



UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS
Programa de Pós-Graduação em Ciência de Alimentos

**DETERMINAÇÃO DA ATIVIDADE ANTIOXIDANTE E
COMPOSIÇÃO FÍSICO-QUÍMICA DE DIFERENTES
PARTES DE QUATRO FRUTAS BRASILEIRAS**

Adriela Albino Rydlewski Ito

**Maringá
2013**

Adriela Albino Rydlewski Ito

**DETERMINAÇÃO DA ATIVIDADE ANTIOXIDANTE E
COMPOSIÇÃO FÍSICO-QUÍMICA DE DIFERENTES
PARTES DE QUATRO FRUTAS BRASILEIRAS**

Dissertação apresentada ao Programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de mestre em Ciência de Alimentos.

**Maringá
2013**

Dados Internacionais de Catalogação na Publicação
(CIP) (Biblioteca Central -UEM, Maringá, PR, Brasil)

I89d Ito, Adriela Albino Rydlewski
Determinação da atividade antioxidante e
composição físico-química de diferentes partes de
quatro frutas brasileiras / Adriela Albino
Rydlewski Ito. -- Maringá, 2013.
36 f. : il., tabs.

Orientador: Prof. Dr. Jesuí Vergílio Visentainer
Dissertação (mestrado) - Universidade Estadual de
Maringá, Centro de Ciências Agrárias, Programa de
Pós-Graduação em Ciências de Alimentos, 2013.

1. Frutas brasileiras. 2. Atividade antioxidante.
3. Compostos bioativos. 4. Ácidos graxos.
I. Visentainer, Jesuí Vergílio, orient. II.
Universidade Estadual de Maringá. Centro de
Ciências Agrárias. Programa de Pós-Graduação em
Ciências de Alimentos. III. Título.

CDD 21.ed. 634.6

ECSL-00976

Orientador:
Professor Dr. Jesu Vergilio Visentainer

BIOGRAFIA

Adriela Albino Rydlewski Ito nasceu na cidade de Cornélio Procópio, no estado do Paraná. Possui graduação em Nutrição pela Universidade Filadélfia (UNIFIL- Londrina/PR) e especialização em Nutrição Clínica pela Universidade Federal do Paraná (UFPR). Possui experiência nas áreas de Administração em Serviços de Alimentação, Nutrição Clínica Funcional no atendimento a pacientes com Diabetes tipo 1 e 2, obesidade, dislipidemia e síndrome metabólica e Nutrição Hospitalar, atuando principalmente nos seguintes temas: Terapia Nutricional Enteral em pacientes críticos e nutrição geriátrica.

Dedico

**Ao meu marido Akio, que passou comigo por todas as fases
do mestrado, me apoiando em todos os momentos
e sempre me incentivando a seguir em frente.**

AGRADECIMENTOS

Agradeço a Deus por me conceder saúde e discernimento, essenciais para que eu pudesse desenvolver as atividades do curso de mestrado e do projeto.

Ao meu marido Akio, por tanto apoio, compreensão da minha ausência, amor e companheirismo.

Aos meus pais: Maria Pureza Albino, minha maior incentivadora, amiga, confidente e orientadora e Claudio Rydlewski "*in memoriam*", meu grande amigo.

Ao professor Dr. Jesuí Vergílio Visentainer, por me dar a oportunidade de fazer parte do grupo CromAlimentos, pelas orientações, confiança e paciência.

Aos parceiros de laboratório Damila Rodrigues de Moraes, Eliza Rotta, Thiago Claus e Paula Montanher, pelos ensinamentos sobre metodologias e dedicação.

As amigas pessoais Aline Gohara e Cassandra Terres, pela amizade e apoio.

A professora Dra. Rosane Peralta, pelo fornecimento de alguns padrões de antioxidantes utilizados para a realização da análise de cromatografia líquida de alta eficiência.

A minha chefe e administradora do Hospital Memorial de Maringá, Maria Cristina Gobbi Marques, por permitir que eu me ausentasse do hospital para realizar as atividades pertinentes ao curso de mestrado durante os dois anos de curso.

A minha equipe de trabalho do hospital: Rosemari da Matta, Neiri Alves Pereira e Maria da Penha Cateli, pela realização de suas atividades, cumprindo com responsabilidade suas atividades e também pela parceria.

Aos amigos do laboratório CromAlimentos pela amizade e companhia durante a realização das análises no laboratório.

Muito obrigada!

APRESENTAÇÃO

Esta dissertação de mestrado está apresentada na forma de um artigo científico.

Rydlewski A. A., Morais D. R., Rotta E. M., Claus T., Visentainer, J. V. Determination of bioactive compounds, antioxidant activity and physical and chemical composition of different parts of four Brazilian fruits. Food Research International.

GENERAL ABSTRACT

INTRODUCTION. Brazil boasts a large number of native and exotic fruits and the interest in exploiting new species during recent years has arisen because many of them are rich in antioxidants and are thus able to protect the human metabolism against oxidative stress. In addition to the significant impact on human health, antioxidants may also have application in the food industry and pharmaceutical industry. Recent research studies of bioactive compounds in peels and seeds of fruits, considered subproducts, found significant levels of these compounds, which strengthens the possibilities of using these parts of the fruit as a source of nutraceutical compounds and application in the food industry to increase stability and shelf life of food products. Jambolan (*Syzygium cumini*), Maria Preta (*Solanum nigrum*), Inga (*Inga edulis*) and Japanese grape (*Hovenia dulcis*) are fruits cultivated in Brazil, but few studies have been published concerning their bioactive compounds and antioxidant activity of parts of these fruits individually. Therefore, need to be properly investigated, in order to evaluate its potential as a source of antioxidants.

AIMS. The aims of this study were to evaluate the antioxidant activity in different parts of Jambolan, Maria Preta, Inga and Japanese grape via the activity of free-radical scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay and quantify the total phenolic compounds, total flavonoids, total anthocyanins and identify fatty acids and phenolic compounds by chromatographic methods.

MATERIAL AND METHODS. Jambolan, Maria Preta, Inga and Japanese grape were obtained from farmers in the region of the state of Paraná. Parts of the fruit were separated, crushed, transferred to plastic bags, subjected to vacuum and stored in a freezer at $-18\text{ }^{\circ}\text{C}$. The methanol extracts were prepared using 10 g of each part of fruit and 100 mL of methanol. Was stirred for 4 h, the extracts were filtered and concentrated on a rotary evaporator. The dried extracts were stored at $-18\text{ }^{\circ}\text{C}$ until analyzed. Analyses of DPPH, FRAP, total phenolics, flavonoids and identification of bioactive compounds by high performance liquid chromatography (HPLC) were performed with methanol extracts. The antioxidant activity of methanolic extracts was evaluated via the activity of free-radical scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH), based on the method of El-Massry, El-Ghorab, and Farouk (2002). The results are expressed as Trolox equivalents ($\mu\text{MTE } 100\text{ g}^{-1}$) and also estimated by the FRAP assay, according to Benzie and Strain (1996). The results are expressed as equivalents in $\mu\text{M FeSO}_4\text{ } 100\text{ g}^{-1}$. The content of total phenolic compounds (TPC) was determined according to Shahidi and Naczki method (1995) and the results were expressed as gallic acid equivalents (GAE 100 mg g^{-1}). The total flavonoid (TF) was determined according Buriol *et al.* (2009) and the results were expressed as mg of quercetin equivalents (QE 100 mg g^{-1}). Extraction of total anthocyanins (TA) was performed according to Lee and Francis (1972) and the results were expressed in $\text{mg } 100\text{ g}^{-1}$ sample. The moisture, ash and crude protein (CP) were determined according to AOAC (Cunniff, 1998). The total lipids (TL) were extracted by the method of Bligh and Dyer (1959). The fatty acid methyl esters were prepared by methylation of total lipids according to Hartman and Lago (1973) and separated by chromatography according to Martin *et al.* (2008). The

identification of fatty acids (FA) were performed by comparison of retention times with a mixture of methyl esters (189-19). The quantification of FAs was performed as described by Joseph and Ackman (1992), using methyl tricosanoato as the internal standard (23:0). Was used a correction factor and a conversion factor to express the results in FAs instead of methyl esters according Visentainer (2012). The results were converted from mg g^{-1} for $\text{g } 100 \text{ g}^{-1}$ of FAs. The identification of phenolic compounds was performed by HPLC according Fratianni *et al.* (2007) and the results were expressed as $\text{mg } 100 \text{ g}^{-1}$. Statistical analysis of the results were presented as mean \pm standard deviation. Data were analyzed by analysis of variance (ANOVA). The Tukey test ($p = 0.05$), was used to evaluate the significant differences between the means of the samples. Pearson's correlation coefficient (r) was calculated for each part of fruit to relate the contents of TPC, TF and TA with DPPH and FRAP.

