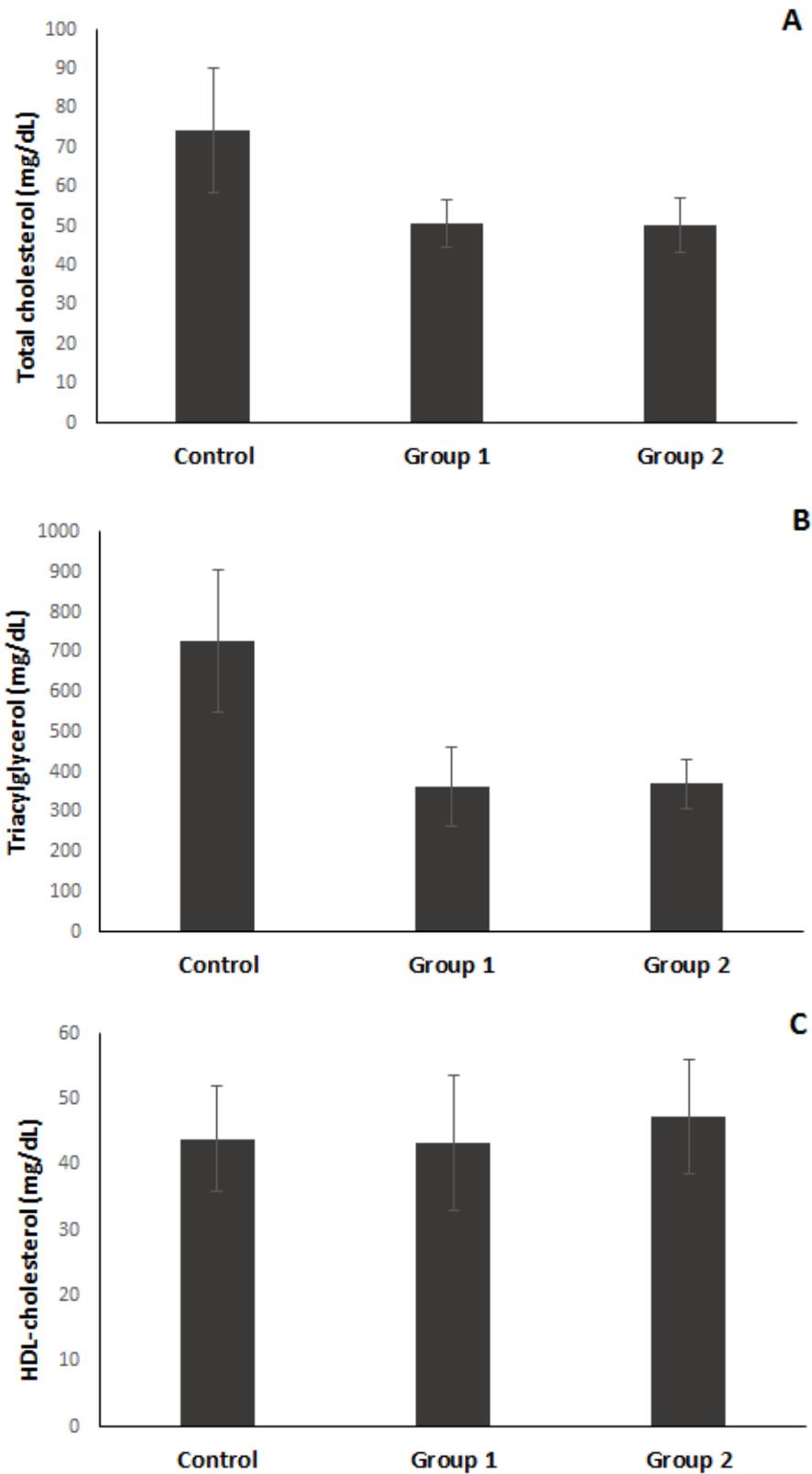
**Figure 1.** (A) Water consumption (mL/day), (B) energy intake (kcal/day) and (C) plasma glucose levels (mg/dL) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving jaboricaba for 40 days by gavage. Values were expressed as mean  $\pm$  SD (n = 12 rats/group). Different letters above the columns indicates statistical difference. The *p* value between supplemented and control groups was expressed as \* (*p* < 0.05) and \*\* (*p* < 0.01).

The mean body weight of the diabetic rats treated with both doses of jaboricaba was not significantly different from the control rats. However, the weight of the liver in proportion to the body weight was lower ( $p < 0.01$ ) in the treated groups (5.3-5.9%) compared to the untreated animals (7.1%), a 21% decrease (**Figure 2**).



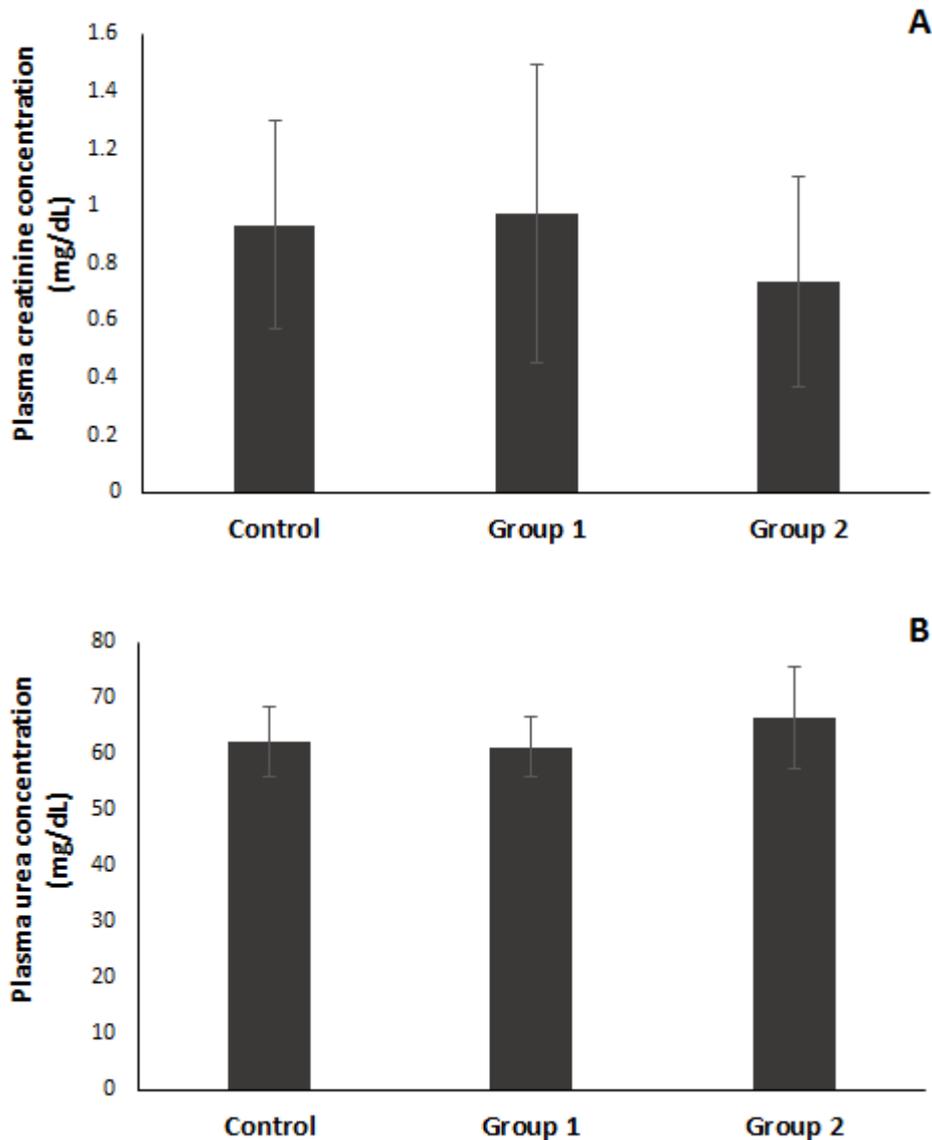
**Figure 2.** (A) Body weight (g) and tissues weight (% body weight), liver (B), brain (C) and kidneys (D) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving jaboricaba for 40 days by gavage. Values were expressed as mean  $\pm$  SD (n = 12 rats/group). Different letters above the columns indicates statistical difference. The  $p$  value between supplemented and control groups was expressed as \* ( $p < 0.01$ ).

