UNIVERSITY OF SÃO PAULO
School of Pharmaceutical Science
Graduate Program in Food Science
Field of Study Bromatologie

Biomarker exposure of phenolic compounds from grumixama (*Eugenia brasiliensis* Lam.) in healthy human model: Metabolomic approach

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Thesis for obtaining DOCTORAL degree in Science

São Paulo
2016
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Corrected version of Thesis according to resolution CoPGr 6018. The original version is available at the Post-Graduation Service of FCF/USP

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São Paulo
2016
Teixeira, Luciane de Lira
T266b  Biomarker exposure of phenolic compounds from grumixama (Eugenia brasiliensis Lam.) in healthy human model: metabolomics approach / Luciane de Lira Teixeira -- São Paulo, 2016. 157p.

Thesis (doctorate) -- School of Pharmaceutical Science of University of São Paulo. Department of Food and Experimental Nutrition.
Advisor: Hassimotto, Neuza Mariko Aymoto.

1. Alimento : Composto fenólico : Ciência dos alimentos
Biomarker exposure of phenolic compounds from grumixama (*Eugenia brasiliensis* Lam.) in healthy human model: Metabolomic approach

Referee Commission
Thesis for obtaining doctoral degree in Science

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1º Examiner

2º Examiner

3º Examiner

4º Examiner

São Paulo, _____ of _____________ of 2016
ACKNOWLEDGMENTS

I would like to thank God to make me strong during my four years of doctoral course.

To my family and friends, including my ex-husband, to encourage me to do my doctoral course at University of São Paulo.

To my advisor, Professor Neuza Mariko Aymoto Hassimotto, for being the best advisor I could have here.

Special thanks to Professor Franco Maria Lajolo, Professor Ernani Pinto, Professor Thomas Prates Ong, PhD Fabiane Dörr, PhD Felipe Dörr and Msc Gabriela Rezende Souza to collaborate with my work.

Special thanks to the laboratory teknichians, PhD Tânia Shiga, Lúcia Helena Justinos and Elias Araújo to the great work and support.

I would like to thank PhD Silas G. Villas-Bôas to advise me about the metabolomic analysis and for the great opportunity of a short internship at University of Auckland (Auckland, New Zealand), as well to all friends that I made in his laboratory. Thanks for Núcleo de Apoio a Pesquisa em Alimentos e Nutrição (NAPAN) for providing the scholarship.

I would like also to thank PhD André Marette, PhD Geneviève Pilon and PhD Yves Desjardans to receive me for an internship at Université Laval (Quebéc, Canadá), specially to Geneviève Chevrier for being a great friend. Thanks for CAPES for providing the scholarship.

Thanks to CNPq for providing scholarship and FAPESP (Forc-CEPID) for financial support.

I learned a lot in all opportunities that I received during my doctoral course and I am sure that these learning made me a better researcher and person.
Resumo


