UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Engenharia de Alimentos

ÂNGELA GIOVANA BATISTA

# BERRY FRUITS: PREVENTIVE ACTION AGAINST METABOLIC DISEASES AND COGNITIVE IMPAIRMENT

# *FRUTAS VERMELHAS: AÇÃO PREVENTIVA CONTRA DOENÇAS METABÓLICAS E DANOS COGNITIVOS*

**CAMPINAS**

**2017**

ÂNGELA GIOVANA BATISTA

# BERRY FRUITS: PREVENTIVE ACTION AGAINST METABOLIC DISEASES AND COGNITIVE IMPAIRMENT

## *FRUTAS VERMELHAS: AÇÃO PREVENTIVA CONTRA DOENÇAS METABÓLICAS E DANOS COGNITIVOS*

Thesis presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food and Nutrition in the area of Experimental Nutrition applied to Food Technology.

Tese apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Alimentos e Nutrição na Área de Concentração de Nutrição Experimental aplicada à Tecnologia de Alimentos.

Orientador: MÁRIO ROBERTO MARÓSTICA JÚNIOR
Coorientadora: MARIA ALICE DA CRUZ HÖFLING

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE A SER DEFENDIDA PELA ALUNA ÂNGELA GIOVANA BATISTA E ORIENTADA PELO PROF. DR. MÁRIO ROBERTO MAROSTICA JÚNIOR E COORIENTADA POR MARIA ALICE DA CRUZ HÖFLING

**CAMPINAS**

**2017**

**Agência(s) de fomento e nº(s) de processo(s):** CAPES; CNPq, 206092/2014-8
**ORCID:** http://orcid.org/0000-0002-1650-6589

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Engenharia de Alimentos
Claudia Aparecida Romano - CRB 8/5816

Batista, Ângela Giovana, 1985-
B32b Berry fruits : preventive action against metabolic diseases and cognitive impairment / Ângela Giovana Batista. – Campinas, SP : [s.n.], 2017.

Orientador: Mário Roberto Maróstica Júnior.
Coorientador: Maria Alice da Cruz Höfling.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos.

1. Obesidade. 2. Hipocampo. 3. Cognição. 4. Compostos bioativos. 5. Compostos fenólicos. I. Maróstica Júnior, Mário Roberto. II. Cruz Höfling, Maria Alice. III. Universidade Estadual de Campinas. Faculdade de Engenharia de Alimentos. IV. Título.

Informações para Biblioteca Digital

**Título em outro idioma:** Frutas vermelhas : ação preventiva contra doenças metabólicas e danos cognitivos
**Palavras-chave em inglês:**
Obesity
Hippocampus
Cognition
Bioactive compounds
Phenolic compounds
**Área de concentração:** Nutrição Experimental e Aplicada à Tecnologia de Alimentos
**Titulação:** Doutora em Alimentos e Nutrição
**Banca examinadora:**
Mário Roberto Maróstica Júnior [Orientador]
Stanislau Bogusz Junior
Elisa de Almeida Jackix
Mariana Battaglin Villas Boas Alvaro
Valéria Helena Alves Cagnon Quitete
**Data de defesa:** 03-05-2017
**Programa de Pós-Graduação:** Alimentos e Nutrição

**COMISSÃO EXAMINADORA**

**Dr. Mário Roberto Maróstica Júnior –** Orientador
Universidade Estadual de Campinas

**Dr. Stanislau Bogusz Junior –** Membro
Universidade de São Paulo

**Dra. Mariana Battaglin Villas Boas Alvaro –** Membro
Universidade Paulista

**Dra. Elisa de Almeida Jackix -** Membro
Pontifícia Universidade Católica de Campinas

**Dra. Valéria Helena Alves Cagnon Quitete -** Membro
Universidade Estadual de Campinas

**Dr. Jaime Amaya Farfán -** Suplente
Universidade Estadual de Campinas

**Dra. Jane Cristina de Souza -** Suplente
Universidade Estadual de Campinas

**Dra. Elisvânia Freitas dos Santos -** Suplente
Universidade Federal do Mato Grosso do Sul

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

Dedico aos animais de laboratório.

## AGRADECIMENTOS

Agradeço ao **Prof. Mário** por ser um orientador sempre presente e por todo incentivo e credibilidade dado a mim e ao meu trabalho. À **Profa. Maria Alice** que abriu as portas do seu laboratório para realização deste trabalho e por todo tempo dedicado à minha tese. Meu obrigada à **Profa. Elin Östman** pela supervisão no período sanduíche e acolhida na Suécia.

Agradeço à agência de fomento **CAPES** pela bolsa concedida e ao **CNPq** pela bolsa do estágio sanduíche no programa Ciências sem Fronteiras. Agradeço também a **UNICAMP**, à **FEA** e à **Universidade de Lund** (Suécia), assim como os funcionários que foram facilitadores da realização do meu trabalho no doutorado. Obrigada ao **Programa de Moradia Estudantil da Unicamp** que permitiu minha estadia em Campinas por 6 anos.

Agradeço aos professores e pesquisadores parceiros neste projeto: **César Sartori, Stanislau Bogusz, Aline Biasoto, Ana Paula Dionísio, Alexandra Frank Sawaya,** e aos profissionais técnicos que com ajuda valiosa foram suporte para muitas atividades: **Soely, Susana, Celis, Francisco, Cidinha** e **Éder** do DEPAN, **Stephanie** e **Cintia** do IB. Obrigada aos **docentes** do programa de pós-graduação e aos demais **funcionários do DEPAN**.

Meus sinceros agradecimentos aos membros da **banca julgadora** por aceitarem o convite e tempo dedicado à exame da minha tese. Também aos **revisores** dos meus artigos por tantas contribuições valiosas.

Agradeço aos meus amigos e parceiros mais próximos na realização do meu projeto de doutorado: **Juliana da Silva, Monique Mendonça, Edilene Siqueira** e **Yoghatama Zanzer**; pessoas sem as quais o trabalho seria mais difícil e entediante. Houve também os amigos que me ajudaram em partes do meu trabalho como **Glaucia Lima**, **Milena Vuolo**, e **Cinthia Betim**. Obrigada **Nathalia Medina** e **Andressa Baseggio** pela ajuda com as formalidades da tese enquanto eu estava fora: foram muitas idas e vindas! Muito obrigada, sobretudo pela amizade de todos vocês! Estar no laboratório com **Cibele, Kemila, Cintia, Gustavo e Maria Helena** deixou também boas lembranças, e por isso também sou muito grata! Agradeço aos demais colegas do Laboratório de Nutrição e Metabolismo e do Laboratório de Ultraestrutura Celular. Obrigada à minha e estagiária do Cotuca **Nayara Une**, e aos bolsistas BIC-Jr pela ajuda nos ensaios biológicos e outras análises.

Agradeço aos meus amigos de fora da Universidade que me ajudaram aliviar muitos momentos de stress e por serem companheiros sempre. Às minhas amigas da Moradia Estudantil e da ICM pela amizade e por me ajudarem sempre.

Meus agradecimentos aos **animais de laboratório** e **voluntários** nos trabalhos experimentais, partes fundamentais do meu trabalho.

Meu agradecimento especial à minha **família** e ao meu **namorado**, que conviveram por muito tempo com a minha ausência, por apoiarem minhas decisões e por serem sempre o porto seguro em todos os momentos da minha vida.

À **Deus** por estar sempre ao meu lado e por todas as conquistas!

*Thank you **berry** much!*

## RESUMO

O resgate da alimentação baseada em frutas, grãos e outros vegetais, tem sido buscada como alternativa para controlar e/ou minimizar efeitos da dieta moderna, que acompanha o crescimento gradual da prevalência da obesidade no mundo. O jambo-vermelho é um fruto nativo de regiões tropicais, pouco estudado sob o ponto de vista químico, e não há relatos até o momento sobre sua utilização como alimento funcional em ensaios *in vivo*. A casca de jabuticaba é conhecida cientificamente pelas suas propriedades no tratamento da resistência à insulina e outras desordens metabólicas. O objetivo desta tese foi investigar compostos bioativos em frutos de jambo-vermelho (*Syzygium malaccense*) e da casca de jabuticaba (*Myrciaria jaboticaba*) e verificar os efeitos destas na prevenção da obesidade, doença hepática gordurosa não-alcoólica, diabetes tipo 2, resistência à insulina central e periférica, e desempenho cognitivo de camundongos alimentados com dietas normo e hiperlipídicas. As frutas de jambo-vermelho e casca de jabuticaba foram secas e submetidas a análises de macronutrientes e, fitoquímicos de referência bioativa, utilizando métodos físico-químicos, colorimétricos e cromatográficos. As farinhas dos frutos foram incorporadas a dietas normo e hiperlipídicas para a realização dos ensaios biológicos. Camundongos adultos consumiram as dietas por 10 semanas, e foram submetidos à testes de tolerância à glicose e insulina, e teste de aprendizado/memória. Após a morte dos animais e coletas de soro, fígado, tecido adiposo, fezes, carcaças e cérebro, avaliou-se a produção de ácidos graxos de cadeia curta, a adiposidade, marcadores inflamatórios, esteatose hepática, dislipidemia, estresse oxidativo, resistência à insulina periférica e via da sinalização da insulina no hipocampo. O jambo-vermelho e a casca de jabuticaba possuem compostos em comum, como a cianidina 3-glicosídeo, e fibras dietéticas totais em concentração em torno de 34%. Ambos os tratamentos com as frutas (HS= dieta hiperlipídica +jambo-vermelho e HM= dieta hiperlipidica + casca de jabuticaba) preveniram a resistência periférica à insulina e o aumento da glicemia de jejum nos animais. No entanto, o tratamento com HS não diferiu do grupo que recebeu dieta hiperlipídica (H) no teste de tolerância à glicose. De forma mais significativa, a ingestão da casca de jabuticaba preveniu o ganho de peso, massa gorda e esteatose hepática nos animais HM. Adicionalmente, as dietas HS e HM promoveram uma maior sensibilidade hipocampal à insulina, desativando a kinase GSK3-β e mantendo a função da proteína estabilizadora de microtúbulos tau. A expressão de TNF-α e interferon-γ estavam diminuídos no hipocampo dos

animais alimentados com dieta HM, assim como a expressão de NeuN (marcador de viabilidade neuronal). A ingestão das dietas HS e HM induziram o melhor aprendizado e memória dos respectivos animais. Finalmente, os animais que receberam dieta HM mostraram maior produção de ácidos graxos de cadeia curta em relação aos animais obesos, com destaque para o butirato. Em conclusão, as dietas HS e HM marcadamente preveniram desenvolvimento de marcadores de doenças metabólicas como obesidade, diabetes tipo 2 e inflamação. A suplementação com as frutas também possui papel neuroprotetor importante, prevenindo déficits cognitivos e fosforilação da tau induzida pela dieta hiperlipídica.

## ABSTRACT

The rescue of old eating habits, based on diets rich in fruits and vegetables, has been attemped as an alternative to control and/ or minimize the effects of the modern diet, which accompany the gradual growth in the prevalence of obesity worldwide. Red-jambo is a fruit native to tropical regions, and little studied from the chemical point of view, and to date, there are no reports on its use as a functional food in *in vivo* trials. The peel of jabuticaba is already known scientifically for its properties on treating insulin resistance and other metabolic disorders. Thus, the aim of this research was to investigate the bioactive compounds of red-jambo fruits (*Syzygium malaccense*) and jaboticaba peel (*Myrciaria jaboticaba*) and verify the effects on the prevention of obesity, non-alcoholic fatty liver disease, type 2 diabetes, peripheral and hippocampal insulin resistance, as well as cognitive performance of mice fed normal and high-fat diets. For this, red-jambo fruit and jaboticaba peel were dried and submitted to macronutrient analysis, bioactive reference phytochemicals, using physicochemical, colorimetric and chromatographic methods. These materials were incorporated into normal and high-fat diets for a mice study. Adult Swiss mice consumed the diets for 10 weeks, in which the animals experienced glucose and insulin tolerance tests, as well as a learning/ memory test. After euthanasia, serum, liver, adipose tissue, feces, carcasses and brain were collected to analyze the production of short chain fatty acids, adiposity, low grade inflammation, hepatic steatosis, dyslipidemia, oxidative stress, peripheral and hippocampal insulin signaling pathway. Red-jambo and jaboticaba peel have in common anthocyanins such as cyanidin 3-glycoside and total dietary fibers, about 34%. Both treatments with the fruits prevented peripheral insulin resistance and lowered fasting glucose of the animals that received high-fat diet (HS= high-fat diet + red-jambo and HM= high-fat diet + jaboticaba peel). However the treatment with HS diet did not differ from the high-fat control group (H) in the glucose tolerance test. More significantly, the HM diet prevented weight gain, fat mass, and hepatic steatosis. Additionally, the intake of HS and HM promoted greater hippocampal sensitivity to insulin, thus deactivating GSK3-β kinase and maintaining the tau microtubule stabilizing function. Expression of TNF-α and interferon-γ decreased in the hippocampus of animals fed HM, as well as NeuN expression (neuronal viability marker). Corroborating these results, the animals treated with the high-fat diet and both fruits

supplemnetation also showed better learning and memory performance in the MWM test. Finally, the animals that received HM showed higher production of short chain fatty acids in relation to obese animals, with emphasis on butyrate. In conclusion, the supplementation of high-fat diet with jaboticaba peel or red-jambo fruit markedly improve markers for metabolic diseases such as obesity, type 2 diabetes and inflammation. Supplementation also has important neuroprotective role, preventing cognitive deficits and phosphorylation of tau induced by insulin resistance.

## SUMÁRIO

# INTRODUÇÃO

A dieta define de forma abrangente a saúde dos povos, como parte da diversa variedade cultural existente no mundo (WHO, 2003). A importância da alimentação para a saúde humana não é, portanto, um novo conceito da nutrição moderna, considerando-se a sentença de Hipocrates (450–380 a.C.) há mais 2000 anos: "Deixe o alimento ser o seu medicamento e o medicamento ser seu alimento". A frase do médico grego inicia o conceito de que a alimentação pode ajudar no controle ou reversão de doenças. No entanto, o consumo habitual de certos alimentos traz também uma forte relação com a prevenção das mesmas (WHO, 2003). Exemplos de como estas afirmativas estão bem fundamentadas podem ser vistas em estudos epidemiológicos mostrando como o estado de saúde de muitas regiões no mundo é marcado pelos seus hábitos alimentares (NG et al., 2014; WHO, 2003).

A dieta do Mediterrâneo, por exemplo, é considerada por muitos cientistas a causa do baixo índice de doenças cardiovasculares, cânceres, doenças neurodegenerativas e mortalidade em geral nos países Europeus banhados pelo Mar Mediterrâneo, principalmente no século 20 (BACH-FAIG et al., 2011; KASTORINI et al., 2016; LASHERAS; FERNANDEZ; PATTERSON, 2000; PANAGIOTAKOS et al., 2015; PANAGIOTAKOS et al., 2003; SOFI et al., 2008; TRICHOPOULOU, A. et al., 2003; TRICHOPOULOU, A. et al., 2000). Tal dieta é caracterizada pela ingestão de uma grande variedade de vegetais, legumes e frutas frescas, cereais, gorduras insaturadas (provenientes principalmente de óleo de oliva), baixo consumo de gorduras saturadas, consumo moderado a alto de peixes, baixo ou moderado consumo de derivados de leite, com preferências para queijos e iogurtes; baixo consumo de carnes vermelhas, consumo regular de vinho durante as refeições (BACH-FAIG et al., 2011; TRICHOPOULOU, ANTONIA et al., 2000). Um estudo epidemiológico de coorte mostrou que a aderência à dieta do Mediterrâneo está também associada com a redução dos riscos da doença de Alzheimer (DA) e previne o avanço de déficits cognitivos independentemente de idade, sexo, educação fatores genéticos ou peso corporal (SCARMEAS et al., 2006). Outro estudo de coorte sugeriu que dos alimentos da dieta mediterrânea os vegetais parecem participar mais ativamente em prevenir o declínio cognitivo especialmente em indivíduos de 75 anos ou mais (TRICHOPOULOU, ANTONIA et al., 2015).

Nos anos 90, o Professor Serge Renaud discutiu o Paradoxo Francês, dada a correlação negativa do consumo de dietas hiperlipídicas e mortalidade por doenças cardiovasculares entre os franceses, devido ao consumo regular e moderado de vinho tinto (RENAUD; DE LORGERIL, 1992). Os benefícios da dieta do Mediterrâneo e o vinho tinto no Paradoxo francês são atribuídos aos polifenóis abundantes nos alimentos da dieta. Estes são metabólitos secundários de plantas, que possuem propriedades antioxidantes, anti-inflamatórias e previnem agregação plaquetária (CRESCENTE et al., 2009). Estudos em animais mostraram que dentre os polifenóis as antocianinas são destacadas pela ação de proteção cardíaca, anti-câncer, e ação limitante do ganho de peso (CERLETTI et al., 2016; CHIVA-BLANCH et al., 2013). No entanto, estudos em humanos são mais complexos e indicam que os efeitos benéficos das antocianinas não são pronunciados em voluntários saudáveis, ressalvo se um desafio de stress como uma dieta hiperlipídica é usado (CERLETTI et al., 2016; CHIVA-BLANCH et al., 2013).

Alguns autores, também sugeriram que o hábito de ingerir frequentemente a cúrcuma (ou açafrão, raiz rica em curcumina e o principal ingrediente do *curry*), seria responsável pela baixa prevalência da DA na Índia (CHANDRA et al., 1998; VAS et al., 2001). A curcumina tem sido proposta como fármaco na terapia da DA, devido ao seu efeito anti-inflamatório e antioxidante e de fato tem demonstrado a diminuição de marcadores da doença de Alzheimer em estudos experimentais (BELKACEMI et al., 2011; MA et al., 2009).

No outro lado do mundo, os Estados Unidos da América (EUA) se destaca por hábitos alimentares pautado em alimentos de alto aporte energético (gorduras e carboidratos simples) (WHO, 2003). Os EUA são hoje o país com maior prevalência de sobrepeso (25< IMC >30) e obesidade (IMC > 30) no mundo. A saber, 70,9% da população americana de homens adultos têm sobrepeso, mulheres representam 61,9% e crianças menores de 20 anos por volta de 30% (NG et al., 2014). A Inglaterra vem logo após os EUA onde 66,6% dos homens e 57,2% das mulheres tem sobrepeso (NG et al., 2014). Apesar de destacados estes países, a obesidade cresce em níveis pandêmicos globalmente desde os anos 80 e a industrialização de alimentos em conjunto com modernização do estilo de vida estão dentre os motivos (NG et al., 2014; WHO, 2003).

O Brasil é conhecido mundialmente pela sua diversidade em vegetais, principalmente pela disponibilidade de frutas devido ao clima e outras condições favoráveis. No entanto, mesmo com tal diversidade o Inquérito Nacional de Alimentação (2008-2009),

mostrou que a banana é a única fruta dentre os vinte alimentos mais consumidos por adultos no Brasil (SOUZA et al., 2013). A situação é ainda pior entre os adolescentes, uma vez que nenhuma fruta foi citada (SOUZA et al., 2013). A alimentação da população brasileira reflete assim a crescente prevalência de obesidade em homens (11,7%) e mulheres (20,6%) adultos, e de menores de 20 anos (~7%). A prevalência de sobrepeso é também alarmante representando mais de 50% da população adulta e mais de 20% de menores de 20 anos (NG et al., 2014).

A prevalência de sobrepeso e obesidade no Brasil retrata assim uma desvalorização de alimentos naturais ricos em agentes protetores contra a obesidade e outras doenças relacionadas (BRASIL, 2015). Neste trabalho, propôs-se o estudo de duas frutas vermelhas típicas de regiões tropicais, como o Brasil: jambo-vermelho e jabuticaba, frutas ricas em fibras dietéticas e polifenóis que são compostos associados com melhoras de parâmetros de saúde como exposto acima. Até o momento, não existem relatos de ensaios *in vivo* com a fruta do jambo-vermelho, e em estudo anterior a casca de jabuticaba demonstrou propriedades protetoras contra o estresse oxidativo no cérebro e fígado de animais obesos (BATISTA et al., 2014). No presente estudo, buscou-se descobrir o potencial destas frutas brasileiras em prevenir marcadores metabólicos responsáveis por obesidade, resistência à insulina além de prevenção de fatores de risco para doença de Alzheimer relacionada ao diabetes do tipo 2 e doença hepática gordurosa não alcoólica.

## OBJETIVOS

O objetivo geral deste trabalho foi identificar os efeitos benéficos de duas frutas vermelhas em relação à prevenção da obesidade, diabetes do tipo 2, resistência à insulina periférica e hipocampal, doença hepática gordurosa não-alcoólica, e contribuir para compreensão de como esses efeitos são mediados.

Especificamente cada artigo desta tese teve como objetivo:

1) Determinar os macronutrientes e compostos bioativos do jambo-vermelho e avaliar a sua capacidade antioxidante.

2) Verificar os efeitos de dietas normo e hiperlipídicas contendo a fruta do jambo-vermelho sobre a prevenção da obesidade e suas implicações sobre a resistência periférica à insulina, inflamação de baixo grau, estresse oxidativo, resistência à insulina no hipocampo e desempenho em desafio de memória/ aprendizagem.

3) Avaliar os efeitos da suplementação com a casca de jabuticaba sobre a prevenção de déficits cognitivos relacionados resistência à insulina periférica e hipocampal induzida pela ingestão de dieta hiperlipídica, além de marcadores inflamatórios e estresse oxidativo.

4) Analisar as respostas fisiológicas de camundongos após suplementação crônica de dietas normo e hiperlipídicas com a casca de jabuticaba, relacionadas à esteatose hepática, adiposidade visceral, e saúde intestinal.

# CAPÍTULO 1 - REVISÃO BIBLIOGRÁFICA

## 1. OBESIDADE: CAUSAS E CONSEQUÊNCIAS

### 1.1. Obesidade e Resistência à insulina

A resistência à insulina (RI) pode ser considerada o elo entre obesidade e diabetes mellitus do tipo 2 (DM2). Definida como estado de menor resposta metabólica aos níveis circulantes de insulina, a RI e o desarranjo metabólico decorrente desencadeiam a falência das células β-pancreáticas em produzir insulina, dando origem ao DM2 (JOHNSON; OLEFSKY, 2013; KAHN; HULL; UTZSCHNEIDER, 2006; MOLLER; KAUFMAN, 2005).

A obesidade é a causa mais comum da RI, o que explica o fato de 80% dos diabéticos tipo 2 nos EUA estarem acima do peso. O DM2 atinge 285 milhões de pessoas (20-79 anos) em todo o mundo (7,5 milhões no Brasil) (SHAW; SICREE; ZIMMET, 2010). Em paralelo, a epidemia da obesidade ainda é mais assustadora, associada com um aumento de 10 e 14% da morbidade e mortalidade entre mulheres e homens adultos, respectivamente (WHO, 2012).

O hábito alimentar e estilo de vida sedentária são hoje as maiores causas do aumento de peso (WHO, 2003). A alimentação de indivíduos obesos está pautada na alta ingestão de gordura saturada além de alimentos de alto índice glicêmico (GARDNER; RHODES, 2009).

### 1.2 Inflamação de baixo grau e resistência à insulina

Evidências indicam a RI no hipotálamo como o desencadeador da obesidade, estimulada pela inflamação de baixo grau instalada no local (POSEY et al., 2009). O bloqueio da sinalização da insulina por intermediários inflamatórios no hipotálamo impediria a transcrição de genes relacionados à produção de neurotransmissores anorexigênicos. Com estímulo orexigênico descontrolado, o indivíduo teria seu metabolismo energético alterado, consumindo alimentos em excesso, e, consequentemente altos níveis de glicose e insulina sanguínea, aumento de massa adiposa, e RI periférica alojada (KAHN et al., 2006; MILANSKI et al., 2012).

O consumo frequente de ácidos graxos saturados (SFA) pode desencadear um processo inflamatório em vários tecidos que possuem receptores toll-like do tipo 4 (TLR4). O

TLR4 está presente: na membrana de macrófagos, células do tecido adiposo, músculo, hipotálamo e hipocampo (ROLLS et al., 2007; SUGANAMI et al., 2007). O estímulo do TLR4 por SFA ou lipopolissacarídeos de membrana de bactérias (LPS), pode ativar moléculas como a c-Jun N-terminal kinase (JNK) e a IKβ quinase (IKK), que por sua vez inibem a sinalização da insulina por fosforilação em serina de substrato 1 do receptor da insulina (IRS-1) (HOTAMISLIGIL, G. S., 2006; MILANSKI et al., 2012; POSEY et al., 2009).

As proteínas IKK e JNK, por sua vez, ativam os fatores de transcrição AP-1 e NF-κB, levando à produção exacerbada de citocinas pró-inflamatórias como interleucinas 1β, 6 (IL-1β, IL-6), e o fator de necrose tumoral α (TNF-α). O TNF-α produzido vai para circulação e também se liga à seus receptores específicos (TNFR) em vários tecidos e células. O TNFR também ativa IKK e JNK, gerando assim um ciclo pró-inflamatório vicioso (JOHNSON; OLEFSKY, 2013; MILANSKI et al., 2012). A obesidade acompanha a expressão anormal e elevada de TNF-α pelo tecido adiposo (HOTAMISLIGIL, GOKHAN S; SHARGILL; SPIEGELMAN, 1993). A ligação entre a RI e a inflamação de baixo grau na obesidade foi descoberta quando modelos de animais obesos com disfunção do TNF-α mostraram melhor sensibilidade à insulina e homeostase da glicose (UYSAL et al., 1997), comprovando a importância da inflamação para o desenvolvimento de doenças metabólicas.

A Figura 1 ilustra o mecanismo de bloqueio da sinalização de citocinas inflamatórias e ácidos graxos saturados em bloquear a propagação do sinal da insulina nas células hipotalâmicas.

### 1.2.1 Papel do tecido adiposo na resistência à insulina e inflamação

Acompanhando o aumento do tecido adiposo e elevação dos níveis inflamatórios, uma infiltração de células imunes ocorre, atraindo macrófagos, que chegam a ocupar 40% das células do tecido. Macrófagos da população M1 (mais ativos e pró-inflamatórios) são os mais numerosos em indivíduos obesos. Estes juntamente com as células adiposas produzem citocinas pró-inflamatórias como as IL-1β e 6, proteína quimioatrativa de monócitos 1 (MCP-1), e TNF-α em excesso. Da mesma forma que no hipotálamo, as citocinas produzidas no tecido adiposo induzem a RI pela ativação da via TNF-α/JNK que impedem a propagação do sinal da insulina pela fosforilação em serina do IRS1. As citocinas produzidas atingem a circulação e podem também prejudicar a propagação do sinal da insulina em outros órgãos como músculos e fígado (HOTAMISLIGIL, G. S., 2006).

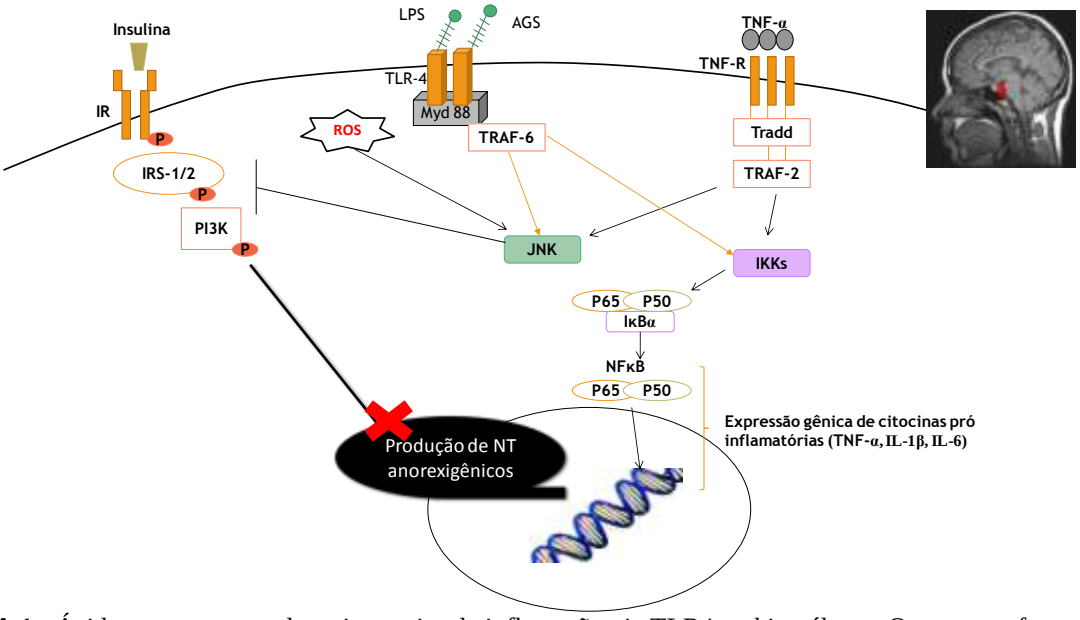



**FIGURA 1** - Ácidos graxos saturados ativam vias de inflamação via TLR4 no hipotálamo. O consumo frequente de ácidos graxos saturados (SFA) pode desencadear um processo inflamatório no cérebro e outros tecidos, uma vez que este possui receptores do tipo *Toll Like* 4 (TLR4). O TLR4 também está presente na membrana de macrófagos, células do tecido adiposo e músculo. O estímulo do TLR4 por SFA ou LPS (lipopolissacarídeo de bactérias gram negativas), pode ativar moléculas como a c-Jun N-terminal kinase (JNK) e a IKβ quinase (IKK), que por sua vez inibem a sinalização da insulina por fosforilação em serina de substrato 1 do receptor da insulina (IRS-1). Baseado em Hotamisligil (2006) e Milanski et al. (2012).