RESULTS. The moisture content of fruits ranged from 4.85% in seeds to 86.35% in pulps of Maria Preta. The highest levels of ash (2:46%), PB (17:08%) and TL (8.73%) were found in Japanese grape seeds. The Jambolan peels excelled in TA content (63.31 $\text{mg } 100 \text{ g}^{-1}$). The highest concentration of TPC (518.18 $\text{mg GAE } 100 \text{ g}^{-1}$) and TF (76.54 $\text{mg QE } 100 \text{ g}^{-1}$) were found in the peel and pulp of Japanese grape. The same parts of the Japanese grape also had the highest antioxidant activity by DPPH (13240.6 $\mu\text{MTE } 100 \text{ g}^{-1}$) and FRAP (690.68 $\mu\text{MFeSO}_4 \text{ } 100 \text{ g}^{-1}$). Pearson's correlation coefficient (r) showed moderate positive results in pulps and peels between of TPC and DPPH analysis, in pulps between TF and DPPH analysis and in pulps between TA and DPPH analysis. In seeds, between TPC and DPPH analysis, in peels and seeds between TF and DPPH, in pulps, peels and seeds between TPC, TF and FRAP and pulps, between TA and FRAP analysis of the results were strongly positive. The correlation was negative in peels and seeds from TA with DPPH and TA with FRAP, respectively. In peels and seeds between TA with DPPH and FRAP, the correlation was zero, respectively. Only the Jambolan seeds showed ellagic acid (36.30 $\text{mg } 100 \text{ g}^{-1}$) and epicatechin (48.14 $\text{mg } 100 \text{ g}^{-1}$) and present high levels of content of gallic acid (571.05 $\text{mg } 100 \text{ g}^{-1}$). Maria Preta peels showed higher concentrations of kaempferol (573.80 $\text{mg } 100 \text{ g}^{-1}$). The FA oleic (18:1 n-9) was found in greater proportion in pulp (18.19 $\text{mg } 100 \text{ g}^{-1}$), peel (257.24 $\text{mg } 100 \text{ g}^{-1}$) and seed (291.01 $\text{mg } 100 \text{ g}^{-1}$) of Jambolan, Inga pulp (97.36 $\text{mg } 100 \text{ g}^{-1}$) and Maria Preta seed (1891.36 $\text{mg } 100 \text{ g}^{-1}$). Linoleic acid (18:2 n-6) stood in Maria Preta pulp (48.09 $\text{mg } 100 \text{ g}^{-1}$) and peel and pulp of Japanese grape (98.69 $\text{mg } 100 \text{ g}^{-1}$). The arachidic acid (20:0) was the major FA in Maria Preta peel (888.22 $\text{mg } 100 \text{ g}^{-1}$). Inga seeds (313.70 $\text{mg } 100 \text{ g}^{-1}$) stood palmitic acid (16:0) and the Japanese grape seed (3985.95 $\text{mg } 100 \text{ g}^{-1}$), alpha-linolenic acid (18:3 n-3). Relations between FAs linoleic and linolenic (n-6/n-3) was greater than 5:1 in pulp and peel of Jambolan (51.01 and 19.54, respectively), in pulp and seed Maria Preta (51.15 and 78.30, respectively) and Inga pulp (38.40). However, relations between the FAs were more adequate in pulp and peel of Japanese grape (2.38), Maria Preta peel (0.24), Jambolan seeds (0.06), Inga seeds (0.16) and Japanese grape seeds (0.48).

CONCLUSIONS. Jambolan peels showed significant concentration of anthocyanins. Phenolic compounds responsible for the antioxidant activity of the extracts showed the highest concentration in Jambolan seeds, followed by Japanese grape, Maria Preta and Inga. The concentration of bioactive compounds identified by HPLC in peel followed the decreasing scale: Maria Preta > Jambolan > Japanese grape and pulps: Japanese grape > Jambolan > Maria Preta > Inga. DPPH and FRAP methods were able to represent the antioxidant activity of the samples with relation to concentration of

bioactive compounds only in fruit pulp. Japanese grape seed has excelled content of alpha-linolenic acid, a FA essential for human health. These results demonstrate that these seeds have significant levels of nutrients, in addition, high levels of bioactive compounds found in peels and pulps of Japanese grape, Maria Preta peels and Jambolan seeds confirm the antioxidant potential of these parts of fruit and may be considered as a source of natural antioxidants.

KEYWORDS: Brazilian fruits, bioactive compounds, antioxidant activity, fatty acids.

RESUMO GERAL

INTRODUÇÃO. O Brasil possui um grande número de frutas nativas e exóticas e o interesse em se estudar espécies pouco conhecidas tem aumentado nos últimos anos, pois muitas destas espécies são ricas em substâncias antioxidantes, capazes de proteger o metabolismo humano contra o stress oxidativo. Além do significativo impacto na saúde humana, os antioxidantes também podem ter aplicação na indústria de alimentos e farmacêutica. Recentes estudos de investigação de compostos bioativos em cascas e sementes de frutas, consideradas subprodutos, tem encontrado níveis significativos destes compostos, o que fortalecem as possibilidades de utilização dessas partes das frutas como fonte de compostos nutracêuticos e aplicação na indústria de alimentos, para aumentar a estabilidade e vida útil de produtos alimentares. Jambolão (*Syzygium cumini*), Maria Preta (*Solanum nigrum*), Ingá (*Ingá edulis*) e Uva japonesa (*Hovenia dulcis*), são frutas cultivadas no Brasil, mas poucos estudos têm sido publicados sobre os seus compostos bioativos e atividade antioxidante das partes destas frutas individualmente. Por isso, necessitam ser adequadamente investigadas, a fim de se analisar o seu potencial como uma fonte de antioxidantes.

OBJETIVOS. Os objetivos deste estudo foram avaliar a atividade antioxidante nas diferentes partes de Jambolão, Maria Preta, Ingá e Uva japonesa pelos métodos de sequestro do radical livre 1,1-difenil 2-picrilhidrazil (DPPH) e por poder de redução do ferro (FRAP), além de quantificar os compostos fenólicos totais, flavonóides totais, antocianinas totais e identificar os ácidos graxos e os compostos fenólicos por métodos cromatográficos.

MATERIAL E MÉTODOS. Jambolão, Maria Preta, Ingá e a Uva japonesa foram obtidas a partir de agricultores da região do estado do Paraná. As partes das frutas foram separadas, trituradas, transferidas para sacos plásticos, submetidas à vacuo e armazenadas em congelador a $-18\text{ }^{\circ}\text{C}$. Os extratos metanólicos foram preparados utilizando-se 10 g de cada parte da fruta e 100 mL de metanol. Agitou-se durante 4 h, posteriormente os extratos foram filtrados e concentrados em evaporador rotativo. Os extratos secos foram armazenados a $-18\text{ }^{\circ}\text{C}$ até serem analisados. As análises de DPPH, FRAP, compostos fenólicos totais, flavonóides totais e a identificação de compostos bioativos por cromatografia líquida de alta eficiência (CLAE), foram realizadas com os extratos metanólicos. A atividade antioxidante dos extratos metanólicos foi avaliada utilizando-se o método de varredura do radical livre 1,1-difenil-2-picrilhidrazil (DPPH), de acordo com El-Massry, El-Ghorab e Farouk (2002). Os resultados foram expressos como equivalente Trolox ($\mu\text{MET } 100\text{ g}^{-1}$) e também pelo ensaio FRAP, de acordo com Benzie e Strain (1996). Os resultados foram expressos como equivalentes $\mu\text{M FeSO}_4$ 100 g^{-1} . O conteúdo de compostos fenólicos totais (CFT) foi determinado de acordo com o método de Shahidi e Naczk (1995) e os resultados foram expressos como equivalentes de ácido gálico (EAG $\text{mg } 100\text{ g}^{-1}$). O teor de flavonóides totais (FT) foi determinado de acordo com Buriol *et al.* (2009) e os resultados foram expressos como equivalentes mg de quercetina (EQ $\text{mg } 100\text{ g}^{-1}$). A extração de antocianinas totais (AT) foi realizada de acordo com Lee e Francis (1972) e os resultados foram expressos em $\text{mg } 100\text{ g}^{-1}$ de amostra. A umidade, as cinzas e o teor de proteína bruta (PB) foram