Lipid profile was also altered in the increased oxidative stress, but the administration of jaboricaba was able to recover lipid levels in diabetic rats. Plasma TAG ( $p < 0.01$ ) and TC ( $p < 0.01$ ) levels were reduced in the treated groups (**Figure 3**). TAG was reduced in 50% and TC in 32% for both doses of jaboricaba.



**Figure 3.** Plasma lipid profile (mg/dL) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving jaboricaba for 40 days by gavage: (A) total cholesterol, (B) triacylglycerol, (C) HDL-cholesterol. Values were expressed as mean  $\pm$  SD (n = 12 rats/group). Different letters above the columns indicates statistical difference. The *p* value between supplemented and control groups was expressed as \* (*p* < 0.01).

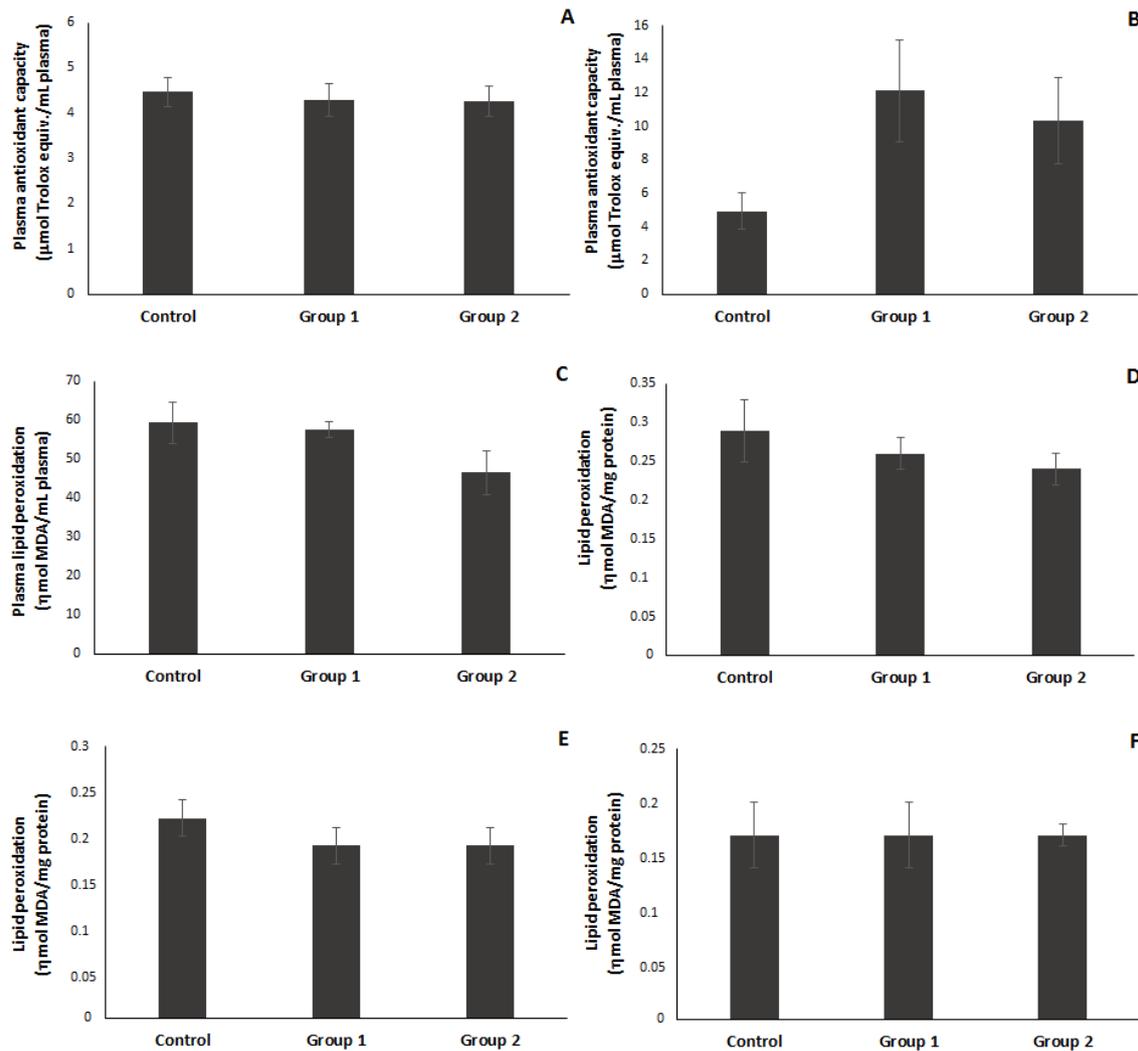
The two important indicators of renal health were evaluated. Creatinine varied from 0.73 to 0.97 mg/dL, and urea ranged between 61 and 67 mg/dL. No effect ( $p > 0.05$ ) was observed after administration of jaboticaba during 40 days, comparing the control with treated groups (**Figure 4**).



**Figure 4.** Plasma creatinine (**A**) and urea (**B**) concentrations (mg/dL) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving jaboticaba for 40 days by gavage: (**A**) total cholesterol, (**B**) triacylglycerol, (**C**) HDL-cholesterol. Values were expressed as mean  $\pm$  SD (n = 12 rats/group). Different letters above the columns indicates statistical difference.

Antioxidant capacity and lipid peroxidation are two of the most relevant parameters of oxidative stress. Antioxidant capacity assessed by FRAP assay significantly increased ( $p < 0.01$ ) from 4.5  $\mu\text{mol TE/ mL}$  plasma in the control rats to 12.1  $\mu\text{mol TE/ mL}$  plasma in the treated rats from Group 1, which represents a 2.5 fold increase. For the group that received the highest dose of jaboticaba, antioxidant capacity was 2-fold higher than in the control rats. Conversely, no significant ( $p = 0.53$ ) effect in the antioxidant capacity assessed by ORAC was observed after treatment (**Figure 5**).