O tecido adiposo é também o responsável pela produção de adipocinas como a leptina, e adiponectina, e na obesidade a produção destas citocinas encontra-se desequilibrada devido a inflamação e hipertrofia do tecido (TILG; MOSCHEN, 2006). A leptina é a adipocina responsável pela sinalização de estímulos anorexigênicos no hipotálamo e possui características pró-inflamatórias (TILG; MOSCHEN, 2006). A hiperleptinemia nos indivíduos obesos está relacionada a falhas na sua sinalização, desencadeando um quadro de resistência à leptina e consequentemente menor liberação de neurotransmissores anorexigênicos (MYERS; COWLEY; MUNZBERG, 2008). A leptina também está associada à produção de citocinas pró-inflamatórias em macrófagos. Já a adiponectina é conhecida como sensibilizadora à insulina e esta diminuída na obesidade, contribuindo ainda mais para o quadro de resistência à insulina (TILG; MOSCHEN, 2006).

### 1.2.2. Obesidade, estresse oxidativo e inflamação

O estresse oxidativo caracteriza-se pela insuficiência do sistema antioxidante fisiológico em equilibrar os níveis de espécies reativas de oxigênio (ROS) e de nitrogênio (RNS) (VALKO et al., 2007). O sistema de defesa antioxidante natural do organismo é

constituído pelas enzimas catalase (CAT), superóxido dismutase (SOD), glutationa peroxidase (GPx) e glutationa redutase (GRd), sendo responsável pela neutralização e regulação dos níveis gerais de ROS para manter a homeostase fisiológica (FIGURA 2) (JACOB, 1995).

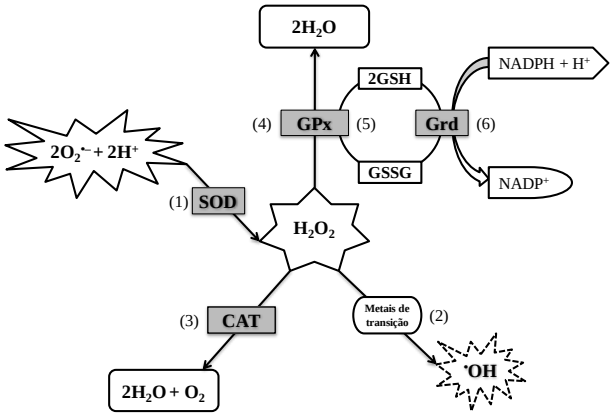



**FIGURA 2 –** Esquema de sistema de defesa antioxidantes enzimático (MEISTER; ANDERSON, 1983; BARBOSA et al., 2010). (1) Superóxido dismutase (SOD) converte $O2^{\bullet-}$ em $H_2O_2$; (2) $H_2O_2$ na presença de metais de transição se converte em radical hidroxila ($^{\bullet}OH$); (3 e 4) catalase (CAT) e glutationa peroxidase (GPx) transformam $H_2O_2$ em água; (5) GPx converte a glutationa reduzida (GSH) à sua forma oxidada GSSG (glutationa oxidada), além de converter $H_2O_2$ em água; (6) a glutationa redutase (Grd) utiliza nicotinamida adenina dinucleotídeo fosfato (NADPH) para converter GSSG em GSH.

O estresse oxidativo é tido como causa e consequências de quadros patológicos como a obesidade, resistência à insulina e inflamação (STYSKAL et al., 2012). Na obesidade, o estado inflamatório de baixo grau estimula a produção de ROS e RNS por macrófagos e monócitos no tecido adiposo por exemplo. Assim, maiores concentrações de citocinas inflamatórias podem ser responsáveis pelo estresse oxidativo. Por outro lado, as espécies reativas também ativam diretamente os fatores de transcrição NF-κB and AP-1, dando continuidade ao ciclo de produção de citocinas pró-inflamatórias (STYSKAL et al., 2012; VALKO et al., 2007) (FIGURA 3).

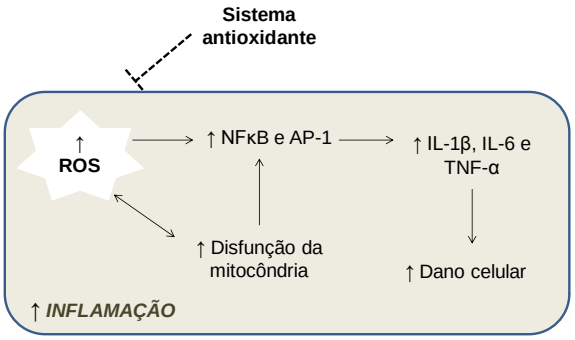



**FIGURA 3-** Esquema de representação de conexões entre o estresse oxidativo e a inflamação na obesidade. Setas para cima: aumentam com a obesidade. Pontilhados: bloqueio.

Adicionalmente, o consumo de dietas hiperlipídicas altera o metabolismo de oxigênio e os depósitos de gordura nos tecidos são vulneráveis a reações de oxidação levando à peroxidação lipídica (POLI; ALBANO; DIANZANI, 1987). O acúmulo de gordura em tecidos como o fígado, por si, pode danificar a célula pressionando-a, e este dano celular levaria à produção de citocinas inflamatórias e consequentemente ROS.

### 1.3. Obesidade, resistência à insulina hepática e doença hepática gordurosa não alcoólica

A insulina tem também ação antilipolítica no tecido adiposo, e a RI culmina assim na liberação de ácidos graxos livres para a circulação de forma intensificada. Concomitantemente, lipo e glicotoxicidade associadas à maior síntese e oxidação de lipídios pelo fígado, corroboram para o desenvolvimento da esteatose hepática e até mesmo esteatohepatite (LOMONACO et al., 2012; MOLLER; KAUFMAN, 2005).

O tecido adiposo supre mais de dois terços de ácidos graxos usados na síntese hepática de triglicerídeos. A disfunção do tecido adiposo pode levar a um influxo elevado de lipídeos no fígado induzindo rapidamente a RI e marcadores da doença do fígado gorduroso não-alcoólica (NAFLD), como a esteatose hepática (KIMURA et al., 2013). A esteatose hepática é o acumulo de triglicerídeos no fígado resultado do maior influxo de lipídeos e síntese lipídica de novo em desbalanço com a oxidação e efluxo de lipídeos. O aumento da síntese lipidica hepática, glicogenólise, gliconeogênese e secreção de VLDL (very-low-density lipoprotein) também corroboram para a NAFLD (KAWANO; COHEN, 2013).

Em indivíduos obesos com a doença hepática gordurosa não-alcoólica, ocorre uma deterioração progressiva de triglicerídeos plasmáticos e HDL-c (high-density lipoprotein cholesterol), RI hepática e fibrose proporcional à RI no tecido adiposo (LOMONACO et al., 2012). No entanto, alguns casos da NAFLD podem ocorrer em indivíduos não obesos, da mesma forma que alguns indivíduos obesos não desenvolvem a NAFLD (KIM et al., 2004).

A Figura 4 ilustra a progressão de NAFLD para esteatohepatite, processo que envolve a disfunção mitocondrial, estresse oxidativo, e ativação de vias pró-inflamatórias.

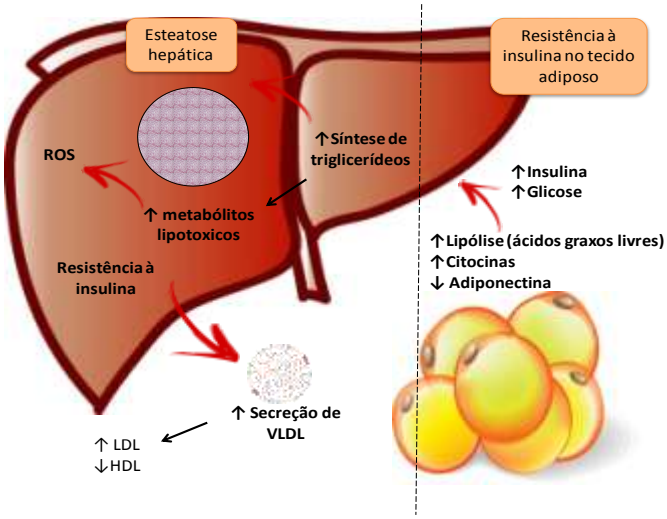



**FIGURA 4 -** Representação esquemática do desenvolvimento da doença do fígado gorduroso (NAFLD) e sua relação com o tecido adiposo em indivíduos obesos. Resistência à insulina periférica desencadeia o estado lipolítico do tecido adiposo em não resposta à insulina. Neste estado ocorre também a liberação de citocinas pró-inflamatórias e pró-resistência à insulina para a circulação, alcançando o fígado. No fígado à deposição de gordura advinda do tecido adiposo, dieta e produção endógena evolui para a esteatose hepática, desencadeando também alta produção de espécies reativas de oxigênio, resistência à insulina hepática, maior liberação de lipoproteínas de densidade muito baixa e consequentemente dislipidemias. O quadro inflamatório também se desenvolve e a NAFLD pode evoluir assim para NASH (esteatohepatite não alcoólica). Adaptado de Cusi (2012).

No presente trabalho, também se investigou o papel do PPAR-α na NAFLD. Os PPAR-α (peroxissome proliferator receptor alpha) são receptores nucleares, altamente expressos no fígado, que se ligam a elementos específicos (ex. ácido linoleico, leucotrieno B4) na região promotora de genes alvo e ativam a transcrição de genes em resposta a esta ligação. Estes receptores estimulam a oxidação de ácidos graxos em várias organelas celulares como a mitocôndria, peroxissomos e microssomos, além de instigar a absorção de ácidos graxos e a síntese de lipoproteínas. Em estados de jejum o PPAR-α estimula assim a oxidação de ácidos graxos à acetil-CoA e corpos cetônicos liberados para circulação (KERSTEN; DESVERGNE; WAHLI, 2000).

Assim, mesmo no estado alimentado, quando o tecido adiposo entra em lipólise devido à resistência à insulina, os ácidos graxos estimulam sua própria absorção e oxidação pelo fígado quando ativam o PPAR-α, ligando-se ao receptor (KERSTEN et al., 2000). A oxidação dos ácidos graxos gera assim a produção de ROS, elevando o estresse oxidativo no fígado e consequentemente mais reações inflamatórias. O aumento de corpos cetônicos advindos da maior oxidação dos ácidos graxos contribui também para acidose metabólica, característica da DM2 (KERSTEN et al., 2000).

## 1.4. Obesidade e saúde intestinal

A causa do desenvolvimento da obesidade ainda é alvo de muitas discussões e estudos. No entanto evidências científicas sugerem que a microbiota humana tem grande influência sobre o desenvolvimento da obesidade e intolerância à glicose (BÄCKHED et al., 2007; JOHNSON; OLEFSKY, 2013; TURNBAUGH et al., 2006). Um experimento com animais *germ-free* mostrou que estes não desenvolviam obesidade e resistência à insulina quando alimentados com dieta hiperlipídica (BÄCKHED et al., 2007), mas o fenômeno acontecia quando a microbiota dos animais foi reconstituída com uma microbiota de um animal normal (BÄCKHED et al., 2004).

A microbiota intestinal está alterada na obesidade e estudos mostram que ocorre um desequilíbrio entre os filos de bactérias *Firmicutes* e *Bacteroidetes*, caracterizando a disbiose (LEY et al., 2006). No entanto, não há um consenso se essas alterações poderiam ser a causa ou consequência da obesidade e RI. Estudos correlacionam a baixa contagem de genes de bactérias intestinais com dislipidemias, resistência à insulina e inflamação (LE CHATELIER et al., 2013). No entanto uma maior gama de gene bacterial no intestino estava associada com uma prevalência de espécies com potencial anti-inflamatório e aumento da produção de ácidos graxos de cadeia curta (AGCC), como o butirato (LE CHATELIER et al., 2013).

A disbiose afeta assim a produção de metabolitos bacteriais como os AGCC, responsáveis pela modulação inflamatória no intestino. Carboidratos não digeridos são fermentados no colón para produzir AGCC, como acetato, propionato e butirato. Estes metabolitos podem se ligar a proteínas acopladas a receptores do tipo G (GPR) e suprimir inflamação, manter a integridade da função da barreira epitelial intestinal e prevenir a resistência à insulina (MASLOWSKI et al., 2009; TREMAROLI; BACKHED, 2012).

Uma das consequências da disbiose instalada na obesidade que destacamos neste trabalho é o aumento da permeabilidade intestinal. Dietas hiperlipídicas promovem uma redução na expressão de proteínas epiteliais *tight-junctions*, permitindo a passagem de bactérias e seus produtos (lipopolissacarídeos de membrana - LPS e DNA) para a circulação, o que é o suficiente para promover inflamação sistêmica e resistência à insulina em indivíduos obesos (CANI et al., 2008; JOHNSON; OLEFSKY, 2013). De fato estudos mostram que LPS de bactérias patogênicas pode chegar à circulação e tecidos via quilomicrons e ativar vias de inflamação ligadas ao do tipo TLR4 e expressão de proteínas de vias pró-inflamatórias (GHOSHAL et al., 2009).

## 1.5. O papel da resistência à insulina no desenvolvimento de marcadores da doença de Alzheimer

O papel da sinalização da insulina no sistema nervoso central vai muito além do controle da fome e gasto energético pela sua sinalização no hipotálamo. Receptores da insulina (IR) e seus substratos também podem ser encontrados em outras regiões do cérebro, como, por exemplo, naquelas relacionadas com a memória e aprendizagem: hipocampo, amígdala e córtex frontal (ZHAO, W. Q.; ALKON, 2001). Nestas regiões, o estímulo da insulina é importante para sobrevida de neurônios, expressão da proteína estabilizante de microtúbulo tau (tau), metabolismo energético, função mitocondrial, atividades sinápticas e processo cognitivo (DE LA MONTE; WANDS, 2008; MESSIER; TEUTENBERG, 2005).

A RI periférica e central pode ser considerada como fator de risco para desenvolvimento da DA, pois a sinalização debilitada da insulina está relacionada com a ocorrência de dois marcadores da doenças e precursores da formação de placas senis e emaranhados fibrilares (NFT): a proteína amiloide β (Aβ) e tau, respectivamente (FIGURA 5) (CASTELLANI; ROLSTON; SMITH, 2010; CORREIA et al., 2012; MA et al., 2009; SCHUBERT, MARKUS et al., 2004).

A proteína tau estabiliza os microtúbulos, que por sua vez são estruturas proteicas que compõem o citoesqueleto de neurônios e axônios predominantemente (HIMMLER et al., 1989). Quando hiperfosforiladas em vários sítios, a tau se desliga do microtúbulo, desestabilizando-o e pode assim se agrupar no citosol, formando os filamentos helicoidais pareados e em sequência formar NFT (FIGURA 5). Com a deterioração do citoesqueleto o axônio se degenera e não é capaz de manter as conexões e sinapses dos neurônios (MATTSON, 1995).

A sinalização da insulina ativa a via da fosfatidil-inositol 3-kinase (PI3K)-proteína kinase B (PKB/AKT) fosforilando glicogênio sintase kinase-3 (GSK3), uma enzima cuja atividade é contra-regulada pela fosforilação em Serina 21 da isoforma GSK3α e Serina 9 de GSK3β (LEE, J. S.; KIM, 2007). Na RI, o bloqueio da propagação do sinal da insulina inativa a kinase AKT, que por sua vez seria responsável em fosforilar GSK3-β quando ativa, bloqueando sua ação em fosforilar a tau e impedir subsequente formação de NFT, deposição de peptídeos Aβ, estresse oxidativo e neurodegeneração (CORREIA et al., 2012; CROSS et al., 1995; JOLIVALT et al., 2008; SCHUBERT, M. et al., 2003). Adicionalmente, falhas na

sinalização da leptina também podem levar à hiperfosforilação da tau e à neurodegeneração (LEE, 2011).

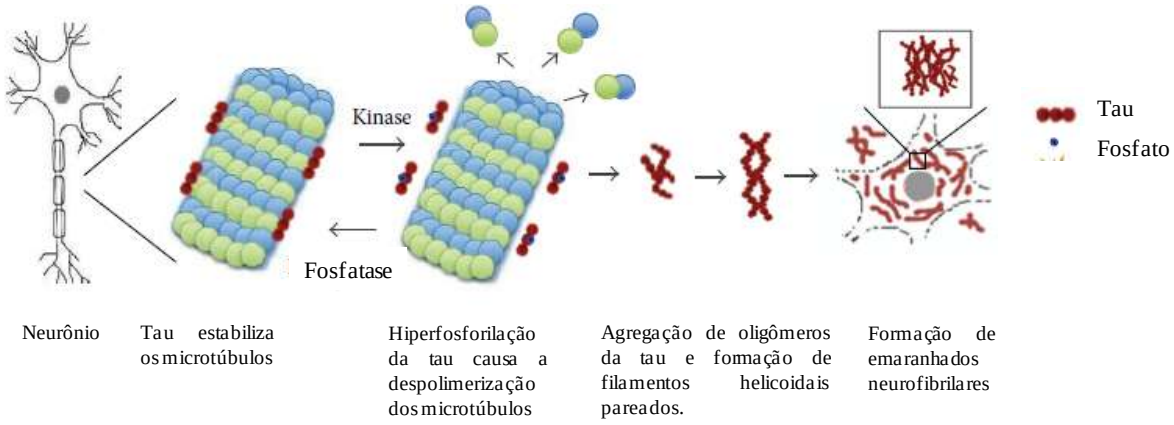



**FIGURA 5** - A estabilização dos microtúbulos pela proteína tau é regulada por kinases e fosfatases. A hiperfosforilação anormal das proteínas tau provoca a despolimerização dos microtúbulos e a formação de oligômeros de tau citoplasmáticos insolúveis, que se agregam formando filamentos helicoidais pareados, que se reúnem formando emaranhados neurofibrilares (NFT). Adaptado de Mokhtar et al. (2013).

Os peptídeos $A\beta_{1-40}$ e $A\beta_{1-42}$ originados da clivagem proteolítica da proteína precursora Aβ (APP) pelas enzimas β e γ-secretases, são os componentes primários das placas senis. Tanto $A\beta_{1-40}$ como $A\beta_{1-42}$ são peptídeos tóxicos, no entanto $A\beta_{1-42}$ é insolúvel e tem maior propensão de formar placas senis no meio extracelular. A insulina pode modular a atividade da γ-secretases, no entanto a enzima degradadora de insulina (IDE), principal degradadora da Aβ, é inibida pela sua ligação à insulina, repercutindo na aglomeração da Aβ, e maior ocorrência de placas senis, estresse oxidativo e neuroinflamação (DE LA MONTE; WANDS, 2008; MESSIER; TEUTENBERG, 2005).

A hiperinsulinemia periférica desenvolvida no DM2 contribui contra-regulando a função da barreira hemato-encefálica e atividade do IR, o que reduz o transporte de insulina para o cérebro, agravando a RI no cérebro (ZHAO, W. Q.; ALKON, 2001). A deficiência da insulina no cérebro concomitantemente à sua resistência já foi reportada por pesquisadores como diabetes tipo 3, uma forma específica de diabetes neste órgão (DE LA MONTE; WANDS, 2008).

De fato, biomarcadores da DA começam a surgir com o desenvolvimento da obesidade, de forma independente da idade (FIGURA 6). Estudos epidemiológicos evidenciam um aumento do crescimento da mortalidade por DA em indivíduos com 45-54 anos a partir do ano 2000 (0,20/100.000 indivíduos), corroborando um aumento proporcional

nos casos de morte por DM2 no mesmo período (13/100.000 indivíduos) (DE LA MONTE, 2011). Dessa forma pode-se inferir que a obesidade e DM2 na meia-idade é um fator de risco para o desenvolvimento de DA.

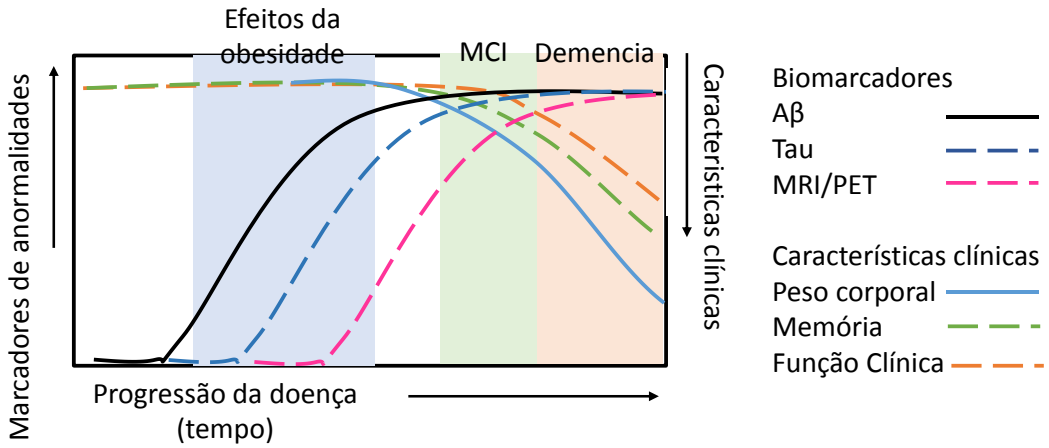



**FIGURA 6.** Modelo da progressão clínica e biomarcador da doença de Alzheimer (DA). Eixo x= progressão da AD; eixo y esquerdo= biomarcadores da DA; e eixo y direito = parâmetros clínicos associados com AD. Os valores dos biomarcadores anormais relacionadas com o peptídeo Aβ (linha preta), indicam que as alterações relacionadas com Aβ pode ser detectado em indivíduos pré-sintomáticos. Consequentemente, há desenvolvimento de alterações na tau (linha pontilhada azul), seguida por alterações na estrutura e função cerebral (MRI/PET= ressonância magnética, linha rosa). O início do declínio da memória (linha pontilhada verde) anuncia a designação clínica de "transtorno cognitivo leve" (MCI), que normalmente é seguido por um declínio evidente em função clínica (linha tracejada laranja) e demência, mais comumente diagnosticado como AD. A associação entre a obesidade e demência também está refletida neste modelo, a qual ocorre na fase pré-sintomática da doença, bem como a associação entre a perda de peso (linha azul claro vazio) e demência que ocorre tipicamente ao fim e durante os últimos estágios de AD. Adaptado de Lee (2011).

## 2. DOENÇA DE ALZHEIMER E MEMÓRIA

### 2.1. Epidemiologia da doença de Alzheimer e implicações na memória

O envelhecimento é o fator de risco número um para desenvolvimento de demências, e a AD representa cerca de 70% dos casos (REITZ; BRAYNE; MAYEUX, 2011). Em 2010 estimou-se que 35,6 milhões de pessoas tinham demência e a progressão para o ano 2030 é que 65,7 milhões de pessoas desenvolvam demência, evoluindo para 115,4 milhões em 2050 (REITZ et al., 2011) e (World Alzheimer Report 2010 [http://www.alz.co.uk/research/files/WorldAlzheimerReport.pdf]).

A doença de Alzheimer é uma desordem neurodegenerativa associada com disfunção cognitiva e comportamental e tem como principais marcadores os emaranhados neurofibrilares causados por hiperfosforilação da tau e placas de proteína amiloide β (REITZ; MAYEUX, 2014). A memória é afetada rapidamente com o desenvolver da doença, uma vez

que o hipocampo é a primeira região atingida pela neurodegeneração (VARGHA-KHADEM et al., 1997).

### 2.1.1. Processo de formação e acesso da memória

A formação de memória é um processo que inclui diferentes fases: a aprendizagem medeia à rápida transcrição de genes, seguida por síntese de proteínas, que desencadeia então alterações na conectividade sináptica em processos de consolidação da memória, gerando estabilidade no alcance da memória (DUDAI, 2012; FRANKLAND; BONTEMPI, 2005; KANDEL, 2001). A recordação/acesso da memória também instiga nova síntese proteica, modificando e reforçando o caminho da memória original. Cita-se assim 4 etapas, ilustradas na Figura 7: a) aprendizagem (ou aquisição de novas informações), b) consolidação, c) armazenamento, e d) recuperação (DUDAI, 2012; FRANKLAND; BONTEMPI, 2005; KANDEL, 2001).

A conversão da memória de curto para longo prazo exige que a síntese de novas proteínas ocorra dentro dos neurônios e de suas sinapses (MARTIN; BARAD; KANDEL, 2000). Portanto, terapias que visem à preservação da memória deve ser tal que ofereça compostos capazes de interagir, e efetivamente modificar as vias de sinalização proteica dentro dos neurônios e sinapses levando a mudanças na eficiência dessa síntese.

Assim, o aprimoramento da memória de curto e longo prazo, controlado por eventos moleculares nos neurônio convergem na ativação de fatores de transcrição de genes como a CREB, responsável pela transcrição de neurotrofinas (p. ex. BDNF), indução da plasticidade sináptica e consequentemente formação de memória de longa-duração (ALONSO et al., 2002). Resumidamente, a plasticidade sináptica é composta de três processos (SPENCER, 2009):

1) Os aumentos na densidade de receptores sinápticos podem resultar da síntese de novo de proteínas neuronais, particularmente neurotransmissores e receptores, que reforçam a força comunicativa entre neurônios, auxiliando assim o fluxo de informação.

2) Aumentos na densidade, volume e morfologia de novas espinhas dendriticas ou de existentes reforçam vias neurais pela ligação entre dois neurônios no trajeto pré-sináptico e pós-sináptico melhorando o circuito de comunicação sináptica.

3) Mudanças moleculares específicas também podem levar à neurogênese ou ao processo pelo qual os neurônios são criados a partir de células-tronco neuronais. Embora a neurogênese seja principalmente ativa durante o desenvolvimento pré-natal, novos neurônios

continuamente nascem durante a idade adulta, podendo ocorrer no bulbo olfatório e no giro denteado do hipocampo (DG) (ZHAO, C.; DENG; GAGE, 2008). O DG é uma região do hipocampo que está criticamente envolvida na memória e, portanto, espera-se que uma estimulação da neurogênese do hipocampo tenha impacto na memória e no aprendizado humano.

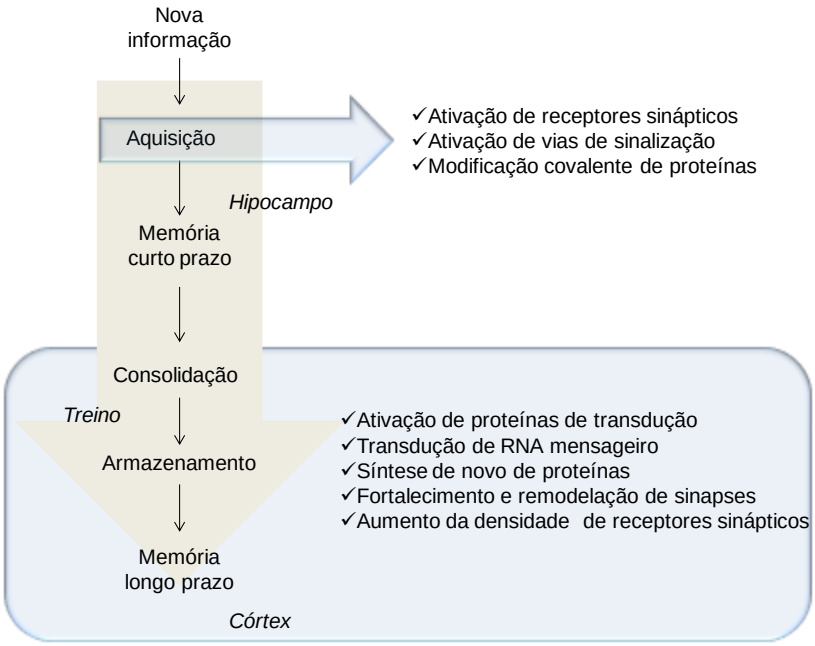



**FIGURA 7-** Vias de sinalização envolvidas no controle da memória e aprendizagem no cérebro humano. Essas vias são críticas no controle da plasticidade sináptica e neurogênese. Adaptado de Spencer (2009).

## 2.2. Morris *water maze* como medida de memória e aprendizado em modelos animais

O labirinto aquático de Morris our Morris *water maze* (MWM) é um teste comportamental que tem sido utilizado para estudos de aprendizagem e da memória espacial de roedores experimentais (MORRIS, 1984). O teste é baseado na tentativa de escape do animal a uma situação estressante como a piscina de água. A piscina contém uma pequena plataforma escondida logo abaixo do nível da água. Esta plataforma permite que os animais possam escapar do estresse de nadar na água. Os animais são treinados previamente ao teste, onde o experimentador apresenta a localização da plataforma. O animal deve se lembrar da localização da plataforma de escape, usando pistas visuais na área de teste. Esta tarefa requer o uso da memória de referência espacial dependente do hipocampo (MORRIS, 1984).

Animais normais ou saudáveis aprendem muito rapidamente a nadar diretamente para a plataforma a partir de qualquer posição de partida na circunferência da piscina seguindo as pistas do ambiente. Os animais com problemas na aprendizagem ou memória

tendem a passar mais tempo procurando pelo escape, nadando aleatoriamente na piscina. A comparação do desempenho de animais normais e tratados é uma maneira de examinar a neurobiologia da aprendizagem espacial (BRANDEIS; BRANDYS; YEHUDA, 1989).

## 3. COMPOSTOS BIOATIVOS EM FRUTAS VERMELHAS

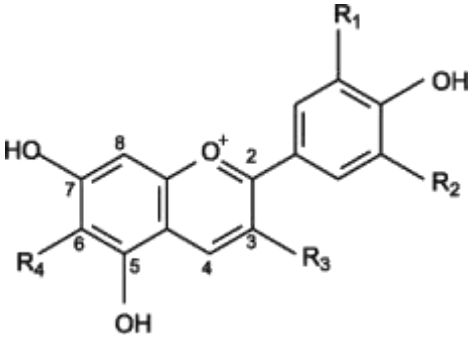
### 3.1. Compostos fenólicos e suas funções na saúde

Os compostos fenólicos representam um grande grupo de metabolitos secundários de plantas contendo um ou mais anéis aromáticos com vários graus de hidroxilação, metoxilação e glicosilação. Muitas classificações de compostos fenólicos existem, e a seguinte classificação é baseada na estrutura química: ácidos fenólicos, lignanas, stilbenos e flavonoides, que também possui subclasses devido a diferenças na sua estrutura química (MANACH et al., 2004). Estes compostos e seus derivados possuem diversas formas de polimerização, glicosilação e complexação formando assim polifenóis.