determinados de acordo com a AOAC (Cunnif, 1998). Os lipídios totais (LT) foram extraídos pelo método de Bligh e Dyer (1959). Os ésteres metílicos de ácidos graxos foram preparados por metilação dos lipídios totais, de acordo com Hartman e Lago (1973) e separados por cromatografia em fase gasosa conforme Martin *et al.* (2008). A identificação dos ácidos graxos (AGs) foi realizada por comparação dos tempos de retenção com um mix de ésteres metílicos (189-19). A quantificação dos AGs foi realizada como descrito por Joseph e Ackman (1992), utilizando-se o tricosanoato de metila como padrão interno (23:0). Foram utilizados um fator de correção e um fator de conversão para expressar os resultados em AGs em vez de ésteres metílicos, de acordo com Visentainer (2012). Os resultados foram convertidos de mg g^{-1} para $\text{g } 100 \text{ g}^{-1}$ de AGs. A identificação dos compostos fenólicos por CLAE foi realizada de acordo com Fratianni *et al.* (2007) e os resultados foram expressos em $\text{mg } 100 \text{ g}^{-1}$. A análise estatística dos resultados foram apresentados como média \pm desvio padrão. Os dados foram analisados por meio da análise de variância (ANOVA). O teste de Tukey ($p = 0.05$), foi utilizado para avaliar as diferenças significativas entre as médias das amostras. O coeficiente de correlação de Pearson (r) foi calculado em cada parte das frutas para relacionar o conteúdo de CFT, FT e AT com DPPH e FRAP.

RESULTADOS. A umidade das frutas variou de 4.85% na semente até 86.35% na polpa de Maria Preta. Os maiores teores de cinzas (2.46%), PB (17.08%) e LT (8.73%) foram encontrados nas sementes de Uva japonesa. As cascas de Jambolão se destacaram no teor de AT ($63.31 \text{ mg } 100 \text{ g}^{-1}$). A maior concentração de CFT ($518.18 \text{ mg EAG } 100 \text{ g}^{-1}$) e FT ($76.54 \text{ mg EQ } 100 \text{ g}^{-1}$) foram encontrados nas cascas e polpas de Uva japonesa. As mesmas partes da Uva japonesa também apresentaram as maiores atividades antioxidante pelo método DPPH ($13240.6 \text{ } \mu\text{MET } 100 \text{ g}^{-1}$) e FRAP ($690.68 \text{ } \mu\text{MFeSO}_4 \text{ } 100 \text{ g}^{-1}$). O coeficiente de correlação de Pearson (r) apresentou resultados positivos moderados nas polpas e cascas entre as análises de CFT e DPPH, nas polpas entre as análises de FT e DPPH e nas polpas entre as análises de AT e DPPH. Nas sementes, entre as análises de CFT e DPPH, nas cascas e sementes entre as análises de FT e DPPH, nas polpas, cascas e sementes entre as análises de CFT, FT e FRAP e nas polpas, entre as análises de AT e FRAP, os resultados foram forte positivos. A correlação foi negativa nas cascas e sementes entre as análises de AT com DPPH e AT com FRAP, respectivamente. Já nas sementes e cascas entre as análises de AT com DPPH e AT com FRAP, a correlação foi nula, respectivamente. Somente as sementes de Jambolão apresentaram ácido elágico ($36.30 \text{ mg } 100 \text{ g}^{-1}$) e epicatequina ($48.14 \text{ mg } 100 \text{ g}^{-1}$), além de se destacarem no conteúdo de ácido gálico ($571.05 \text{ mg } 100 \text{ g}^{-1}$). As cascas de Maria Preta apresentaram os maiores valores de kaempferol ($573.80 \text{ mg } 100 \text{ g}^{-1}$). O AG oleico (18:1n-9) foi encontrado em maior proporção na polpa ($18.19 \text{ mg } 100 \text{ g}^{-1}$), casca ($257.24 \text{ mg } 100 \text{ g}^{-1}$) e semente ($291.01 \text{ mg } 100 \text{ g}^{-1}$) de Jambolão, na polpa de Ingá ($97.36 \text{ mg } 100 \text{ g}^{-1}$) e na semente de Maria Preta ($1891.36 \text{ mg } 100 \text{ g}^{-1}$). O ácido linoleico (18:2n-6) se destacou nas polpas de Maria Preta ($48.09 \text{ mg } 100 \text{ g}^{-1}$) e nas cascas e polpas de Uva japonesa ($98.69 \text{ mg } 100 \text{ g}^{-1}$). O ácido araquídico (20:0) foi o AG majoritário encontrado nas cascas de Maria Preta ($888.22 \text{ mg } 100 \text{ g}^{-1}$). Nas sementes de Ingá ($313.70 \text{ mg } 100 \text{ g}^{-1}$) se destacou o ácido palmítico (16:0) e nas sementes de Uva japonesa ($3985.95 \text{ mg } 100 \text{ g}^{-1}$), o ácido alfa-linolênico (18:3n-3). As relações entre os AGs linoleico e linolênico (n-6/n-3) foi maior que 5:1 na polpa e casca de Jambolão (51.01 e 19.54, respectivamente), na polpa e semente de Maria Preta (51.15 e 78.30, respectivamente) e nas polpas de Ingá (38.40). Porém, as relações entre os AGs foram mais adequadas nas polpas e cascas de Uva japonesa (2.38), cascas de

Maria Preta (0.24), sementes de Jambolão (0.06), de Ingá (0.16) e de Uva japonesa (0.48).

CONCLUSÕES. As cascas de Jambolão apresentaram concentração significativa de antocianinas. Os compostos fenólicos responsáveis pela atividade antioxidante dos extratos apresentaram a maior concentração nas sementes de Jambolão, seguido da Uva japonesa, Maria Preta e Ingá. A concentração de compostos bioativos identificados por cromatografia líquida de alta eficiência nas cascas seguiram a seguinte escala decrescente: Maria Preta > Jambolão > Uva japonesa e nas polpas: Uva japonesa > Jambolão > Maria Preta > Ingá. Os métodos DPPH e FRAP foram capazes de representar a atividade antioxidante das amostras com relação a concentração de compostos bioativos apenas nas polpas das frutas. As sementes de Uva japonesa se destacaram no conteúdo de ácido alfa-linolênico, um AG essencial para a saúde humana. Estes resultados demonstram que estas sementes possuem consideráveis níveis de nutrientes, além disso, os altos níveis de compostos bioativos encontrados nas polpas e cascas de Uva japonesa, nas cascas de Maria Preta e sementes de Jambolão, confirmam o potencial antioxidante destas partes das frutas e podem ser consideradas como uma fonte de antioxidantes naturais.

PALAVRAS- CHAVE: Frutas brasileiras, compostos bioativos, atividade antioxidante, ácidos graxos.

Determination of antioxidant activity and physical and chemical composition of different parts of four Brazilian fruits

Adriela Albino Rydlewski^{a*}, Damila Rodrigues Morais^b, Eliza Mariane Rotta^b, Thiago Claus^b, Jesuí Vergílio Visentainer^b

^aAgricultural Center of Science, ^bDepartment of Chemistry, State University of Maringá, Av. Colombo, 5790, CEP 87020-900, Maringá, Paraná State, Brazil

ABSTRACT

Antioxidant activity by DPPH and FRAP, proximate composition, total phenolic, total flavonoid, total anthocyanins contents, identification by chromatographic methods of phenolic compounds and fatty acids were determined in different parts of Jambolan (*Syzygium cumini*), Maria Preta (*Solanum nigrum*), Inga (*Inga edulis*) and Japanese grape (*Hovenia dulcis*). The values moisture ranged from 4.85% to 86.35% in seeds and pulps of Maria Preta, respectively. Japanese grape seeds showed the highest values of ash (2.46%), crude protein (17.80%) and the total lipids (8.73%). The Japanese grape pulp and peel had the greatest potential for antioxidant by DPPH (13240.6 $\mu\text{MTE } 100 \text{ g}^{-1}$) and by FRAP (690.68 $\mu\text{MFeSO}_4 \text{ } 100 \text{ g}^{-1}$). Phenolic and flavonoid contents was also greater in this part of fruit (518.18 mg GAE 100 g^{-1} and 76.54 mg QE 100 g^{-1} , respectively). Anthocyanins have excelled in Jambolan peels (63.31 mg 100 g^{-1}). The kaempferol concentration was extremely high in Maria Preta peels (573.80 mg 100 g^{-1}), Jambolan seeds presented 571.05 mg 100 g^{-1} of gallic acid and only this seeds presented epicatechin (48.14 mg 100 g^{-1}) and ellagic acid (36.30 mg 100 g^{-1}). Japanese grape seeds presented 3985.95 mg 100 g^{-1} sample of fatty acid alpha-linolenic (18:3n-3), representing a total of 53% of total lipids. These results show that these seeds has considerable levels of nutrients, apart from high levels of bioactive compounds found in Maria Preta peel and Jambolan seeds, confirming their antioxidant potential and that they might be considered as a source of natural antioxidants.