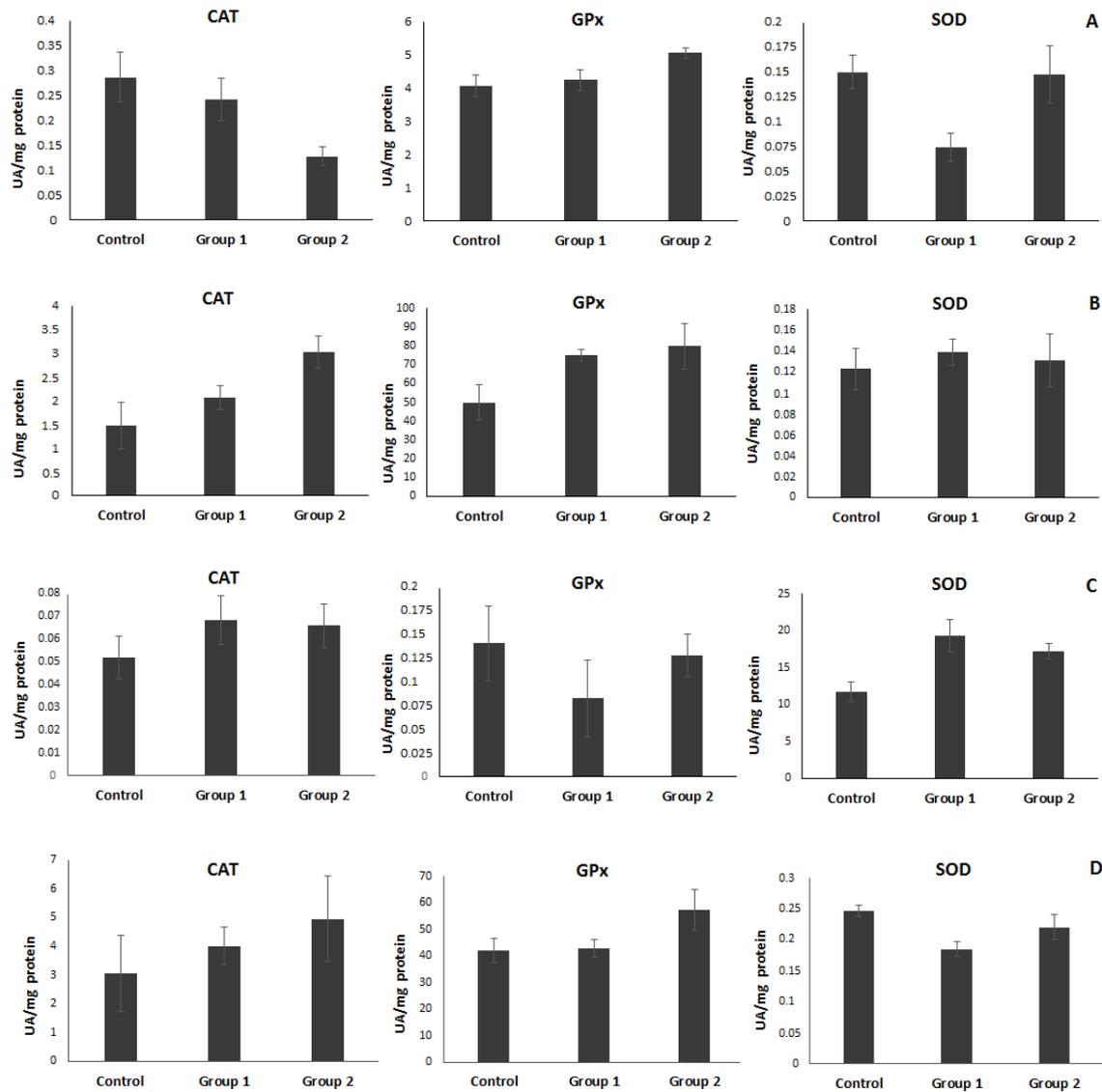
High concentration of MDA in tissues and/or in plasma is a known biomarker of oxidative damage. Untreated diabetic rats showed elevated levels of MDA in both plasma and brain, when compared to treated groups (**Figure 5**). Daily administration of jaboticaba significantly decreased lipid peroxidation in plasma ( $p < 0.01$ ) and brain ( $p = 0.01$ ), although no effect was observed in liver ( $p = 0.07$ ) and kidney ( $p = 0.54$ ). Our findings suggest that polyphenols from jaboticaba protected the STZ-diabetic rats against oxidative damage.



**Figure 5.** Plasma antioxidant capacity ( $\mu\text{mol Trolox equivalent/mL plasma}$ ) assessed by ORAC (A) and FRAP (B) assays, and lipid peroxidation ( $\eta\text{mol MDA/mg protein}$ ) of plasma (C), brain (D), kidneys (E) and liver (F) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving jaboticaba for 40 days by gavage. Values were expressed as mean  $\pm$  SD ( $n = 12$  rats/group). Different letters above the columns indicates statistical difference. The  $p$  value between supplemented and control groups was expressed as \* ( $p < 0.01$ ).

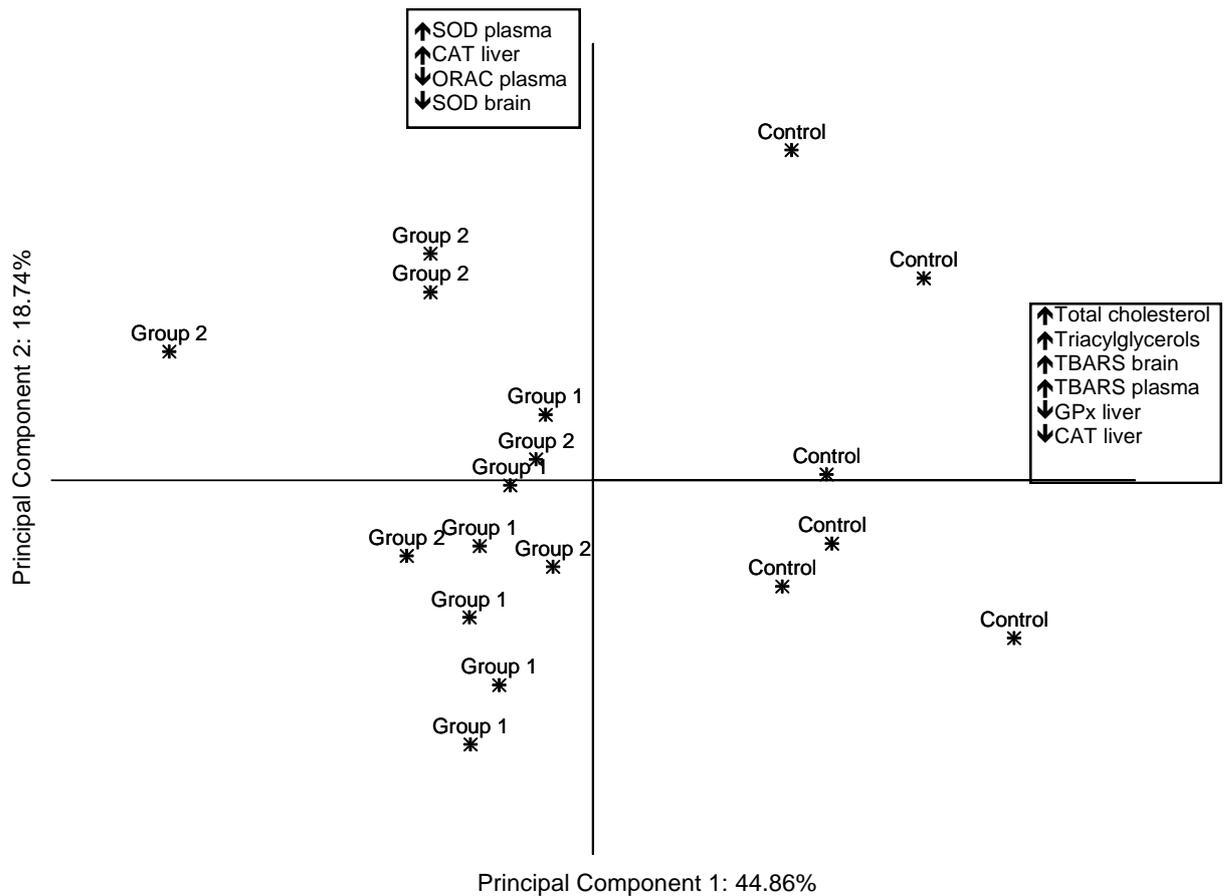
The activities of enzymatic antioxidants (SOD, CAT, GPx) were evaluated in plasma and tissues. SOD activity in the brain of treated diabetic rats increased significantly (about 55%) as compared to the control rats ( $p = 0.001$ ). Administration of jaboticaba to STZ-induced diabetic rats significantly increased ( $p < 0.01$ ) or restored the CAT and GPx activities

in kidney and liver. In plasma, GPx activity was 25% higher after treatment with the highest dose of jaboticaba than observed in control rats (Figure 6).



**Figure 6.** Activity of antioxidant enzymes (UA/mg protein) CAT, SOD and GPx in plasma (A), kidneys (B), brain (C) and liver (D) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving jaboticaba for 40 days by gavage. Values were expressed as mean  $\pm$  SD (n = 12 rats/group). Different letters above the columns indicates statistical difference. The *p* value between supplemented and control groups was expressed as \* (*p* < 0.01) and \*\* (*p* < 0.001).

A multivariate approach using principal component analysis was proposed (**Figure 7**). By analyzing the results, a total of 63.60% of data variability was explained by the first two principal components. It is possible to observe that rats treated with jaboticaba (Group 1 and Group 2) were separated from the control group using the nine selected oxidative stress biomarkers as responses, indicating that the supplementation was effective and this *in vivo* model was suitable to assess the oxidative stress and lipid profile of STZ-induced diabetic Wistar rats treated with jaboticaba. The control group was separated from both Group 1 and Group 2 due to differences (higher contents) in cholesterol, triacylglycerols, TBARS (brain and plasma), and also because of low activity of CAT (liver) and GPx (liver). In a comparison between the two experimental groups, Group 1 was separated from Group 2 based on its lower activity of SOD (plasma) and CAT (liver) and higher antioxidant capacity of plasma (ORAC) and activity of SOD (brain).