#### 3.1.1. Flavonoides e antocianinas

Os flavonoides, presentes em vários vegetais, são uma classe de compostos fenólicos que tem chamado muita atenção devido aos seus efeitos benefícios à saúde. São compostos de baixo peso molecular e estão geralmente ligados a açúcares. Podem ser classificados em antocianinas, flavonóis, flavonas, flavanóis e isoflavonas (KING; YOUNG, 1999; MANACH et al., 2004). As antocianinas, por exemplo, presentes na maioria dos vegetais de pigmentação vermelha, azul e violeta de flores, frutos, folhas, sementes e raízes se destacam neste trabalho (KONG et al., 2003).

Antocianina é a forma glicosilada de antocianidina e possui um cátion *flavilium*, o que as faz estáveis em meios ácidos (FIGURA 8). A sua estrutura química consiste de dois anéis de benzeno e um anel heterocíclico central contendo oxigênio. Possui 15 carbonos e uma ou mais ligações com moléculas de açúcares que podem se ligar às hidroxilas em diferentes posições (KONG et al., 2003). Algumas antocianinas de interesse neste trabalho estão representadas na Figura 8. São compostos de rápida absorção e metabolização, associados não só a ações antioxidantes, como a ações anti-inflamatórias, anti-obesogênicas e sensibilizadora à insulina *in vivo* (DRAGANO et al., 2013; TSUDA et al., 2003). No entanto pouco se sabe sobre os mecanismos envolvidos em tais efeitos benéficos.




| Antocianidina | R1 | R2 | R3 | R4 | PM |
|---|---|---|---|---|---|
| Pelargonidina | H | H | OH | H | 271 |
| Cianidina | OH | H | OH | H | 287 |
| Peonidina | $OCH_3$ | H | OH | H | 301 |
| Delfinidina | OH | OH | OH | H | 303 |
| Petunidina | $OCH_3$ | OH | OH | H | 317 |
| Malvidina | $OCH_3$ | $OCH_3$ | OH | H | 331 |

| Antocianina | R1 | R2 | R3 | R4 | 5 | PM |
|---|---|---|---|---|---|---|
| Cianidina-3-O-glicosídeo | OH | H | Glc | H | OH | 449 |
| Cianidina-3,5-O-diglicosídeo | OH | H | Glc | H | Glc | 611 |
| Peonidina-3-O-glicosídeo | $OCH_3$ | H | Glc | H | OH | 463 |
| Delfinidina-3-O-glicosídeo | OH | OH | Glc | H | OH | 465 |

**FIGURA 8-** Cátion *flavilium* e estrutura de antocianidinas e formas glicosiladas de relevância para este trabalho. PM= peso molecular.

### 3.1.1.1. Mecanismos de ação contra a resistência à insulina

Recentemente, tem-se comprovado que os flavonoides podem interferir em importantes cascatas de sinalização celular em diferentes tecidos: sinalização da insulina (PI3K-AKT-FOXO), sinalização de citocinas inflamatórias (TNF-α – NFκB), e outras (citocinas-JNK, AMPK-AS160) (DRAGANO et al., 2013; GUO; GUO et al., 2012; GUO; XIA et al., 2012; TSUDA, 2008)

A cianidina 3-glicosídeo (C3G), antocianina mais abundante na natureza, (DENEV et al., 2012) pode regular a função dos adipócitos na obesidade (GUO; GUO et al., 2012). A C3G pode ativar a *AMP-activated protein kinase* (AMPK), uma kinase serina-treonina que é fosforilada após o aumento da razão adenosina monofosfato: adenosina trifosfato (AMP: ATP). Assim, ao ativar AMPK, a C3G bloqueia a lipólise via desativação da transcrição de enzimas lipolíticas (*adipose triglyceride lipase)* pela FoxO1, melhorando a lipotoxicidade (GUO; GUO et al., 2012; GUO; LIU et al., 2012). Outro trabalho (TSUDA, 2008) também mostrou que a AMPK é ativada por C3G independentemente do aumento de AMP:ATP e da fosforilação em tirosina de IR ou IRS1 e AKT (FIGURA 9).

O aumento da produção hepática de glicose é de grande relevância no diabetes, e existem relatos da interferência positiva da C3G neste quadro (GUO; XIA et al., 2012). A ingestão de C3G reduz a expressão de TNF-α e outras citocinas pró-inflamatórias no fígado e tecido adiposo em paralelo com a inibição da ativação de JNK. A menor expressão das citocinas reduz também a sinalização destas para ativação de NFκB. A C3G também pode

aumentar a fosforilação de AKT (FIGURA 9). Dessa forma, há um decréscimo na produção hepática de glicose pela diminuição de FoxO1 no núcleo dos adipócitos e hepatócitos (DRAGANO et al., 2013; GUO; XIA et al., 2012). Sob estímulo da C3G, AKT pode ainda ativar a proteína AS160, a qual ativa a translocação da GLUT4 até a membrana para captação da glicose em células dos tecidos adiposo e muscular (LETO; SALTIEL, 2012). Antocianinas de um modo geral podem também doar elétrons, neutralizando radicais livres, o que também pode impedir a ativação de JNK pelo estresse oxidativo (PRIOR, 2003).

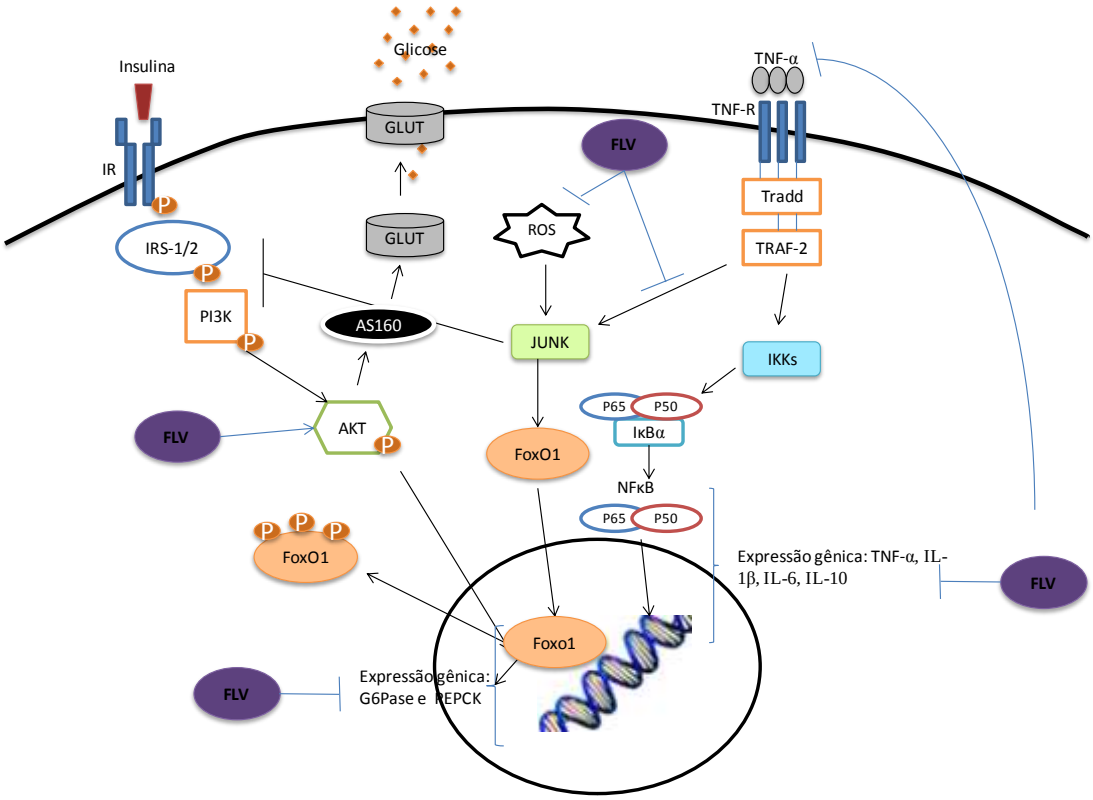



**FIGURA 9**- Via de sinalização da insulina no tecido adiposo e hepático e participação das antocianinas. A insulina se liga ao seu receptor (IR) que autofosforila seus resíduos tirosina. A seguir, ocorre a fosforilação dos substratos do receptor de insulina (IRS1/2) e da PI3K, que por sua vez ativa AKT. A AKT ativa a proteína AS160 que sensibiliza proteínas do transportador de glicose GLUT4, que se transloca até a membrana para captar a glicose extracelular. A AKT pode também se dirigir ao núcleo onde fosforila FoxO1, que assim deixa o núcleo, cessando a sua função na expressão de G6Pase e PEPCK. A AMPK também pode atuar diretamente na sensibilização da AS160 quando ativada. Na obesidade e resistência a insulina, o processo inflamatório propicia que a TNF-α se ligue ao seu receptor específico na célula (TNFR) que se fosforila desencadeando sinais que fosforilam IKK e JNK em serina. Estas fosforilam IRS-1 em serina impedindo a sinalização de AKT para FoxO1, que permanece no núcleo, induzindo a gliconeogênese com a expressão de G6Pase e PEPCK. Além disso, a IKK pode ativar NFκB, outro fator de transcrição que induz à expressão de mais citocinas pro-inflamatórias. Os flavonoides (FLV) podem melhorar o quadro de resistência a insulina auxiliando na ativação da AKT, que aumenta a fosforilação da FoxO1 e impede a transcrição de enzimas gliconeogênicas. FLVs podem também impedir o estresse oxidativo e ativação de JNK corroborando para presença de FoxO1 no núcleo. As FLVs reduzem a expressão de citocinas por NFκB, diminuindo a ativação da via do TNF- α.

### 3.1.1.2. Mecanismos de ação de flavonoides e antocianinas em núcleos centrais de cognição

Trabalhos anteriores sugerem que frutas vermelhas ricas em antocianinas atenuaram a peroxidação lipídica e elevaram o status antioxidante no cérebro de animais obesos, mesmo com diminuição na massa cerebral (ALEZANDRO; GRANATO; GENOVESE, 2013; BATISTA, A. G. et al., 2014). Os flavonoides e seus metabólitos podem atravessar a barreira hematoencefálica e promover efeitos benéficos como melhora da inflamação e cognição (CHEN et al., 2015; KALT et al., 2008; YOUDIM et al., 2003).

Camundongos e ratos tratados com extratos de mirtilo mostraram melhoras no desempenho de testes de memória e aprendizado, como o *passive-avoidance test* (PAPANDREOU et al., 2009) e o *14-unit T-maze*, além de maior contagem de neurônios no hipocampo (DUFFY et al., 2008). Um trabalho sugeriu que a ingestão de 2% de extrato (poli)fenólico de mirtilo na dieta foi capaz de aumentar a neurogênese e plasticidade neural de ratos, ativando kinases responsáveis pela ativação da CREB (CASADESUS et al., 2004) corroborando outros estudos (WILLIAMS, C. M. et al., 2008). Estes mesmos animais mostraram melhor desempenho em um labirinto-desafio para memória e aprendizagem ou *radial arm water maze* (CASADESUS et al., 2004). Outros compostos fenólicos, como o resveratrol (stilbeno), também estão associados com melhoras nos desempenhos nos testes cognitivos *passive-avoidance test* e MWM (GACAR et al., 2011; JEON et al., 2012).

Alguns mecanismos de atuação dos flavonoides podem explicar estes efeitos. Pesquisadores acreditam que os flavanoides podem atravessar a barreira hematoencefálica de forma dependente da lipofilicidade de seus metabolitos (YOUDIM et al., 2003), e assim ter uma ação direta em vias sinalizadoras que ativam receptores de membrana, kinases, fatores de transcrição, e expressão de genes; além de indiretamente, como por exemplo, melhorando a integridade vascular e o metabolismo como um todo (CASADESUS et al., 2004; RENDEIRO, CATARINA; RHODES; SPENCER, 2015; SPENCER, 2009; WILLIAMS, R. J.; SPENCER, 2012). Existem evidências de que a ingestão de flavonoides e suas fontes aumentam a expressão de neurotrofinas (p. ex. BDNF) pela ativação da via ERK-CREB (CASADESUS et al., 2004; RENDEIRO, CATARINA et al., 2012; RENDEIRO, C. et al., 2013; SPENCER, 2009). Os flavonoides possuem também efeito neuroprotetor ativando vias de proteção contra apoptose e de fosforilação da tau via GSK3β, mantendo a função dos axônios (WILLIAMS, R. J.; SPENCER, 2012). Outros autores atribuem estes efeitos ao poder

antioxidante destes compostos (BATISTA, A. G. et al., 2014; DUFFY et al., 2008; PAPANDREOU et al., 2009).

Os flavonoides também podem atuar reduzindo a produção de Aβ por melhorar a atividade de α-secretase, inibir β-secretase e agregação de Aβ via quelação de metais. Eles também estão associados a um aumento do fluxo sanguíneo cerebral, o que permite maior *clearance* de Aβ (WILLIAMS, R. J.; SPENCER, 2012). As vias de ativação da inflamação, da adiponectina e AMPK nos centros nervoso, também são outro ponto de ação dos compostos fenólicos nos sítios de controle da cognição, uma vez que podem sensibilizar a ação da insulina, e aumentar a taxa de fosforilação da GSK3-β e impedir a fosforilação da tau (JEON et al., 2012).

### 3.1.2. Taninos

Taninos são fenólicos solúveis em água que pesam entre 500 a 3000 Daltons, possuem muitas hidroxilas e outros grupos funcionais, podendo se ligar à proteínas e outras macromoléculas. Os taninos se dividem em duas classes: hidrolisáveis (galotaninos e elagitaninos) e não-hidrolisáveis ou condensados (polímeros de flavon-3-ois e flavon-3,4-diois) (HASLAM, 2007).

Os taninos hidrolisáveis são centralizados por moléculas de glicose e grupos hidroxilas e quando são hidrolisados formam glicose e ácido gálico (galotaninos) ou glicose, ácido elágico e ácido gálico (elagitaninos). Os taninos condensados são polímeros de catequinas e seus isômeros ligados por ligações carbônicas. Devido à sua reatividade, facilmente formam complexos com proteínas, metais e polissacarídeos (HASLAM, 2007). Os dímeros de catequinas são conhecidos como procianidinas e são de grande ocorrência em cascas e sementes de frutas vermelhas (DÉCORDÉ et al., 2009).

#### 3.1.2.1 Elagitaninos

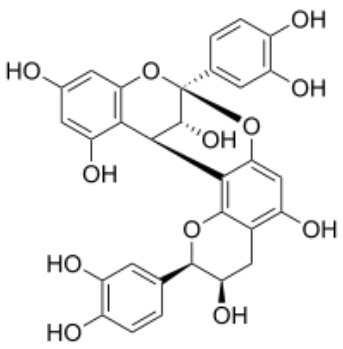
Os elagitaninos são formados de unidades de hexahidroxidifenoila (HHDP) unidos por ligações éster (YANG et al., 2012). Estes compostos não são absorvidos pelo intestino em sua forma natural, no entanto as diferenças de pH do trato gastrointestinal e a microbiota intestinal podem hidrolisá-los em ácido elágico e em sequência urolitinas, que são compostos mais lipofílicos e possuem maior absorção pelo intestino (LANDETE, 2011).

Os efeitos benéficos dos elagitaninos são citados resumidamente como anti-inflamatórios e prébióticos, inibindo crescimento de bactérias patogênicas e estimulando

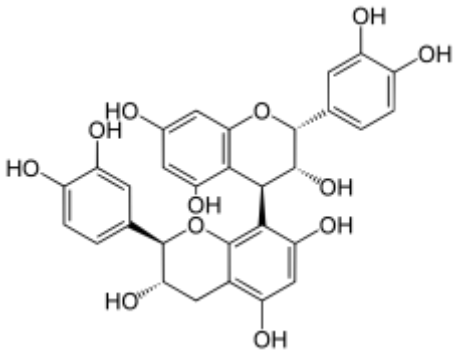
crescimento de bactérias benéficas ao intestino do hospedeiro (LANDETE, 2011). Assim também contribuem para efeitos benéficos na prevenção de doenças metabólicas. A casca de romã, por exemplo, é tida como uma referência em fonte de elagitaninos e está associada a funções anti-obesogênicas e anti-inflamatórias (AL-MUAMMAR; KHAN, 2012; FARIA; CALHAU, 2011). Adicionalmente, quando testadas em células adiposas e hepatócitos, as urolitinas A, C e D diminuíram acúmulo de triglicerídeos e aumentaram a oxidação de ácidos graxos (KANG et al., 2016).

### 3.1.3. Flavanóis e Procianidinas

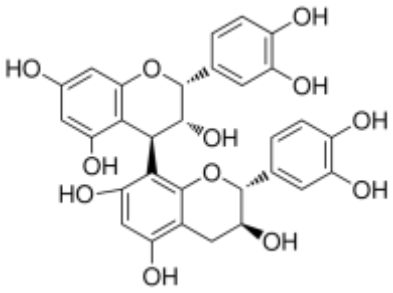
Os monômeros das procianidinas são: (+)-catequina, (-)-epicatequina e (-)-epicatequina 3-galato (quando esterificadas com ácido gálico). Estes flavanóis formam os dímeros chamados procianidinas (HASLAM, 2007), das quais se destacam neste trabalho (FIGURA 10): Procianidina A2; Procianidina B1, Procianidina B2: dímero epicatequina - (4β→8)-epicatequina.



**Procianidina A2: epicatequina-(2β→7, 4β→8)-epicatequina**



**Procianidina B1: epicatequina-(4β→8)-catequina**



**Procianidina B2: epicatequina -(4β→8)- epicatequina**

**FIGURA 10** - Estrutura química de procianidinas de interesse.

Os monômeros e dímeros dos flavanóis podem ser absorvidos rapidamente pelo intestino, mas seus polímeros passam intactos pelo trato gastrointestinal e são transformados

pela microbiota, podendo ser hidrolisados em monômeros e dímeros e/ou outros metabolitos e são então absorvidos no cólon (APPELDOORN; VINCKEN; AURA et al., 2009; MONAGAS et al., 2010). No entanto, mesmo que os dímeros sejam absorvidos em uma menor taxa que os monômeros, um estudo comprovou que o dímero do tipo A2, por exemplo, não é conjugado ou metilado por enzimas de fase 2, conservando assim atividade antioxidante (APPELDOORN; VINCKEN; GRUPPEN et al., 2009). Um estudo com animais provou que alimentos fontes de procianidinas possuem importante efeito anti-inflamatório no intestino, contribuindo para prevenção de doenças relacionadas (LARROSA et al., 2009).

### 3.1.4 Mecanismos de ação na microbiota intestinal

Os compostos fenólicos são absorvidos no jejuno e íleo, no entanto uma grande parte chega ao colón, onde podem sofrer metabolização pelas enzimas liberadas pela microbiota intestinal. Os metabolitos podem então ser absorvidos e atuar como anti-inflamatórios locais, ou ainda no lúmen podem atuar como antimicrobianos contra bactérias patogênicas gram-negativas (ex. *Enterobacteriaea*) (SHIMIZU, 2017).

Estudos em roedores obesos comprovaram que o uso de *berries*, como a lingonberry, diminuiu a permeabilidade intestinal e a razão *Firmicutes/Bacteriodetes*, aumentando, principalmente, a população de bactéria do genêro *Akkermansia* (MATZIOURIDOU et al., 2016). As bactérias do genêro *Akkermansia* estão associadas à mucina e se relacionam com uma mucosa saudável, sendo de grande importância para reversão da endotoxemia induzida pelo consumo de dietas hiperlipídicas, inflamação no tecido adiposo e resistência à insulina (ANHÊ et al., 2015; MATZIOURIDOU et al., 2016). A suplementação de dieta hiperlipídica com *cranberries* também apresentou resposta similar: espécies de *Akkermansia* aumentaram significativamente com a "*berry*-terapia" em animais alimentados com dieta hiperlipídica e hiperglicídica (ANHÊ et al., 2015). Os autores também demonstraram menores índices de LPS no plasma de animais alimentados com a *berry* (ANHÊ et al., 2015). Assim, as bactérias do gênero *Akkermansia* assim como a permeabilidade intestinal e endotoxemia podem ser biomarcadores importantes para o impacto positivo da ingestão de frutas vermelhas na intervenção nutricional.

Apesar dos benefícios citados acima, existe o relato de que ingestão de frutas vermelhas poderia diminuir a produção de AGCC devido à presença de compostos fenólicos no lúmen intestinal (MATZIOURIDOU et al., 2016). Ao contrário do poder prebiótico das fibras, os polifenóis podem inibir e selecionar o crescimento de algumas bactérias produtoras

de AGCC de uma forma geral ou específica (LEE, H. C. et al., 2006). Dessa forma, o balanço entre fibras e compostos fenólicos deve ser aquele que não impeça a produção destes metabólitos de grande importância para saúde intestinal.

## 4. JAMBO-VERMELHO

O jambo-vermelho (*Syzygium malaccense*, família: *Myrtaceae*), tem origem na Malásia, e por isso é também conhecido internacionalmente como *Malay apple*. É comum em regiões tropicais, e no Brasil é encontrado nos estados da região Norte, Nordeste e nas regiões quentes do Sudeste (FALCÃO; PARALUPPI; CLEMENT, 2002).

A árvore alcança de 12 a 15 m de altura, possui tronco reto e copa piramidal. Os frutos (cerca de 80 g) são piriformes, carnosos, contendo uma única semente (cerca de 14 g) e casca (cerca 8%) de cor variando do rosa, vermelho, vermelho-escuro a vermelho bem escuro, dependendo do grau de maturação. A polpa (67 ou 80%) é esbranquiçada e suculenta de sabor ácido, semelhante à maçã (FIGURA 11). O aroma do jambo-vermelho é semelhante a uma mistura de rosas e herbáceas (AUGUSTA et al., 2010).

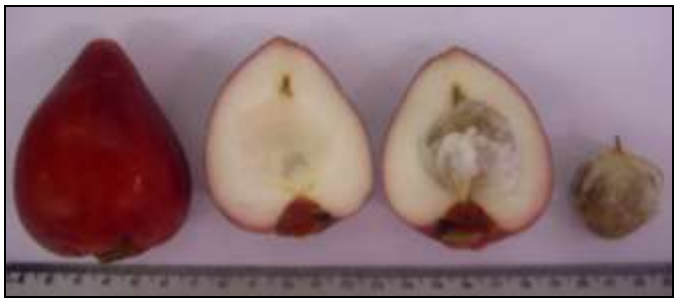


**FIGURA 11 -** Frutos de jambo-vermelho. Da esquerda para direita: fruta inteira, corte longitudinal da fruta (parte sem e com semente), semente. Foto: AG Batista.

O jambeiro é cultivado como planta ornamental devido a sua beleza, frutifica em dois períodos do ano (março, julho-agosto) e os frutos são consumidos na maioria dos casos *in natura* ou na forma de produtos artesanais, como compotas, sucos e geleias. Grande parte dos frutos é desperdiçada na época da safra, em virtude da alta produção por árvore, da perecibilidade e da falta de informação da viabilidade tecnológica para a sua utilização pela indústria (CARDOSO, 2008).

O fruto possui características químicas que incitam pesquisas que explorem seus efeitos funcionais (LAKO et al., 2007). Alguns compostos bioativos, como fibras e fenólicos já foram encontrados na fruta (LAKO et al., 2007; MAISUTHISAKUL; PASUK;

RITTHIRUANGDEJ, 2008; NUNES et al., 2016; REYNERTSON et al., 2008). Entretanto, não há relatos de experimentos *in vivo* que possam comprovar a biodisponibilidade e bioatividade de tais compostos.

## 5. JABUTICABA

A planta de jabuticaba pertence à família *Myrtaceae* e está amplamente distribuída na região sul e sudeste do Brasil. A principal espécie é a *Plinia* ou *Myrciaria jaboticaba*, popularmente conhecida como jabuticaba sabará, encontrada no sudeste; mas existem outras espécies, como a *Plinia* ou *Myrciaria cauliflora*.

Esta planta (*Myrciaria* spp.) produz frutos globosos com uma casca de cor púrpura escura. Os frutos nascem diretamente nos troncos e ramos principais das árvores, ou seja, uma planta cauliflora. O diâmetro dos frutos inteiros é de cerca de 3 a 4 cm, com 1 a 4 sementes grandes (4% de fruta inteira) e uma casca grossa púrpura (20%) que cobre uma polpa branca doce e gelatinosa (76%) (CLERICI; CARVALHO-SILVA, 2011; DESSIMONI-PINTO et al., 2011; REYNERTSON et al., 2008) (FIGURA 12).

**FIGURA 12 -** Fruta e casca de jabuticaba sabará. Foto: JK da Silva.

A fruta é muito popular no Brasil devido ao seu gosto sub-ácido e sabor doce. O principal período de produção dos frutos no Brasil é de setembro a outubro. Como resultado de sua alta perecibilidade, seu período de comercialização pós-colheita é bastante curto, atingindo apenas dois a três dias, quando podem ser observadas mudanças na aparência do fruto, perda de água intensa e fermentação da polpa.

O fruto é comumente consumido *in natura*, mas apesar de sua alta sazonalidade, pode ser usado em sucos, geléias, doces, licor, vinagre, vinhos e molhos. De um modo geral, a casca dos frutos não é extensivamente consumida, mas recentemente algumas novas formulações parecem incluir este subproduto da fruta (ASQUIERI; SILVA; CÂNDIDO,

2009; DESSIMONI-PINTO et al., 2011). As cascas de jabuticaba também podem ser secas para prolongar sua vida útil e proporcionar uma alternativa ao consumo da parte mais nutritiva do fruto.

As cascas de jabuticaba concentram a maior quantidade de polifenóis como taninos, ácido elágico, quercetina, antocianinas e tem uma atividade antioxidante expressiva (ABE; LAJOLO; GENOVESE, 2012; LEITE-LEGATTI et al., 2012; LIMA et al., 2011). A casca parece ter a mais quantidade de antocianinas e compostos polifenólicos na fruta, no entanto, muitas vezes é descartada. A casca de jabuticaba sabará (*M. jaboticaba*) contém cerca de 205 e 66 vezes maior quantidade de antocianina monomérica do que a polpa e sementes, respectivamente (LIMA et al., 2011).

Um estudo com *M. cauliflora* mostrou o isolamento de um novo composto fenólico a partir dos extratos de frutos: um depsídeo chamado jaboticabin (methyl 2-[(3,4-dihydroxybenzoyloxy)-4,6-dihydroxyphenyl] acetate). Depsídeos são um tipo de compostos polifenólicos formado por duas ou mais unidades aromáticas monocíclicas ligadas por uma ligação éster. Outros fenólicos foram também identificados: 2-*O*-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid, piranocinina B, quercetina, isoquercitrina, quercimeritrina, quercitrina, rutina, mirricitrina, ácido cinâmico, ácido *O*-cumárico, ácido gálico ácido protocatecuico, protocatecuato de metila e ácido elágico. Estes compostos estão relacionados com atividade antirradical, anti-inflamatória e citotóxica (REYNERTSON et al., 2006)

Os polifenóis da espécie sabará ou *M. jaboticaba* não foram amplamente explorados. Contudo, alguns compostos bioativos já foram descritos: antocianinas (cianidina e delfinidina 3-glucosídeo), elagitaninos (casuarinina, casuarictina, pedunculagina, casuariina, telimagrandina I e II), galotanino (pentagaloil hexose), ácido elágico e ácido elágico pentosídeo, quercitrina, limoneno, terpenos, fibras dietéticas e outros (DA SILVA et al., 2017; PLAGEMANN, 2012; PLAZA et al., 2016).

Elagitaninos e galotaninos representam 18% dos polifenóis da casca de *M. jaboticaba*, e contribuem para 43% da capacidade antioxidante (PLAZA et al., 2016). Enquanto que antocianinas são os compostos fenólicos majoritários (71% dos polifenóis) e contribuem com apenas 38% da capacidade antioxidante total da casca de *M. jaboticaba.* O ácido elágico e derivados representam 5% da casca de *M. jaboticaba* e contribuem com 6% da capacidade antioxidante total (PLAZA et al., 2016). Dessa forma, embora muita atenção seja dada às antocianinas da casca de jabuticaba até o momento (DRAGANO et al., 2013; LEITE-LEGATTI et al., 2012; LEITE et al., 2011), os elagitaninos e o ácido elágico livre também

merecem o devido destaque em estudos com a casca de jabuticaba (ABE et al., 2012; ALEZANDRO et al., 2013; BATISTA, A. G. et al., 2014; DA SILVA et al., 2017).

A tabela 1 ilustra os principais efeitos *in vivo* da casca de jabuticaba.

**Tabela 1 –** Síntese de pesquisas relevantes sobre o efeito da casca de jabuticaba *in vivo*.

| Modelo | Tratamento | S | Principais resultados |
|---|---|---|---|
| Ratos *Wistar* adultos saudáveis (1) | Dietas normolipídicas suplementadas com 2 e 4% de casca de jabuticaba (*M. jaboticaba*). | 4 | ↑ capacidade antioxidante no plasma. |
| Camundongos *Swiss.* Dieta hiperlipídica (60% calorias do VET) (2) | Dietas hiperlipídicas suplementadas com 2 e 4% de casca de jabuticaba (*M. jaboticaba*) depois da obesidade induzida. | 6 | ↓ resistência à insulina periférica, no fígado e tecido adiposo; ↓ IL-1β no tecido adiposo e fígado. |
| Ratos *Sprague-Dawley*. Dieta hiperlipídica (60% calorias do VET) (3-5) | Dietas hiperlipídicas suplementadas com 2 e 4% de casca de jabuticaba (*M. jaboticaba*) depois da obesidade induzida. | 6 | ↑ colesterol HDL; ↓ indicador de resistência a insulina HOMA-IR.<br>↑ excreção de lipídeos; ↓ índice de peróxido no fígado.<br>↓ácidos graxos saturados metilados; ↓ estresse oxidativo no fígado e cérebro; ↑ capacidade antioxidante e atividade de enzimas antioxidante no plasma e fígado. |
| Ratos *Wistar*. Diabetes induzida por streptozotocina (6) | Suplementação com 1 e 2 g $kg^{-1}$ peso corporal de casca de jabuticaba (*M. cauliflora*) diluída em água administrado por gavage. | 6 | ↓ colesterol plasmático; ↑ FRAP e atividade de Gpx no plasma; ↑atividade de enzimas do sistema antioxidante no rim, cérebro e fígado; ↓ estresse oxidativo no plasma e cérebro. |
| Ratos *Wistar*. Dieta hiperlipídica (24% calorias do VET) (7) | Dietas hiperlipídicas suplementadas com 7 e 10 e 15% de casca de jabuticaba jabuticaba (*M. cauliflora*) depois da obesidade induzida. | 4 | ↓ colesterol total sérico; ↓ colesterol total hepático; ↓ triglicerídeos totais séricos; ↓ glicose sérica |

S= semanas; 1 - (LEITE et al., 2011); 2 - (DRAGANO et al., 2013); 3 -(LENQUISTE et al., 2012); 4 - (BATISTA, A. G. et al., 2013); 5 - (BATISTA, A. G. et al., 2014); 6 - (ALEZANDRO et al., 2013); 7- (ARAÚJO et al., 2013).