Keywords: Brazilian fruits, bioactive compounds, antioxidant activity, fatty acids.

1. Introduction

Brazil boasts a large number of native and exotic fruits and the interest in exploiting new species during recent years has arisen because many of them are rich in antioxidants and are thus able to protect the human body by preventing oxidative stress and the appearance of degenerative diseases (Alves, Brito, Rufino, & Sampaio, 2008; Schreckinger, Lotton, Lila, & Gonzalez de Mejia, 2010). Antioxidants have a significant impact on the status of human health, but are also useful as nutraceuticals and phytochemicals (Noguchi & Nikki, 2000). Therefore, the physical and chemical characterisation of fruits and the quantification of their bioactive components are important for the understanding of their nutritional value and for increasing the quality and value of the final product (Mattietto, Lopes, & Menezes, 2010).

Potential sources of natural antioxidants have been investigated in different types of plant material such as leaves, seeds, peel and fruit (Rababah, Hettiarachy, & Horax, 2004). Recent studies that have investigated the bioactive compounds in seeds and peels of fruits, which are considered as sub-products, have found high levels of various substances that strengthen the possibility of using these fruit parts as a source of nutraceutical compounds and potential application in the food industry for increasing the stability and shelf life of food products (Gorinstein et al., 2011; Noguchi & Nikki, 2000). Moreover, the consumption of fruits has significantly increased in domestic and international markets due to their attractive sensory properties and a growing recognition of their nutritional and therapeutic value (Gonzalez-Aguilar, Villa-Rodriguez, Ayala-Zavala, & Yahia, 2010; Rufino, Alvez, Brito, Pérez-Jimenez, Saura-Calixto, & Mancini-Filho, 2010).

Jambolan (*Syzygium cumini*), Maria preta (*Solanum nigrum*), Inga (*Inga edulis*) and Japanese grape (*Hovenia dulcis*), are fruits cultivated in Brazil, but few studies have been published concerning their bioactive compounds and antioxidant activity, and the literature contains no studies evaluating the individual parts of these fruits, which need to be adequately investigated to analyse their potential as a source of antioxidants. Therefore, the aim of this study was to evaluate the antioxidant activity in pulp, peel and seeds of Jambolan, Maria Preta, Inga and Japanese grape by DPPH and FRAP, total phenolic contents, total flavonoid, total anthocyanin, fatty acid compounds and the identification and quantification of phenolics by HPLC.

2. Materials and methods

2.1. Materials

2.1.1. Sampling, chemical and standards

Jambolan, Maria Preta, Inga and Japanese grape were obtained from farmers in the region of Paraná state, Brazil, at three different periods, separated by 10 days. Inga peel was discarded, and Japanese grape peel and pulp were combined. Maria preta and Jambolan were separated into different parts. Parts of fruits were crushed and homogenized in a multiprocessor, stored in plastic bags, subjected to a vacuum and stored in a freezer at -18°C .

Fatty acid methyl ester standard (mixture 189 – 19), tricosanoic fatty acid methyl ester (23:0), Folin–Ciocalteu's phenol reagent, gallic acid, kaempferol, ellagic acid, epicatechin, quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxyl acid (Trolox), 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ), methanol and acetonitrile grade HPLC were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). All other chemicals and standards were of analytical grade.

2.2. Methods

2.2.1. Antioxidant extraction

Methanolic extracts were prepared using 10 g parts of each fruit in 100 mL methanol (in a ratio of 1:10 w/v) and stirred for 4 h with a magnetic bar. The extracts were filtered and concentrated on a rotary evaporator at 40°C. The dried extracts were stored at -18°C until analysed. Analyses of DPPH, FRAP, total phenolic compounds, total flavonoids and the identification of bioactive compounds by HPLC were performed with the methanolic extracts.

2.2.2. Antioxidant analysis

2.2.2.1. DPPH (free-radical scavenging) and ferric reducing antioxidant power (FRAP) assay.

The antioxidant activity of methanolic extracts was evaluated via the activity of free-radical scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH), based on the method of El-Massry, El-Ghorab, and Farouk (2002). The results are expressed as Trolox equivalents ($\mu\text{M ET } 100 \text{ g}^{-1}$). Antioxidant activity was also estimated by the FRAP assay, according to Benzie and Strain (1996). The results are expressed as equivalents in $\mu\text{M FeSO}_4 100 \text{ g}^{-1}$.

2.2.2.2. Total phenolic and total flavonoid contents

The total phenolic content of methanolic extracts was determined according to the method of Shahidi and Naczk (1995). The results were evaluated by comparison with a calibration curve of the gallic acid standard ($r^2 = 0.986$) and expressed as gallic acid equivalents ($\text{mg GAE } 100 \text{ g}^{-1}$). Total flavonoid content was determined according

to Buriol et al. (2009). Results were evaluated by comparison with a calibration curve of the quercetin standard ($r^2 = 0.997$) and expressed as mg quercetin equivalents (mg QE 100 g⁻¹).

2.2.2.3. Total anthocyanin content

The extraction of anthocyanins was performed according to Lee and Francis (1972). Fruits (50 g) were weighed and homogenized for 2 min with 50 mL solvent (70% ethanol acidified to pH 2.0 with 0.1% HCl). The volume was adjusted to 200 mL in a volumetric flask, covered with parafilm and stored at 4°C for 12 h. The material was filtered through a Büchner funnel and the filtrate received in a Kitassato flask. An aliquot (125 mL) of the filtrate was removed and added to 250 mL solvent. Triplicate aliquots of 2 mL were again removed and the volume was adjusted to 100 mL in a volumetric flask. The material was incubated for 2 h in the dark. The absorbance at 535 nm was measured in a spectrophotometer and the results were expressed in mg 100 g⁻¹.

2.2.3. Proximate composition and fatty acid composition

Moisture, ash and crude protein content were determined according to AOAC (Cunniff, 1998). Total lipids (TL) were extracted by the method of Bligh and Dyer (1959).

Fatty acid methyl esters (FAME) were prepared by methylation of total lipids as described by Hartman and Lago (1973). Methyl esters were separated by gas chromatography in a Varian model 3380 equipped with flame ionization and a cyanopropyl capillary column (100 m x 0.25 i.d., 0.25 µm film thickness, CP-7420 Varian, EUA) (Martin, Oliveira, Visentainer, Matsushita, & Souza, 2008). The injector and detector temperatures were 220°C and 240°C, respectively. The gas flow rates used

were 1.2 mL min⁻¹ carrier gas (H₂), 30 mL min⁻¹ make-up gas (N₂), 35 and 300 mL min⁻¹ flame gases (H₂ and synthetic air, respectively). The sample splitting rate was 1:80. The operating parameters were as follows: the column temperature was held at 185°C for 10 min, programmed to increase the temperature of 4°C min⁻¹ to 235°C and maintained at this temperature for 4.5 min; the total run time was 25 min. The peak areas were determined by the Workstation 5.0 (Varian) data acquisition program. For fatty acid (FA) identification, retention times were compared with those of standard methyl esters.

Quantification (in mg FA g⁻¹ of total lipids) was performed against tricosanoic acid methyl ester as an internal standard (23:0), as described by Joseph and Ackman (1992). Theoretical FID (flame ionization detector) correction factor (Visentainer, 2012) values were used to obtain concentration values. FA content was calculated in mg g⁻¹ of total lipids by using equation 1:

$$\text{FA (mg g}^{-1} \text{ of TL)} = \frac{A_X W_{IS} CF_X}{A_{IS} W_X CF_{AE}} \times 100 \quad (1)$$

where FA is expressed as mg g⁻¹ total lipids, A_X is the peak area (FAs), A_{IS} is the peak area of the internal standard (IS) methyl ester of tricosanoic acid (23:0), W_{IS} is the IS weight (mg) added to the sample (in mg), W_X is the sample weight (in mg), CF_X is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of FAs rather than as methyl esters. The results were converted from mg g⁻¹ to g 100 g⁻¹ FA.