**Figure 7.** Dispersion (PC1 vs PC2) of Wistar rats identified by the experimental groups (Control, Group 1 and Group 2) using nine biomarkers of oxidative stress.

#### 4. Discussion

Experimental models carried out with the use of diabetic animals have demonstrated that oxidative stress, caused by persistent hyperglycemia, impairs the antioxidant defense system and generates reactive oxygen species by auto-oxidation of glucose. In experiments with streptozotocin-induced diabetes, both hyperglycemia and oxidative stress are involved in the etiology and pathology of disease-related complications (Baynes & Thorpe, 1997).

Streptozotocin acts causing damage to DNA, after entering into the  $\beta$ -cells via GLUT 2, a glucose transporter. The alkylation of DNA induces activation of poly ADP-ribosylation,

which is more relevant for diabetes induction than DNA damage itself. Poly ADP-ribosylation brings on depletion of cellular  $\text{NAD}^+$  and ATP, providing a substrate for xanthine oxidase, which results in the formation of superoxide radicals. Hydrogen peroxide and hydroxyl radicals are also generated, thereafter. Moreover, streptozotocin releases high amounts of nitric oxide, which is toxic and inhibits aconitase activity and participates in DNA damage.  $\beta$ -cells undergo the destruction by necrosis, as a consequence of the streptozotocin action (Szkudelski, 2001).

Many fruits belonging to the Myrtaceae family have displayed an important role in controlling the oxidative stress damage related to chronic diseases, such as diabetes, in animal model. Organic extracts from *Psidium guajava* Linn. and *Eugenia jambolana* were shown to protect against lipid peroxidation in tissue (islet  $\beta$ -cells), restore the activities of antioxidant enzymes, including GPx, CAT, and SOD, as well as to ameliorate plasma lipid profile and hyperglycemia (Huang, Yin & Chiu, 2011; Sharma, Balomajumder & Roy, 2008).

Jaboticaba also belongs to the Myrtaceae family and among the Brazilian native fruits is the most popular and largely studied in the recent years. It is very attractive not only because of the distinct flavor and high production and commercialization, but also due to its singular chemical composition (Abe et al., 2012). Our findings showed that jaboticaba is rich in phenolic compounds, mainly proanthocyanidins, ellagic acid derivatives and tannins. Previous results demonstrated that a high concentration of anthocyanins is present in the skin of the fruit (Alezandro et al., 2013). For being an important source of a wide variety of polyphenols, the effects of chronic administration of this fruit on oxidative stress in this study were evaluated.

Alterations caused by STZ are very well established (Lenzen, 2008; Szkudelski, 2001). Here the objectives were to evaluate the effect of jaboticaba under pathological stress, and in this way only animals presenting glucose levels upper than 200 mg/dL were selected to

be supplemented. Results from the literature consistently demonstrate that, besides hyperglycemia, there is an increase in triacylglycerol and total cholesterol levels in plasma, high concentrations of thiobarbituric acid reactive substances in liver and kidneys. In these tissues, the weight of the organ in proportion to the body weight is also increased (Silva, Lima, Silva, & Pedrosa, 2011).

In diabetes, polyuria, polyphagia and polydipsia are usual symptoms, which are evident since the beginning of the disease. Polyphagia is associated with the absence or resistance to insulin action that hinders glucose entry into cells. Additionally, hyperglycemia is responsible for blood hyperosmolarity, which causes an osmotic diuresis, known as polyuria. Consequently, there is an excessive loss of water leading to a dehydration and activation of thirst centre, resulting in polydipsia (Okon, Owo, Udokang, Udobang, & Ekpennyong, 2012). The chronic administration of jaboticaba ameliorates those symptoms, reducing the water and energy intakes after 40 days of supplementation.

However, since the cell injuries caused by STZ are irreversible, the treatment with jaboticaba was not able to recover the  $\beta$ -cells and insulin secretion, being difficult to control hyperglycemia (Szkudelski, 2001). Moreover, diabetes also leads to a significant weight loss related to the incapability of cells to produce energy from glucose. Once gluconeogenesis is activated, muscle protein and fats are excessively mobilized for energy production, which contributes for the weight loss, along with the dehydration caused by polyuria (Okun et al., 2012). An increase in the weight of the liver in proportion to the body weight (liver to body weight ratio) is also usual (Maritim, Dene, Sanders, & Watkins, 2003), and although jaboticaba administration had no effect ( $p > 0.05$ ) on weight during 40 days, this ratio was reduced from 7.1% in the control groups to 5.3-5.9% in the treated rats, a 21% decrease.

Lipids play an important role in the development of diabetes, and increased concentrations of lipids in plasma represent a risk factor for coronary heart diseases (He &

King, 2004). The reduction of total cholesterol and triacylglycerol levels could be related to the biological activities of jaboticaba polyphenols, which were shown to be responsible for inhibition of pancreatic lipase, a key enzyme for lipid absorption. It is well-known that pancreatic lipase is responsible for dietary fat break down before it could be absorbed from the intestine (McDougall, Kulkarni, & Stewart, 2009). Hypercholesterolemia in animals that received streptozotocin is caused by a higher intestinal absorption and increased cholesterol biosynthesis (Silva et al., 2011). The lipoproteins in diabetic rats are oxidized and may be cytotoxic, which is reversed by treatment with antioxidants (Mathe, 1995). Our results demonstrated that jaboticaba recovered lipid profile of diabetic rats, reducing both triacylglycerol and total cholesterol concentrations in plasma.

Nephropathy is one of the most severe complications of diabetes and often leads to end-stage chronic renal failure. Diabetic individuals usually have high contents of nitrogen compounds in plasma and urine, such as creatinine and urea, as a result of decreased protein synthesis and increased muscle proteolysis (Gray & Cooper, 2011). In this study, no effect was observed in the concentrations of creatinine and urea in plasma, and these data are similar to the results obtained by Oliveira and Genovese (2013) after treating STZ-diabetic rats with cupuassu and cocoa liquors.

Indeed, antioxidant capacity is decreased in the plasma of untreated diabetic animals, as a consequence of a higher requirement of antioxidants to regulate the ROS homeostasis (Posuwan et al., 2013). However, increased plasma antioxidant capacity along with reduced lipid peroxidation could be achieved after regular intake of rich sources of antioxidant compounds (Torabian, Haddad, Rajaram, Banta & Sabaté, 2009).

Anthocyanins, such as cyanidin-3-O-glucoside and delphinidin-3-O-glucoside, found in jaboticaba skin, were shown to be responsible for an increase in the antioxidant capacity of plasma in an animal model (Leite, Malta, Riccio, Eberlin, Pastore & Maróstica-Júnior, 2011).

Ellagic acid, in addition, can inhibit ROS formation (Larrosa, García-Conesa, Espín & Tomás-Barberán, 2010). Quercetin prevents oxidant injury and cell death by scavenging oxygen radicals, protecting against lipid peroxidation and by chelating metal ions (Coskun, Kanter, Korkmaz & Oter 2005).

ROS can be initially eliminated by essential scavenger enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Posuwan et al., 2013). When the activity of these antioxidant enzymes is reduced, the superoxide anion and hydrogen peroxide are exceedingly available in biological systems, stimulating the ROS production and the propagation of lipid peroxidation. Generally, the tissues of diabetic individuals showed decreased CAT activity (Posuwan et al., 2013). Hepatic SOD and GPx are also affected by increasing ROS generation and their activities are diminished, which were confirmed by our results. The effect of exogenous antioxidants, such as polyphenols from jaboticaba, would change according to the intervention period, in other words, if the administration started before the diabetes induction, concomitantly or after well-established diabetes (Maritim, Sanders & Watkins, 2003).

Results from the literature consistently demonstrate that the activities of antioxidant enzymes can be differently affected by dietary administration of phenolics, depending on the tissue considered and physiopathological condition. We previously showed that isoflavones even caused a decrease in SOD activity in the liver, which could be a compensation for the increased antioxidant capacity of the plasma, in normal condition (Barbosa, Lajolo, & Genovese, 2011). Additionally, Maritim et al. (2003), in a review of the effects of STZ and STZ plus antioxidant on the activity of antioxidant enzymes in animals (mice and rats), had already reported that there is not total agreement about the effects of diabetes on the activities of these enzymes.