## 6. REFERÊNCIAS

ABE, L. T.; LAJOLO, F. M.; GENOVESE, M. I. Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (Myrciaria jaboticaba (Vell.) Berg). **Journal of the Science of Food and Agriculture,** v. 92, n. 8, p. 1679-1687, Jun 2012.

AL-MUAMMAR, M. N.; KHAN, F. Obesity: The preventive role of the pomegranate (Punica granatum). **Nutrition,** v. 28, n. 6, p. 595-604, 2012.

ALEZANDRO, M. R.; GRANATO, D.; GENOVESE, M. I. Jaboticaba (Myrciaria jaboticaba (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. **Food Research International,** v. 54, n. 1, p. 650-659, 2013.

ALONSO, M.; VIANNA, M. R. M.; IZQUIERDO, I.; MEDINA, J. H. Signaling Mechanisms Mediating BDNF Modulation of Memory Formation In Vivo in the Hippocampus. **Cellular and Molecular Neurobiology,** v. 22, n. 5, p. 663-674, 2002.

ANHÊ, F. F.; ROY, D.; PILON, G.; DUDONNÉ, S.; MATAMOROS, S.; VARIN, T. V. *et al.* A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. **Gut,** v. 64, n. 6, p. 872-883, June 1, 2015 2015.

APPELDOORN, M. M.; VINCKEN, J.-P.; AURA, A.-M.; HOLLMAN, P. C. H.; GRUPPEN, H. Procyanidin Dimers Are Metabolized by Human Microbiota with 2-(3,4-Dihydroxyphenyl)acetic Acid and 5-(3,4-Dihydroxyphenyl)-γ-valerolactone as the Major Metabolites. **Journal of Agricultural and Food Chemistry,** v. 57, n. 3, p. 1084-1092, 2009/02/11 2009.

APPELDOORN, M. M.; VINCKEN, J.-P.; GRUPPEN, H.; HOLLMAN, P. C. H. Procyanidin Dimers A1, A2, and B2 Are Absorbed without Conjugation or Methylation from the Small Intestine of Rats. **The Journal of Nutrition,** v. 139, n. 8, p. 1469-1473, August 1, 2009 2009.

ARAÚJO, C. R.; ESTEVES, E. A.; DESSIMONI-PINTO, N. A.; BATISTA, Â. G. Myrciaria cauliflora peel flour had a hypolipidemic effect in rats fed a moderately high-fat diet. **Journal of Medicinal Food,** v. 17, n. 2, p. 262-267, 2013.

ASQUIERI, E. R.; SILVA, A. G. D. M. E.; CÂNDIDO, M. A. Aguardente de jabuticaba obtida da casca e borra da fabricação de fermentado de jabuticaba. **Ciência e Tecnologia de Alimentos,** v. 29, p. 896-904, 2009.

AUGUSTA, I. M.; RESENDE, J. M.; BORGES, S. V.; MAIA, M. C., A.; ; COUTO, M. A. P. G. Caracterização física e química da casca e polpa de jambo vermelho (Syzygium

malaccensis, (L.) Merryl & Perry). **Ciencia e Tecnologia de Alimentos,** v. 30, n. 4, p. 928-936, 2010.

BÄCKHED, F.; DING, H.; WANG, T.; HOOPER, L. V.; KOH, G. Y.; NAGY, A. *et al.* The gut microbiota as an environmental factor that regulates fat storage. **Proceedings of the National Academy of Sciences of the United States of America,** v. 101, n. 44, p. 15718-15723, November 2, 2004 2004.

BÄCKHED, F.; MANCHESTER, J. K.; SEMENKOVICH, C. F.; GORDON, J. I. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. **Proceedings of the National Academy of Sciences,** v. 104, n. 3, p. 979-984, January 16, 2007 2007.

BATISTA, A. G.; LENQUISTE, S. A.; CAZARIN, C. B. B.; DA SILVA, J. K.; LUIZ-FERREIRA, A.; BOGUSZ, S. *et al.* Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. **Journal of Functional Foods,** v. 6, p. 450-461, Jan 2014.

BATISTA, A. G.; LENQUISTE, S. A.; MOLDENHAUER, C.; GODOY, J. T.; REIS, S. M. P. M.; MARÓSTICA JÚNIOR, M. R. Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats. **Brazilian Journal of Nutrition,** v. 26, n. 5, p. 571-581, 2013.

BRANDEIS, R.; BRANDYS, Y.; YEHUDA, S. The Use of the Morris Water Maze in the Study of Memory and Learning. **International Journal of Neuroscience,** v. 48, n. 1-2, p. 29-69, 1989/01/01 1989.

CANI, P. D.; BIBILONI, R.; KNAUF, C.; NEYRINCK, A. M.; NEYRINCK, A. M.; DELZENNE, N. M. *et al.* Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. **Diabetes,** v. 57, n. 6, p. 1470-1481, Jun 2008.

CARDOSO, R. L. **Estabilidade da cor de geléia de jambo (Eugenia malaccensis, l.) sem casca armazenada aos 25 ºC e 35 ºC na presença e ausência de luz Ciência e Agrotecnologia,** v. 32, n. 5, p. 1563-1567, 2008.

CASADESUS, G.; SHUKITT-HALE, B.; STELLWAGEN, H. M.; ZHU, X.; LEE, H.-G.; SMITH, M. A. *et al.* Modulation of Hippocampal Plasticity and Cognitive Behavior by Short-term Blueberry Supplementation in Aged Rats. **Nutritional Neuroscience,** v. 7, n. 5-6, p. 309-316, 2004/10/01 2004.

CASTELLANI, R. J.; ROLSTON, R. K.; SMITH, M. A. Alzheimer Disease. **Disease-a-month : DM,** v. 56, n. 9, p. 484-546, 2010.

CHEN, T.-Y.; KRITCHEVSKY, J.; HARGETT, K.; FELLER, K.; KLOBUSNIK, R.; SONG, B. J. *et al.* Plasma bioavailability and regional brain distribution of polyphenols from apple/grape seed and bilberry extracts in a young swine model. **Molecular Nutrition & Food Research,** v. 59, n. 12, p. 2432-2447, 2015.

CLERICI, M. T. P. S.; CARVALHO-SILVA, L. B. Nutritional bioactive compounds and technological aspects of minor fruits grown in Brazil. **Food Research International,** v. 44, n. 7, p. 1658-1670, 2011.

CORREIA, S. C.; SANTOS, R. X.; CARVALHO, C.; CARDOSO, S.; CANDEIAS, E.; SANTOS, M. S. *et al.* Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. **Brain Research,** v. 1441, p. 64-78, Mar 2 2012.

CROSS, D. A. E.; ALESSI, D. R.; COHEN, P.; ANDJELKOVICH, M.; HEMMINGS, B. A. Inhibition of Glycogen-Synthase Kinase-3 by Insulin-Mediated by Protein-Kinase-B. **Nature,** v. 378, n. 6559, p. 785-789, Dec 21 1995.

DA SILVA, J. K.; BATISTA, Â. G.; CAZARIN, C. B. B.; DIONÍSIO, A. P.; DE BRITO, E. S.; MARQUES, A. T. B. *et al.* Functional tea from a Brazilian berry: Overview of the bioactives compounds. **LWT - Food Science and Technology,** v. 76, Part B, p. 292-298, 2017.

DE LA MONTE, S. M.; WANDS, J. R. Alzheimer's disease is type 3 diabetes-evidence reviewed. **J Diabetes Sci Technol,** v. 2, n. 6, p. 1101-13, Nov 2008.

DÉCORDÉ, K.; TEISSÈDRE, P. L.; SUTRA, T.; VENTURA, E.; CRISTOL, J. P.; ROUANET, J. M. Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers. **Molecular Nutrition & Food Research,** v. 53, n. 5, p. 659-666, 2009.

DENEV, P. N.; KRATCHANOV, C. G.; CIZ, M.; LOJEK, A.; KRATCHANOVA, M. G. Bioavailability and Antioxidant Activity of Black Chokeberry (Aronia melanocarpa) Polyphenols: in vitro and in vivo Evidences and Possible Mechanisms of Action: A Review. **Comprehensive Reviews in Food Science and Food Safety,** v. 11, n. 5, p. 471-489, Aug 2012.

DESSIMONI-PINTO, N. A. V.; MOREIRA, W. A.; CARDOSO, L. D. M.; PANTOJA, L. A. Jaboticaba peel for jelly preparation: an alternative technology. **Ciência e Tecnologia de Alimentos,** v. 31, p. 864-869, 2011.

DRAGANO, N. R. V.; MARQUES, A. C.; CINTRA, D. E. C.; SOLON, C.; MORARI, J.; LEITE-LEGATTI, A. V. *et al.* Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. **British Journal of Nutrition,** v. 110, n. 3, p. 447-55, 2013.

DUDAI, Y. The Restless Engram: Consolidations Never End. **Annual Review of Neuroscience, Vol 35,** v. 35, p. 227-247, 2012.

DUFFY, K. B.; SPANGLER, E. L.; DEVAN, B. D.; GUO, Z. H.; BOWKER, J. L.; JANAS, A. M. *et al.* A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. **Neurobiology of Aging,** v. 29, n. 11, p. 1680-1689, Nov 2008.

FALCÃO, M. A.; PARALUPPI, N. D.; CLEMENT, C. R. Fenologia e produtividade do jambo (*Syzigium malaccensis*) na Amazônia central. **Acta Amazônica,** v. 32, n. 1, p. 3-8, 2002.

FARIA, A.; CALHAU, C. The Bioactivity of Pomegranate: Impact on Health and Disease. **Critical Reviews in Food Science and Nutrition,** v. 51, n. 7, p. 626-634, 2011/08/01 2011.

FRANKLAND, P. W.; BONTEMPI, B. The organization of recent and remote memories. **Nat Rev Neurosci,** v. 6, n. 2, p. 119-130, 2005.

GACAR, N.; MUTLU, O.; UTKAN, T.; CELIKYURT, I. K.; GOCMEZ, S. S.; ULAK, G. Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and Morris water maze tests in rats. **Pharmacology Biochemistry and Behavior,** v. 99, n. 3, p. 316-323, Sep 2011.

GARDNER, D. S.; RHODES, P. Developmental Origins of Obesity: Programming of Food Intake or Physical Activity? **Early Nutrition Programming and Health Outcomes in Later Life: Obesity and Beyond,** v. 646, p. 83-93, 2009.

GHOSHAL, S.; WITTA, J.; ZHONG, J.; DE VILLIERS, W.; ECKHARDT, E. Chylomicrons promote intestinal absorption of lipopolysaccharides. **Journal of Lipid Research,** v. 50, n. 1, p. 90-97, January 1, 2009 2009.

GUO, H. H.; GUO, J. B.; JIANG, X. W.; LI, Z.; LING, W. H. Cyanidin-3-O-beta-glucoside, a typical anthocyanin, exhibits antilipolytic effects in 3T3-L1 adipocytes during hyperglycemia: Involvement of FoxO1-mediated transcription of adipose triglyceride lipase. **Food and Chemical Toxicology,** v. 50, n. 9, p. 3040-3047, Sep 2012.

GUO, H. H.; LIU, G. L.; ZHONG, R. M.; WANG, Y.; WANG, D. A.; XIA, M. Cyanidin-3-O-beta-glucoside regulates fatty acid metabolism via an AMP-activated protein kinase-dependent signaling pathway in human HepG2 cells. **Lipids in Health and Disease,** v. 11, Jan 13 2012.

GUO, H. H.; XIA, M.; ZOU, T.; LING, W.; LIU, G. L.; ZHONG, R. M. *et al.* Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. **Journal of Nutritional Biochemistry,** v. 23, p. 349–360, 2012.

HASLAM, E. Vegetable tannins – Lessons of a phytochemical lifetime. **Phytochemistry,** v. 68, n. 22–24, p. 2713-2721, 2007.

HIMMLER, A.; DRECHSEL, D.; KIRSCHNER, M. W.; MARTIN, D. W. Tau Consists of a Set of Proteins with Repeated C-Terminal Microtubule-Binding Domains and Variable N-Terminal Domains. **Molecular and Cellular Biology,** v. 9, n. 4, p. 1381-1388, Apr 1989.

HOTAMISLIGIL, G. S. Inflammation and metabolic disorders. **Nature,** v. 444, n. 7121, p. 860-867, Dec 14 2006.

HOTAMISLIGIL, G. S.; SHARGILL, N. S.; SPIEGELMAN, B. M. Adipose Expression of Tumor Necrosis Factor- : Direct Role in Obesity-Linked Insulin Resistance. **SCIENCE-NEW YORK THEN WASHINGTON-,** v. 259, p. 87-87, 1993.

JACOB, R. A. The Integrated Antioxidant System. **Nutrition Research,** v. 15, n. 5, p. 755-766, May 1995.

JEON, B. T.; JEONG, E. A.; SHIN, H. J.; LEE, Y.; LEE, D. H.; KIM, H. J. *et al.* Resveratrol Attenuates Obesity-Associated Peripheral and Central Inflammation and Improves Memory Deficit in Mice Fed a High-Fat Diet. **Diabetes,** v. 61, n. 6, p. 1444-1454, Jun 2012.

JOHNSON, A. M. F.; OLEFSKY, J. M. The Origins and Drivers of Insulin Resistance. **Cell,** v. 152, n. 4, p. 673-684, Feb 14 2013.

JOLIVALT, C. G.; LEE, C. A.; BEISWENGER, K. K.; SMITH, J. L.; ORLOV, M.; TORRANCE, M. A. *et al.* Defective Insulin Signaling Pathway and Increased Glycogen Synthase Kinase-3 Activity in the Brain of Diabetic Mice: Parallels With Alzheimer's Disease and Correction by Insulin. **Journal of Neuroscience Research,** v. 86, n. 15, p. 3265-3274, Nov 15 2008.

KAHN, S. E.; HULL, R. L.; UTZSCHNEIDER, K. M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. **Nature,** v. 444, n. 7121, p. 840-846, Dec 14 2006.

KALT, W.; FOOTE, K.; FILLMORE, S. A. E.; LYON, M.; VAN LUNEN, T. A.; MCRAE, K. B. Effect of blueberry feeding on plasma lipids in pigs. **British Journal of Nutrition,** v. 100, n. 1, p. 70-78, Jul 2008.

KANDEL, E. R. The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses. **Science,** v. 294, n. 5544, p. 1030-1038, 2001.

KANG, I.; KIM, Y.; TOMÁS-BARBERÁN, F. A.; ESPÍN, J. C.; CHUNG, S. Urolithin A, C, and D, but not iso-urolithin A and urolithin B, attenuate triglyceride accumulation in human cultures of adipocytes and hepatocytes. **Molecular Nutrition & Food Research,** v. 60, n. 5, p. 1129-1138, 2016.

KAWANO, Y.; COHEN, D. E. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. **Journal of Gastroenterology,** v. 48, n. 4, p. 434-441, 2013.

KERSTEN, S.; DESVERGNE, B.; WAHLI, W. Roles of PPARs in health and disease. **Nature,** v. 405, n. 6785, p. 421-424, 2000.

KIM, H.; LEE, K.; KIM, D. J.; KIM, S. K.; AHN, C. W.; LIM, S.-K. *et al.* Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. **Archives of Internal Medicine,** v. 164, n. 19, p. 2169-2175, 2004.

KIMURA, I.; OZAWA, K.; INOUE, D.; IMAMURA, T.; KIMURA, K.; MAEDA, T. *et al.* The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. **Nature Communications,** v. 4, p. 1829, 2013.

KING, A. M. Y.; YOUNG, G. Characteristics and Occurrence of Phenolic Phytochemicals. **Journal of the American Dietetic Association,** v. 99, n. 2, p. 213-218, 1999.

KONG, J.-M.; CHIA, L.-S.; GOH, N.-K.; CHIA, T.-F.; BROUILLARD, R. Analysis and biological activities of anthocyanins. **Phytochemistry,** v. 64, n. 5, p. 923-933, 2003.

LAKO, J.; TRENERRY, V. C.; WAHLQVIST, M.; WATTANAPENPAIBOON, N.; SOTHEESWARAN, S.; PREMIER, R. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. **Food Chemistry,** v. 101, n. 4, p. 1727-1741, 2007.

LANDETE, J. M. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. **Food Research International,** v. 44, n. 5, p. 1150-1160, 2011.

LARROSA, M.; LUCERI, C.; VIVOLI, E.; PAGLIUCA, C.; LODOVICI, M.; MONETI, G. *et al.* Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. **Molecular Nutrition & Food Research,** v. 53, n. 8, p. 1044-1054, 2009.

LE CHATELIER, E.; NIELSEN, T.; QIN, J.; PRIFTI, E.; HILDEBRAND, F.; FALONY, G. *et al.* Richness of human gut microbiome correlates with metabolic markers. **Nature,** v. 500, n. 7464, p. 541-546, 2013.

LEE, E. B. Obesity, leptin, and Alzheimer's disease. **Year in Diabetes and Obesity,** v. 1243, p. 15-29, 2011.

LEE, H. C.; JENNER, A. M.; LOW, C. S.; LEE, Y. K. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. **Research in Microbiology,** v. 157, n. 9, p. 876-884, 2006.

LEE, J. S.; KIM, M. S. The role of GSK3 in glucose homeostasis and the development of insulin resistance. **Diabetes Research and Clinical Practice,** v. 77, p. S49-S57, Sep 2007.

LEITE-LEGATTI, A. V.; BATISTA, A. G.; DRAGANO, N. R. V.; MARQUES, A. C.; MALTA, L. G.; RICCIO, M. F. *et al.* Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. **Food Research International,** v. 49, p. 596-603, 2012.

LEITE, A. V.; MALTA, L. G.; RICCIO, M. F.; EBERLIN, M. N.; PASTORE, G. M.; MAROSTICA, 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, n. 6, p. 2277-2283, Mar 23 2011.

LENQUISTE, S. A.; BATISTA, A. G.; MARINELI, R. D.; DRAGANO, N. R. V.; MAROSTICA, M. R. Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. **Food Research International,** v. 49, n. 1, p. 153-160, Nov 2012.

LETO, D.; SALTIEL, A. R. Regulation of glucose transport by insulin: traffic control of GLUT4. **Nature Reviews Molecular Cell Biology,** v. 13, n. 6, p. 383-396, Jun 2012.

LEY, R. E.; TURNBAUGH, P. J.; KLEIN, S.; GORDON, J. I. Microbial ecology: Human gut microbes associated with obesity. **Nature,** v. 444, n. 7122, p. 1022-1023, 2006.

LIMA, A. D. B.; CORREA, A. D.; SACZK, A. A.; MARTINS, M. P.; CASTILHO, R. O. Anthocyanins, Pigment Stability and Antioxidant Activity in Jabuticaba [Myrciaria Cauliflora (Mart.) O. Berg]. **Revista Brasileira De Fruticultura,** v. 33, n. 3, p. 877-887, Sep 2011.

LOMONACO, R.; ORTIZ-LOPEZ, C.; ORSAK, B.; WEBB, A.; HARDIES, J.; DARLAND, C. *et al.* Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. **Hepatology,** v. 55, n. 5, p. 1389-1397, 2012.

MA, Q.-L.; YANG, F.; ROSARIO, E. R.; UBEDA, O. J.; BEECH, W.; GANT, D. J. *et al.* β-Amyloid Oligomers Induce Phosphorylation of Tau and Inactivation of Insulin Receptor Substrate via c-Jun N-Terminal Kinase Signaling: Suppression by Omega-3 Fatty Acids and Curcumin. **The Journal of Neuroscience,** v. 29, n. 28, p. 9078-9089, 2009.

MAISUTHISAKUL, P.; PASUK, S.; RITTHIRUANGDEJ, P. Relationship between antioxidant properties and chemical composition of some Thai plants. **Journal of Food Composition and Analysis,** v. 21, n. 3, p. 229-240, May 2008.

MANACH, C.; SCALBERT, A.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Polyphenols: food sources and bioavailability. **The American Journal of Clinical Nutrition,** v. 79, n. 5, p. 727-747, May 1, 2004 2004.

MARTIN, K. C.; BARAD, M.; KANDEL, E. R. Local protein synthesis and its role in synapse-specific plasticity. **Current Opinion in Neurobiology,** v. 10, n. 5, p. 587-592, 2000.

MASLOWSKI, K. M.; VIEIRA, A. T.; NG, A.; KRANICH, J.; SIERRO, F.; DI, Y. *et al.* Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. **Nature,** v. 461, n. 7268, p. 1282-1286, 2009.

MATTSON, M. P. Degenerative and protective signaling mechanisms in the neurofibrillary pathology of AD. **Neurobiology of Aging,** v. 16, n. 3, p. 447-457, 1995.

MATZIOURIDOU, C.; MARUNGRUANG, N.; NGUYEN, T. D.; NYMAN, M.; FÅK, F. Lingonberries reduce atherosclerosis in Apoe-/- mice in association with altered gut microbiota composition and improved lipid profile. **Molecular Nutrition & Food Research,** v. 60, n. 5, p. 1150-1160, 2016.

MESSIER, C.; TEUTENBERG, K. The role of insulin, insulin growth factor, and insulin-degrating enzyme in brain and Alzheimer's disease. **Neural Plasticity,** v. 12, n. 4, p. 311-328, 2005.

MILANSKI, M.; ARRUDA, A. P.; COOPE, A.; IGNACIO-SOUZA, L. M.; NUNEZ, C. E.; ROMAN, E. A. *et al.* Inhibition of Hypothalamic Inflammation Reverses Diet-Induced Insulin Resistance in the Liver. **Diabetes,** v. 61, n. 6, p. 1455-1462, Jun 2012.

MOKHTAR, S. H.; BAKHURAYSAH, M. M.; CRAM, D. S.; PETRATOS, S. The Beta-Amyloid Protein of Alzheimer’s Disease: Communication Breakdown by Modifying the Neuronal Cytoskeleton. **International Journal of Alzheimer’s Disease,** v. 2013, p. 15, 2013.

MOLLER, D. E.; KAUFMAN, K. D. Metabolic syndrome: A clinical and molecular perspective. **Annual Review of Medicine,** v. 56, p. 45-+, 2005.

MONAGAS, M.; URPI-SARDA, M.; SANCHEZ-PATAN, F.; LLORACH, R.; GARRIDO, I.; GOMEZ-CORDOVES, C. *et al.* Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. **Food & Function,** v. 1, n. 3, p. 233-253, Dec 2010.

MORRIS, R. Developments of a Water-Maze Procedure for Studying Spatial-Learning in the Rat. **Journal of Neuroscience Methods,** v. 11, n. 1, p. 47-60, 1984.

MYERS, M. G.; COWLEY, M. A.; MUNZBERG, H. Mechanisms of leptin action and leptin resistance. **Annual Review of Physiology,** v. 70, p. 537-556, 2008.

NUNES, P. C.; AQUINO, J. D. S.; ROCKENBACH, I. I.; STAMFORD, T. L. M. Physico-Chemical Characterization, Bioactive Compounds and Antioxidant Activity of Malay Apple [Syzygium malaccense (L.) Merr. & L.M. Perry]. **Plos One,** v. 11, n. 6, p. e0158134, 2016.

PAPANDREOU, M. A.; DIMAKOPOULOU, A.; LINARDAKI, Z. I.; CORDOPATIS, P.; KLIMIS-ZACAS, D.; MARGARITY, M. *et al.* Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. **Behavioural Brain Research,** v. 198, n. 2, p. 352-358, Mar 17 2009.

PLAGEMANN, I., KRINGS, I., BERGER, R. G., & MARÓSTICA JÚNIOR., M. R. Volatile constituents of jabuticaba (Myrciaria jaboticaba (Vell.) O. Berg) fruits. **Journal of Essential Oil Research,** v. 24, n. 1, p. 45-51, 2012.

PLAZA, M.; BATISTA, Â. G.; CAZARIN, C. B. B.; SANDAHL, M.; TURNER, C.; ÖSTMAN, E. *et al.* Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. **Food Chemistry,** v. 211, p. 185-197, 2016.

POLI, G.; ALBANO, E.; DIANZANI, M. U. The role of lipid peroxidation in liver damage. **Chemistry and Physics of Lipids,** v. 45, n. 2, p. 117-142, 1987/11/01 1987.

POSEY, K. A.; CLEGG, D. J.; PRINTZ, R. L.; BYUN, J.; MORTON, G. J.; VIVEKANANDAN-GIRI, A. *et al.* Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. **American Journal of Physiology - Endocrinology And Metabolism,** v. 296, n. 5, p. E1003-E1012, 2009.

PRIOR, R. L. Fruits and vegetables in the prevention of cellular oxidative damage. **American Journal of Clinical Nutrition,** v. 78, n. 3, p. 570s-578s, Sep 2003.

REITZ, C.; BRAYNE, C.; MAYEUX, R. Epidemiology of Alzheimer disease. **Nat Rev Neurol,** v. 7, n. 3, p. 137-152, 2011.

REITZ, C.; MAYEUX, R. Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. **Biochemical Pharmacology,** v. 88, n. 4, p. 640-651, 2014.

RENDEIRO, C.; RHODES, J. S.; SPENCER, J. P. E. The mechanisms of action of flavonoids in the brain: Direct versus indirect effects. **Neurochemistry International,** v. 89, p. 126-139, 2015.

RENDEIRO, C.; VAUZOUR, D.; KEAN, R. J.; BUTLER, L. T.; RATTRAY, M.; SPENCER, J. P. E. *et al.* Blueberry supplementation induces spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. **Psychopharmacology,** v. 223, n. 3, p. 319-330, 2012.

RENDEIRO, C.; VAUZOUR, D.; RATTRAY, M.; WAFFO-TEGUO, P.; MERILLON, J. M.; BUTLER, L. T. *et al.* Dietary Levels of Pure Flavonoids Improve Spatial Memory Performance and Increase Hippocampal Brain-Derived Neurotrophic Factor. **Plos One,** v. 8, n. 5, May 28 2013.

REYNERTSON, K. A.; WALLACE, A. M.; ADACHI, S.; GIL, R. R.; YANG, H.; BASILE, M. J. *et al.* Bioactive depsides and anthocyanins from jaboticaba (Myrciaria cauliflora). **J Nat Prod,** v. 69, n. 8, p. 1228-30, Aug 2006.

REYNERTSON, K. A.; YANG, H.; JIANG, B.; BASILE, M. J.; KENNELLY, E. J. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. **Food Chemistry,** v. 109, n. 4, p. 883-890, Aug 15 2008.

ROLLS, A.; SHECHTER, R.; LONDON, A.; ZIV, Y.; RONEN, A.; LEVY, R. *et al.* Toll-like receptors modulate adult hippocampal neurogenesis. **Nat Cell Biol,** v. 9, n. 9, p. 1081-1088, 2007.

SCHUBERT, M.; BRAZIL, D. P.; BURKS, D. J.; KUSHNER, J. A.; YE, J.; FLINT, C. L. *et al.* Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. **Journal of Neuroscience,** v. 23, n. 18, p. 7084-7092, Aug 6 2003.

SCHUBERT, M.; GAUTAM, D.; SURJO, D.; UEKI, K.; BAUDLER, S.; SCHUBERT, D. *et al.* Role for neuronal insulin resistance in neurodegenerative diseases. **Proceedings of the National Academy of Sciences of the United States of America,** v. 101, n. 9, p. 3100-3105, March 2, 2004 2004.

SHAW, J. E.; SICREE, R. A.; ZIMMET, P. Z. Global estimates of the prevalence of diabetes for 2010 and 2030. **Diabetes Research and Clinical Practice,** v. 87, n. 1, p. 4-14, Jan 2010.

SHIMIZU, M. Multifunctions of dietary polyphenols in the regulation of intestinal inflammation. **Journal of Food and Drug Analysis,** v. 25, n. 1, p. 93-99, 2017.

SPENCER, J. P. E. The impact of flavonoids on memory: physiological and molecular considerations. **Chemical Society Reviews,** v. 38, n. 4, p. 1152-1161, 2009.

STYSKAL, J.; VAN REMMEN, H.; RICHARDSON, A.; SALMON, A. B. Oxidative stress and diabetes: What can we learn about insulin resistance from antioxidant mutant mouse models? **Free Radical Biology and Medicine,** v. 52, n. 1, p. 46-58, 2012.

SUGANAMI, T.; TANIMOTO-KOYAMA, K.; NISHIDA, J.; ITOH, M.; YUAN, X.; MIZUARAI, S. *et al.* Role of the Toll-like Receptor 4/NF-κB Pathway in Saturated Fatty Acid–Induced Inflammatory Changes in the Interaction Between Adipocytes and Macrophages. **Arteriosclerosis, Thrombosis, and Vascular Biology,** v. 27, n. 1, p. 84-91, 2007.

TILG, H.; MOSCHEN, A. R. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. **Nature Reviews Immunology,** v. 6, n. 10, p. 772-783, Oct 2006.

TREMAROLI, V.; BACKHED, F. Functional interactions between the gut microbiota and host metabolism. **Nature,** v. 489, n. 7415, p. 242-249, 2012.

TSUDA, T. Regulation of adipocyte function by anthocyanins; Possibility of preventing the metabolic syndrome. **Journal of Agricultural and Food Chemistry,** v. 56, n. 3, p. 642-646, Feb 13 2008.

TSUDA, T.; HORIO, F.; UCHIDA, K.; AOKI, H.; OSAWA, T. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. **Journal of Nutrition,** v. 133, n. 7, p. 2125-2130, Jul 2003.