2.2.4. Quantitative identification of polyphenols by HPLC

The chromatographic determination of polyphenols was performed by HPLC (Fратиани, Tucci, Palma, Pepe, & Nazzaro, 2007) using a chromatograph Thermo Scientific, model Tinnigan Surveyor PDA, with a manual injection valve, a photodiode array detection (DAD), connected to the software CromQuest 5.0 and a column Macherey-Nagel EC 250/4.6 Nucleodur 100-5 C18ec (250 mm x 4.6mm). The mobile phase included Milli-Q water (containing 1% acetic acid), in phase A and acetonitrile (containing 1% acetic acid) in phase B. The running started with 90% of A and amounted to 60% of A in 25 min. After 26 min, phase A returned to 90% and remained at this rate until 35 min. The total running time was 35 min. The flow rate was 0.7 mL min⁻¹, the injection volume was 50 µL and the detection wavelength was set at 243 nm and 270 nm. Phenolic compounds were identified from a comparison of the retention times with the standards of Sigma and UV spectra of individual compounds.

2.2.5. Statistical analysis

Results were reported as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA), using the software Statistica 5.1 (StatSoft, 1996). The Tukey test, at $p = 0.05$, was used to assess significant differences between means for samples. Pearson's correlation coefficient (r) was calculated for each part of the fruits for total phenolic content, total flavonoids and total anthocyanins, comparing DPPH and FRAP.

3. Results and discussion

3.1. Proximate composition

The proximate compositions of the different parts of fruit are shown in Table 1. The moisture content of fruits ranged from 4.85% in seeds to 86.35% in Maria Preta

pulp, values which were close to those for Italy grape (85%), Rubi grape (86.1%) and jaboticaba (83.6%) TACO (2011).

The pulp and peel of Japanese grape contained 2.63% crude protein, less than that found by Bampi, Bicudo, Fontoura, and Ribani (2010) (3.74%) and seeds of this fruit had the largest proportion of ash (2.46%). The lowest crude protein values were found in Maria Preta pulp (0.74%) and largest in Japanese grape seeds (17.08%). Values of total lipids ranged from 0.15% in Jambolan pulp to 8.73% in Japanese grape seeds.

3.2. Antioxidant analysis and Pearson's correlation coefficient (*r*)

The total flavonoid content in pulp and peel of Japanese grape was significantly different to that in the pulps of other fruits (Table 2) and pulp and peel of this fruit also presented the highest content of flavonoid kaempferol identified by HPLC. This flavonoid was found in all methanolic extracts of parts of the fruits (Table 3). Faria et al. (2011), found 91.2 mg 100 g⁻¹ of total flavonoids in Jambolan. In this study, Jambolan pulps (59.0 mg QE 100 g⁻¹) and peel (65.51 mg QE 100 g⁻¹) had a lower content of flavonoids. However, in this study the parts of this fruit were evaluated separately.

Jambolan peel was notable amongst all other fruit parts for its high anthocyanin content (Table 2), with levels comparable to jaboticaba (58.1 mg 100 g⁻¹) and camu-camu (42.2 mg 100 g⁻¹) found in a study by Rufino et al. (2010), but lower than those for puçá-preto (103 mg 100 g⁻¹) and murta (143 mg 100 g⁻¹), in the same study.

The concentration of phenolics of different parts of the fruits are shown in Table 2. Maria Preta pulp showed phenolic concentrations of 412.71 mg GAE 100 g⁻¹. Due to the lack of studies with this fruit, these values can be compared to those of jaboticaba

(440 mg GAE 100 g⁻¹) and assaí (454 mg GAE 100 g⁻¹) (Rufino et al., 2010). Vasco, Ruales, and Kamal-Eldin (2008) classified fruits into three categories: low (< 100mg GAE 100g⁻¹), medium (100–500 mg GAE 100g⁻¹) or high (> 500 mg GAE 100g⁻¹), based on fresh matter. Classifying the samples in this study this manner, all parts of Inga, peel and seeds of Maria Preta and Japanese grape seeds had a low amount of phenolics, all parts of Jambolan and Maria Preta pulp had medium quantities, and pulp and peel of Japanese grape had high amounts.

The lowest antioxidant activity by DPPH method was observed in Maria Preta peel, followed of Maria Preta seed, Inga seed, Inga pulp, Maria Preta pulp, Jambolan peel, Jambolan pulp, Japanese grape seed, Jambolan seed and and Japanese grape pulp and peel (Table 2). Despite Maria Preta peel has presented the minor antioxidant activity among all samples, found a high amount of kaempferol in the peel by HPLC (Table 3). Furthermore, except for Japanese grape seeds, samples with higher values of phenolic compounds also showed the highest values of antioxidant activity, indicating that the phenolic compounds present in methanolic extracts were able to sequester free radical DPPH. Compared to others, Jambolan seeds showed considerably higher antioxidant activity measured by this method. In this study, the phenolic compounds responsible for antioxidant activity were gallic and ellagic acids and also the flavonoids kaempferol and epicatechin (Table 3). These results indicate that these fruit seeds might potentially serve as natural sources for diet phenolic compounds.

Organised in crescent order of antioxidant activity by the FRAP method, the parts of fruit are ranked as follows: Maria Preta seed, Inga seed, Inga pulp, Maria Preta peel, Japanese grape seed, Jambolan seed, Maria Preta pulp, Jambolan pulp, Jambolan peel and Japanese grape pulp and peel. The antioxidant activity measured by the FRAP did not showed the same results by DPPH method, but showed direct correlation with

phenolic and flavonoid compounds from all parts of the four fruits, although this was less in the case of Jambolan seeds (Table 2), despite significant amounts of gallic and ellagic acids, kaempferol and epicatechin. The correlation between bioactive compounds and antioxidant activity depends on the methodology used as well as the sample type and variations might be due to different antioxidant components contributing to antioxidant activity (Ismail, Marjan, & Foong, 2004). Gallic acid has a low solubility and requires a longer time to complete the reaction with the oxidant (Noguchi & Nikki, 2000). Probably the lowest result of FRAP in Jambolan seeds in relation to the other parts of the fruit might relate to the significant amount of gallic acid present (Table 3).

The antioxidant activity measured by the FRAP method was lower in Japanese grape seeds than in pulp and peel (Table 2). The total lipid content of seeds of this fruit was higher than in pulp and peel (Table 1). According to Rufino et al. (2010), the FRAP method is generally suitable for hydrophilic compounds. Therefore, higher amounts of total lipids in Japanese grape seeds might have influenced these results.

Variations in Pearson's correlation coefficient (r) between different methods to measure the antioxidant activity of a sample, suggest that a single test alone might not be reliable, therefore, the antioxidant activity of phenolics and flavonoids was compared using either DPPH or FRAP method for each fruit part. Moderate and strong positive correlations between results from the DPPH and FRAP methods are shown in Table 4. Although Jambolan peel has a high anthocyanin content, the correlation between this compound in peel and the antioxidant activity was negative with results from DPPH, and not correlated with those of FRAP, because the correlations included all the fruit peel. This result can be attributed to the low content of anthocyanins in Maria Preta peel and Japanese grape peel. The absence of correlation between the content of

anthocyanins with DPPH in seed and the negative correlation between anthocyanins with FRAP in seed can be attributed to low levels of this compound in all seeds.

3.3. Identification of phenolic compounds by HPLC

The phenolic compounds responsible for the antioxidant activity of methanolic extracts of samples were gallic acid, which produced a peak at a retention time of 3.6 min, kaempferol at 5.4 min, epicatechin at 14.7 min and ellagic acid at 19.1 min. The gallic and ellagic acids showed a λ_{\max} at 270 nm, characteristic of phenolic acids derived from hydroxybenzoic acid (Faria, Marques, & Mercadante, 2011). The flavonoid epicatechin also showed a λ_{\max} at 270 nm, but kaempferol showed a λ_{\max} at 243 nm.