O consumo de frutas e verduras na dieta está associado à redução da incidência de doenças crônicas não transmissíveis entre eles câncer, síndrome metabólica e doenças cardiovasculares. A atividade biológica atribuída à ingestão desses alimentos é relacionada principalmente à presença de compostos bioativos, entre eles os compostos fenólicos, tais como flavonoides e elagitaninos. Contudo, a biodisponibilidade e a influência destes compostos no metabolismo humano não estão estabelecidas. Assim, o objetivo geral do presente estudo foi investigar as alterações no metaboloma humano decorrente da ingestão de uma fonte rica em compostos fenólicos, o suco da grumixama roxa (Eugenia brasiliensis Lam.), buscando identificar possíveis pontos de regulação do metabolismo. Para isto, a grumixama, variedades amarela e roxa, foram caracterizadas quanto ao seu perfil de compostos fenólicos e administradas na forma de suco, em dose única, a voluntários saudáveis. A grumixama roxa se mostrou rica em antocianinas e elagitaninos, principalmente cianidina 3-O-glicosídeo e a strictinina, respectivamente. Para o ensaio clínico, 15 voluntários saudáveis consumiram, em dose única, suco de grumixama roxa (10 ml de suco/kg de peso corporal). Amostras de plasma e urina foram coletadas em diferentes tempos durante 24 h após ingestão e analisados por CG-MS e LC-ESI-MS/MS. Os metabólitos exógenos excretados na urina foram identificados como urolitinas e ácidos fenólicos, derivados da degradação, principalmente, pela microbiota dos elagitaninos e das antocianinas, respectivamente. Quatro urolitinas (A, B, C e D) foram encontradas na urina, principalmente como metabólitos de fase II, detectados a partir de 4 h após a ingestão do suco com aumento na concentração observado até 24h. Além disso, quatro ácidos fenólicos foram identificados, destes o ácido hipúrico como majoritário. 114 metabólitos, entre eles, 17 aminoácidos, 47 ácidos orgânicos, 7 outras classes de compostos e 43 compostos desconhecidos foram identificados por CG-MS, para os tempos de coleta de urina antes da ingestão de suco de grumixama (T0) e para os períodos de 1-2 h e 2-4 h após a ingestão. A OPLS-DA foi utilizada para descobrir os metabólitos alterados pela ingestão de suco de grumixama. A análise das vias metabólicas mostrou que a ingestão do suco de grumixama influenciou principalmente em três vias metabólicas: metabolismo do glicoxilato e dicarboxilato (up-regulated), da betalalanina (down-regulated) e da fenilalanina (up-regulated), sendo essas direcionadas ao metabolismo energético. Além disso, os extratos de grumixama roxa e amarela também foram testados em modelo animal (camundongo C57BL/6J) de obesidade e resistência à insulina induzido por uma dieta rica em lipídios e açúcares. O tratamento com os extratos, concomitante à dieta e durante 8 semanas, promoveu modulação significativa do metabolismo lipídico. Como conclusões, a grumixama roxa mostrou ser uma boa fonte de antocianinas e elagitaninos, e a interação entre metabólitos oriundos da ingestão do fruto e dos metabólitos endógenos podem estar relacionados com alterações nos metabolismos de aminoácidos e energético. No entanto, mais estudos são necessários para elucidar e validar as hipóteses geradas.

Palavras-chave: grumixama, antocianinas, elagitaninos, urolitinas, aminoácidos, ácidos orgânicos, metabôlica.
ABSTRACT

TEIXEIRA, L.L. Biomarker exposure of phenolic compounds from grumixama (Eugenia brasiliensis Lam.) in healthy human model: Metabolomic approach. 2016. 157f. Tese (Doutorado) - School of Pharmaceutical Sciences, University of São Paulo, São Paulo, 2016.

The fruits and vegetables intake has been associated to the reduction of chronic non-communicable disease incidence, such as cancer, metabolic syndrome and cardiovascular diseases. The biological activities attributed to them has been related mainly to the phenolic compounds, such as flavonoids and ellagitannins, presents in their composition. However, the bioavailability and influence of these compounds under human metabolism still unclear. Thus, the objective of the present study was investigate changes in human metaboloma as a result of the acute intake of the polyphenol-rich source from purple grumixama juice (Eugenia brasiliensis Lam.), searching to identify possible sites of metabolic regulation. In this way, purple and yellow grumixama varieties were characterized to polyphenol profile, and a single dose of the purple grumixama juice was administered to healthy human. The purple grumixama showed be a good source of anthocyanins and ellagitannins, mainly cyanidin 3-O-glucoside and strictinin, respectively. In the clinical trial, 15 healthy subjects intake a single dose of purple grumixama juice (10 ml of juice/kg of body mass). Plasma and urine samples were collected, before and after intake (over 24 h), and analyzed by GC-MS and LC-MS. The exogenous metabolites excreted and identified in urine samples by LC-MS were identified as urolithins and phenolic acids, gut microbiota catabolites of ellagitannins and anthocyanins, respectively. Four urolithins were detected beginning excretion 4 h after juice intake, increasing over 24 h. Furthermore, four phenolic acids were identified, being the hippuric acid the majority of them. 114 metabolites were identified to urine collection points before and after intake (1-2 h and 2-4 h) by CG-MS, being 17 amino acids, 7 other classes, 47 organic acids and 43 unknown compounds. A OPLS-DA discriminated the metabolites changed by the grumixama juice intake. The pathway analysis showed that juice intake influenced mainly three metabolic pathways: glyoxylate and dicarboxylate metabolism (up-regulated), beta-alanine metabolism (down-regulated), and phenylalanine metabolism (up-regulated), being these pathways related to energetic metabolism. Furthermore, the purple and yellow grumixama fruits extracts were evaluated in animal model of obesity and insulin resistance (C57BL/6J mice) induced by high fatty and high sugar diet. The treatment, during 8 weeks, promoted lipid metabolism modulation. As conclusions, purple grumixama showed to be a good source of anthocyanins and ellagittanins, and the interaction among the metabolites from fruits and endogenous metabolites can be related to changes in energetic metabolism and amino acid metabolism. However, more studies are necessary to elucidate and validate these hypotheses.