TURNBAUGH, P. J.; LEY, R. E.; MAHOWALD, M. A.; MAGRINI, V.; MARDIS, E. R.; GORDON, J. I. An obesity-associated gut microbiome with increased capacity for energy harvest. **Nature,** v. 444, n. 7122, p. 1027-131, 2006.

UYSAL, K. T.; WIESBROCK, S. M.; MARINO, M. W.; HOTAMISLIGIL, G. S. Protection from obesity-induced insulin resistance in mice lacking TNF-[alpha] function. **Nature,** v. 389, n. 6651, p. 610-614, 1997.

VALKO, M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M. T. D.; MAZUR, M.; TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. **The International Journal of Biochemistry & Cell Biology,** v. 39, n. 1, p. 44-84, 2007.

VARGHA-KHADEM, F.; GADIAN, D. G.; WATKINS, K. E.; CONNELLY, A.; VAN PAESSCHEN, W.; MISHKIN, M. Differential Effects of Early Hippocampal Pathology on Episodic and Semantic Memory. **Science,** v. 277, n. 5324, p. 376-380, 1997.

WHO. **Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation**. Geneva: World Health Organization 2003.

_____. **World health statistics 2012**. Geneva: WHO Press**:** 176 p. 2012.

WILLIAMS, C. M.; EL MOHSEN, M. A.; VAUZOUR, D.; RENDEIRO, C.; BUTLER, L. T.; ELLIS, J. A. *et al.* Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. **Free Radical Biology and Medicine,** v. 45, n. 3, p. 295-305, 2008.

WILLIAMS, R. J.; SPENCER, J. P. E. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. **Free Radical Biology and Medicine,** v. 52, n. 1, p. 35-45, Jan 1 2012.

YANG, B.; KORTESNIEMI, M.; LIU, P.; KARONEN, M.; SALMINEN, J.-P. Analysis of Hydrolyzable Tannins and Other Phenolic Compounds in Emblic Leafflower (Phyllanthus emblica L.) Fruits by High Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometry. **Journal of Agricultural and Food Chemistry,** v. 60, n. 35, p. 8672-8683, 2012/09/05 2012.

YOUDIM, K. A.; DOBBIE, M. S.; KUHNLE, G.; PROTEGGENTE, A. R.; ABBOTT, N. J.; RICE-EVANS, C. Interaction between flavonoids and the blood–brain barrier: in vitro studies. **Journal of Neurochemistry,** v. 85, n. 1, p. 180-192, 2003.

ZHAO, C.; DENG, W.; GAGE, F. H. Mechanisms and Functional Implications of Adult Neurogenesis. **Cell,** v. 132, n. 4, p. 645-660, 2008.

ZHAO, W. Q.; ALKON, D. L. Role of insulin and insulin receptor in learning and memory. **Molecular and Cellular Endocrinology,** v. 177, n. 1-2, p. 125-134, May 25 2001.

# CAPÍTULO 2

**Artigo Original: JAMBO-VERMELHO (*Syzygium malaccense*): COMPOSTOS BIOATIVOS EM FRUTAS E FOLHAS**

Batista, Ângela Giovana; Da Silva, Juliana Kelly; Betim Cazarin, Cinthia B.; Biasoto, Aline Camarão Telles ; Prado, Marcelo Alexandre ; Maróstica Júnior, Mário Roberto. **Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves.** Lebensmittel-Wissenschaft + Technologie / Food Science + Technology, v. 76, p. 284-291, 2017.

Este artigo foi reproduzido sob autorização da Editora *Elsevier* (ANEXO 1).

LWT - FOOD SCIENCE AND TECHNOLOGY

# RED-JAMBO *(Syzygium malaccense)*: BIOACTIVE COMPOUNDS IN FRUITS AND LEAVES

Running title: Bioactive compounds of red-jambo

Ângela Giovana Batista[a], Juliana Kelly da Silva[a], Cinthia B. Betim Cazarin[a], Aline Camarão Telles Biasoto[b], Alexandra Christine Helena Frankland Sawaya[c], Marcelo Alexandre Prado[a], Mário Roberto Maróstica Júnior[a,*].

[a]School of Food Engineering, University of Campinas (UNICAMP), P.O. Box 6121, 13083-862 Campinas-SP, Brazil.

[b]Brazilian Agricultural Research Corporation, Embrapa Tropical Semi-arid, Petrolina, Pernambuco 56302-970, Brazil.

[c]Plant Biology Department, Institute of Biology, UNICAMP, Campinas, SP, Brazil.

*Corresponding author:

Mário Roberto Maróstica Júnior

Contact details: Departamento de Alimentos e Nutrição. Rua Monteiro Lobato, 80. Cidade Universitária. Campinas-SP, Brasil. E-mail: mario@fea.unicamp.br

## ABSTRACT

*Syzygium malaccense* is poorly studied regarding the bioactive compounds of fruits and leaves. The aim of this study was to determine proximate composition, phenolic compounds, carotenoids, and antioxidant capacity of the fruit parts and leaves of *S. malaccense*. The samples were extracted with different solvents in order to analyze phenolic compounds content (Folin-Ciocalteau and HPLC-DAD/FLD), flavonoids (reaction with $AlCl_3$ and HPLC-DAD/FLD), anthocyanins (differential pH and HPLC-DAD, UPLC-ESI-MS/MS), total carotenoids (colorimetric method) and antioxidant capacity (hydrophilic and lipophilic-ORAC, FRAP, DPPH). The pulp demonstrated high amounts of soluble fibers and reducing sugars. The peel, seeds and leaves of *S. malaccense* showed great contents of phenolic compounds, flavonoids and carotenoids as well as antioxidant capacity. The anthocyanins found in the fruit were cyanidin-3-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, and peonidin-3-*O*-glucoside. Polar bioactive compounds showed strong correlation to hydrophilic antioxidant capacity, while carotenoids did not correlate to lipophilic-ORAC.


**Keywords:** *Myrtaceae*; Pomerac; Malay apple; Hydrolysis; Polyphenols.

**Abbreviations:**

BHT (2,6-di-tert-butyl-4-methylphenol); CE: catechin equivalents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FLD: fluorescence detector; FPP: fresh pulp + peel; FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; H-MetOH: hydrolyzed 80% methanol extract; H-ORAC: hydrophilic ORAC; L-ORAC: lipophilic ORAC; MetOH: 80% methanol extract; ORAC: oxygen radical absorbance capacity; PCA: principal component analysis; PP: pulp+ peel; SS: soluble solids; TA: titratable acidity; TE: trolox equivalents; T-ORAC: total ORAC.

## 1. Introduction

Several berries are of great scientific interest due to their antioxidant power, antitumoral and anti-inflammatory effects (Nile & Park, 2014; Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010). *Syzygium malaccense* (syn. *Eugenia malaccensis, Jambos malaccensis*) belongs to the *Myrtaceae* family and is an original plant from Malaysia, known as Malay apple. Although, the plant was widespread throughout tropical regions, where was also named as pomerac, mountain-apple or red-jambo (Morton, 1987).

The tree reaches 12- 15 m height, shows straight trunk and pyramidal canopy. The red-jambo tree could be used as ornamental plant due to the beauty of its fallen flowers, and offers berry fruits two times per year: February-March and October-November. The fruits are pear-shaped, succulent, containing a single seed, and thin, smooth, waxy peel varying from the rose, crimson, and dark red colors, depending on the stage of maturation or harvest conditions. The pulp is whitish and juicy with acid flavor, similar to apples (Morton, 1987). The aroma of red-jambo is similar to a mixture of roses and herbaceous plants (Pino, Marbot, Rosado, & Vazquez, 2004). The fruits are eaten fresh or in form of handmade products. Although, much of the fruit is wasted during harvest time due to high production, perishability and lack of technological feasibility information for the use by industries.

The scientific literature reports antioxidant capacity of the edible part of red-jambo fruits (Lako et al., 2007; Reynertson, Yang, Jiang, Basile, & Kennelly, 2008), anti-inflammatory and antioxidant effects of leaves use (Andersson Dunstan et al., 1997; Arumugam, Manaharan, Heng, Kuppusamy, & Palanisamy, 2014), cytotoxic power of leaves (Savitha, Padmavathy, & Sundhararajan, 2011), and glycemia /cholesterolemia-lowering effects of the trunk bark extract (Bairy, Sharma, & Shalini, 2005). Thus, each tissue of the

plant has different chemical characteristics that encourage research to explore their functional effects in separated way, since this data is previously rare in literature.

The aim of this work was to explore different tissues of *S. malaccense* (pulp, peel, seed and leaf) in relation to nutrients composition and phytochemicals with bioactive function, such as phenolic compounds and carotenoids, and to determine antioxidant capacity of the different plant tissues.

## 2. Material and methods

### 2.1 Standards

Benzoic acid standard was purchased from Chem Service (West Chester, USA). Kaempferol-3-*O*-glucoside, (+)-catechin, cyanidin-3,5-*O*-diglucoside-chloride (cyanin chloride), cyanidin-3-*O*-glucoside-chloride (kuromanin chloride), (−)-epicatechin, (−)-epicatechin gallate, isorhamnetin-3-*O*-glucoside, peonidin-3-*O*-glucoside chloride, procyanidin A2, procyanidin B1, procyanidin B2, quercetin (dihydrate), isoquercitrin, and rutin standards were obtained from Extrasynthese (Genay, France). *p*-Coumaric acid was purchased from Sigma (UK).

### 2.2 Samples

Ripe red-jambo fruits were harvested in Araçatuba-SP in February 2013 and purchased from a local market in Campinas-SP, Brazil.

**Physical-chemical determinations:** Ten ripe fresh fruits were randomly chosen (Suppl. 1), cleaned and pulp, peel, pulp+ peel (PP) and seeds were separated and weighed. Titratable acidity (TA), soluble solids and pH were determined in the edible part (fresh pulp+ peel= FPP). The TA was determined by titration with standardized 0.01 N NaOH and the

results were expressed as grams of citric acid. Soluble solids (SS) were measured in a manual refractometer. The pH was evaluated using a digital pH meter (Ion Meter 450).

The leaves were collected in the main campus of the University of Campinas (February 2013) and cleaned. The leaves and the parts of the fruits were dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil) at a range from -40 to 25 °C, 300 μm Hg for 95 h, crushed, homogenized and frozen at -18 ± 5 °C.

### *2.3 Macronutrient determinations*

Total nitrogen was determined using a NDA 701 Dumas Nitrogen analyzer (VelpScientifica, Usmate, Italy). Moisture and ash were analyzed according to standard methods (AOAC, 2002) and lipids according to Bligh and Dyer (Bligh & Dyer, 1959). Carbohydrates were calculated by difference. Total and insoluble dietary fibers were quantified by the enzymatic–gravimetric method (AOAC, 2002). Soluble fibers were calculated by difference.

In order to determine reducing sugars, a 70% ethanol solution was added to the samples and allowed to react during 60 min at 100 ºC. After ethanol evaporation, the extracts were filled with water and then analyzed according to the Somogyi-Nelson method, using a Synergy HT, Biotek microplate reader (Winooski, USA), with readings set at 520 nm (Nelson, 1944).

### 2.4 Bioactive compounds and antioxidant capacity measurements

All the absorbance and fluorescence readings for the analyses were determined in a Synergy HT, Biotek microplate reader (Winooski, USA) with Gen5™ 2.0 data analysis software.

### *2.4.1 Sample treatments*

Extracts (40g $L^{-1}$ for freeze-dried samples and 120 g $L^{-1}$ for FPP) were made in duplicate as follows:

**80% Methanol extract:** The samples were extracted with 80% methanol (MetOH:$H_2O$, v:v) at 37 °C for 3 h in a shaking water bath, centrifuged at 2000 g, for 10 min, and stored in amber flasks at 4 - 8 °C (Batista et al., 2016).

**Hydrolyzed 80% methanol extract:** A HCl and BHT (2,6-di-tert-butyl-4-methylphenol) solutions were added to the aforementioned methanol extract, to result in a final concentration of 1.2 mol $L^{-1}$ HCl, 0.26 g $L^{-1}$ BHT in 50% methanol (H-MetOH:$H_2O$, v:v) in the extract. In order to complete the hydrolysis, the extracts were allowed to react in water bath at 90 °C for 30 min, with refrigerated reflux condenser.

**Lipophilic extract:** The samples were extracted by successive maceration with hexane at room temperature to obtain a non-polar extract. After solvent evaporation, samples were resuspended with acetone (Prior et al., 2003).

All extracts were stored at 4 $^{o}$C until analyzed and new extracts were made each 5 days.

### *2.4.2 Bioactive compounds*

**Total phenolic compounds or Folin-Ciocalteau reagent reducing substances method:** Folin-Ciocalteau reagent and sodium carbonate were added to water-diluted extract, and after 2h in the dark at room temperature, the absorbance of samples and standard curve was read at 725 nm. Gallic acid was used as standard and results were expressed as gallic acid equivalents (mg GAE) (Batista et al., 2016).

**Total flavonoids:** Solutions, such as 5% sodium nitrite, 10% aluminum chloride and 1 mol $L^{-1}$ sodium hydroxide were added to water-diluted extracts (Zhishen, Mengcheng, &

Jianming, 1999). A precipitation was observed after the addition of NaOH solution to the leaf extract, and because of that the mix was centrifuged at 2000 g, 10 min. A calibration curve was made using (+)-catechin. The samples and standard were read at 510 nm. The results were expressed as catechin equivalents (mg CE).

**Monomeric anthocyanin method:** The extracts were diluted using 0.025 mol $L^{-1}$ potassium chloride buffer (pH 1.0) according to sample absorbance (0.4 to 0.6), and in 0.4 mol $L^{-1}$ sodium acetate buffer (pH 4.5) in the same proportions (1:2, 1:6 and 1:15 dilution factors for FPP, PP and peel, respectively). After addition of 250 µL in the microplate, the absorbance was read at 520 and 700 nm (Wrolstad, 1976). The absorbance was calculated using Equation 1:

$$A = [(A_{520\ nm} - A_{700\ nm})\ pH = 1.0] - [(A_{520\ nm} - A_{700\ nm})\ pH = 4.5] \quad (1)$$

The anthocyanin content (mg $100g^{-1}$) was calculated as cyanidin-3-*O*-glucoside (PM = 449.2) using Equation 2:

$$C\ (mgC3G\ 100g^{-1}) = A.MW.DF/\xi.L \quad (2)$$

Where C= concentration, C3G= cyanidin-3-*O*-glucoside, $\xi$ = molar absorptivity (26900 mol $L^{-1}$), L = pathlength (cm), MW= molecular weight and DF= dilution factor.

**HPLC analysis:** The analyses of the fruit extracts were performed using a HPLC system Waters e2695 Separation Module Alliance equipped with a quaternary solvent pump and an automatic injector. For the phenolic compounds determination, a diode array detector (DAD) Waters model 2998 and a fluorescence detector (FLD) Waters model 2475 were employed. Acquisition and processing of data were carried out using the Waters Empower™ 2 software (Milford, USA).

The extracts were filtered through 0.45 µm nylon membranes (Allcrom-Phenomenex, USA). The injection volume was 10 µL. The Gemini NX C-18 column (150 mm × 4.6 mm × 3 µm) (Phenomenex, USA) was maintained at 40 °C. The mobile phase consisted of a

gradient mixture of solvent A (0.85% aqueous phosphoric acid solution) and solvent B (acetonitrile), with 0.5 mL $min^{-1}$ flow-rate. The gradient was started with 100% solvent A and adjusted for 93% A and 7% B at 10 min; 90% A and 10% B at 20 min; 88% A and 12% B at 30 min; 77% A and 23% B at 40 min; 65% of solvent A and 35% of solvent B at 45 min; and 100% B at 55 min.

Fluorescence detector was used at 320 nm emission for identification of the following compounds: (+)-catechin, procyanidin B2, procyanidin A2 and (−)-epicatechin. The DAD was set in four wavelengths: 280 nm for identification of gallic acid and tannins: (−)-epicatechin gallate and procyanidin B1; 320 nm for phenolics acids; 360 nm for flavonols and 520 nm for anthocyanins.

**UPLC-ESI-MS/MS analysis:** After successive extractions with 1% aqueous formic acid, the anthocyanins from the freeze-dried peel were extracted using a solid-phase - manifold apparatus and methanol, water, ethyl acetate and 1% formic acid in methanol as solvents, concentrated under gas nitrogen atmosphere, resuspended in water, filtered through 0.22 μm syringes and analyzed by ultra-high performance chromatography – mass spectrometry (UPLC–MS). An Acquity UPLC system (Waters, Milford, MA, USA) was used coupled with UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm particle size) at 30 °C and 3 μL of the extract were injected. Mobile phase was used as follows: a gradient of (A) deionized purified water with 1% aqueous formic acid and (B) acetonitrile starting with 5% B and ramping to 100% B at 8 min, maintained until 8.5 min, then returning to initial conditions and stabilizing by 10 min. Detection in positive ion modes was achieved on an Acquity TQD mass spectrometer (Micromass Waters, Milford, MA, USA) with capillary – 3000V, Cone – 30V, source temperature 150 $^{o}$C; desolvation temperature 350 °C.

The phenolic compounds in the leaves of *S. malaccenses* were performed using a HPLC-DAD method as described previously (Batista et al., 2014).

**Total carotenoids method:** The lipophilic extract was shaken in an Erlenmeyer flask containing isopropanol and hexane, transferred to a separation funnel and gently washed with water, 3 times. The hexane extract was filtered in sodium sulfate and filled with hexane (Higby, 1962). The samples and blank (hexane) were read at 450 nm in the microplate reader. The calculations were performed as follows (Equation 3):

$$C\ (mg\ 100g^{-1}) = (A.\ 100)/\ (250.\ L.\ W) \quad (3)$$

Where C= concentration, A= absorbance, L= wells pathlength (cm), W= sample weight $mL^{-1}$ final solution.

In order to eliminate possible interferences of chlorophyll content, the same extract used on carotenoid measurements was read at 649 and 665 nm and proper calculations were made (Total chlorophyll: $(6.45.\ A_{665}) + (17.72.\ A_{649})$ (Wellburn, 1994).

### *2.4.3 Antioxidant capacity*

Antioxidant capacity was assessed using FRAP, DPPH and ORAC methods as described previously (Batista et al., 2016), with some adaptations:

**FRAP method:** Samples and trolox standard curve were read at 595 nm. The results were expressed as trolox equivalents (μm TE).

**DPPH-$IC_{50}$**: The reading time of the extracts in DPPH analysis were 15 min for leaves, seeds and peel, 60 min for PP, 3h for pulp and 24h for FPP, respectively. The concentration of samples required in order to reduce the 50% DPPH radical ($IC_{50}$) was calculated by linear regression.

**ORAC:** The H-ORAC (hydrophilic oxygen radical absorbance capacity) and L-ORAC (lipophilic-ORAC) tests were carried out according to previous descriptions (Batista et al., 2016). In addition, the total-ORAC (T-ORAC) was assessed by the sum of H-ORAC values of the MetOH extracts and L-ORAC values.

### 2.5 Statistics

Due to peculiarities in chemical composition, no statistical tests were used to assess differences among the different tissues of *S. malaccense*. The comparison between the data of methanol and hydrolyzed extracts was evaluated using Student's *t*-test, considering $p < 0.05$. The correlations were made considering Pearson's coefficient ($p < 0.05$). GraphPad Prism 5.0 software (GraphPad, Inc. La Jolla, CA, USA) was used for the aforementioned analyses.

The Principal Component Analysis (PCA) was made using the SAS software, version 9.4 (SAS Institute Inc., Cary, USA).

All results were expressed as g or 100g of wet weight of freeze-dried samples (PP, peel, pulp, leaves and seeds), as well as wet weight of fresh pulp+ peel .

## 3. Results

Nutrients, bioactive compounds and antioxidant capacity of *S. malaccense* fruit parts and leaves were explored in this study. The PP, fresh and dried, was also studied since it is the usual eaten or most studied form. Phenolic compounds and lipophilic bioactive compounds were related with great antioxidant power in all tissues of *S. malaccense*, as detailed below.

The whole fresh fruit weighed 75.86 ± 16.26 g. The proportion of FPP in the fruits was 64.58 ± 20.85%, showing high variation in the fresh pulp part (47.21 ± 20.95%), but not in the fresh peel (17.37 ± 1.03%), and seeds (18.94 ± 2.48%) (Suppl. 1). The pH of the FPP was 3.72 ± 0.01, the SS 4.67 ± 0.2% and TA reached 0.628 ± 0.01 g citric acid $100g^{-1}$ wet weight (SS/TA ratio= 7.37 ± 0.3).



| Fruits | Total (g) | Peel (g) | Pulp (g) | Seed (g) | Peel % | Pulp % | PP % | Seeds % |
|---|---|---|---|---|---|---|---|---|
| 1 | 91.19 | 14.25 | 58.43 | 15.56 | 15.63 | 64.08 | 79.70 | 17.06 |
| 2 | 63.45 | 11.74 | 34.73 | 13.96 | 18.50 | 54.74 | 73.24 | 22.00 |
| 3 | 48.58 | 8.55 | 29.34 | 9.08 | 17.60 | 60.40 | 78.00 | 18.69 |
| 4 | 69.30 | 11.43 | 43.22 | 11.74 | 16.49 | 62.37 | 78.86 | 16.94 |
| 5 | 92.15 | 16.66 | 62.64 | 16.06 | 18.08 | 67.98 | 86.06 | 17.43 |
| 6 | 69.49 | 11.23 | 37.00 | 15.63 | 16.16 | 53.25 | 69.41 | 22.49 |
| 7 | 71.76 | 13.40 | 40.74 | 15.97 | 18.67 | 56.77 | 75.45 | 22.25 |
| 8 | 88.74 | 15.16 | 15.16 | 13.89 | 17.08 | 17.08 | 34.17 | 15.65 |
| 9 | 64.09 | 11.06 | 11.06 | 12.35 | 17.26 | 17.26 | 34.51 | 19.27 |
| 10 | 99.84 | 18.16 | 18.16 | 17.57 | 18.19 | 18.19 | 36.38 | 17.60 |
| Mean | 75.86 | 13.16 | 35.05 | 14.18 | 17.37 | 47.21 | 64.58 | 18.94 |
| SD | 16.26 | 2.92 | 17.32 | 2.53 | 1.03 | 20.95 | 20.85 | 2.48 |
| CV% | 21.43 | 22.20 | 49.42 | 17.85 | 5.94 | 44.38 | 32.29 | 13.12 |

**Suppl. 1**. Red-jambo fruits used in the physicochemical analyses (A). Longitudinal section of red-jambo fruit (B). The table shows the physical characterization of the 10 fruits.

*S. malaccense* contains high amounts of water and reducing sugars (Table 1). The peel of the red-jambo concentrated more protein (total N) and lipids, but less reducing sugars than the pulp. The dietary fiber content was higher in the peel, although the pulp has shown higher soluble fiber amounts (Table 1).

**Table 1**. Proximate composition (%) of *S. malaccense* fruit parts.[a]

| Composition | FPP | Pulp[b] | PP[b] | Peel[b] |
|---|---|---|---|---|
| Moisture | 93.09 ± 0.24 | 4.81 ± 0.13 | 4.83 ± 0.22 | 4.27 ± 0.06 |
| Protein (N x 6.25) | 0.45 ± 0.014 | 3.10 ± 0.13 | 5.23 ± 0.07 | 5.57 ± 0.10 |
| Lipids | 0.20 ± 0.008 | 1.24 ± 0.05 | 2.62 ± 0.04 | 5.92 ± 0.12 |
| Ash | 0.31 ± 0.006 | 3.39 ± 0.083 | 3.85 ± 0.12 | 5.06 ± 0.131 |
| Total carbohydrates | 6.19 ± 0.51 | 87.65 ± 0.32 | 83.73 ± 0.16 | 78.59 ± 0.19 |
| Reducing sugars | 2.25± 0.04 | 64.98 ± 3.69 | 49.55 ± 0.91 | 48.42 ± 2.09 |
| Total dietary fibers | 1.54 ± 0.02 | 28.50 ± 0.89 | 33.67 ± 0.52 | 36.16± 0.006 |
| Insoluble dietary fibers | 1.28 ± 0.03 | 20.94 ± 1.55 | 27.92 ± 0.006 | 32.78 ± 0.46 |
| Soluble dietary fibers | 0.25 ± 0.01 | 8.00 ± 0.54 | 5.62 ± 0.22 | 3.53 ± 0.17 |

[a] Expressed as wet weight of samples. [b] Freeze-dried samples. PP: pulp + peel, FPP: fresh PP.

The level of phenolic compounds varied depending on the plant tissue type and different sample treatment methods. The leaves, peel and seeds showed the highest amounts of total phenolic compounds and flavonoids (Table 2). The acid hydrolysis of MetOH extracts allowed the release of phenolic monomers, increasing the quantification of total phenolic compounds found in the PP (2.85 times) and leaves (1.67 times) (Table 2). Phenolic compound levels were significantly correlated with hydrophilic antioxidant power of *S. malaccense* (phenolic compounds x ORAC: $r$=0.9841, $P$<0.0001; flavonoids x ORAC: $r$=0.9876, $p$<0.0001). The Folin-Ciocalteau method was also performed on the BHT solution used for the acid hydrolysis at the same conditions as the samples, and it was found a value of 26.98 ± 0.73 mg GAE 100 $g^{-1}$ for the final solution.

The peel of red-jambo also concentrated the monomeric anthocyanins from the edible part (Table 2), which corroborates its higher hydrophilic antioxidant capacity (Table 3). In addition, due to the low pH, methods for quantification of total flavonoids and monomeric anthocyanins in the hydrolyzed extracts were not done.

**Table 2.** Bioactive compounds in *S. malaccense* fruit and leaves analyzed by colorimetric methods.

| | Extracted samples | mg $100g^{-1}$[a] |
|---|---|---|
| Total polyphenols | MetOH FPP | 14.81 ± 1.14 |
| | MetOH Pulp | 28.40 ± 1.92 |
| | MetOH PP | 158.3 ± 4.23*** |
| | H-MetOH PP | 232.2 ± 14.80 |
| | MetOH Peel | 392.9 ± 6.38 |
| | H-MetOH Peel | 394.2 ± 33.42 |
| | MetOH Leaves | 5377.08 ± 129.06*** |
| | H-MetOH Leaves | 8873.03 ± 407.71 |
| | MetOH Seed | 1063.81 ± 68.45 |
| Total flavonoids | MetOH FPP | 12.86 ± 0.41 |
| | MetOH Pulp | 13.12 ± 1.029 |
| | MetOH PP | 81.89 ± 5.52 |
| | MetOH Peel | 267.84 ± 9.43 |
| | MetOH Leaves | 810.22 ± 46.75 |
| | MetOH Seed | 154.37 ± 25.74 |
| Monomeric anthocyanins | MetOH FPP | 12.90 ± 0.63 |
| | MetOH PP | 126.95 ± 0.42 |
| | MetOH Peel | 424.82 ± 0.42 |
| Total carotenoids | Non-polar Pulp | 0.015 ± 0.001 |
| | Non-polar PP | 0.046 ± 0.007 |
| | Non-polar Peel | 0.158 ± 0.011 |
| | Non-polar Leaves | 3.93 ± 0.55[b] |
| | Non-polar Seed | 0.16 ± 0.008 |

FPP: fresh pulp + peel, PP: pulp + peel.
[a] Expressed as wet weight of samples
[b] Value discounted of chlorophyll amount in the carotenoid extract (6.53 ± 0.27 mg $100g^{-1}$).
*Indicates statistical differences from the respective hydrolyzed extracts according to Student's *t*-test (*** $p < 0.001$).

The hydrolyzed extracts of peel and leaves showed similar FRAP values compared to the MetOH extracts (Fig 1). Indeed, the phenolic compounds content was not increased in

hydrolyzed peel extract like as in PP, which corroborated the FRAP values (FRAP x phenolic compounds: $p<0.0001$, $r=0.9696$). Although, hydrolysis released a significant portion of radical scavenging capacity according to the ORAC values, since it was around 3 times higher in all the H-MetOH extracts (Table 3).

The samples showed good linearity ($r^2 >0.95$) in the concentration ranges studied in DPPH-$IC_{50}$ and H-ORAC (Table 3). Regarding the DPPH-$IC_{50}$ values, the antioxidant capacity was higher in the following crescent order FPP< Pulp< PP< Peel< Seeds< Leaves (Table 3).

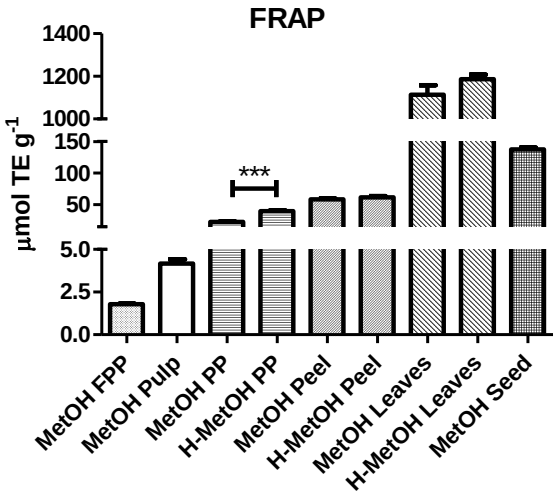



**Fig. 1.** Antioxidant capacity by FRAP assay in fruits and leaves of red-jambo. Samples were extracted with MetOH and acid hydrolysis (H-MetOH). PP: pulp +peel, FPP: fresh PP. Hydrolyzed extracts were compared to their respective MetOH using Student's *t*-test (***$p<0.001$).

The non-polar extract of the peels showed higher L-ORAC value, followed by seeds and leaves, which had approximately the same value (Table 3). Although, due to the H-ORAC, T-ORAC values were higher in the dried leaves (528.93± 16.44 µmol TE $g^{-1}$), followed by the peel (135.47± 9.47 µmol TE $g^{-1}$), seeds (108.78 ± 7.99 µmol TE $g^{-1}$), PP (70.16 ± 3.49 µmol TE $g^{-1}$), and pulp (14.20 ± 0.14 µmol TE $g^{-1}$).

Total carotenoids were high in leaves (Table 2), but the data was not correlated with the lipophilic-ORAC, since the highest L-ORAC value was found in the peel and not in the leaves as in the carotenoids assay.