The phenolic concentration in each part of the fruits is shown in Table 3. Gallic acid was the greatest contributor to the antioxidant activity of Jambolan seeds (63.98%) and Japanese grape seeds (54.60%). The amount of gallic acid in pulp and peel of Jambolan was comparable to that raspberry (30 mg 100 g⁻¹) and strawberry (80 mg 100 g⁻¹) (Agar, Streif, & Bangerth, 1997). The flavonoid kaempferol was identified in all parts of the fruit, representing 99.38% in Maria Preta peel. Epicatechin was present only in Jambolan seeds at a concentration similar to that of dark chocolate (58 mg 100 g⁻¹) (Spadafranca, Martínez-Conesa, Sirini, & Testolin, 2010). This is the first report of the presence of epicatechin and ellagic acid in Jambolan seeds. However, Faria et al. (2011) found gallic acid in Jambolan fruit previously identified by HPLC-DAD-MS/MS.

Flavonols such as kaempferol and epicatechin and hydroxybenzoic acids (gallic and ellagic acids) might contribute to the neutralization of cell-damaging free radicals and to the maintenance of coronary health in humans and prevent the appearance of cancer (Noguchi & Nikki, 2000; Souza, Souza Neto, & Maia, 2003).

3.4. Fatty acids

A total of eight FAs were detected in pulp, peel and seeds of all fruits (Table 5). The proportion of alpha-linolenic acid (LNA, 18:3 n-3) in Japanese grape seeds was 53.07% of the TL content (8.73%), and differed significantly from the content of the seeds (Table 1). Apart from LNA, the FAs of Japanese grape seeds contained 4.35% palmitic acid (16:0), 2.14% stearic acid (18:0), 13.24% oleic acid (18:1 n-9) and 25.76% linoleic acid (18:2 n-6). These seeds showed relative values of FAs similar to those of flaxseeds. According to Rinaldi, Garcia, Marciniuk, Rossi and Schuchardt (2007), flaxseed contains 5%, 4%, 13–37%, 2–3% and 26–58% palmitic, stearic, oleic, linoleic and linolenic acid, respectively. Linolenic acid favors the synthesis of odd-series eicosanoids such as PGE₃, TXA₃ and LTB₅ and has anti-inflammatory properties in human metabolism contributing to the prevention of chronic diseases (Souza et al., 2003).

The FA found at the highest concentration in all parts of Jambolan was oleic acid (18:1 n-9), representing 56.26% in pulp, 59.15% in peel and 96% in seeds. In Inga pulp and Maria Preta seeds, oleic acid was also abundant (74.09% and 62.32%, respectively). Linoleic acid (18:2 n-6) was found at a higher concentration in pulp and peel of Japanese grape (45.22%) and in Maria Preta pulp (71.60%). Palmitic acid in Inga seeds (PA, 16:0) represented 35.34%, and in Maria Preta peel the arachidic acid (20:0) represented 63.72% of the total FA content. The differences in individual FA contents are reflected in the total saturated fatty acid (SFA), total mono-unsaturated fatty acid (MUFA) and total poly-unsaturated FA (PUFA) contents (Table 5). SFA dominated in Maria Preta peel (87%), MUFA in Jambolan seeds (96.55%) and PUFA in Japanese grape seeds (78.32%).

The omega-6/omega-3 (n-6/n-3) ratio varied from 2.38 to 51.15 in pulps, 0.06 to 19.54 in peels and from 0.06 to 78.30 in seeds. The amount of omega-6 FA in foods has increased and omega-3 has decreased during human evolution, from an estimated ratio of 1:1 of n-6/n-3 to 10:1 or 20–25:1. The ratio balanced between of fatty acids in the human diet can reduce the chances of developing several diseases therefore, nutrition societies recommend a ratio of 5:1 to n-6/n-3 for a healthy diet (Simopoulos, 1991).

4. Conclusion

The Jambolan peels showed high concentrations of anthocyanins differed significantly from the other parts of fruits. DPPH and FRAP showed that the antioxidant activity of the samples accurately represented the concentration of bioactive compounds only in fruit pulp. The other fruit parts did not show a direct correlation of antioxidant activity with the amount of phenolics. Phenolic compounds responsible for the antioxidant activity of methanolic extracts had the highest concentration in Jambolan, followed by Japanese grape, Maria Preta and Inga seeds. The concentration of bioactive compounds identified by HPLC in peel followed the decreasing scale: Maria Preta > Jambolan > Japanese grape and for pulp: Japanese grape > Jambolan > Maria Preta and Inga. Japanese grape seeds showed significant amounts of alpha-linolenic acid (18:3 n-3), a FA essential for human health. These results show that these seeds has considerable levels of nutrients, apart from high levels of bioactive compounds found in Japanese grape pulp and peel, Maria Preta peel and Jambolan seeds, confirming their antioxidant potential and that they might be considered as a source of natural antioxidants.

5. Acknowledgements

We gratefully acknowledge the Laboratório Crom Alimentos.

6. References

- Agar, I. T., Streif, J., & Bangerth, F. (1997). Effect of high CO₂ and controlled atmosphere on the ascorbic and dehydroascorbic acid content of some berry fruits. *Postharvest Biology and Technology*, *11*, 47–55.
- Alves, R. E., Brito, E. A., Rufino, M. S. M., & Sampaio, C. G. (2008). Antioxidant activity measurement in tropical fruits: A case study with acerola. *Acta Horticulturae*, *773*, 299–305.
- Bampi, M., Bicudo M. O. P., Fontoura P. S. G., & Ribani, R. H. (2010). Composição centesimal do fruto, extrato concentrado e da farinha da uva do japão. *Ciência Rural*, *40*, 2361–2367.
- Benzie, I. F. F., & Strain, J. J. (1996). Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, *239*, 70-76.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.
- Buriol, L., Finger D., Schmidt E. M., Santos, J. M. T., Rosa, M. R., Quinaia, S. P., et al (2009). Composição química e atividade biológica de extrato oleoso de própolis: uma alternativa ao extrato etanólico. *Quimica Nova*, *32*, 296–302.
- Cunniff, P. A. (1998). Official methods of analysis of AOAC international. *Association of Official Analytical Chemists*, (16th ed).

El-Massry, K. F. E., El-Ghorab, A. H. E., & Farouk, A. (2002). Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. *Food Chemistry*, *79*(3), 331–336.

Faria, A. F., Marques, M. C., & Mercadante, A. Z. (2011). Identification of bioactive compounds from jambolão (*Syzygium cumini*) and antioxidant capacity evaluation in different pH conditions. *Food Chemistry*, *126*, 1571–1578.

Fратиани F., Tucci, M., Palma M., Pepe, R. & Nazzaro, F. (2007). Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). *Food Chemistry*, *104*, 1282–1286.

Gonzalez-Aguilar, G., Villa-Rodriguez, J., Ayala-Zavala, J., & Yahia, E. (2010). Improvement of the antioxidant status of tropical fruits as a secondary response to some postharvest treatments. *Trends in Food Science and Technology*, *21*, 475–482.

Gorinstein, S., Poovarodom, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Vearasilp, S., Haruenkit, R., Ruamsuke, P., Katrich, E. & Tashma, Z. (2011). Antioxidant properties and bioactive constituents of some rare exotic Thai fruits and comparison with conventional fruits: *In vitro* and *in vivo* studies. *Food Research International*, *44*(7), 2222–2232.

Hartman, L., & Lago, R. C. A. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice*, *22*(8), 475–476, 494.

Ismail, A., Marjan, Z. M., & Foong, C.W. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry*, 87, 581–586.

Joseph, J. D., & Ackman, R. G. (1992). Capillary column gas chromatography method for analysis of encapsulated fish oil and fish oil ethyl esters: collaborative study. *Journal Association of Official Analytical Chemists*, 75(3), 488–506.

Lee, D. H., & Francis, F. J. (1972). Standardization of pigment analyses in cranberries. *HortScience*, 7, 83–84.

Mattietto, R. A., Lopes, A. S., & Menezes, H. C. (2010). Caracterização física e físicoquímica dos frutos da cajazeira (*Spondias mombin* L.) e de duas polpas obtidas por dois tipos de extrator. *Brazilian Journal of Food Technology*, 13, 156–164.

Martin, C. A., Oliveira, C. C., Visentainer, J. V., Matsushita, M., & Souza, N. E. (2008). Optimization of the selectivity of a cyanopropyl stationary phase for the gas chromatographic analysis of trans fatty acids. *Journal of Chromatography A*, 1194, 111–117.