Key-words: grumixama, anthocyanins, ellagitannins, amino acids, organic acids, metabolimic.
1 INTRODUCTION

The healthy conditions have been associated to the food quality intake during the life. The intake of fruits and vegetables has been related to the reduction of incidence of chronic diseases such as cancer, metabolic syndrome and cardiovascular diseases (PAREDES-LÓPEZ et al., 2010). The health benefits associated to fruits and vegetables-rich diets have been attributed to bioactive compounds that can be essential nutrients, such as ω3-fatty acids and vitamin C, and non-essential nutrients, such as carotenoids and phenolic compounds (CLIFFORD and BROWN, 2006; SCALBERT et al., 2014).

Nutritional intervention studies have been looking for explanations to biological activities attributed to nutrients and non-nutrients bioactive compounds and their role on health promotion (SCALBERT et al., 2014). These bioactive compounds can interact with human organism playing an important role by modulating enzyme systems (SZOTAKOVA et al., 2013), transcription factors (SALAMONE et al., 2016), proteins expression, signaling cascade (EDWARDS et al., 2015) and showing more directly antioxidant activity (DÍAZ-RUBIO et al., 2015).

The metabolomic approach shows to be a powerful tool to helpful to explain these biological activities, attributed to nutrients and non-nutrients compounds.

Among the “omics” (Proteomic, Genomic, Metabolomic and Transcriptomic), the metabolomic approach showed to be the most directly related to possible biological effects of the nutrition intervention (HERRERO et al., 2012; SCALBERT et al., 2014), due to it search for metabolites, which could express an end product of the biological system. Metabolomic studies are used to identify biomarker of exposure from food and endogenous metabolome, trying to understand the interaction among them (SCALBERT et al., 2014) and their interaction with also gut microbiota (VERNONCCHI et al., 2012). Usually, the metabolites are separated in endogenous and exogenous compounds (SCALBERT et al., 2014), but it also possible to identify metabolites that can be both or be formed from gut microbiota (VERNOCCHI et al., 2012, JACOBS et al., 2012). The metabolomic studies can be classified in targeted and untargeted studies, being the search for metabolites specific and unspecific, respectively. This classification is a direct consequence of sample preparation and extraction which will direct the findings to specific (targeted) or unspecific metabolites (untargeted).

Among the non-nutrients, bioactive compounds, the phenolic compounds present in high amount in fruits and vegetables have been associated to the biological activities, such as
flavonoids and ellagitannins (ETs) (PAREDES-LÓPEZ et al., 2010, LLORACH et al., 2014; DÍAZ-RUBIO et al., 2015). Flavonoids have showed modulate some important pathways as amino acid metabolism (JACOBS et al. 2012) and steroid biosynthesis (MEDINA et al., 2013). However, most of the studies involving metabolomic approach search for exogenous metabolites (LLORACH et al., 2014).