Regarding the phenolic compounds profile, tannin amounts were smaller after hydrolysis of red-jambo fruit extracts, mainly the (-)-epicatechin gallate (non-detected), procyanidins B1 (non-detected) and A2 contents. The MetOH PP extract showed higher contents of (+)-catechin and (-)-epicatechin in comparison to the peel. However, the amount of such compounds was increased in the H-MetOH peel extracts (Table 4).

**Table 3.** DPPH $IC_{50}$, H- and L-ORAC values and linearity of *S. malaccense* fruit parts and leaves.

| | Extracts | Mean ± SD[a] | Conc[b] (g $L^{-1}$) | Slope | intercept | $R^2$ |
|---|---|---|---|---|---|---|
| DPPH $IC_{50}$ (g $L^{-1}$) | MetOH FPP | 674.75 ± 40.03 | 100-750 | 0.0361 | 25.764 | 0.9740 |
| | MetOH Pulp | 61.36 ± 0.93 | 21-73 | 0.5933 | 13.587 | 0.9910 |
| | MetOH PP | 35.95 ± 0.49 | 5.0-40 | 0.9888 | 14.453 | 0.9904 |
| | MetOH Peel | 29.88 ± 0.52 | 5.0-40 | 1.1761 | 14.873 | 0.9869 |
| | MetOH Leaves | 0.67 ± 0.01 | 0.2-1.0 | 50.329 | 16.981 | 0.9581 |
| | MetOH Seed | 2.47± 0.05 | 1.0-4.0 | 14.8315 | 13.352 | 0.9921 |
| H-ORAC (µmol TE $g^{-1}$) | MetOH FPP | 7.50 ± 0.46 | 1.0-5.5 | 5.146 | 3.272 | 0.9937 |
| | MetOH Pulp | 13.62 ± 0.11 | 2.0-5.0 | 7.732 | 5.7611 | 0.9912 |
| | MetOH PP | 59.99 ± 5.93*** | 0.3-0.9 | 46.406 | -0.159 | 0.9957 |
| | H-MetOH PP | 168.53 ± 42.26 | 0.05-0.3 | 134.19 | 1.9084 | 0.9939 |
| | MetOH Peel | 108.75 ± 13.01* | 0.1-0.6 | 93.566 | -0.3837 | 0.9958 |
| | H-MetOH Peel | 362.52 ± 42.26 | 0.025-0.125 | 174.51 | 1.3157 | 0.9959 |
| | MetOH Leaves | 502.28 ± 33.88*** | 0.01-0.15 | 263.42 | 9.294 | 0.9975 |
| | H-MetOH Leaves | 1374.419 ± 125.06 | 0.015-0.035 | 825.9 | 6.3483 | 0.9651 |
| | MetOH Seed | 88.31 ± 8.79 | 1.6-8.0 | 7.024 | 1.5221 | 0.9980 |
| L-ORAC (µmol TE $g^{-1}$) | Non-polar Pulp | 0.55 ± 0.031 | 2.0-5.0 | $y=-0.0008x^2+ 0.1199x -0.0563$ | | 0.829 |
| | Non-polar PP | 10.50 ± 0.10 | 5.0-20.0 | $y=-0.0025x^2 +0.1617x -11.663$ | | 0.938 |
| | Non-polar Peel | 26.47 ± 1.57 | 2.8-8.0 | $y=-0.3118x^2 +4.3886x -1.9594$ | | 0.951 |
| | Non-polar Leaves | 12.88 ± 0.27 | 2.8-13.3 | $y = -0.1501x^2 + 3.2613x - 2.978$ | | 0.991 |
| | Non-polar Seed | 13.91 ± 0.40 | 2.8-13.3 | $y = 1.4773x + 1.9641$ | | 0.969 |

FPP: fresh pulp + peel, PP: pulp + peel. [a] Expressed as wet weight of samples.
[b]Concentration ranges.
*Indicates statistical differences from the respective hydrolyzed extracts according to Student's *t*-test (*$p < 0.05$; *** $p < 0.001$).

Phenolic acids were also increased with the hydrolysis of the PP extract. In the peel, benzoic acid was detected only in MetOH and p-coumaric only in H-MetOH (Table 4).

**Table 4-** Polyphenols of *S. malaccence* fruit parts analyzed by HPLC-DAD/FLD expressed as mg $100g^{-1}$ wet weight of freeze-dried samples.

| | Polyphenols | MetOH PP | H-MetOH PP | MetOH Peel | H-MetOH Peel |
|---|---|---|---|---|---|
| | **Phenolic acid** | | | | |
| *1* | *p*-Coumaric acid | ND | 0.999 ± 0.000 | ND | 1.160 ± 0.001 |
| 2 | Benzoic acid | 0.724 ± 0.064 | ND | 1.517 ± 0.094 | ND |
| | **Flavanols and Procyanidins** | | | | |
| 3 | (−)-Epicatechin gallate | 1.374 ± 0.247 | ND | 1.21 ± 0.15 | ND |
| 4 | (+)-Catechin | 1.124 ± 0.087 | 0.500 ± 0.01*** | 0.224 ± 0.029 | 1.243 ± 0.096*** |
| 5 | (−)-Epicatechin | 1.374 ± 0.029 | 1.332 ± 0.083 | 0.808 ± 0.047 | 2.321 ± 0.167*** |
| 6 | Procyanidin A2 | 1.736 ± 0.246 | 1.221 ± 0.192* | 0.932 ± 0.025 | 0.622 ± 0.083*** |
| 7 | Procyanidin B1 | 1.311 ± 0.246 | ND | 2.072 ± 0.189 | ND |
| 8 | Procyanidin B2 | 0.450 ± 0.001 | 0.541 ± 0.083 | 1.206 ± 0.024 | 1.160 ± 0.192 |
| | **Flavonols** | | | | |
| 9 | Isorhamnetin-3-*O*-glucoside | 3.334 ± 0.205 | ND | 7.143 ± 0.141 | ND |
| 10 | Isoquercitrin | 0.549 ± 0.000 | ND | 2.014± 0.094 | ND |
| 11 | Quercetin | 0.200 ± 0.000 | 1.332 ± 0.136*** | 0.298 ± 0.000 | 3.646 ± 0.164*** |
| 12 | Kaempferol-3-*O*-glucoside | 1.315 ± 0.161 | 3.538 ± 0.250*** | 2.967± 0.077 | 1.878 ± 0.254** |
| 13 | Rutin | 0.549 ± 0.100 | 1.082 ± 0.288* | 0.448 ± 0.000 | 0.580 ± 0.096 |
| | **Anthocyanins[a]** | | | | |
| 14 | Cyanidin-3,5-*O*-diglucoside[1] | 5.519 ± 0.247 | 6.050 ± 0.535 | 11.698 ± 0.667 | 14.971 ± 0.671** |
| 15 | Cyanidin-3-*O*-glucoside[2] | 42.77 ± 3.36 | 63.60 ± 0.99*** | 155.29 ± 3.76 | 226.54 ± 6.81*** |
| 16 | Peonidin-3-*O*-glucoside[3] | 0.512 ± 0.025 | 0.888 ± 0.096*** | 1.724 ± 0.124 | 3.066 ± 0.165*** |
| | Sum | 63.53 ± 5.10 | 81.02 ± 1.55** | 189.53 ± 3.17 | 257.21 ± 6.14*** |

[a]Identifications of the anthocyanins: [1][M+] 611 m/z, MS/MS 449, 287 m/z, for cyanidin 3,5-*O*-diglucoside (TR=1.6min); [2][M+] 449 m/z, MS/MS 287 m/z, for cyanidin 3-*O*-glucoside (TR= 2.15 min) and [3][M+] 463 m/z, MS/MS 301 m/z, for peonidin and hexose. ND: non-detected. PP: pulp +peel. Hydrolyzed extracts were compared to their respective 80% MetOH extract using Student's *t*-test (**p*<0.05, ** *p*<0.01, and ****p*<0.001).

In relation to flavonols, isorhamnetin, quercetin and isoquercitrin, the amounts were higher in peel of red-jambo compared to the PP. Rutin and kaempferol-3-*O*-glucoside amounts were higher in H-MetOH PP extract. Both hydrolyzed extracts increased the

quantification of the aglycone quercetin, probably because of hydrolysis of its glycosylated compounds (Table 4).

In addition, linear combinations among the phenolic compounds found in the extracts of PP and peel of red-jambo were separated out in different clusters by PCA (Fig. 2). The non-hydrolyzed peel extract could be grouped by the condensed tannins ((−)-epicatechin gallate and procyanidin B1), benzoic acid, isorhamnetin-3-*O*-glucoside and isoquercitrin contents, and the PP by procyanidin A2. In the other hand, the H-MetOH PP was characterized for the content of kaempferol-3-*O*-glucoside and rutin, corroborating aforementioned results; but the H-MetOH peel was correlated with the amounts of p-coumaric, procyanidin B2, (+)-catechin, (−)-epicatechin, quercetin and anthocyanins (Fig. 2).

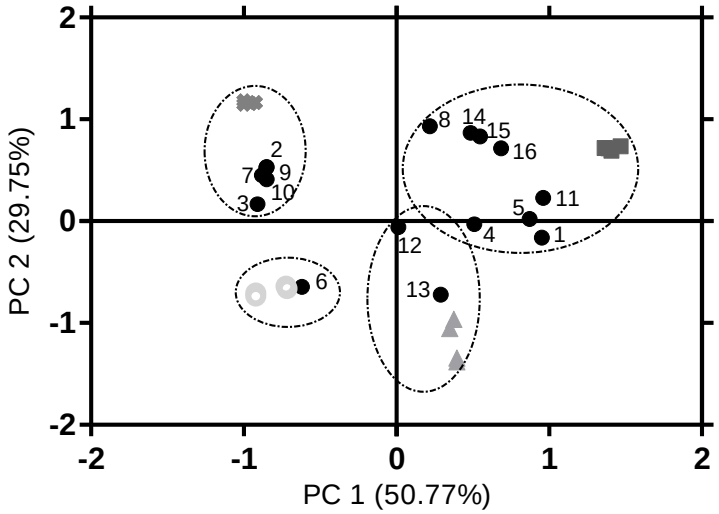



**Fig. 2-** Plot of the first and second PC score vectors of the 16 phenolic compounds found in the red-jambo fruit parts. The grouping indicates a relationship between the compounds marked as numbers: ●1: p-Coumaric, 2: benzoic acid, 3: (−)-epicatechin gallate, 4: (+)-catechin, 5: (−)-epicatechin, 6: procyanidin A2, 7: procyanidin B1, 8: procyanidin B2, 9: isorhamnetin-3-*O*-glucoside, 10: isoquercitrin, 11: quercetin, 12: kaempferol-3-*O*-glucoside, 13: rutin, 14: cyanidin-3,5-*O*-diglucoside, 15: cyanidin-3-*O*-glucoside, 16: peonidin-3-*O*-glucoside, and the method of extraction used for pulp+ peel and peel, marked as ○for MetOH PP, ▲H-MetOH PP, xMetOH Peel and ■H-MetOH Peel extracts.

Results showed that anthocyanins were the major class of phenolic compounds in the edible portion of red-jambo, representing more 75% of the total amount determined by HPLC (Table 4). Five anthocyanins were found in red-jambo peel, but two minor ones could not be

identified. The three anthocyanins identified by UPLC-MS analysis in the positive ion mode were: Cyanidin-3,5-*O*-diglucoside ([M+] *m*/*z* 611, MS/MS fragment ions *m*/*z* 449, 287); cyanidin-3-*O*-glucoside ([M+] *m/z* 449, MS/MS fragment ions *m*/*z* 287), and peonidin + hexose ([M+] *m*/*z* 463, MS/MS fragment ions *m*/*z* 301). However, using HPLC-DAD and comparing retention time and elution order with identical standards, the hexose of peonidin was confirmed to be a 3-*O*-glucoside (Table 4). Cyanidin-3-*O*-glucoside was the most abundant anthocyanin quantified by HPLC-DAD, followed by cyanidin-3,5-*O*-diglucoside. In general, the hydrolysis increased quantification of anthocyanins in the fruit, most of them due their stability in acid conditions (Table 4, Fig. 2).

The H-MetOH extracts of the leaves of *S. malaccence* showed 464.42 ± 27.54 mg 100 $g^{-1}$, 377.91 ± 32.34 mg 100 $g^{-1}$, and 508.05 ± 10.40 mg 100 $g^{-1}$ of (+)-catechin, (-)-epicatechin and quercetin, respectively.

**4. Discussion**

This study demonstrated that *S. malaccense* fruits and leaves have bioactive compounds with antioxidant capacity, such as phenolic compounds, carotenoids, as well as high amounts of dietary fibers.

Dietary fibers are associated with control of metabolic diseases, like obesity, diabetes, cardiovascular and inflammatory disorders (Kaczmarczyk, Miller, & Freund, 2012). This is the first study to investigate the fractions of fibers in *S. malaccense* fruit, which is rich in insoluble fibers, being the soluble ones concentrated in the pulp (Table 1). The amount of total fibers found in a previous study (Maisuthisakul, Pasuk, & Ritthiruangdej, 2008) was lower than that shown in this study, probably because of different methods used, conditions of harvest, or even sample treatment. For instance, it is known that freeze-drying method, used in the present study, increases the share of the soluble fraction of fibers (Milala et al., 2013).

The Folin-Ciocalteau method has shown a poor correlation with HPLC quantifications of phenolic compounds, since it could be affected by organic acids and reducing sugars (Kapasakalidis, Rastall, & Gordon, 2006). The red-jambo showed high acidity and amounts of reducing sugars, this might have influenced the result of total phenolic compounds by this method. The error could be minimized by using other techniques such as solid-phase extraction, although it could also reduce the final content of phenolic compounds (Palma, Pineiro, & Barroso, 2002). Particularly in this study, Folin-Ciocalteau reagent showed mild reaction with BHT, synthetic antioxidant used to reduce the heating damage when the extracts were hydrolyzed. Furthermore, previous works demonstrated no differences for Folin-Ciocalteau reagent reducing substances, ORAC (Batista et al., 2014) and FRAP assays (Batista et al., 2016) after acid hydrolysis of the MetOH fruit extracts containing 0.26 g $L^{-1}$ BHT in the final solution. Based on this, we concluded that the use of BHT did not interfere on the statistical differences found for total phenolic compounds or antioxidant capacity values, since its reducing power was small.

Acid hydrolysis is a common technique used previously to phenolic compounds quantification by HPLC. The conditions used to hydrolyze extracts have been cited as: 1.2-1.5 mol $L^{-1}$ HCl solution under 90 $^{o}$C, during 60-120 min (Careri, Elviri, Mangia, & Musci, 2000; Kapasakalidis et al., 2006; Pyrzynska & Biesaga, 2009). These studies have shown release of phenolic monomers, anthocyanidins (aglycone forms of anthocyanins) and other flavonoid aglycone forms after hydrolysis. On the other hand, some deglycosylated phenolic compounds are labile molecules and may be degraded upon exposure to heat (Careri et al., 2000). Using 30 min, this study showed complete hydrolysis of some compounds, such as isorhamnetin-3-O-glucoside, isoquercitrin, (−)-epicatechin gallate and procyanidin B1; supported by the increased amounts of quercetin, (+)-catechin and (-)-epicatechin as aglycones/ monomers of the last three compounds. Corroborating this data, the hydrolyzed

peel extract showed high and positive correlation with these monomers as well as with anthocyanins, when PCA was used (Fig. 2).

The hydrolyzed extracts showed higher amounts of anthocyanins, probably due to their stability in acid conditions. As demonstrated in another study (Lee, Rennaker, & Wrolstad, 2008), the pH differential method for monomeric anthocyanins quantification in this work showed strong correlation with HPLC results ($r$=0.997, $P$<0.001); however, the first method showed around 2.5 times higher values. Hence, even with more stability of anthocyanins in the H-MetOH extract, the quantification was possibly underestimated, since hydrolysis to anthocyanidin forms might have occurred concomitantly.

Even though, there are no previous works about the anthocyanins profile of red-jambo fruit by HPLC or MS. A study with *Myrtaceae* fruits (Reynertson et al., 2008) only showed the content of cyanidin 3-glucoside in the freeze-dried PP of *S. malaccense*, in lower amounts than that found in the present work. Furthermore, in agreement with our data (Table 4), quercetrin and rutin were also found in such study (Reynertson et al., 2008), but not ellagic acid.

There are no data about phenolic compounds in the separated parts of red-jambo. For example, analyzing the peel and PP separately, it was possible to infer, that from the edible part, the pulp has the highest association with procyanidin A2 and kaempferol-3-*O*-glucoside and rutin, as shown by PCA (Table 4, Fig. 2).

Proanthocyanidins or condensed tannins were found in red-jambo, particularly in the non-hydrolyzed extract of the peel, as indicated by the PCA analysis (Fig 2). They represent oligomers or polymers of mainly (-)-epicatechin and (+)-catechin, which occur naturally in berries and represent the flavonoids more consumed every day. They could be absorbed as native form or metabolized by gut microbiota, linked with prebiotic, antioxidant, antitumor

and anti-inflammatory activities (Ou & Gu, 2014). In the PP of red-jambo, flavanols and procyanidins represent 11% of the phenolic compounds (Table 4).

The seed of red-jambo fruit is common wasted; however, it could be used for further investigations due to its antioxidant capacity and bioactive compounds, which were not previously studied. The interest in the use of byproducts, such as peels and seeds of fruits, is raising since they have shown higher antioxidant power and phenolic compounds content than in edible portions (Soong & Barlow, 2004). Our results of the seed analyses corroborated these findings.

The leaves of *S. malaccence* are known by their anti-inflammatory power (Andersson Dunstan et al., 1997) and this could be related to its elevated content of flavonoids, like myricitrin (Arumugam et al., 2014), and quercetin, found in the present study. Due to its several electron-donating groups, quercetin also pronounces antioxidant activity and could inhibit inflammation, likewise anthocyanins (Nair et al., 2006). Sources of quercetin, were associated with cardiovascular, antioxidant, and cognitive improvements (Williamson & Manach, 2005). The intake of edible red-jambo may well also be implicated with such effects, since quercetin was also found in the fruit.

Measurements of antioxidant capacity stimulate the consumption of plant-based foods, since they could predict ‘in vivo’ effects against oxidative stress (Prior et al., 2003; Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010)(Batista et al., 2014). However, due to peculiar characteristics of each method (e.g. pH, temperature, and radical), they often show different results. In this work, among the methods used to determine antioxidant capacity, the ORAC assay (pH 7.4, 37 $^{o}$C, peroxyl radical) was more sensible to the changes associated to the hydrolysis in comparison to the FRAP assay (pH 3.6, 37 $^{o}$C, ferric reducing power). In addition, the use of DPPH assay (room temperature, DPPH radical)

for berries may have controversial outcomes since the color of anthocyanins could influence the absorbance of the DPPH solution.

Finally, lipophilic compounds, such as carotenoids (Lako et al., 2007), and terpenes (Karioti, Skaltsa, & Gbolade, 2007; Pino et al., 2004) also contribute to the antioxidant effects of red-jambo fruit and leaves. However, the composition of carotenoids in red-jambo fruit remain unclear, since some studies either found β-carotene in the edible parts (Lako et al., 2007), either not (Khoo, Ismail, Mohd-Esa, & Idris, 2008). Nevertheless, the total carotenoids results indicate the importance of these compounds in the fruit (Khoo et al., 2008). The peel of red-jambo fruit might concentrate carotenoids and other lipids from the fruit (as suggested by total lipids, Table 1), which could explain the highest value found in the lipophilic antioxidant capacity.

This work showed that *S. malaccense* contains several bioactive compounds that could be used in the search of dietary therapies to help prevent certain diseases or maintain oxidative balance.

## 5. Conclusions

The fresh pulp and peel of red-jambo showed acid characteristics, elevated moisture content, and solid part containing bioactive compounds with nutritional and pharmacological interest. Each part of *S. malaccense* could be highlighted for different chemical characteristics: the pulp was a rich source of soluble fibers and reducing sugars; the peel concentrated the insoluble fibers, lipid content, lipophilic/hydrophilic antioxidant power, and anthocyanins; the seeds could be also highlighted by both lipophilic and hydrophilic antioxidant power; and finally the leaves that provide large amounts of catechins, quercetin, carotenoids, and great antioxidant capacity.

**Acknowledgments**

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #300533/2013-6, #142493/2013-9), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and scholarship, and to Luiz Claudio Correa for helpful collaboration.

## 6. References

Batista, A. G., Lenquiste, S. A., Cazarin, C. B. B., da Silva, J. K., Luiz-Ferreira, A., Bogusz, S., Hantao, L. W., de Souza, R. N., Augusto, F., Prado, M. A., & Marostica, M. R. (2014). Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *Journal of Functional Foods, 6*, 450-461.

Andersson Dunstan, C., Noreen, Y., Serrano, G., Cox, P. A., Perera, P., & Bohlin, L. (1997). Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosynthesis and rat ear oedema assays. *Journal of Ethnopharmacology, 57*(1), 35-56.

AOAC. (2002). *Official methods of analysis of AOAC International*. Gaithersburg, Md.: AOAC International.

Arumugam, B., Manaharan, T., Heng, C. K., Kuppusamy, U. R., & Palanisamy, U. D. (2014). Antioxidant and antiglycemic potentials of a standardized extract of Syzygium malaccense. *LWT - Food Science and Technology*, *59*(2, Part 1), 707-712.

Bairy, K. L., Sharma, A., & Shalini, A. (2005). *Evaluation of the Hypoglycemic, Hypolipidaemic and Hepatic Glycogen Raising Effects of Syzygium malaccense Upon Streptozotocin Induced Diabetic Rats*.

Batista, Â. G., Ferrari, A. S., da Cunha, D. C., da Silva, J. K., Cazarin, C. B. B., Correa, L. C. (2016). Polyphenols, antioxidants, and antimutagenic effects of Copaifera langsdorffii fruit. *Food Chemistry, 197, Part B*, 1153-1159.

Bligh, E. G., & Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911-917.

Careri, M., Elviri, L., Mangia, A., & Musci, M. (2000). Spectrophotometric and coulometric detection in the high-performance liquid chromatography of flavonoids and optimization of sample treatment for the determination of quercetin in orange juice. *Journal of Chromatography A, 881*(1–2), 449-460.

Higby, W. K. (1962). A Simplified Method for Determination of Some Aspects of the Carotenoid Distribution in Natural and Carotene-Fortified Orange Juice. *Journal of Food Science, 27*(1), 42-49.

Kaczmarczyk, M. M., Miller, M. J., & Freund, G. G. (2012). The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism-Clinical and Experimental, 61*(8), 1058-1066.

Kapasakalidis, P. G., Rastall, R. A., & Gordon, M. H. (2006). Extraction of Polyphenols from Processed Black Currant (Ribes nigrum L.) Residues. [doi: 10.1021/jf052999l]. *Journal of Agricultural and Food Chemistry, 54*(11), 4016-4021.

Karioti, A., Skaltsa, H., & Gbolade, A. A. (2007). Analysis of the leaf Oil of Syzygium malaccense Merr. et Perry from Nigeria. [doi: 10.1080/10412905.2007.9699290]. *Journal of Essential Oil Research, 19*(4), 313-315.

Khoo, H., Ismail, A., Mohd-Esa, N., & Idris, S. (2008). Carotenoid Content of Underutilized Tropical Fruits. *Plant Foods for Human Nutrition, 63*(4), 170-175.

Lako, J., Trenerry, V. C., Wahlqvist, M., Wattanapenpaiboon, N., Sotheeswaran, S., & Premier, R. (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chemistry, 101*(4), 1727-1741.

Lee, J., Rennaker, C., & Wrolstad, R. E. (2008). Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods. *Food Chemistry, 110*(3), 782-786.

Maisuthisakul, P., Pasuk, S., & Ritthiruangdej, P. (2008). Relationship between antioxidant properties and chemical composition of some Thai plants. *Journal of Food Composition and Analysis, 21*(3), 229-240.

Milala, J., Kosmala, M., Sójka, M., Kołodziejczyk, K., Zbrzeźniak, M., & Markowski, J. (2013). Plum pomaces as a potential source of dietary fibre: composition and antioxidant properties. *Journal of Food Science and Technology, 50*(5), 1012-1017.

Morton, J. F. (1987). Malay apple. In J. F. Morton (Ed.), *Fruits of warm climates* (pp. xii, 505 p., 524 p. of plates). Miami, FL: Creative Resources Systems

Nair, M. P., Mahajan, S., Reynolds, J. L., Aalinkeel, R., Nair, H., Schwartz, S. A. (2006). The Flavonoid Quercetin Inhibits Proinflammatory Cytokine (Tumor Necrosis Factor Alpha) Gene Expression in Normal Peripheral Blood Mononuclear Cells via Modulation of the NF-κβ System. *Clinical and Vaccine Immunology, 13*(3), 319-328.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry, 153*(2), 375-380.

Nile, S. H., & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. *Nutrition, 30*(2), 134-144.

Ou, K. Q., & Gu, L. W. (2014). Absorption and metabolism of proanthocyanidins. *Journal of Functional Foods, 7*, 43-53.

Palma, M., Pineiro, Z., & Barroso, C. G. (2002). In-line pressurized-fluid extraction-solid-phase extraction for determining phenolic compounds in grapes. *Journal of Chromatography A, 968*(1-2), 1-6.

Paredes-López, O., Cervantes-Ceja, M., Vigna-Pérez, M., & Hernández-Pérez, T. (2010). Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life—A Review. *Plant Foods for Human Nutrition, 65*(3), 299-308.

Pino, J. A., Marbot, R., Rosado, A., & Vazquez, C. (2004). Volatile constituents of Malay rose apple [Syzygium malaccense, (L.) Merr. & Perry]. *Flavour and Fragrance Journal, 19*(1), 32-35.

Prior, R. L., Hoang, H., Gu, L. W., Wu, X. L., Bacchiocca, M., Howard, L. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry, 51*(11), 3273-3279.

Pyrzynska, K., & Biesaga, M. (2009). Analysis of phenolic acids and flavonoids in honey. *TrAC Trends in Analytical Chemistry, 28*(7), 893-902.

Reynertson, K. A., Yang, H., Jiang, B., Basile, M. J., & Kennelly, E. J. (2008). Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. *Food Chemistry, 109*(4), 883-890.

Savitha, R. C., Padmavathy, S., & Sundhararajan, A. (2011). Invitro antioxidant activities on leaf extracts of syzygium malaccense (L.) merr and perry. *Anc Sci Life, 30*(4), 110-113.

Soong, Y.-Y., & Barlow, P. J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry, 88*(3), 411-417.

Williamson, G., & Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *The American Journal of Clinical Nutrition, 81*(1), 243S-255S.

Wrolstad, R. E. (Ed.). (1976). *Color and pigment analysis in fruit products*. Corvallis: Oregon Agricultural Experimental Station.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555-559.

Wellburn A.R. (1994). The spectral determination of chlorophyll-a and chlorophyll-b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *J Plant Physiol* 144: 307–313.

## CAPÍTULO 3

**Artigo Original: SUPLEMENTAÇÃO COM A FRUTA DE JAMBO -VERMELHO (*Syzygium malaccense*) PROTEGE O CÉREBRO DE ANIMAIS CONTRA DANOS CAUSADOS POR DIETA HIPERLIPÍDICA E MELHORA FUNÇÕES COGNITIVAS**

Ângela Giovana Batista, Monique Culturato P. Mendonça, Edilene Siqueira Soares, Juliana Kelly da Silva, Cesar R. Sartori, Maria Alice da Cruz-Höfling and Mário Roberto Maróstica Júnior. ***Syzygium malaccense* fruit supplementation protects mice brain against high-fat diet impairment and improves cognitive functions.** A ser submetido à revista ***Nutrition Research,*** Elsevier, ISSN: 0271-5317.

Vide autorização CEUA no Anexo 2.

Research article

# *Syzygium malaccense* fruit supplementation protects mice brain against high-fat diet impairment and improves cognitive functions

Ângela Giovana Batista[1], Monique Culturato P. Mendonça[2,3], Edilene Siqueira Soares[3], Juliana Kelly da Silva[1], Cesar R. Sartori[4], Maria Alice da Cruz-Höfling[2,3] and Mário Roberto Maróstica Júnior[1,*].

[1]School of Food Engineering, Department of Food and Nutrition, University of Campinas, Campinas, SP, Brazil.
[2]Faculty of Medical Sciences, Department of Pharmacology, University of Campinas, Campinas, SP, Brazil.
[3]Institute of Biology, Department of Biochemistry and Tissue Biology, University of Campinas, Campinas, SP, Brazil.
[4] Institute of Biology, Department of Department of Structural and Functional Biology, University of Campinas, Brazil.

*Corresponding author:
Mário Roberto Maróstica Júnior
Contact details: Departamento de Alimentos e Nutrição. Rua Monteiro Lobato, 80. Cidade Universitária. Campinas, SP, Brasil. Zip Code: 13083-862. E-mail: mario@fea.unicamp.br

**Abbreviations:** AD: Alzheimer's disease; AKT: protein kinase B; CAT: catalase; EAT: epidiymal adipose tissue; GSH: reduced glutathione; GSK3-β: Glucogen synthase kinase 3-β; GPx: glutathione peroxidase; GRd: glutathione reductase; IRS: insulin receptor substrate; MAT: mesenteric adipose tissue; MWM: Morris water maze; NFT: neurofibrillary tangles; RAT: retroperitoneal adipose tissue; SMF: *Syzygium malaccense* fruit SOD: superoxide dismutase; Tau: microtubule-associated protein tau; TBARS: thiobarbituric acid reactive substances.