Using one-way ANOVA it was possible to observe statistical differences among diabetic rats that received jaboticaba and the control group, for each biomarker evaluated in this study. However, when many responses are assessed, it is preferable to observe the results for both oxidative stress biomarkers and animal groups simultaneously. This would facilitate the visualization of the experimental results and inferences about the supplementation of jaboticaba to STZ-induced diabetic rats could be easily drawn. In this work, a multivariate statistical approach composed of principal component analysis was used to highlight differences among groups receiving jaboticaba and the control using nine biomarkers of oxidative stress. Using a two-dimensional plot (PC1 x PC2) more than 63% of the variability in the experimental data could be explained by the proposed statistical approach, which is a very interesting and desired fact once *in vivo* assays naturally present a high variability within groups. Herein, the assessment of oxidative stress of diabetic Wistar rats supplemented with jaboticaba using PCA was highly effective.

## 5. Conclusion

This study demonstrated that jaboticaba administration provided beneficial health effects in diabetic rats by improving lipid profile and reducing oxidative stress. Besides reducing water (8-14%) and energy intake (6-13%), both doses of jaboticaba were responsible for decrease in total cholesterol (32%) and triacylglycerol (50%), increase in the antioxidant capacity of plasma (2-2.5 times) along with diminished lipid peroxidation in plasma (22%) and brain (10-17%). The activity of the antioxidant enzymes SOD was increased in brain, CAT and GPx in kidneys and liver, and GPx in plasma. The findings obtained here support the recommendations for including at least five portions of fruit and vegetables daily as part of a healthy diet, preventing development or complications of non-transmissible chronic

diseases. However, further studies are essential to elucidate the exact mechanism of this modulatory effect and also to evaluate the jaboticaba potential therapeutic effects.

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### **References**

Albrecht, W., & William, B. J. (1981). Glutathione peroxidase. *Methods in Enzymology*, 77, (pp. 325–333): Academic Press.

Alezandro, M. R., Dubé, P., Desjardins, Y., Lajolo, F. M., & Genovese, M. I. (2013). Comparative analysis of chemical and phenolic composition of two species of jaboticaba: *Myrciaria jaboticaba* (Vell.) Berg and *Myrciaria cauliflora* (Mart.) O. Berg. *Food Research International*, submitted.

AOAC. (2005). *Official Methods of Analysis*. (18th ed.). Washington: Association of Official Analytical Chemists.

Arabbi, P. R., Genovese, M. I., & Lajolo, F. M. (2004). Flavonoids in vegetable foods commonly consumed in Brazil. *Journal of Agricultural and Food Chemistry*, *52*, 1124-1131.

Barbosa, A. C. L., Lajolo, F. M., & Genovese, M. I. (2011). Effect of free or protein-associated soy isoflavones on the antioxidant status in rats. *Journal of the Science of Food and Agriculture*, *91*, 721-731.

Baynes, J. W., & Thorpe, S. R. (1997). The role of oxidative stress in diabetic complications. *Current Opinion in Endocrinology, Diabetes and Obesity*, *3*, 277-284.

Benzie, I. F. F., & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a measure of ‘‘Antioxidant Power’’: The FRAP Assay. *Analytical Biochemistry*, *239*, 70-76.

Coskun, O., Kanter, M., Korkmaz, A., & Oter, S. (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and b-cell damage in rat pancreas. *Pharmacological Research*, *51*, 117–123.

Donadio, L. C. (2000). *Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.* Jaboticabal: FUNEP, 55 pp.

Dragano, N. R. V., Marques, A. C., Cintra, D. E. C., Solon, C., Morari, J., Leite-Legatti, A. V., Velloso, L. A., & Maróstica-Júnior, M. R. (2013). Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. *British Journal of Nutrition*, *1*, 1-9.

Durackova, Z. (2010). Some current insights into oxidative stress. *Physiological Research*, 59, 459-469.

Granato, D., Katayama, F. C. U., & Castro, I. A. (2012). Characterization of red wines from South America based on sensory properties and antioxidant activity. *Journal of the Science of Food and Agriculture*, 92, 526-533.

Gray, S. P., & Cooper, M. E. (2011). Diabetic nephropathy in 2010: Alleviating the burden of diabetic nephropathy. *Nature Reviews Nephrology*, 7, 71-73.

Gunzler, W. A., Steffens, G. J., Grossmann, A., Kim, S. M. A., Otting, F., Wendel, A., et al. (1984). The amino-acid sequence of bovine glutathione peroxidase. *Hoppe-Seyler Zeitschrift fur Physiologische Chemie*, 365, 195–212.

Hagerman, A. E., Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry*, 26, 809–812.

He, Z., & King, G. L. (2004). Microvascular complications of diabetes. *Endocrinology and Metabolism Clinics of North America*, 33, 215–238.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplatefluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry*, 50, 4437–4444.

Huang, C. S., Yin, M. C., Chiu, L. C. (2011). Antihyperglycemic and antioxidative potential of *Psidium guajava* fruit in streptozotocin-induced diabetic rats. *Food and Chemical Toxicology*, *49*, 2189-2195.

Hugo, A., & Lester, P. (1984). Catalase in vitro. *Methods in Enzymology*, Vol. 105. (pp. 121–126): Academic Press.

Jacks, T. J., Kircher, H. W. (1967). Fluorometric assay for the hydrolytic activity of lipase using fatty acyl esters of 4-methylumbelliferone. *Analytical Biochemistry*, *21*, 279-285.

Kwon, Y. I., Vатtem, D. A., & Shetty, K. (2006). Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. *Asia Pacific Journal of Clinical Nutrition*, *15*, 107-118.

Larrosa, M., García-Conesa, M. T., Espín, J. C., Tomás-Barberán, F. A. (2010). Ellagitannins, ellagic acid and vascular health. *Molecular Aspects of Medicine*, *31*, 513-539.

Leite, A. V., Malta, L. G., Riccio, M. F., Eberlin, M. N., Pastore, G. M., & Maróstica Júnior, M. R. (2011). Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticaba* Vell Berg). *Journal of Agricultural and Food Chemistry*, *59*, 2277-2283.

Lenquiste, S. A., Batista, A. G., Marineli, R. S., Dragano, N. R. V., & Maróstica Júnior, M. R. (2012). Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. *Food Research International*, *49*, 153,160.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Lenzen, S. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*, 216-226.

Macedo, L. F. L., Rogero, M. M., Guimarães, J. P., Granato, D., Lobato, L. P., & Castro, I. A. (2013). Effect of red wines with different in vitro antioxidant activity on oxidative stress of high-fat diet rats. *Food Chemistry*, *137*, 122-129.

Maritim, A., Dene, B. A., Sanders, R. A., & Watkins, J. B. (2003). Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats. *Journal of Biochemical and Molecular Toxicology*, *17*, 193–199.

Maritim, A. C., Sanders, R. A., & Watkins, J. B. (2003). Diabetes, oxidative stress, and antioxidants: A review. *Journal of Biochemistry and Molecular Toxicology*, *17*, 24–38.

Mathé, D. (1995). Dyslipidemia and diabetes: animal models. *Diabetes & Metabolism*, *21*, 106-11.

McDougall, G. J., Kulkarni, N. & Stewart, D. (2009). Berry polyphenols inhibit pancreatic lipase activity *in vitro*. *Food Chemistry*, *115*, 193–199.

Mullarkey, C. J., Edelstein, D., & Brownlee, M. (1990). Free radical generation by early glycation products: A mechanism for accelerated atherogenesis in diabetes. *Biochemical and Biophysical Research Communications*, *173*, 932–939.

Mullen, W., McGinn, J., Lean, M. E., Maclean, M. R., Gardner, P., Duthie, G. G., Yokota, T., & Crozier, A. (2002). Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. *Journal of Agricultural and Food Chemistry*, *50*, 5191-5196.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*, 351–358.

Okon, U. A., Owo, D. U., Udokang, N. E., Udobang, J. A., & Ekpenyong, C. E. (2012). Oral administration of aqueous leaf extract of *ocimum gratissimum* ameliorates polyphagia, polydipsia and weight loss in streptozotocin-induced diabetic rats. *American Journal of Medicine and Medical Sciences*, *2*, 45-49.