Noguchi, C., & Nikki, E. (2000). Phenolic antioxidants: A rationale for design and evaluation of novel antioxidant drugs for atherosclerosis. *Free Radical Biology and Medicine*, 28, 1538–1546.

Rababah, T. M., Hettiarachy, N. S., & Horax, R. (2004). Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary,

gotu kola, and ginkgo extracts, vitamin E, and terbutylhydroquinone. *Journal of Agriculture and Food Chemistry*, 52, 5183–5186.

Rinaldi, R., Garcia, C., Marciniuk, L. L., Rossi, A. V., & Schuchardt, U. (2007). Síntese de biodiesel: Uma proposta contextualizada de experimento para laboratório de química geral. *Química Nova*, 30 (5), 1374–1380.

Rufino, M. S. M., Alves, R. E., Brito, E. S., Pérez-Jimenez, J., Saura-Calixto, F. & Mancini-Filho, J. (2010). Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. *Food Chemistry*, 121(4), 996–1002.

Schreckinger, M. E., Lotton, J., Lila, M. A., & Gonzalez de Mejia, E. (2010). Berries from South America: a comprehensive review on chemistry, health potential, and commercialization. *Journal of Medicinal Food*, 13, 233–246.

Shahidi, F., & Naczk, M. (1995). *Food phenolics: Sources, chemistry, effects, applications* (p. 331). Technomic Publishing Co. Inc., USA. Lancaster.

Simopoulos, A. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition*. 54, 438–463.

Souza, P. H. M., Souza Neto, M. H., & Maia, G. A. (2003). Componentes funcionais nos alimentos. *Boletim da SBCTA*. 37, 127–135.

Spadafranca, A., Martínez-Conesa, C., Sirini, S., & Testolin, G. (2010). Effect of dark chocolate on plasma epicatechin levels, DNA resistance to oxidative stress and total antioxidant activity in healthy subjects. *British Journal of Nutrition*, *103*, 1008–1014.

StatSoft (1996). Statistica 5.1 Software. Tucksá: Statsoft.

TACO *Tabela brasileira de composição de alimentos*. (2011). Núcleo de estudos e pesquisas em alimentação (p. 113). Unicamp.

Vasco, C., Ruales, J., & Kamal-Eldin, A. (2008). Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry*, *111*(4), 816–823.

Visentainer, J. V. (2010). Aspectos analíticos da resposta do detector de ionização em chama para ésteres de ácidos graxos em biodiesel e alimentos. *Química Nova*, *35*, 274-279.

Table 1
Proximate composition in different parts of fruits.

Test	Part of fruit	Jambolan	Maria Preta	Inga	*Japanese grape
Moisture (%)	Pulp	79.38 ± 0.54 ^{abA}	86.35 ± 0.34 ^{aA}	77.23 ± 0.74 ^{bA}	54.33 ± 0.31 ^{cA}
	Peel	79.14 ± 2.87 ^{aA}	73.98 ± 2.53 ^{aB}	nm	54.33 ± 0.31 ^{bA}
	Seed	42.67 ± 1.87 ^{bb}	4.85 ± 0.72 ^{cC}	67.64 ± 0.97 ^{aB}	8.01 ± 0.17 ^{cB}
Ash (%)	Pulp	0.37 ± 0.09 ^{cB}	0.91 ± 0.03 ^{bC}	0.43 ± 0.28 ^{cB}	1.50 ± 0.04 ^{aB}
	Peel	0.47 ± 0.36 ^{bb}	1.29 ± 0.71 ^{aB}	nm	1.50 ± 0.04 ^{aB}
	Seed	1.27 ± 0.08 ^{ba}	2.44 ± 0.27 ^{aA}	1.34 ± 0.31 ^{ba}	2.46 ± 0.02 ^{aA}
Crude Protein (%)	Pulp	1.66 ± 0.54 ^{bC}	0.74 ± 0.18 ^{cC}	1.32 ± 0.38 ^{bb}	2.63 ± 0.59 ^{aB}
	Peel	2.51 ± 0.10 ^{bb}	4.28 ± 0.79 ^{aA}	nm	2.63 ± 0.59 ^{bb}
	Seed	3.87 ± 0.18 ^{ca}	2.62 ± 0.75 ^{db}	6.75 ± 0.40 ^{ba}	17.08 ± 1.12 ^{aA}
Total Lipid (%)	Pulp	0.15 ± 0.11 ^{dc}	0.21 ± 0.05 ^{cC}	0.28 ± 0.04 ^{bb}	0.41 ± 0.02 ^{aB}
	Peel	0.81 ± 0.19 ^{ba}	1.93 ± 0.49 ^{aB}	nm	0.41 ± 0.02 ^{cB}
	Seed	0.60 ± 0.16 ^{db}	3.72 ± 0.23 ^{ba}	1.37 ± 0.11 ^{ca}	8.73 ± 0.68 ^{aA}

Results expressed as mean ± SD. Means followed by different small letters in the same line are significantly different between the same parts of different fruits ($P \leq 0.05$). Means followed by different capital letters in the same column are significantly different between the different parts of same fruit ($P \leq 0.05$). nm: not measured. * Peel and pulp were combined.

Table 2
Total phenolic content, total flavonoids, total anthocyanins, antioxidant activity by DPPH and FRAP in different parts of fruits.

Test	Part of fruit	Jambolan	Maria Preta	Inga	*Japanese grape
Total phenolic compounds (mg GAE 100g ⁻¹)	Pulp	313.66 ^{cb} ± 8.93	410.71 ^{ba} ± 9.42	72.06 ^{da} ± 7.36	518.18 ^{aA} ± 30.89
	Peel	331.83 ^{bb} ± 7.84	21.89 ^{cb} ± 1.38	nm	518.18 ^{aA} ± 30.89
	Seed	411.01 ^{aA} ± 9.46	19.27 ^{cb} ± 1.06	66.32 ^{ba} ± 8.68	85.56 ^{bb} ± 7.28
Total flavonoids (mg QE 100g ⁻¹)	Pulp	59.0 ^{bb} ± 2.36	55.42 ^{ba} ± 5.95	5.88 ^{ca} ± 1.01	76.54 ^{aA} ± 5.47
	Peel	65.51 ^{ba} ± 7.34	43.39 ^{bb} ± 5.44	nm	76.54 ^{aA} ± 5.47
	Seed	38.97 ^{ac} ± 3.37	10.24 ^{cc} ± 1.42	2.59 ^{db} ± 0.69	18.60 ^{bb} ± 4.36
Total anthocyanins (mg 100g ⁻¹)	Pulp	9.26 ^{ab} ± 0.41	5.41 ^{ba} ± 1.09	0.06 ^{ca} ± 0.05	10.76 ^{aA} ± 1.14
	Peel	63.31 ^{aA} ± 1.64	4.63 ^{ca} ± 0.12	nm	10.76 ^{aA} ± 1.14
	Seed	0.7 ^{bc} ± 0.18	1.4 ^{ab} ± 0.28	nd	1.51 ^{ab} ± 0.25
DPPH (μMTE 100g ⁻¹)	Pulp	2810.9 ^{bb} ± 114.56	1726.6 ^{bcA} ± 105.83	447.08 ^{ca} ± 9.54	13240.6 ^{aA} ± 309.61
	Peel	2010.9 ^{bb} ± 32.49	68.66 ^{cb} ± 1.27	nm	13240.6 ^{aA} ± 309.61
	Seed	10396.4 ^{aA} ± 639.45	95.12 ^{cb} ± 1.15	173.20 ^{cb} ± 12.43	4877.50 ^{bb} ± 195.27
FRAP (μMFeSO ₄ 100g ⁻¹)	Pulp	323.5 ^{ba} ± 10.30	300.32 ^{ca} ± 12.54	41.27 ^{da} ± 3.61	690.68 ^{aA} ± 9.43
	Peel	341.95 ^{ba} ± 6.08	48.36 ^{cb} ± 3.38	nm	690.68 ^{aA} ± 9.43
	Seed	209.3 ^{ab} ± 3.77	22.93 ^{cc} ± 1.11	30.13 ^{cb} ± 3.46	75.22 ^{bb} ± 1.22

Results expressed as mean ± SD. Means followed by different small letters in the same line are significantly different between the same parts of different fruits ($P \leq 0.05$). Means followed by different capital letters in the same column are significantly different between the different parts of same fruit ($P \leq 0.05$). nm: not measured. nd: not detected. * Peel and pulp were combined.

Table 3
Quantification of phenolic compounds in different parts of fruits.