**ABSTRACT**

Berries contain flavonoids involved in several mechanisms related to prevention of cognitive deficit, obesity, and insulin resistance. Accumulating evidence suggests that insulin resistance is the link associating obesity and Alzheimer's disease (AD). In this study, a high-fat diet was used to induce obesity and insulin resistance in mice and compared to a normal-fat standard diet. We have supplemented the both diets with 5% powder of freeze-dried *Syzygium malaccense* fruit (SMF) and investigated proofs of peripheral insulin resistance, hippocampal insulin sensitivity, learning/memory performance, tau protein stability, and brain frontal lobe oxidative stress. The high-fat diet supplemented with SMF (HS group) did not prevent weight gain but showed peripheral insulin sensitivity, improved insulin signaling in the hippocampus, which consequently reduced GSK3-β activity and lowered tau phosphorylation. The results from Morris water maze cognitive test were consistent with the finding, once the HS group showed better performance on learning/ memory tasksrelated to the hippocampus. Brain frontal lobe antioxidant enzyme activities were down-regulated when high-fat diet was consumed but the supplementation with SMF prevented lipid peroxidation and improved activities of catalase, superoxide dismutase, and glutathione peroxidase. SMF intake also elevated serum antioxidant defenses. The therapeutic intervention with SMF was effective in minimizing cognitive dysfunctions and in preventing risk factors associated to AD development in mice fed high-fat diet without changing energy metabolism.

Keywords: *Myrtaceae*; Malay apple; tauopathies; anthocyanins; metabolic diseases.

## 1. Introduction

Accumulating evidence suggests that obesity is associated with a higher risk of dementia and Alzheimer's disease (AD) occurrence in middle or late life [1-3]; and insulin resistance may be the causative link between them [2]. Furthermore, mechanistic studies report that diet-induced obesity enhances AD-associated pathological processes increasing tau (microtubule-associated tau protein) phosphorylation in hippocampus and diminishes cognitive function due to insulin resistance [4-7].

In brief, impaired signaling of insulin in neurons involves phosphorylation of tau, leading to the formation of neurofibrillary tangles (NFT), a marker of neuronal alterations in AD [7]. On the other hand, when insulin is able to bind its receptor in neurons, the signaling cascade phosphorylates GSK3-β in Serine 9, blocking tau phosphorylation, and then, preventing the formation of NFT and further propagation to neuron losses [8-9]. Peripheral hyperinsulinemia also impairs insulin transit through the blood-brain barrier, exacerbating brain insulin resistance ref. Accordingly, a study showed a 56% increased risk of AD in patients with history of diabetes compared with non-diabetic subjects [10]. Consequently, AD was already believed to represent a form of diabetes mellitus that affects the brain as result of insulin resistance [11].

In the present study, we focus on how diets can play a role in preventing tau phosphorylation through obesity-related metabolic changes. For instance, the supplementation of diets with berries is related to better performance of cognitive behavioral tasks and increased hippocampal plasticity [12-14]. Berry fruits contain flavonoids involved in several mechanisms related to prevention of insulin resistance, obesity and cognitive deficit [12, 13, 15]. *Syzygium malaccense* (*Myrtaceae* family) fruit contains flavonoids such as cyanidin 3-glucoside [16], an anthocyanin compound related with antioxidant activity, anti-obesogenic and anti-diabetogenic effects [15].

A study has shown that mice fed a high-fat diet plus an isolated flavonoid present in grapes had lower levels of tau phosphorylation in hippocampus and improved performance in Morris water maze task [6]. However, it remains unclear whether berry fruit intake, rather than its isolated (poly)phenolic compounds, can modulate tau-related pathological processes in mice in an obesogenic condition. In the present study we evaluated the effect of normal and high-fat diets supplemented with *Syzygium malaccense* fruit (SMF) on peripheral and hippocampal insulin resistance associated with phosphorylation of tau. Furthermore, central oxidative stress, learning and memory were investigated.

## 2. Material and Methods

### 2.1. Fruits' characteristics

*S. malaccense* fruits were obtained and characterized as described in a previous study [16] and below. Fruits were harvested in Araçatuba - SP and purchased from a local market in Campinas-SP, Brazil. Peel and pulp were sliced and freeze-dried in order to obtain a powder [16]. The freeze-dried peel + pulp was characterized as follows: 33.7% total dietary fibers, from which 27.9% are insoluble and 5.6% soluble dietary fibers. Among the 16 (poly)phenols already indentified in pulp + peel of SMF (total of 81 mg 100 $g^{-1}$), we highlight cyanidin 3-glucoside, cyanidin 3,5-diglucoside, isorhamnetin 3-glucoside, kaempferol 3-glucoside and procyanidins [16].

### 2.2. Animal proceedings

All *in vivo* experiments were carried out in accordance with the Brazilian Society of Laboratory Animal Science (SBCAL) guidelines and approved by the Institutional Committee for Ethics in Animal Use (CEUA/IB/UNICAMP, protocol #3157-1).

Forty male Swiss mice (*Mus musculus*, 8-weeks old, 37.6 ± 3.5 g) acquired from the Multidisciplinary Center of Biological Investigation (CEMIB/UNICAMP) were housed individually in cages under 22 ± 2 °C, 50 - 60% humidity and standard inverted 12/12 h dark/light cycles. During 10 weeks of experiment, the animals had free access to water and semi-purified based-diets (AIN93-M) [17]: i) a normal-fat diet (NF); ii) normal-fat diet containing 5% (w/w) of *S. malaccense* fruit (NS); iii) a high-fat diet (HF), in which 60% total calories were fat; and iv) the high-fat diet containing 5% (w/w) of *S. malaccense* fruit (HS) (Table 1). The dose of SMF in the diet was chosen in order to offer about 0.6 mg of total (poly)phenols and 14 mg of total soluble dietary fiber [16] as supplementation. The animals were divided into 4 groups in accordance to the diets NF, NS, HF or HS (*n*=10/diet). Body weight and diet consumption were checked weekly.

**Table 1**- Composition of experimental diets (g $kg^{-1}$) by mice groups.

| Ingredient | NF | NS | HF | HS |
|---|---|---|---|---|
| Casein (83.95% protein) | 141.75 | 141.75 | 141.75 | 141.75 |
| Corn starch | 464.56 | 441.27 | 264.25* | 240.96 |
| Maltodextrin | 154.62 | 146.87 | 87.95* | 80.20 |
| Sucrose | 99.76 | 94.76 | 56.74* | 51.74 |
| Soy oil | 40.00 | 40.00 | 40.00 | 40.00 |
| Lard | - | - | 310.00 | 310.00 |
| Cellulose | 50.00 | 36.04** | 50.00 | 36.04** |
| Mineral mix | 35.00 | 35.00 | 35.00 | 35.00 |
| Vitaminic mix | 10.00 | 10.00 | 10.00 | 10.00 |
| L-Cystine | 1.80 | 1.80 | 1.80 | 1.80 |
| Choline bitartrate | 2.50 | 2.50 | 2.50 | 2.50 |
| *tert*-Butylhydroquinone | 0.008 | 0.008 | 0.008 | 0.008 |
| *S. malaccense* fruit | - | 50.00 | - | 50.00 |
| Calories[a] | 3786.18 ± 2.69 | 3773.90 ± 7.27 | 5464.62 ± 12.89 | 5426.25 ± 33.22 |

*The carbohydrate ingredients were changed according to the addition of red-jambo fruit powder and/or lard content. **The cellulose content was discounted according to the amount of insoluble dietary fibers of red-jambo pulp+peel (BATISTA, Â. G. et al., 2017). [a]Calories values (kcal $kg^{-1}$) were calculated considering the conversion factors of Atwater. NF= group that received normal-fat diet; NS= group that received normal-fat diet containing 5% SMF; HF= high-fat diet group; HS= high-fat diet supplemented with 5% SMF.

### 2.2.1. Cognitive test - Morris Water Maze (MWM)

Spatial learning and memory based on hippocampal functions were assessed at the week 8 of treatment using an adaptation of the MWM test [18]. The maze consisted of a circular pool (120 cm in diameter, 50 cm high) filled with water (25 ± 1$^{o}$C) that was made opaque with nontoxic grey paint. The pool was located in a 3 m$^{2}$ room with extra-maze visual cues in the walls, red-light lamp bulbs, a video camera positioned 2 m above the center and the experimenter. The pool was divided into four quadrants: three quadrants were used as release positions and their order was systematically varied throughout the experiment sessions. A circular plastic escape platform (9 cm in diameter) was placed in one quadrant into the pool approximately at 1 cm below the water surface. Mice were trained in MWM over five consecutive daily sessions, the acquisition phase. During the acquisition sessions, each mouse participated in two trials per day with at least 30 min interval in-between them. A trial was started when the animal was released from one of three randomly chosen start positions. The escape latency was recorded (maximum of 60 s) when the mice found and climbed onto the platform, being there for more than 10 s. If the animal had not climbed onto the platform in 60 s, the experimenter guided the animal by hand to the platform and an escape latency of 60 s was recorded and kept there for 20 s. Twenty-four hours after the last acquisition session (day 6), a probe trial was used to assess spatial retention of the location of the hidden platform. For this purpose, the platform was removed from the maze, and each mouse was allowed to swim freely to search for the platform for 60 s.

During the probe test, the circular area surrounding the location where the platform was hidden previously was delimited three times larger and used as counting zone to determine the times crossing the area. The time of swimming into the quadrant where the platform was previously hidden was also recorded for each mouse in order to determine memory retention.

### 2.2.2 Insulin resistance assessment

*Glucose tolerance test (GTT):* The GTT was performed injecting a D-glucose solution (2 g $kg^{-1}$) via intraperitoneal (ip) in the 6h-fasted mice. Blood glucose was measured via tail vein at fasting, 30, 60, 90 and 120 min after the injection. The test was carried out in the 9th week of experiment using a glucometer and respective test strips (FreeStyle Lite, Abbott, Alameda, CA, USA).

*Insulin tolerance test (ITT):* For the ITT a 0.9% saline solution containing 0.75 units $kg^{-1}$ insulin (Novolin R, Novo Nordisk Bagsvaerd, DK) was ip-injected in the 6 h-fasted mice and blood glucose was measured via tail vein at fasting, 10, 20, 30, 45, and 60 min after the injection. The ITT was done in the $10^{th}$ week of treatment using the glucometer and test strips.

*Fasting glucose and insulin:* In the last day of experiment the fasting (6 h) blood glucose was measured via tail vein using the glucometer. Insulin was assessed in serum using a specific ELISA kit (Cat. #EZRMI-13K, Millipore, St. Charles, Missouri, USA).

### 2.2.3 Sampling

At the end of the experiment, the animals were anesthetized (100 mg/kg ketamine chloride: 10 mg/kg xylazine chloride (Fortvale, Valinhos, SP, Brazil)) and euthanized. The blood samples were obtained by cardiac puncture and collected in serum separator gel tube.

After euthanizing, brains were quickly removed, washed in a 0.9% saline solution, weighed, frozen in liquid nitrogen and kept at -80°C. Later, to dissect the hippocampus, the frozen brain was placed in a clean Petri dish over ice and covered with buffer. Using a scalpel, the hindbrain and olfactory bulb were removed. Next and carefully, the cerebral cortex was detached from hippocampus using tweezers. Due to the presence of ventricles between midbrain and neocortex, the hippocampus could be easily separated [19]. The tissue was homogenized in anti-protease cocktail (Section 2.2.5). The frontal lobe of the brain was taken

out and were homogenized in phosphate buffer (PB), pH 7.4 (approximately 100 mg $mL^{-1}$) and the homogentaes used for antioxidant status analyses (Section 2.2.4).

Epididymal adipose tissue (EAT), retroperitoneal adipose tissue (RAT), and mesenteric adipose tissue (MAT) of mice were rapidly dissect, washed in 0.9% saline solution, dried in appropriated tissue paper and weighed. The EAT was further used to analyze the expression of inflammatory marker in section 2.2.6.

**2.2.4 Antioxidant status analyses**

The measurements of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GRd), and superoxide dismutase (SOD) values in serum and brain frontal lobe homogenates were conducted as detailed below. Brain frontal lobe has close relation to prospective memory and correlates with hippocampal memory function [20-21].

The TBARS values were determined using the method described by Ohkawa, Ohishi and Yagi [22], with adaptations. Serum or homogenate's supernatant were mixed in 8.1% sodium dodecyl sulphate, and 0.53% TBA (2-thiobarbituric acid) solution in 20% acetic acid and 5% sodium hydroxide. After heating at 95 °C for 60 min, samples were cooled in an ice bath for 10 min and then centrifuged at 10,000 g, 10 min, 4 $^{o}$C. The resulting MDA-TBA and related adducts were read at 532 nm using a 96-well microplate. A standard curve was prepared using the MDA standard (1,1,3,3-tetramethoxypropane, Sigma-Aldrich, St Louis MO, USA).

The SOD activity was measured by mixing the homogenate's supernatant or serum with working solution (0.1 mmol $L^{-1}$ hypoxanthine, 0.07 U xanthine oxidase and 0.6 mmol $L^{-1}$ NTB in PB in 1: 1: 1 proportions) and read at 560 nm for 10 min. The SOD activity was expressed as U $mg^{-1}$ protein [23].

GPx working reagent (10 mmol $L^{-1}$ reduced glutathione, 4 mmol $L^{-1}$ NADPH (β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate) and 1 U glutathione reductase) was added to PB-homogenate's supernatant or serum and mixed with 0.25 mmol $L^{-1}$ $H_2O_2$ [24]. The decrease in absorbance was monitored at 365 nm and the results were expressed as nmol NADPH consumed $min^{-1}$ $mg^{-1}$ protein.

GRd activity was measured in PB-homogenate's supernatant [25], following the decrease in absorbance at 340 nm induced by oxidized glutathione in the presence of NADPH in phosphate buffer. The results were expressed as nmol NADPH consumed $min^{-1}$ $mg^{-1}$ protein.

For catalase (CAT) activity**,** the method of Johansson [26] was adapted. Brain frontal lobes homogenates and serum were diluted in 25 mmol $L^{-1}$ PB (pH 7.5, containing 1 mmol $L^{-1}$ EDTA and 0.1% BSA) and added to 96-well microplate containing 100 mM PB (pH 7.0) and methanol. Briefly, 10.5 mmol $L^{-1}$ $H_2O_2$ was added to the microplate and allowed to react for 20 min in a microplate shaker in dark conditions. After, 7.8 mol $L^{-1}$ KOH and 34.2 mmol $L^{-1}$ Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) solution were added to the microplate and allowed to react for 10 min in a shaker; and then a 65.2 mmol $L^{-1}$ potassium periodate solution were added and mixed during 5 min before reading at 540 nm. Formaldehyde was used as standard and results were expressed as nmol of formaldehyde formation per minute at 25 $^{o}$C.

### 2.2.5 Western Blotting

Western blotting was performed in hippocampal homogenates (*n* = 3-5/group) from the same animals used for antioxidant enzyme activity analyses (Section 2.2.4). Briefly, frozen hippocampi were homogenized in an extraction cocktail (10 mmol $L^{-1}$ EDTA, 2 mmol $L^{-1}$ phenylmethane sulfonyl-fluoride, 100 mmol $L^{-1}$ NaF, 10 mmol $L^{-1}$ sodium pyrophosphate,

10 mmol $L^{-1}$ $NaVO_4$, 10 μg $mL^{-1}$ aprotinin and 100 mmol $L^{-1}$ Tris, pH 7.4), except for 8% used to phospho-IRS and total IRS. After electrotransfer, the membranes were prior incubated with 5% bovine serum albumin to block non-specific antigenic sites followed by washing with TBS-T (0.1% Tris-buffered saline with 0.05% Tween 20, pH 7.4). Subsequently, the membranes were incubated with primary antibodies as follows: p-IRS-1 (Tyr 632)-R, IRS-1 (E-12), p-AKT 1/2/3 (Ser 473)-R, AKT 1/2/3 (H-136), p-GSK-3β (Ser 9), GSK-3β (H-76), p-tau (Thr 205), and tau (TAU-5) from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Then, the membranes were washed with TBS-T and incubated with appropriate secondary antibody.

The proteins were visualized using an enhanced chemiluminescence substrate (Thermo Scientific, Waltham, MA, USA) and blot band densitometry was acquired using the NIH Image J 1.45s software (Wayne Rasband, NIH, Bethesda, MD, USA). All proteins were normalized by β-actin (Sigma-Aldrich) internal control and the results were expressed as a ratio of p-proteins/ total proteins in the case of the phosphorylated ones.

### 2.2.6. RNA extraction and quantitative real-time PCR

Total RNA was extracted from EAT of non-fasted mice ($n$ = 5) using a commercial acid phenol reagent, Trizol (Invitrogen, CA, USA). RNA integrity was confirmed by non-denaturing agarose gel electrophoresis. First strand complementary DNA was synthesized using SuperScript III RT and random hexamer primers, as described in the manufacturer's protocol (Invitrogen). Quantitative PCR was run to determine the expression of IL-1β (Mm00434228_m1; Applied Biosystems, CA, USA) and IL-6 (Mm00446190_m1; Applied Biosystems) and TNF-α (Mm00443258_m1). The reference gene was glyceraldehyde-3-phosphate dehydrogenase (#4352339E; Applied Biosystems). Real-time PCR analysis of gene expression was carried out in an ABI Prism 7500 sequence detection system (Applied

Biosystems). Each PCR contained 25 ng of reverse-transcribed RNA and was run according to the manufacturer's recommendations using the TaqMan PCR master mix. Real-time data were analyzed using the Sequence Detector System 1.7 (Applied Biosystems). Results were expressed as relative transcript amounts.

### 2.3 Statistics

Weekly weight gain and MWM acquisition were analyzed using two-way ANOVA followed by Bonferroni test ($p < 0.05$). Student's *t* test ($p < 0.05$) was used to compare group pairs according to the fat content in the diets (NF × HF; NF × NS and HF × HS). All tests were carried out using GraphPad Prism 5.0 software (GraphPad, Inc. La Jolla, CA, USA).

## 3. Results

### 3.1. *Syzygium malaccense* supplementation did not affect body weight gain and brain weight

The HF group had the lowest value of food intake, but the highest for calorie intake among the groups reflecting its higher body weight and weight gain in comparison to the N group (Figure 1). The body weight of HF animals was higher than in NF since the second week of experiment. The supplementation of the normal and high-fat diets with SMF did not cause significant impact on the food/calorie intake nor in body weight gain as shown in Figure 1.

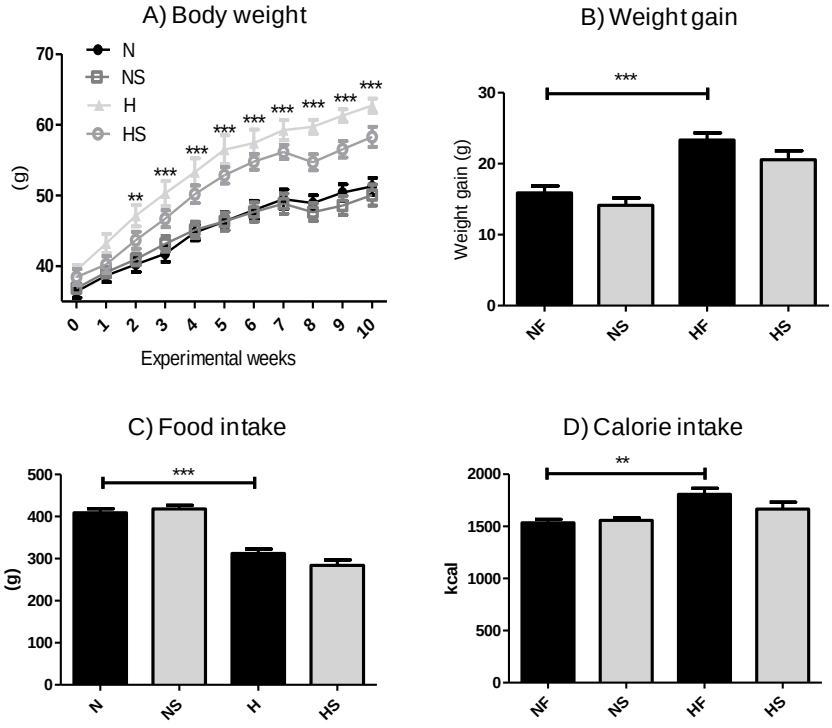



**Figure 1** - Weight growing curves and feeding parameters. A) Body weight during 10 experimental weeks; B) total weight gain; C) total food intake; and D) total calorie intake. NF= group that received normal-fat diet; NS= group that received normal-fat diet containing 5% SM; HF= high-fat diet group; HS= high-fat diet with 5% SM. In A the weight x time interactions was assessed by two-way ANOVA and Bonferroni test. In B, C and D Student's *t* test was applied. *Indicates $p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, $n = 10$.

Although, even if the body weight did not change for the groups fed the SM-containing diets, the EAT and RAT were smaller in the group HS when compared to HF (Table 2). The MAT weight was higher in HF in comparison to NF, but no differences were found in NS or HS (Table 2).

**Table 2.** Percentual tissue ratio of body weight.

| | NF | NS | HF | HS |
|---|---|---|---|---|
| EAT | 4.18 ± 0.24 | 3.75 ± 0.24 | 5.28 ± 0.43* | 3.51 ± 0.32** |
| RAT | 1.72 ± 0.21 | 1.29 ± 0.12 | 2.69 ± 0.27* | 1.49± 0.17** |
| MAT | 1.95 ± 0.10 | 1.74± 0.40 | 2.84 ± 0.19** | 1.98 ± 0.31* |
| Brain | 0.614 ± 0.018 | 0.658 ± 0.040 | 0.532 ± 0.011** | 0.538 ± 0.019 |

NF= group that received normal-fat diet; NS= group that received normal-fat diet containing 5% SM; HF= high-fat diet group; HS= high-fat diet with 5% SM. EAT=epididymal adipose tissue; RAT retroperitoneal adipose tissue; MAT= mesenteric adipose tissue *Indicates $p < 0.05$ and **$p < 0.01$ according to Student's *t* test between groups (NF × HF and HF × HS) , $n = 10$.

The total brain weight was inversely correspondent with body weight. The relative brain weight of the SMF-fed animals did not change in relation to their control groups.

### 3.2 *Syzygium malaccense* fruit intake improves peripheral insulin sensitivity

In the GTT and ITT tests, the HF group had higher response to both glucose and insulin injection when compared to the NF group (Figure 2 A-D). Although not statistically significant ($p > 0.05$), the blood glucose level was lower in the HS group than in the HF group in the end of the glycemia curve (Figure 2 A), corroborating to a trend toward to lower AUC in the HS group in comparison to HF ($p = 0.09$) (Figure 2 B). Blood glucose response after insulin injection in ITT drives to similar results, showing better insulin sensitivity in HS *versus* HF: lower glycemia curve and AUC values (Figures 2 C and D). Fasting glucose in the last experimental day was also higher in HF in comparison to NF and HS groups ($p < 0.05$). No statistical differences were found in circulating insulin ($p > 0.05$).

When real time -PCR was used to investigate inflammatory cytokine m-RNA levels in the EAT, HS group showed lower transcription of IL-6 and TNF-α relative to HF ($p < 0.05$; Figure 2).

### 3.3 *Syzygium malaccense* fruit intake increases serum and brain antioxidant defense

The antioxidant defenses in serum did not change with high-fat feeding, except for lower SOD activity in serum of HF mice in comparison to NF (Figure 3). However, the addition of SM in normal-fat diet increased GRd, GPx, SOD and CAT in serum. When added to the high-fat diet, SM revealed to improve GRd, GPx, SOD and CAT activities in serum, besides of reducing lipid peroxidation in comparison to the HF group.

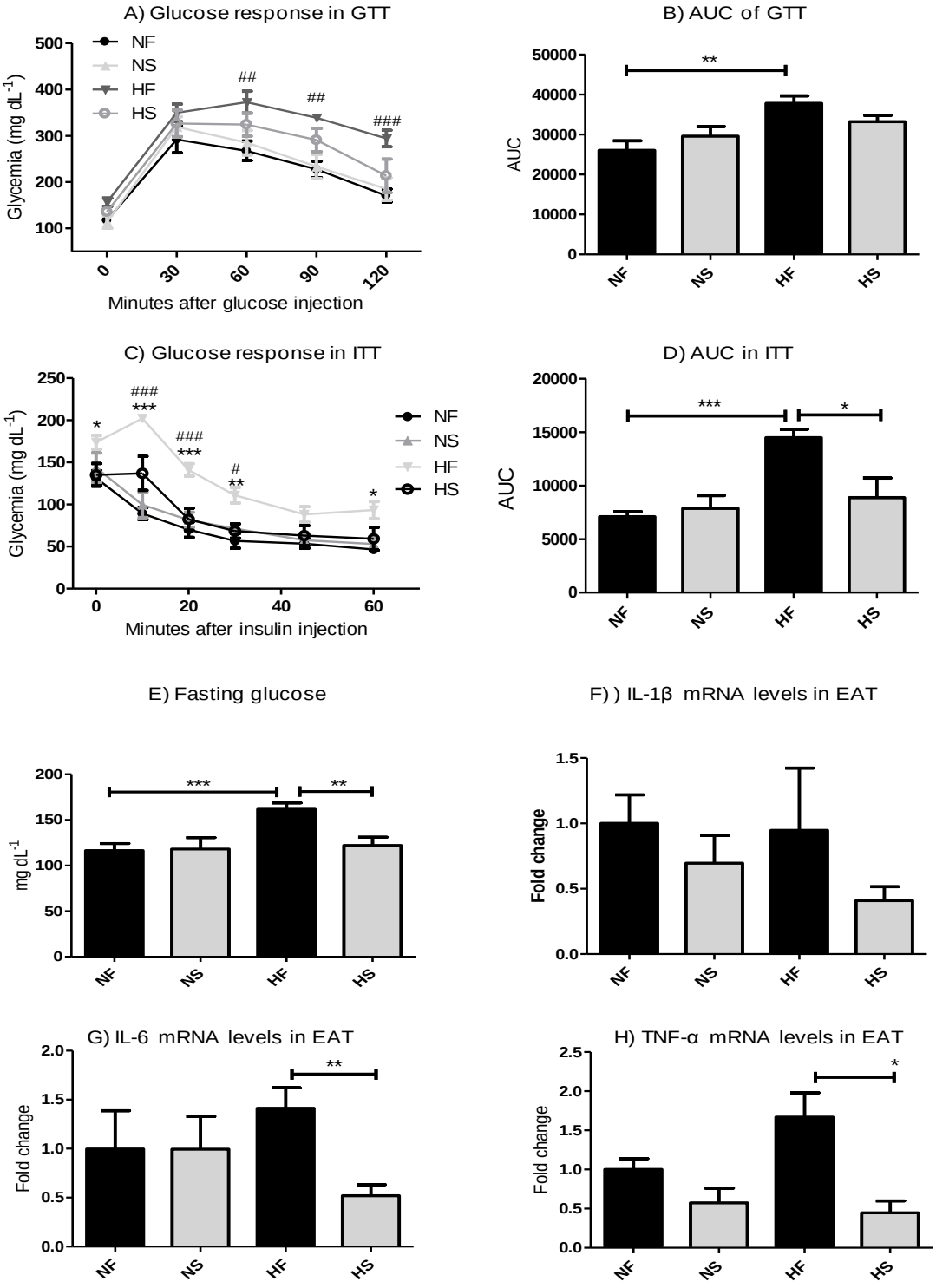



**Figure 2 -** Peripheral insulin resistance measurements, glucose and insulin levels in the mice. A) Glycemic response in GTT test; B) AUC response in GTT test; C) Glycemic response in ITT test; D) AUC response in ITT test; E) Fasting blood glucose levels; F) mRNA levels of IL-1β; G) mRNA levels of IL-6 and H) TNF-α in EAT. NF= group that received normal-fat diet; NS= group that received normal-fat diet containing 5% SM; HF= high-fat diet group; HS= high-fat diet with 5% SM. In A and C the glycemia × time interactions was assessed by two-way ANOVA and Bonferroni test (*indicates differences between NF × HF and #HF × HS). In B and D-G Student's *t* test was applied. *Indicates $p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, $n = 5$.

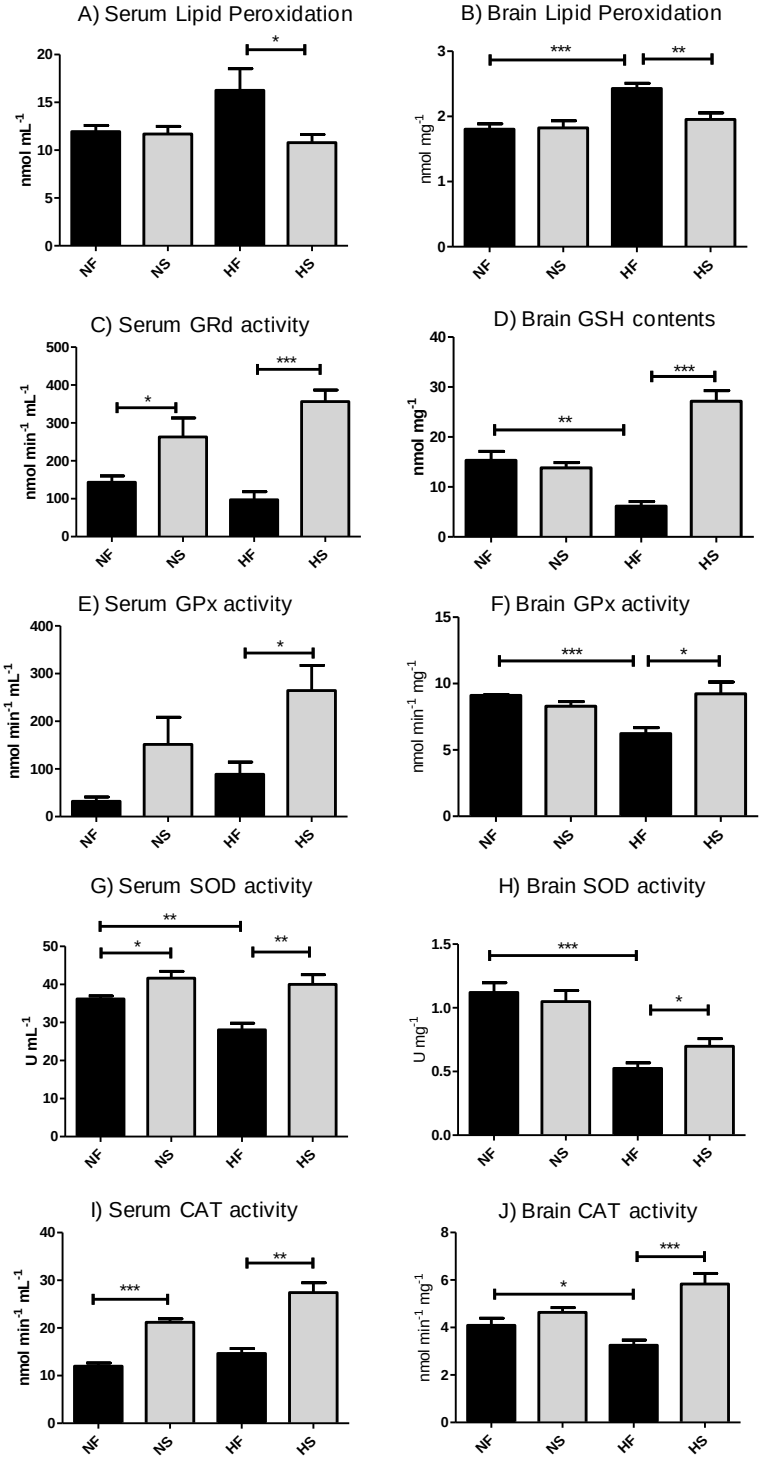



**Fig 3.** *S. malaccense* fruit intake reduced lipid peroxidation and increased antioxidant enzyme activities in brain frontal lobe. A) Malondialdehyde values; B) GSH contents; C) GPx , D) GRd, E) SOD, and F) CAT activities. Data in C) and D) were expressed as nmol $min^{-1}$ consumed NADPH mg protein and in F) nmol $min^{-1}$ formed formaldehyde mg protein. *$p < 0.05$; ***$p < 0.01$; ***$p < 0.001$ indicate statistical differences according to Student's *t* test, $n = 5$.