Oliveira, T. B., & Genovese, M. I. (2013). Chemical composition of cupuassu (*Theobroma grandiflorum*) and cocoa (*Theobroma cacao*) liquors and their effects on streptozotocin-induced diabetic rats. *Food Research International*, *51*, 929-935.

Pinto, M. S., Lajolo, F. M., & Genovese, M. I. (2008). Bioactive compounds and quantification of total ellagic acid in strawberries (*Fragaria ananassa* Duch.). *Food Chemistry*, *107*, 1629-1635.

Porter, L. J., Hrstich, L. N., & Chan, B. G. (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, 25, 223-230.

Posuwan, J., Prangthip, P., Leardkamolkarn, V., Yamborisut, U., Surasiang, R., Charoensiri, R., & Kongkachuichai, R. (2013). Long-term supplementation of high pigmented rice bran oil (*Oryza sativa* L.) on amelioration of oxidative stress and histological changes in streptozotocin-induced diabetic rats fed a high fat diet; Riceberry bran oil. *Food Chemistry*, 138, 501-508.

Priyadarsini, K. I., Khopde, S. M., Kumar, S. S., & Mohan, H. (2002). Free radical studies of ellagic acid, a natural phenolic antioxidant. *Journal of Agricultural and Food Chemistry*, 50, 2200-2206.

Raza, H., & John, A. (2012). Streptozotocin-induced cytotoxicity, oxidative stress and mitochondrial dysfunction in human hepatoma HepG2 cells. *International Journal of Molecular Sciences*, 13, 5751–5767.

Sharma, B., Balomajumder, C., & Roy, P. (2008). Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food and Chemical Toxicology*, 46, 2376-2383.

Silva, M., Lima, W. G., Silva, M. E., & Pedrosa, M. L. (2011). Effect of streptozotocin on the glycemic and lipid profiles and oxidative stress in hamsters. *Arquivos Brasileiros de Endocrinologia & Metabologia*, 55, 46-53.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.

Song, Y., Wang, J., Li, X. K., & Cai, L. (2005). Zinc and the diabetic heart. *Biometals*, 18, 325-32.

Srinivasan, P., Vadhanam, M. V., Arif, J. M., & Gupta, R. C. (2002). A rapid screening assay for antioxidant potential of natural and synthetic agents in vitro. *International Journal of Oncology*, 20, 983-986.

Strobel, N. A., Fassett, R. G., Marsh, S. A., & Coombes, J. S. (2010). Oxidative stress biomarkers as predictors of cardiovascular disease. *International Journal of Cardiology*, 147, 191-201.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, 50, 537-46.

Tas, S., Celikler, S., Ziyank-Ayvalik, S., Sarandol, E., & Dirican, M. (2011). *Ulva rigida* improves carbohydrate metabolism, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. *Cell Biochemistry and Function*, 29, 108-113.

Torabian, S., Haddad, E., Rajaram, S., Banta, J., & Sabaté, J. (2009). Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation. *Journal of Human Nutrition and Dietetics*, 22, 64–71.

You, Q., Chen, F., Wang, X., Jiang, Y., & Lin, S. (2012). Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. *LWT – Food Science and Technology*, *46*, 164-168.

Yu, Y. M., Chang, W. C., Wu, C. H., & Chiang, S.Y. (2005). Reduction of oxidative stress and apoptosis in hyperlipidemic rabbits by ellagic acid. *The Journal of Nutritional Biochemistry*, *16*, 675–681.

Zafar, M., & Naqvi, S. N. (2010). Effects of STZ-induced diabetes on the relative weights of kidney, liver and pancreas in albino rats: A comparative study. *International Journal of Morphology*, *28*, 135–142.

#### 4. CONCLUSÕES

- ✓ A composição centesimal e de minerais das duas variedades é similar. No entanto, a jabuticaba Paulista apresenta teores de Na e K maiores, enquanto Zn e Mn estão presentes em maior quantidade na Sabará;
- ✓ Existem diferenças significativas nos teores de compostos bioativos entre as cultivares de jabuticaba analisadas. Antocianinas e os derivados de ácido elágico são os principais compostos fenólicos presentes, sendo a variedade Paulista a melhor fonte destes compostos;
- ✓ A variedade Sabará apresentou os maiores valores para capacidade antioxidante e proantocianidinas e a semente foi a porção do fruto com maior concentração de compostos fenólicos;
- ✓ O fruto verde apresentou a maior capacidade antioxidante e o maior teor de ácido elágico e houve redução destes valores com a maturação. O fruto maduro é rico em antocianinas, as quais estão concentradas na casca;
- ✓ A jabuticaba apresentou capacidade inibitória das enzimas  $\alpha$ -amilase e  $\alpha$ -glicosidase, sendo o fruto maduro e a semente as amostras com maior potencial;
- ✓ As frações fenólicas da jabuticaba foram capazes de inibir a produção de óxido nítrico em hepatócitos e macrófagos, e aumentar a captação de glicose pelo músculo, em culturas de células;
- ✓ A administração de jabuticaba aos ratos com diabetes induzida por estreptozotocina foi eficiente no aumento da atividade de enzimas antioxidantes, especialmente nos rins e cérebro dos animais, além de auxiliar no controle dos níveis plasmáticos de colesterol, o que sugere

que os compostos bioativos da jabuticaba promoveram um efeito benéfico sobre o estresse oxidativo dos animais;

✓ O ensaio de fermentação *in vitro* permitiu identificar a formação de metabólitos a partir dos compostos fenólicos da jabuticaba pela ação da microbiota colônica. Os principais compostos detectados após a fermentação com o inóculo fecal foram os ácidos *p*-hidroxibenzóico e *p*-hidroxifenilacético;

✓ No modelo de biodisponibilidade *in vivo*, foi possível verificar que os metabólitos e compostos derivados dos polifenóis da jabuticaba foram absorvidos, atingiram o plasma e foram detectados em tecidos como rins, fígado, músculo e cérebro;

Em resumo, os extratos de jabuticaba, por apresentar altos teores de compostos antioxidantes, foram efetivos em combater o estresse oxidativo gerado tanto nos modelos animais quanto em células. O consumo da fruta e/ou do suco de jabuticaba pode ser considerado uma alternativa efetiva e promissora na proteção do organismo contra os danos oxidativos, portanto, o seu consumo deve ser estimulado. Acredita-se que a redução no risco de desenvolvimento de doenças crônicas se dá pela combinação de micronutrientes, antioxidantes, fitoquímicos e fibras presentes nos alimentos.

## REFERÊNCIAS BIBLIOGRÁFICAS

- ABE, L. T.; LAJOLO, F. M.; GENOVESE, M. I. Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg). **Journal of the Science of Food and Agriculture**, v. 92, p. 1679-87, 2012.
- AMAKURA, Y.; OKADA, M.; TSUJI, S.; TONOGAI, Y. High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits. **Journal of Chromatography A**, v. 896, p. 87-93, 2000.
- ANDERSON, K. J. ; TEUBER, S. S.; GOBEILLE, A.; CREMIN, P.; WATERHOUSE, A. L.; STEINBERG, F. M. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. **Journal of Nutrition**, v. 131, p. 2837-2842, 2001.
- ARABBI, P. R.; GENOVESE, M. I.; LAJOLO, F. M. Flavonoids in vegetable foods commonly consumed in Brazil. **Journal of Agricultural and Food Chemistry**, v. 52, p. 1124-1131, 2004.
- BASTOS, D. H. M.; ROGERO, M. M.; ARÊAS, J. A. G. Mecanismos de ação de compostos bioativos dos alimentos no contexto de processos inflamatórios relacionados à obesidade. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 53, p. 646-656, 2009.
- BATE-SMITH, E. C. Detection and determination of ellagitannins. **Phytochemistry**, v. 11, p. 1153-1156, 1972.
- BEATTIE, J.; CROZIER, A.; DUTHIE, G. G. Potential health benefits of berries. **Current Nutrition & Food Science**, v. 1, p. 71-86, 2005.
- BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Legislação. Resolução RDC n. 2, de 7 de janeiro de 2002. Aprova o regulamento técnico de substâncias bioativas e probióticos isolados com alegação de propriedades funcional e ou de saúde. **Diário Oficial da União**, Brasília, 17 julho 2002.
- BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Legislação. Resolução n. 18, de 30 de abril de 1999. Aprova o Regulamento Técnico que estabelece as diretrizes básicas para análise e comprovação de propriedades funcionais e ou de saúde alegadas em rotulagem de alimentos, **Diário Oficial da União**, Brasília, 3 maio 1999.
- CAO, G.; RUSSELL, R. M.; RISCHNER, N.; PRIOR, R. L. Serum antioxidant capacity is increased by consumption of strawberry, spinach, red wine or vitamin C in elderly woman. **Journal of Nutrition**, v. 128, p. 2383-90, 1998.
- CERDÁ, B.; ESPÍN, J. C.; PARRA, A.; MARTÍNEZ, P.; TOMÁS-BARBERÁN, F. A. The potent in vitro antioxidant ellagitannins from pomegranate juice are metabolized into bioavailable but poor antioxidant hydroxy-6H-dibenzopyran-6-one derivatives by the

colonic microflora of healthy humans. **European Journal of Nutrition**, v. 43, p. 205–220, 2004.