Several biological activities have been attributed to phenolic compounds, including anti-inflammatory, antioxidant and anti-proliferative activities and apoptosis (LI et al., 2009; LI et al., 2011). However, the biological relevance of anthocyanin and ETs intake have been discussed extensively due to the low bioavailability of the native structure, and the beneficial effects have been attributed to the catabolites formed by gut microbiota degradation (KAY, KROON and CASSIDY, 2009; WILLIAMSON and CLIFFORD, 2010). The main catabolites associated with anthocyanins and ETs metabolism are phenolic acids (VITAGLIONE et al., 2007; CZANK et al., 2013) and urolithins (GONZALEZ-BARRIO et al., 2010; GARCÍA-VILLALBA et al., 2013; GARCÍA-MUÑOZ et al., 2014), respectively, which shows the importance of gut microbiota in polyphenol bioavailability assessment.

Anthocyanin bioavailability has been found to be less than 1% of intake (DEL RIO et al., 2013) (considering 690 mg intake) (WU et al., 2002). Overall, 70% disappeared from the gastrointestinal tract after 4 h through gut microbiota action and also by spontaneous degradation by neutral pH (VITAGLIONE et al., 2007; FANG, 2014). When the catabolites, such as phenolic acids, were considered, the bioavailability increased to approximatly 12% (CZANK et al., 2013).

Similarly, ETs were not bioavailable and were metabolized by gut microbiota to form dibenzopyranone compounds, which are also known as urolithins (GONZALEZ-BARRIO et al., 2010; GARCÍA-VILLALBA et al., 2013; TULIPANI et al., 2012; SELMA et al., 2014; LUDWIG et al., 2015). The urolithins can be transformed by the ellagic acid (EA) that is already present in the food matrix or by the EA formed by the action of gut microbiota under the ETs (GARCÍA-VILLALBA et al., 2013; GARCÍA-MUÑOZ et al., 2015).

The grumixama (Eugenia brasilienses Lam.) is a cherry, approximately 2.0 cm in diameter, containing one or many seeds, and is somewhat sweet. It belongs to the Myrtacea family and is a native fruit of the southern and southeastern regions of the Atlantic Forest of Brazil. Three varieties were recognized by Cambessèdes (1832–1833) according to their fruit colors: The α-variety or purple fruit is the most common and is known as the Brazilian cherry (MORENO et al., 2007; FLORES et al., 2012). The β-variety has red fruit, and the γ-variety has white fruit, although it was also described by Mattos (1984) as being yellow (MORENO
et al., 2007). Despite belonging to the same species, the yellow and purple varieties show distinct chemotypes with respect to the terpene profiles in the leaves and fruits (MORENO et al., 2007). Grumixama is rarely consumed as a fresh fruit, but is commonly used to produce fruit juice and frozen pulp, with the pulp frequently containing a mixture of the purple and yellow forms. Although still limited, commercial cultivation has begun in recent years, and this plant represents economic potential due to the attractive sensory attributes and phenolic compounds in its composition, including ellagitannins and flavonoids (REYNERTSON et al., 2008; FLORES et al., 2012; ABE et al., 2012).

In general, fruits belonging to this family are known to be good sources of bioactive compounds (REYNERTSON et al., 2008; FRACASSETTI et al., 2013). The purple grumixama fruit was shown to be rich in anthocyanins, mainly cyanidin-3-glucoside, and carotenoids, mainly monohydroxy carotenoids such as all-trans-β-cryptoxanthin (Flores et al., 2012; SILVA et al., 2014). High antioxidant capacity (REYNERTSON et al., 2008) and anti-inflammatory activity (FLORES et al., 2012) have been demonstrated. However, no information about phenolic composition, the antioxidant capacity, or other biological activities of the yellow fruit was previously available.