Phenolic Compounds	Part of fruit	λ_{\max} (nm)	Jambolan	Maria Preta	Inga	*Japanese grape
Phenolic acids						
Gallic acid (mg 100g ⁻¹)	Pulp		55.95 ^a ± 0.09	8.26 ^b ± 0.08	nd	5.6 ^b ± 0.21
	Peel	270	96.39 ^a ± 0.04	5.37 ^b ± 0.02	nm	5.6 ^b ± 0.21
	Seed		571.05 ^a ± 5.67	4.07 ^c ± 0.04	8.12 ^c ± 0.46	346.17 ^b ± 9.84
Ellagic acid (mg 100g ⁻¹)	Pulp		nd	nd	nd	nd
	Peel	270	nd	nd	nm	nd
	Seed		36.30 ± 4.13	nd	nd	nd
Flavonoids						
Kaempferol (mg 100g ⁻¹)	Pulp		142.95 ^b ± 5.67	166.24 ^b ± 5.49	114.55 ^c ± 6.34	224.98 ^a ± 7.84
	Peel	243	183.21 ^c ± 7.98	573.80 ^a ± 12.46	nm	224.98 ^b ± 7.84
	Seed		236.98 ^b ± 5.63	218.79 ^b ± 6.92	122.44 ^c ± 4.13	287.79 ^a ± 5.25
Epicatechin (mg 100g ⁻¹)	Pulp		nd	nd	nd	nd
	Peel	270	nd	nd	nm	nd
	Seed		48.14 ± 0.57	nd	nd	nd

Results expressed as mean ± SD. Means followed by different small letters in the same line are significantly different between the same parts of different fruits ($P \leq 0.05$). nm: not measured, nd: not detected. * Peel and pulp were combined.

Table 4

Pearson's correlation coefficients (r) between analysis in different parts of fruits.

Correlation (r)	Total phenolic content versus DPPH	Total flavonoid versus DPPH	Total anthocyanins versus DPPH	Total phenolic content versus FRAP	Total flavonoids versus FRAP	Total anthocyanins versus FRAP
Pulps	0.67	0.76	0.63	0.92	0.91	0.89
Peels	0.79	0.99	-0.40	0.98	0.91	0.04
Seeds	0.93	0.97	0.02	0.98	0.97	-0.18

Correlation $0.5 \leq r < 0.8$: moderate positive; $0.8 \leq r < 1$: strong positive; 0.0: no correlation; $-0.1 < r < -0.5$: negative correlation.

Table 5Fatty acid composition in different parts of fruits (mg 100 g⁻¹ sample).

Fatty acids	Pulp				Peel			Seed			
	Jambolan	Maria Preta	Inga	*Japanese grape	Jambolan	Maria Preta	*Japanese grape	Jambolan	Maria Preta	Inga	*Japanese grape
16:0	2.19 ^c ± 0.03	1.05 ^c ± 0.15	11.66 ^b ± 4.74	58.28 ^a ± 9.95	29.66 ^c ± 4.92	299.55 ^a ± 13.92	58.28 ^b ± 9.95	1.56 ^c ± 0.45	37.90 ^b ± 5.37	313.70 ^a ± 52.29	326.76 ^a ± 34.29
18:0	6.38 ^c ± 0.05	7.88 ^b ± 0.27	13.93 ^a ± 3.46	8.10 ^b ± 0.24	83.33 ^a ± 5.28	24.43 ^b ± 0.99	8.10 ^c ± 0.24	0.95 ^d ± 0.10	15.14 ^c ± 1.06	299.17 ^a ± 6.13	161.06 ^b ± 7.89
18:1n-7	0.26 ^c ± 0.01	8.63 ^b ± 0.23	0.07 ^c ± 0.01	9.02 ^a ± 0.20	2.64 ^c ± 0.78	34.41 ^a ± 2.35	9.02 ^b ± 0.20	0.24 ^d ± 0.12	2.67 ^b ± 0.38	1.09 ^c ± 0.04	66.69 ^a ± 7.56
18:1n-9	18.19 ^b ± 0.17	0.20 ^d ± 0.09	97.36 ^a ± 33.8	2.14 ^c ± 0.23	257.24 ^a ± 27.03	1.69 ^b ± 0.02	2.14 ^b ± 0.23	291.01 ^c ± 41.70	1891.36 ^a ± 123.18	192.85 ^d ± 9.98	994.30 ^b ± 24.54
18:2n-6	5.10 ^d ± 0.03	48.09 ^b ± 6.02	8.45 ^c ± 10.56	98.69 ^a ± 10.47	58.44 ^b ± 3.55	1.46 ^c ± 0.20	98.69 ^a ± 10.47	0.45 ^c ± 0.05	957.72 ^b ± 9.50	9.84 ^c ± 0.04	1935.12 ^a ± 120.17
18:3n-3	0.10 ^d ± 0.01	0.94 ^b ± 0.23	0.22 ^c ± 0.01	41.36 ^a ± 8.58	2.99 ^c ± 0.34	6.04 ^b ± 0.82	41.36 ^a ± 8.58	7.08 ^d ± 0.16	12.23 ^c ± 6.40	60.76 ^b ± 0.42	3985.95 ^a ± 652.26
20:0	0.04 ^c ± 0.01	0.27 ^b ± 0.38	0.05 ^c ± 0.01	0.36 ^a ± 0.02	0.12 ^b ± 0.07	888.22 ^a ± 43.50	0.36 ^b ± 0.02	0.40 ^c ± 0.15	116.47 ^a ± 2.41	0.60 ^c ± 0.04	38.84 ^b ± 4.92
20:1n-9	0.08 ^d ± 0.01	0.10 ^c ± 0.03	0.47 ^a ± 0.05	0.25 ^b ± 0.01	1.46 ^b ± 0.17	138.01 ^a ± 32.20	0.25 ^c ± 0.01	1.55 ^c ± 0.43	1.07 ^c ± 0.08	9.41 ^b ± 0.03	50.72 ^a ± 2.75
∑Sfa	8.61 ^c ± 0.08	9.20 ^c ± 0.74	25.64 ^b ± 2.92	66.74 ^a ± 2.26	112.11 ^b ± 10.13	1212.20 ^a ± 56.44	66.74 ^c ± 2.26	2.91 ^d ± 0.26	169.51 ^c ± 96.71	613.47 ^a ± 82.29	526.66 ^b ± 5.82
∑Mufa	18.52 ^b ± 0.10	8.93 ^d ± 0.93	97.90 ^a ± 12.39	11.41 ^c ± 0.32	261.34 ^a ± 9.33	174.11 ^b ± 12.19	11.41 ^c ± 0.32	292.79 ^c ± 9.38	1895.10 ^a ± 176.61	203.35 ^d ± 3.57	1111.71 ^b ± 2.28
∑Pufa	5.20 ^d ± 0.03	49.03 ^b ± 6.25	8.67 ^c ± 0.12	140.05 ^a ± 19.30	61.43 ^b ± 7.52	7.50 ^c ± 0.23	140.05 ^a ± 19.30	7.53 ^d ± 0.21	969.95 ^b ± 14.68	70.60 ^c ± 2.10	5921.07 ^a ± 786.25
Pufa/Sfa	0.60 ^c ± 0.01	5.32 ^a ± 0.23	0.33 ^d ± 0.05	2.09 ^b ± 0.17	0.54 ^b ± 0.18	0.01 ^c ± 0.01	2.09 ^a ± 0.17	2.58 ^c ± 0.13	5.72 ^b ± 0.34	0.11 ^d ± 0.01	11.24 ^a ± 1.22
n-6/n-3	51.01 ^a ± 0.24	51.15 ^a ± 1.41	38.40 ^b ± 5.68	2.38 ^c ± 0.14	19.54 ^a ± 1.79	0.24 ^c ± 0.02	2.38 ^b ± 0.14	0.06 ^d ± 0.01	78.30 ^a ± 3.04	0.16 ^c ± 0.01	0.48 ^b ± 0.04

Results expressed as mean ± SD. Means followed by different letters in the same line are significantly different between the same parts of different fruits ($P \leq 0.05$).

Comparison was made between the same parts of the different fruits. Pufa = Polyunsaturated fatty acid, Mufa = Monounsaturated fatty acid, Sfa = Saturated fatty acid, n-6 = Omega 6 fatty acid, n-3 = Omega 3 fatty acid. Peel of Inga not measured. * Peel and pulp were combined.