CERDÁ, B.; LLORACH, R.; CERON, J. J.; ESPIN, J. C.; TOMAS-BARBERAN, F. A. Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. **European Journal of Nutrition**, v. 42, p. 18–28, 2003.

CERDÁ, B.; TOMÁS-BARBERÁN, F. A.; ESPÍN, J. C. Metabolism of chemopreventive and antioxidant ellagitannins from strawberries, raspberries, walnuts and oak-aged wines in humans: identification of biomarkers and individual variability. **Journal of Agricultural and Food Chemistry**, v. 53, p. 227–235, 2005.

CLIFFORD, M. N.; SCALBERT, A. Ellagitannins - nature, occurrence and dietary burden. **Journal of the Science of Food and Agriculture**, v. 80, p. 1118-1125, 2000.

COOK, N. C.; SAMMAN, S. Flavonoids: chemistry, metabolism, cardio-protective effects, and dietary sources. **Nutritional Biochemistry**, v. 7, p. 66- 76, 1996.

COZZOLINO, S. M. F. **Biodisponibilidade de Nutrientes**, 4a ed., Manole: Barueri, 2012.

CROZIER, A.; DEL RIO, D.; CLIFFORD, M. N. Bioavailability of dietary flavonoids and phenolic compounds. **Molecular Aspects of Medicine**, v. 31, p. 446-467, 2010.

DANIEL, E. M.; KRUPNICK, A. S.; HEUR, Y. H.; BLINZLER, J. A.; NIMS, R. W.; STONER, G. D. Extraction, stability and quantitation of ellagic acid in various fruits and nuts. **Journal of Food Composition and Analysis**, v. 2, p. 338-349, 1989.

DANIEL, E. M.; STONER, G. D. The effects of ellagic acid and 13-cis-retinoic acid on N-nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats. **Letters**, v. 56, p. 117-124, 1991.

D'ARCHIVIO, M.; FILESI, C.; DI BENDETTO, R.; GARGIULO, R.; GIOVANNINI, C.; MASELLA, R. Polyphenols, dietary sources and bioavailability. **Annali dell'Istituto Superiore di Sanità**, v. 43, p. 348-361, 2007.

DEL RIO, D.; COSTA, L. G.; LEAN, M. E. J.; CROZIER, A., Polyphenols and health: What compounds are involved? **Nutrition, Metabolism and Cardiovascular Diseases**, v. 20, p. 1-6, 2010.

DONADIO, L. C. **Jaboticaba (Myrciaria jaboticaba (Vell.) Berg)**. Jaboticabal: Funep, 2000. 55p.(Serie Frutas Nativas, 3).

DONOVAN, J.L.; MANACH, C.; FAULKS, R.M.; KROON, P.A., Absorption and metabolism of dietary secondary metabolites. In: Crozier, A., Clifford, M.N., Ashihara, H. (Eds.), **Plant Secondary Metabolites**. Occurrence, Structure and Role in the Human Diet. Blackwell Publishing, Oxford, New York, pp. 303–351, 2006.

- FRASSETTO, L.; MORRIS, R. C.; SELLMAYER, D. E. ; TODD, K.; SEBASTIAN, A. Diet, evolution and aging - The pathophysiologic effects of the post-agricultural inversion of the potassium-to-sodium and base-to-chloride ratios in the human diet. **European Journal of Nutrition**, v. 40, p. 200-213, 2001.
- GOBBO-NETO, L.; LOPES, N. P. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. **Química Nova**, v. 30, p. 374-381, 2007.
- GONZALEZ-BARRIO, R.; TRUCHADO, P.; GARCÍA-VILLALBA, R.; HERVAS, G.; FRUTOS, P.; ESPÍN, J. C.; TOMAS-BARBERAN, F. A. Metabolism of oak leaf ellagitannins and urolithin production in beef cattle. **Journal of Agricultural and Food Chemistry**, v. 60, n. 12, p. 3068-3077, 2012.
- HAKKINEN, S.; HEINONEN, M.; KARENLAMPI, S.; MYKKANEN, H.; RUUSKANEN, J.; TORRONEN, R. Screening of selected flavonoids and phenolic acids in 19 berries. **Food Research International**, v. 32, p. 345-353, 1999.
- HAKKINEN, S. H.; KARENLAMPI, S. O.; MYKKANEN, H. M.; HEINONEN, M. I.; TORRONEN, A. R. Ellagic acid content in berries: influence of domestic processing and storage. *European Food Research and Technology*, v. 212, p. 75–80. 2000.
- HANNUM, S. M. Potencial impact of strawberry on human health: a review of the science. **Critical Reviews in Food Science and Nutrition**, v. 44, p. 1-17, 2004.
- HARBORNE, J. B.; WILLIAMS, C. A. Advances in flavonoid research since 1992. **Phytochemistry** 2000, 55, 481-504.
- HASLAM, E. **Plant polyphenols, vegetable tannins revisited**, Cambridge University Press, Cambridge, 1989.
- HELDT, H.; **Plant Biochemistry and Molecular Biology**, University Press: Oxford, 1997. 522 p.
- HOLLMAN, P. C.; KATAN, M. B. Dietary flavonoids: intake, health effects and bioavailability. **Food and Chemical Toxicology**, v. 37, p. 937-942, 1999.
- IBGE – Instituto Brasileiro de Geografia e Estatística. **Produção Agrícola Municipal 2010**. Disponível em: <http://www.ibge.gov.br/home/estatistica/economia/pam/2010/default.shtm>. Acesso em 20 setembro 2012.
- JACOBS, D. R.; TAPSELL, L. C. Food, not nutrients, is the fundamental unit in nutrition. **Nutrition Reviews**, v. 65, p. 439-50, 2007.
- KAWANISHI, S.; MURATA, M.; TAKAHASHI, A.; SAITO, I. The role of metals in site-specific DNA damage with reference to carcinogenesis. **Free Radical Biology and Medicine**, v. 32, p. 822-32, 2002.
- KEMPERMAN, R. A.; BOLCA, S.; ROGER, L. C.; VAUGHAN, E. E. Novel approaches for analysing gut microbes and dietary polyphenols: challenges and opportunities. **Microbiology**, v. 156, p. 3224-3231, 2010.