As a member of the Myrtaceae family, in addition to the flavonoids, grumixama would also be expected to be rich in ETs and EA derivates, another class of phenolic compounds. This class of phenolics is relatively uncommon in dietary fruits and vegetables, although a few berries, such as strawberry, raspberry, blackberry, and pomegranate, have substantial contents of these compounds (KASIMSETTY et al., 2010; LANDETE et al., 2011; SANGIOVANNI et al., 2013). It has been suggested that ETs as well as flavonoids may prevent chronic diseases such as cancer and cardiovascular diseases. These protective activities are attributed to both classes of phytochemicals, which are thought to provide antiproliferative (ASCACIO-VALDÉS et al., 2011), anti-inflammatory, and antioxidant activities (SANGIOVANNI et al., 2013; MORTON et al., 2000; NIJVELDT et al., 2001; CLIFFORD and BROWN, 2006) and to function as glycemic regulators (ASCACIO-VALDÉS et al., 2011; ŞTEFĂNUŢ et al., 2013; SEPÚLVEDA et al., 2011).

Several biological activities have been attributed to both phenolic compounds, including anti-inflammatory, antioxidant and anti-proliferative activities and apoptosis (LI et al., 2009; LI et al., 2011). Among the biological activities, the antiproliferative activity of metabolites in breast cancer cells and the effects of poplyphenols against non-alcoholic fatty liver disease had been explored in this thesis.
The anti-tumor activity of anthocyanins and ETs from many fruit and vegetable extracts have been explored in mammal cells. Berries such as blackberry, raspberry, blueberry and strawberry have demonstrated inhibitory activity and apoptosis in oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), and prostate (LNCaP) cancer cells (SEERAM et al., 2006). Anthocyanin has been found in raspberry extract, but the anti-proliferative activity on HeLa cancer cells was mainly associated with ETs (ROSS et al., 2007). In addition, EA also demonstrated anti-proliferative activity against lung (NCI 460), breast (MCF-7) and ovary (OVCAR03) cancer cells, and the activity was more efficient compared to strawberry and strawberry’ ETs extracts (PINTO et al., 2010). Additionally, urolithins strongly inhibited both androgen-dependent and androgen-independent prostate cancer cells (CaP) (KASIMSETTY et al., 2010).

Non-alcoholic fatty liver disease (NAFLD) is extensively reported worldwide and it is related to metabolic syndrome associated to insulin resistance and obesity (ROY et al., 2013; CUI et al., 2014). NAFLD can carry out to development of liver inflammation, steatohepatitis, hepatic fibrosis and cirrhosis (PAN et al., 2015). The pathogenesis of NAFLD has showed be deeply associated to metabolism disorders that promote the energy storage as triglycerides in steatotic hepatocytes in adipocyte-packed vacuoles. Insulin resistance and fatty acid metabolism disorders and inflammatory disorders have been usually associated with NAFLD (BERLANGA et al., 2014).

Polyphenol-rich sources can be helpful to inhibit NAFLD disease progression as well as health melioration (SALAMONE et al., 2012; ANHÊ et al., 2014). Phenolic compounds can act by modulation the antioxidant enzymatic systems (ANHÊ et al., 2014), modulation of lipogenesis (SALAMONE et al., 2012), inflammation inhibition (SALAMONE et al., 2016), directly action as antioxidant (DIAZ-RUBIO et al., SALAMONE et al., 2016) and also by change gut microbiota. The gut microbiota composition has been also appointed as an important factor in NAFLD development. Awhere, a gut microbiota more responsive to a high fatty diet increasing de-novo lipogenesis and triglycerides in liver (ROY et al., 2013). However, the mechanisms of polyphenol activity in NAFLD prevention and inhibition progression still under-recognized with much unknowns.
7 CONCLUSION

The grumixama showed to be a good source of flavonoids and ellagitannins and the interaction among exogenous and endogenous metabolites following an acute intake can be related to changes in amino acids and energetic metabolisms.

In addition, the polyphenols metabolites had also demonstrated potential antiproliferative activity for human breast cancer cells, as well as induced protection against non-alcoholic fatty liver disease in animal model.
8 REFERENCES


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