- KRESTY, L. A.; MORSE, M. A.; ADAMS, C. A.; LU, J.; STONER, G. D. Inhibitory effect of lyophilized black raspberries on esophageal tumorigenesis and O6-methylguanine levels in the F344 rat. **Proceedings of the Annual Meeting of the American Association for Cancer Research.**, A120, 1998.
- KWON, Y.I.; VATTEM, D. A.; SHETTY, K. Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. **Asia Pacific Journal of Clinical Nutrition**, v. 15, p. 107-18, 2006.
- LAGUERRE, M.; LECOMTE, J., VILLENEUVE, P. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. Review. **Progress in Lipid Research**, v. 46, p. 244-282, 2007.
- LANDETE, J. M. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. **Food Research International**, v. 44, p. 1150-1160, 2011.
- LEITE, A. V.; MALTA, L. G.; RICCIO, M. F.; EBERLIN, M. N.; PASTORE, G. M.; MARÓSTICA JÚNIOR, M. R. Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel (*Myrciaria jaboticaba* Vell Berg). **Journal of Agricultural and Food Chemistry**, v. 59, p. 2277-2283, 2011.
- LIMA, A. J. B.; CORRÊA, A. D.; ALVES, A. P. C.; ABREU, C. M. P.; DANTAS-BARROS, A. M. Caracterização química do fruto jaboticaba (*Myrciaria cauliflora* Berg) e de suas frações. **Archivos Latinoamericanos de Nutrición**, v. 58, p. 416-421, 2008.
- MAAS, J. L.; WANG, S. Y.; GALLETTA, G. J. Evaluation of strawberry cultivars for ellagic acid content. **HortScience**, v. 26, n.1, p. 66-68, 1991.
- MANACH, C.; DONOVAN, J. L., Pharmacokinetics and metabolism of dietary flavonoids in humans. **Free Radical Research**, v. 38, p.771-785, 2004.
- MANACH, C.; SCALBERT, A.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Polyphenols: food sources and bioavailability. **American Journal of Clinical Nutrition**, v. 79, p. 727-47, 2004.
- MANDAL, S.; STONER, G. D. Inhibition of N-nitrosobenzylamine-induced esophageal tumorigenesis in rats by ellagic acid". **Carcinogenesis**, v. 11, p. 55-61, 1990.
- MATTOS, J. R. **Frutíferas Nativas do Brasil: Jaboticabeiras**; Porto Alegre, 1983; 92 p.
- MCBAIN, A.; MACFARLANE, G. Investigations of bifidobacterial ecology and oligosaccharide metabolism in a three-stage compound continuous culture system. **Scandinavian journal of gastroenterology**. Supplement, v. 222, p. 32, 1997.
- MCDUGALL, G. J.; STEWART, D. The inhibitory activity effects of berry polyphenols on digestive enzymes. **Biofactors**, v. 23, p. 189-195, 2005.

- MCLEAN, J. A.; KARADAS, F.; SURAI, P.; McDEVITTI, R.; SPEAKE, B. Lipid-soluble and water-soluble antioxidant activities of the avian intestinal mucosa at different sites along the intestinal tract. **Comparative Biochemistry and Physiology**, v. 141, p. 366-372, 2005.
- MEYER, A. M. B. S.; HEINONEN, M.; FRANKEL, E. N. Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin, and ellagic acid on human LDL oxidation. **Food Chemistry**, 61, 71-75, 1998.
- MULLEN, W.; MCGINN, J.; LEAN, M.E.; MACLEAN, M. R.; GARDNER, P.; DUTHIE, G. G.; YOKOTA, T.; CROZIER, A. Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. **Journal of Agricultural and Food Chemistry**, v. 50, p. 5191-5196, 2002.
- NARAYANAN, B. A.; GEOFFROY, O.; WILLINGHAM, M. C.; RE, G. G.; NIXON, D. W. p53/p21(WAF1/CIP1) expression and its possible role in G1 arrest and apoptosis in ellagic acid treated cancer cells. **Cancer Letters**, v. 136, p. 215-221, 1999.
- NOGUEIRA, A. K. M.; SANTANA, A. C. Análise de sazonalidade de preços de varejo de açaí, cupuaçu e bacaba no estado do Pará. **Revista de Estudos Sociais** - ano 11, v. 21, n. 1, 2009. 22p.
- OLIVEIRA, D. M.; BASTOS, D. H. M. Biodisponibilidade de ácidos fenólicos. **Química Nova**, v. 34, p. 1051-1056, 2011.
- ORDOVAS, J. M.; KAPUT, J.; CORELLA, D. Nutrition in the genomics era: cardiovascular disease risk and the Mediterranean diet. **Molecular Nutrition and Food Research**, v. 51, p. 1293-9, 2007.
- PIMENTEL, C. V. M. B.; FRANCKI, V. M.; GOLLÜCKE, A. P. B. **Alimentos funcionais: introdução às principais substâncias bioativas em alimentos**. São Paulo: Varela; 2005.
- PINTO, M. S.; LAJOLO, F. M.; GENOVESE, M. I. Bioactive compounds and quantification of total ellagic acid in strawberry (*Fragaria x ananassa* Duch). **Food Chemistry**, v. 107, p. 1629-1635, 2008.
- POSSEMIERS, S.; BOLCA, S.; VERSTRAETE, W.; HEYERICK, A. The intestinal microbiome: A separate organ inside the body with the metabolic potential to influence the bioactivity of botanicals. **Fitoterapia**, v. 82, p. 53-66, 2011.
- PRIYADARSINI, K. I.; KHOPDE, S. M.; KUMAR, S. S.; MOHAN, H.; Free radical studies of ellagic acid, a natural phenolic antioxidant. **Journal of Agricultural and Food Chemistry**, v. 50, p. 2200-2206, 2002.
- PUUPPONEN-PIMIA, R.; NOHYNEK, L.; HARTMANN-SCHMIDLIN, S.; KAHKONEN, M.; HEINONEN, M.; MAA TTA-RIIHINEN, K.; OKSMAN-CALDENTY, K. M. Berry phenolics selectively inhibit the growth of intestinal pathogens. **Journal of Applied Microbiology**, v. 98, p. 991-1000, 2005.

- RECHNER, A. R.; SMITH, M. A.; KUHNLE, G.; GIBSON, G. R.; DEBNAM, E. S.; SRAI, S. K. S.; MORRE, K. P.; RICE-EVANS, C. A. Colonic metabolism of dietary polyphenols: influence of structure on microbial fermentation products. **Free Radical Biology & Medicine**, v. 36, p. 212-225, 2004.
- SANTOS-BUELGA, C.; SCALBERT, A. Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. **Journal of the Science of Food and Agriculture**, v. 80, p. 1094-1117, 2000.
- SAURA-CALIXTO, F.; GONI, I. Definition of the Mediterranean diet based on bioactive compounds. **Critical Review in Food Science and Nutrition**, v. 49(2), p. 145-52, 2009.
- SELMA, M. A. V.; ESPÍN, J. C.; TOMÁS-BARBERÁN, F. A. Interaction between phenolics and gut microbiota: role in human health. **Journal of Agricultural and Food Chemistry**, v. 57, p. 6485-6501, 2009.
- SETCHELL, K. D. R.; BROWN, N. M.; LYDEKING-OLSEN, E. The clinical importance of the metabolite equol—a clue to the effectiveness of soy and its isoflavones. **The Journal of Nutrition**, v.132, p. 3577-3584, 2002.
- SIKORA, E.; CIESLIK, E.; LESZCZYNSKA, T.; FILIPIAK-FLORKIWUACZ, A.; PISULEWSKI, P. M. The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. **Food Chemistry**, v. 107, p. 50-55, 2008.
- SONG, Y.; WANG, J.; LI, X. K. ; CAI, L. Zinc and the diabetic heart. **Biometals**, v. 18, p. 325-32, 2005.
- SRINIVASAN, P.; VADHANAM, M. V.; ARIF, J. M.; GUPTA, R. C. A rapid screening assay for antioxidant potential of natural and synthetic agents in vitro. **International Journal of Oncology**, v. 20, p. 983-986, 2002.
- TAPAS, A. R.; SAKARKAR, D. M.; KAKDE, R. B. Flavonoids as Nutraceuticals: A Review. **Tropical Journal of Pharmaceutical Research**, v. 7, p. 1089-1099, 2008.
- VASCO, C.; RUALES, J.; KAMAL-ELDIN, A. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. **Food Chemistry**, v. 111, p. 816-823, 2008.
- WHITLEY, A. C.; STONER, G. D.; DARBY, M. V.; WALLE, T. Intestinal epithelial cell accumulation of the cancer preventive polyphenol ellagic acid--extensive binding to protein and DNA. *Biochemical Pharmacology*, v. 66, p. 907-915, 2003.
- XU, X.; HARRIS, K. S.; WANG, H.-J.; MURPHY, P. A.; HENDRICH, S. Bioavailability of soybean isoflavones depends upon gut microflora in women. **The Journal of Nutrition**, v. 125, p. 2307-2315, 1995.
- YUAN, J. P.; WANG, J. H.; LIU, X. Metabolism of dietary soy isoflavones to equol by human intestinal microflora--implications for health. **Molecular Nutrition and Food Research**, v. 51, p. 765-781, 2007.