The HF diet increased lipid peroxidation in the frontal lobe of brain as well as decreased the GSH levels (Fig.3). These findings are in agreement with previous studies linking high-fat diets or metabolic diseases to lower antioxidant defenses in total brain [26]. In contrast, the HS diet prevented lipid peroxidation as shown by the TBARS values (Fig.3) and enhanced the GSH values in the frontal lobe, showing benefits of SMF intake (Fig.3).

The activities of GPx, SOD and CAT in the frontal brain of mice were reduced with high-fat feeding, as shown by the differences between NF and HF groups (Fig.3). However, the GPx activity was higher in the brain of the HS group when compared to HF, as well as the activity of the antioxidant enzymes SOD and CAT (Fig.3). There were no differences among the groups for GRd activity (Fig. 3).

### 3.4 Hippocampal insulin resistance is minimized in *S. malaccense* fruit-fed animals

The insulin signaling pathway was studied in the hippocampus of the mice, a region of the brain involved in learning and memory. The higher phosphorylation of IRS in the groups NF and HS relative to the HF group, showed that the daily supplementation with 5% SMF for 10 weeks prevented the impairment of insulin signaling caused by the high-fat diet in the hippocampus, normalizing the values (Fig. 4.A). Following the signal cascade, the phosphorylation of AKT in the hippocampus of the HS animals was also higher than in the HF group, corroborating to the inactivation of GSK3-β and lower phosphorylation of tau in the group fed HS (Fig. 4).

GSK3-β phosphorylation in serine 9 was reduced in the HF-group's hippocampus when compared to NF counterpart, indicating higher activity of this kinase on phosphorylating tau in the mice. These findings suggest that the addition of SMF to the high-fat diet may protect mice against insulin resistance and hyperphosphorylation of tau mediated by GSK3-β activity (Fig. 4).

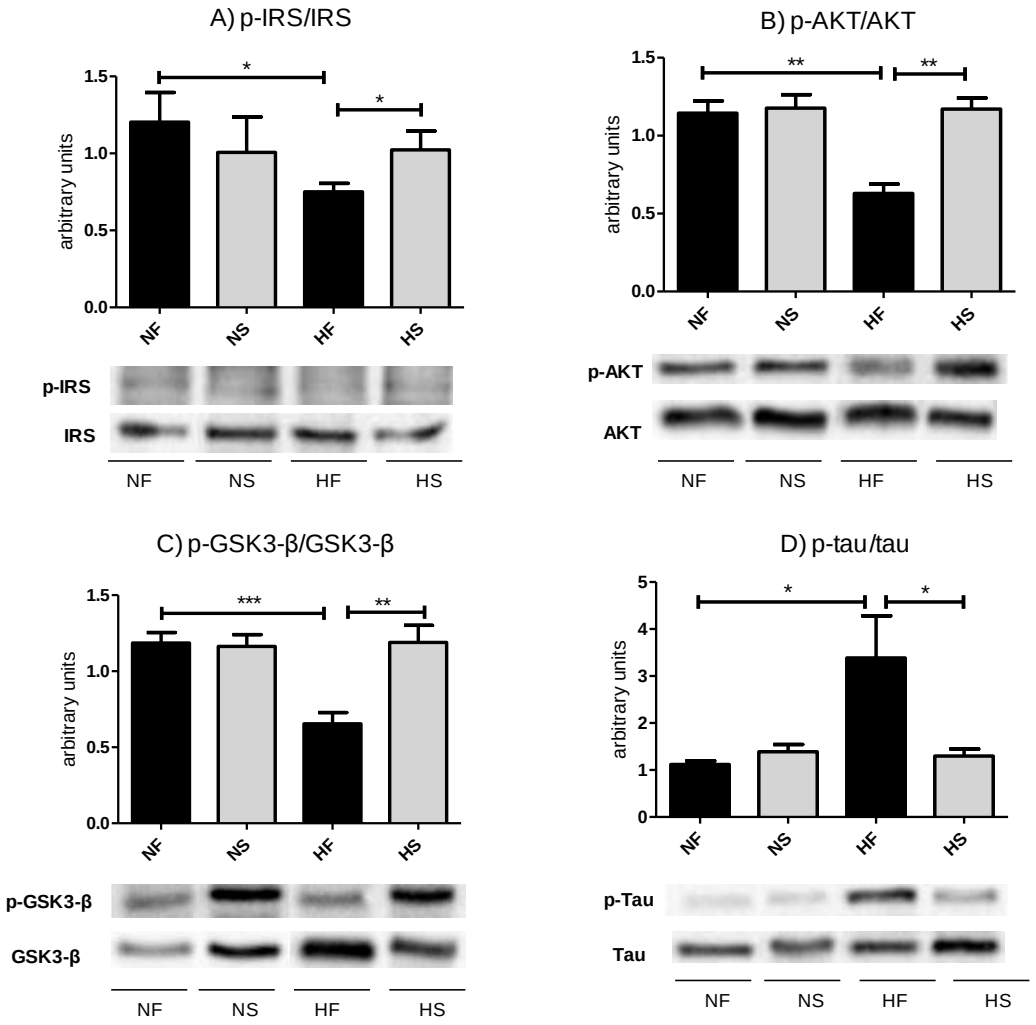



**Fig. 4.** *S. malaccense* fruit intake prevents tau hyperphosphorylation via hippocampal insulin signaling pathway (IRS-GSK3-β-Tau) and low-grade inflammation. Western blotting data for A) IRS phosphorylation; B) AKT phosphorylation; C) GSK3-β phosphorylation; D) Tau phosphorylation. * $p < 0.05$; *** $p < 0.01$; *** $p < 0.001$ indicate statistical differences

### 3.5. *Syzygium malaccense* fruit intake improved learning and memory

The Morris Water Maze cognitive test showed that the animals fed HF-diet had worse performance in the acquisition phase being this significantly different from the observed in the normal-fat groups on sessions 4 and 5, and HS animals at session 3 (Figure 5.A). Thus, the supplementation with SMF improved learning on the acquisition phase, corroborating to better memory performance proven by the probe test (Fig 5.B and C). The finding can be expressed by the longer time spent and crosses searching for the hidden platform found for the

groups fed normal-fat diets and the high-fat diet supplemented with SMF in comparison to HF group.

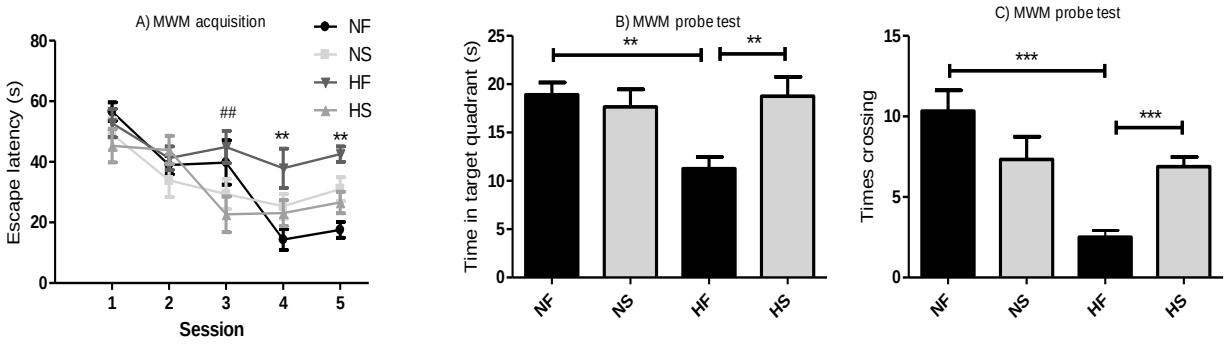



**Fig. 5.** *S. malaccense* fruit improves learning and memory assessed by the Morris Water Maze (MWM) test. A) Acquisition of memory on MWM test; b) MWM probe test as time spent in the target quadrant; and c) MWM probe test as times crossing the couting zone where the platform was hidden. Fig. 4.a) *Indicates statistical differences between NF × HF groups, and #HF × HS according to two-way ANOVA and Bonferroni. Fig. 4.b and c) *$p$< 0.05; ***$p$< 0.01; ***$p$< 0.001 indicate statistical differences according to Student's $t$ test, $n$=8-10.

## 4. Discussion

In our study, we observed that long-term intake of SMF-supplemented high-fat diet may prevent oxidative stress, transcription of pro-inflammatory cytokines, peripheral and hippocampal insulin resistance and hippocampal tau hyperphosphorylation with no association to weight loss.

Previous report proved that SMF is rich in (poly)phenols and mainly anthocyanins like cyanidin 3-glucoside [16]. Cyanidin 3-glucoside is one of the most common anthocyanins found in berries [28]. The peel of SMF contains great value of cyanidin 3-glucoside, claimed for its role in ameliorating obesity and diabetes metabolic markers [15]. However, in the present study the intake of the cyanidin-rich fruit was not able to prevent excessive weight gain linked to high-fat diet intake (Table 1).

Recent attention has been given to neuronal injuries caused by long-term intake of high-fat diets [4]. Among such injuries, neuron loss, oxidative stress, insulin resistance,

increment of inflammatory markers and cognitive deficits can be cited [4, 6]. We have proved that a high-fat diet (60% total calories from fats) promoted a reduction in brain weight and increased lipid peroxidation [27]. The present data showed that SMF addition to the diets did not prevent brain weight loss, but decreased lipid peroxidation, signal of oxidative stress in the brain frontal lobe of the animals when compared to the group fed HF diet. Food components like (poly)phenols and other antioxidant compounds, are metabolized and distributed in tissues, such as brain, where they may also play a role neutralizing free-radicals [27].

The antioxidant enzymes are known to protect the cells against oxidative stress, a state that could trigger a low grade inflammation, insulin resistance and cell death [28]. The biological antioxidant defenses was also increased in the serum and brain frontal lobe of HS animals, as it can be seen by the increase of GSH content and the activities of the antioxidant enzymes GPx, SOD and CAT (Fig. 3). A possible beneficial effect relating berry intake with these findings, is the interference of (poly)phenols activating proteins in pathways responsible for synthesis of antioxidant genes such as NRF2 [30], insulin signaling cascade proteins [31]. Such compounds may also prevent release of inflammatory cytokines via NFκB inactivation [31-33].

Previous report has shown that (poly)phenol-rich berry intake was a protection factor against insulin resistance [31]. Obese Swiss mice received for 6 weeks a high-fat diet containing jaboticaba Brazilian berry and showed higher peripheral insulin sensitivity via insulin tolerance test and activation of IRS-AKT-FoxO1 cascade [31]. A cross-sectional study has also shown that the intake of anthocyanin-rich foods was associated with lower insulin resistance, better economy on insulin release and lower inflammation levels in twin women [34]. Besides of enhanced peripheral insulin sensitivity observed with the supplementation of high-fat diet with anthocyanin-rich fruit, we have also shown improved hippocampal insulin

sensitivity in our study. After 10-weeks supplementation of the diet with SMF, obese mice had the IRS-AKT-GSK3-β activated and the phosphorylation of tau (Thr 205) suppressed in their hippocampus, without preventing weight gain. Same pattern was found previously when a high-fat diet supplemented with an isolated stilbene (resveratrol, 200 mg/kg) was given to mice for 20 weeks [6].

Together with β-amyloid, phosphorylation of tau is one of the most investigate marker of AD [34]. GSK3-β activating tau hyperphosphorylation via insulin signaling in hippocampus may be a link between high-fat diet consumption and memory impairment [4-5, 8, 36]. The present work suggests benefits of SMF supplementation of high-fat diet on preventing hipocampal tau phosphorylation and enhancing memory as seen in the MWM test. A study has also demonstrated that animals fed high-fat based-diet containing resveratrol, but not the supplementation of the normal-fat, were more sensitive to insulin and showed lower tau phosphorylation in the hippocampus and had learning and memory improved [6].

The underlying mechanisms for the berry fruit intake's effect on improvement in cognitive functions consist on indirect effects, like adjustments on inflammatory cytokines release [38], and lower oxidative stress [37, 39], supported by our findings. Moreover, other mechanisms may exist, since studies reported increased neurogenesis in the hippocampus of aged mice and increase of neurotrofins expression in aged and young rats after berry consumption [12, 14].

Taken together, our results showed that the supplementation of the high-fat diet with anthocyanin-rich *S. malaccense* fruit was able to improve the antioxidant defenses, peripheral and hippocampal insulin sensitivity, and lower phosphorylation of tau. The cognitive test corroborated the findings, since the high-fat diet containing the fruit prevented the detrimental effects of the high-fat diets on learning and memory.

**Funding:** The authors thank Brazilian foundations CNPq (#300533/2013-6 and #305099/2011-6) and CAPES for scholarship and financial support.

## 5. References

[1] Profenno LA, Porsteinsson AP, Faraone SV. Meta-Analysis of Alzheimer's Disease Risk with Obesity, Diabetes, and Related Disorders. Biological Psychiatry. 2010;67:505-12.

[2] Profenno LA, Faraone SV. Diabetes and overweight associate with non-APOE4 genotype in an alzheimer's disease population. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2008;147B:822-9.

[3] Emmerzaal TL, Kiliaan AJ, Gustafson DR. 2003-2013: A Decade of Body Mass Index, Alzheimer's Disease, and Dementia. Journal of Alzheimer's Disease. 2015;43:739-55.

[4] Ledreux A, Wang X, Schultzberg M, Granholm A-C, Freeman LR. Detrimental effects of a high fat/high cholesterol diet on memory and hippocampal markers in aged rats. Behav Brain Res. 2016;312:294-304.

[5] Koga S, Kojima A, Kuwabara S, Yoshiyama Y. Immunohistochemical analysis of tau phosphorylation and astroglial activation with enhanced leptin receptor expression in diet-induced obesity mouse hippocampus. Neuroscience Letters. 2014;571:11-6.

[6] Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, Kim HJ, et al. Resveratrol Attenuates Obesity-Associated Peripheral and Central Inflammation and Improves Memory Deficit in Mice Fed a High-Fat Diet. Diabetes. 2012;61:1444-54.

[7] Schubert M, Brazil DP, Burks DJ, Kushner JA, Ye J, Flint CL, et al. Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. J Neurosci. 2003;23:7084-92.

[8] Jolivalt CG, Lee CA, Beiswenger KK, Smith JL, Orlov M, Torrance MA, et al. Defective Insulin Signaling Pathway and Increased Glycogen Synthase Kinase-3 Activity in the Brain of Diabetic Mice: Parallels With Alzheimer's Disease and Correction by Insulin. J Neurosci Res. 2008;86:3265-74.

[9] Cross DAE, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of Glycogen-Synthase Kinase-3 by Insulin-Mediated by Protein-Kinase-B. Nature. 1995;378:785-9.

[10] Gudala K, Bansal D, Schifano F, Bhansali A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. Journal of Diabetes Investigation. 2013;4:640-50.

[11] de la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. J Diabetes Sci Technol. 2008;2:1101-13.

[12] Rendeiro C, Vauzour D, Kean RJ, Butler LT, Rattray M, Spencer JPE, et al. Blueberry supplementation induces spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. Psychopharmacology. 2012;223:319-30.

[13] Shukitt-Hale B, Bielinski DF, Lau FC, Willis LM, Carey AN, Joseph JA. The beneficial effects of berries on cognition, motor behaviour and neuronal function in ageing. British Journal of Nutrition. 2015;114:1542-9.

[14] Casadesus G, Shukitt-Hale B, Stellwagen HM, Zhu X, Lee H-G, Smith MA, et al. Modulation of Hippocampal Plasticity and Cognitive Behavior by Short-term Blueberry Supplementation in Aged Rats. Nutritional Neuroscience. 2004;7:309-16.

[15] Tsuda T, Horio F, Uchida K, Aoki H, Osawa T. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. J Nutr. 2003;133:2125-30.

[16] Batista ÂG, da Silva JK, Betim Cazarin CB, Biasoto ACT, Sawaya ACHF, Prado MA, et al. Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves. LWT - Food Science and Technology. 2017;76, Part B:284-91.

[17] Reeves PG, Nielsen FH, Fahey GC. Ain-93 Purified Diets for Laboratory Rodents - Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the Ain-76a Rodent Diet. J Nutr. 1993;123:1939-51.

[18] Morris R. Developments of a Water-Maze Procedure for Studying Spatial-Learning in the Rat. J Neurosci Meth. 1984;11:47-60.

[19] Paxinos G, Franklin KB. The mouse brain in stereotaxic coordinates: Gulf Professional Publishing; 2004.

[20] McFarland CP, Glisky EL. Frontal lobe involvement in a task of time-based prospective memory. Neuropsychologia. 2009;47:1660-9.

[21] Takahashi H, Kato M, Hayashi M, Okubo Y, Takano A, Ito H, et al. Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. NeuroImage. 2007;34:1643-9.

[22] Ohkawa H, Ohishi N, Yagi K. Assay for Lipid Peroxides in Animal-Tissues by Thiobarbituric Acid Reaction. Anal Biochem. 1979;95:351-8.

[23] Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med. 1975;85:337-41.

[24] Flohe L, Gunzler WA. Assays of Glutathione-Peroxidase. Method Enzymol. 1984;105:114-21.

[25] Carlberg I, Mannervik B. Glutathione-Reductase. Method Enzymol. 1985;113:484-90.

[26] Johansson LH, Borg LAH. A Spectrophotometric Method for Determination of Catalase Activity in Small Tissue Samples. Anal Biochem. 1988;174:331-6.

[27] Batista AG, Lenquiste SA, Cazarin CBB, da Silva JK, Luiz-Ferreira A, Bogusz S, et al. Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. Journal of Functional Foods. 2014;6:450-61.

[28] Veberic R, Slatnar A, Bizjak J, Stampar F, Mikulic-Petkovsek M. Anthocyanin composition of different wild and cultivated berry species. LWT - Food Science and Technology. 2015;60:509-17.

[29] Styskal J, Van Remmen H, Richardson A, Salmon AB. Oxidative stress and diabetes: What can we learn about insulin resistance from antioxidant mutant mouse models? Free Radical Bio Med. 2012;52:46-58.

[30] Cho BO, Ryu HW, Jin CH, Choi DS, Kang SY, Kim DS, et al. Blackberry Extract Attenuates Oxidative Stress through Up-regulation of Nrf2-Dependent Antioxidant Enzymes in Carbon Tetrachloride-Treated Rats. J Agr Food Chem. 2011;59:11442-8.

[31] Dragano NRV, Marques AC, Cintra DEC, Solon C, Morari J, Leite-Legatti AV, et al. Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. British Journal of Nutrition. 2013;110:447-55.

[32] Li L, Wang LY, Wu ZQ, Yao LJ, Wu YH, Huang L, et al. Anthocyanin-rich fractions from red raspberries attenuate inflammation in both RAW264.7 macrophages and a mouse model of colitis. Sci Rep-Uk. 2014;4.

[33] Lee SG, Kim B, Yang Y, Pham TX, Park Y-K, Manatou J, et al. Berry anthocyanins suppress the expression and secretion of proinflammatory mediators in macrophages by inhibiting nuclear translocation of NF-κB independent of NRF2-mediated mechanism. The Journal of Nutritional Biochemistry. 2014;25:404-11.

[34] Jennings A, Welch AA, Spector T, Macgregor A, Cassidy A. Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. J Nutr. 2014;144:202-8.

[35] Ma Q-L, Yang F, Rosario ER, Ubeda OJ, Beech W, Gant DJ, et al. β-Amyloid Oligomers Induce Phosphorylation of Tau and Inactivation of Insulin Receptor Substrate via c-Jun N-Terminal Kinase Signaling: Suppression by Omega-3 Fatty Acids and Curcumin. The Journal of Neuroscience. 2009;29:9078-89.

[36] Schubert M, Gautam D, Surjo D, Ueki K, Baudler S, Schubert D, et al. Role for neuronal insulin resistance in neurodegenerative diseases. Proceedings of the National Academy of Sciences of the United States of America. 2004;101:3100-5.

[37] Duffy KB, Spangler EL, Devan BD, Guo ZH, Bowker JL, Janas AM, et al. A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. Neurobiol Aging. 2008;29:1680-9.

[38] Poulose SM, Fisher DR, Larson J, Bielinski DF, Rimando AM, Carey AN, et al. Anthocyanin-rich Açai (Euterpe oleracea Mart.) Fruit Pulp Fractions Attenuate Inflammatory Stress Signaling in Mouse Brain BV-2 Microglial Cells. J Agr Food Chem. 2012;60:1084-93.

[39] Papandreou MA, Dimakopoulou A, Linardaki ZI, Cordopatis P, Klimis-Zacas D, Margarity M, et al. Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. Behav Brain Res. 2009;198:352-8.

## CAPÍTULO 4

**Artigo Original: CASCA DE JABUTICABA PREVINE A FOSFORILAÇÃO DA TAU INDUZIDA PELA RESISTÊNCIA À INSULINA EM CAMUNDONGOS**

Ângela G Batista, Edilene S Soares, Monique C P Mendonça, Juliana K da Silva, Ana Paula Dionísio, Cesar R Sartori, Maria Alice da Cruz-Höfling and Mário R Maróstica Júnior. **Jaboticaba berry peel intake prevents insulin resistance-induced tau phosphorylation in mice.** Em processo de revisão na revista ***Molecular Nutrition & Food Research***, Wiley Online Library, ISSN: 1613-4133. Submetido em 30 de setembro de 2016. Último status atualizado em 06 de março de 2017 (ANEXO 3).

Vide autorização CEUA no Anexo 2.

Research Article

**Jaboticaba berry peel intake prevents insulin resistance-induced tau phosphorylation in mice**

Ângela G Batista[1], Edilene S Soares[2], Monique C P Mendonça[2,3], Juliana K da Silva[1], Ana Paula Dionísio[4], Cesar R Sartori[5], Maria Alice da Cruz-Höfling[2,3], Mário R Maróstica Júnior[1],*

[1]Faculty of Food Engineering, Department of Food and Nutrition, University of Campinas, Campinas, SP, Brazil

[2]Institute of Biology, Department of Biochemistry and Tissue Biology, University of Campinas, Campinas, SP, Brazil

[3]School of Medical Sciences, Department of Pharmacology, University of Campinas, Campinas, SP, Brazil

[4]Brazilian Agricultural Research Corporation, Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil.

[5]Institute of Biology, Department of Structural and Functional Biology, University of Campinas, Brazil

Correspondence: Mário Roberto Maróstica Júnior

(mario@fea.unicamp.br).

**Keywords:** Antioxidants; bioactive compounds; cognition; obesity; oxidative stress.

**Abbreviations: AD,** Alzheimer's disease; **AKT,** protein kinase B; **AUC,** area under the curve; **CAT,** catalase; **GPx,** glutathione peroxidase; **GRd,** glutathione reductase; **GSH,** reduced glutathione; **GSK3-β,** Glycogen synthase kinase 3-β; **GTT,** glucose tolerance test; **IRS,** insulin receptor substrate; **ITT,** insulin tolerance test; **MJP,** *Myrciaria jaboticaba* berry peel; **MWM,** Morris water maze; **NeuN,** neuronal nuclear antigen; **NFT,** neurofibrillary tangles; **ORAC,** oxygen radical absorbance capacity; **PB,** phosphate buffer; **SOD,** superoxide dismutase; **Tau,** microtubule-associated protein tau; **TBARS,** thiobarbituric acid reactive substances.

ABSTRACT

The hyperphosphorylation of tau in the hippocampus can be caused by central and peripheral insulin resistance and these alterations are related to the development of tauopathies, such as Alzheimer's disease. In this study, we used a high-fat diet to induce obesity and insulin resistance in adult Swiss mice and checked whether supplementation with *Myrciaria jaboticaba* berry peel for 10 weeks could improve insulin sensitivity, learning/memory performance and prevent tau phosphorylation in the hippocampus. Furthermore, adipocytokines, inflammatory markers, and oxidative stress were assessed. *M. jaboticaba* peel has phenolic compounds (e.g. cyanidin, ellagic acid), dietary fiber and carotenoids, which contribute to great antioxidant capacity. Supplementation of the high-fat diet with 4% *M. jaboticaba* peel prevented fat weight gain and reduced peripheral insulin resistance. The treated group also showed lower tau phosphorylation in the hippocampus corroborating better learning/memory performance in the Morris water maze test. Maintenance of neuronal viability, lower levels of hippocampal inflammatory markers and improved brain antioxidant defenses were also related to the consumption of *M. jaboticaba* peel. These findings contribute to a better understanding of how a high-fat diet supplemented with jaboticaba berry peel counteracts the impairment of cognitive functions caused by high-fat diet intake and diet-induced insulin resistance.

## 1 Introduction

In the last decade, evidence supports the association between overweight, insulin resistance and Alzheimer's disease (AD) [1-2]. AD is a progressive neurodegenerative disease characterized by neuron and synapses damage, memory loss and brain atrophy [3]. Neurofibrillary tangles (NFT), formed by aggregates of paired helical filaments of abnormally phosphorylated microtubule-associated tau protein (Tau) account for the disease, as well as senile plaques of β-amyloid [4]. Tau role relates to the stabilization and binding of microtubules in neurons, but highly phosphorylated forms of tau detach from microtubules and self-aggregate in the cytosol, forming NFT and causing tauopathies such as found in AD [4-5]. Glycogen synthase kinase 3-β (GSK3-β) is one of the main kinases responsible for tau phosphorylation and can be inactivated when phosphorylated at Ser 9 by other kinases, such as AKT (protein kinase B) [6].

Insulin resistance can be fundamental for tau phosphorylation, since proper insulin signaling phosphorylates the insulin receptor substrates (IRS), which in turn phosphorylates AKT avoiding thentau phosphorylation by GSK3-β inactivation [6]. Furthermore, the insulin signaling cascade in hippocampus is very important for cellular metabolism and neuron survival and is correlated positively with memory and cognitive functions [6]. In contrast, inflammation and impaired insulin signaling are related to neurodegeneration, cognitive and behavioral impairments [7].

If, on the one hand, dietary components, such as saturated fatty acids and simple sugars impair insulin stimuli and the cognitive processes, on the other, dietary antioxidants, such as phenolic compounds and dietary fiber, improve insulin sensitivity and neurogenesis [8-12]. In fact, the direct or indirect protective properties of flavonoids show enormous potential in counteracting oxidative stress, weight gain, insulin resistance, inflammation and some aspects of cognitive deficits [13-14].

Addition of berries, like jaboticaba (*Myrciaria jaboticaba*), to the diet has been related to health benefits due to their high levels of bioactive compounds [15]. The peel of jaboticaba concentrates the majority of phenolic compounds (such as ellagitannins and anthocyanins) and dietary fiber from the fruit, and some studies have indicated this part of the fruit as a promising food to prevent insulin resistance [16-20]. In addition, we have recently found that supplementation with *M. jaboticaba* berry peel (MJP) in the diet of obese rat was able to prevent brain oxidative stress in a dose-dependent response [18]. These data led us to investigate whether memory deficit and cognitive impairment resulting from high-fat diet intake are prevented in mice fed a high-fat diet containing 4% MJP. Furthermore, we assessed the possible benefits of MJP supplementation on other peripheral and central markers of obesity and related diseases, such as oxidative stress, insulin resistance and inflammatory markers.

## 2 Material and Methods

### 2.1 Jaboticaba chemical composition

Ripe jaboticaba fruits were harvested in October 2013 in Lagoa Branca, SP, Brazil. They were cleaned and peeled and the peel of the fruit was dried in an oven at 40 °C with forced air circulation for 96 h. Then, the peel was ground, homogenized and packed in plastic dark flasks protected from light and frozen at −18 ± 5 °C until use.

The proximate composition of MJP was determined, and analysis of dietary fiber fractions, reducing sugars, total polyphenols, total flavonoids, monomeric anthocyanins, total carotenoids and oxygen radical absorbance capacity tests (hydrophilic-ORAC and lipophilic-ORAC) was carried out as described in a previous study [21].

### 2.1.1 HPLC-DAD-ESI/MS analysis

The MJP was extracted with methanol: water: acetic acid (85: 15: 0.5, v $v^{-1}$) according to previous report [22]. Liquid chromatography (Varian 250 HPLC; Varian, Lake Forest, CA, USA) coupled with DAD, ESI and MS 500-MS IT (Varian) was used for identification and quantification of some polyphenol compounds in MJP [23]. A Symmetry C18 column (3 μm, 250 × 2 mm; Varian) was used at a 0.4 mL $min^{-1}$ flow rate and the oven temperature set at 30 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) solutions. The gradient varied linearly from 10% to 26% B (v $v^{-1}$) in 40 min, to 65% B at 70 min, finally to 100% B at 71 min and was held at 100% B to 75 min. The DAD was set at 270 and 512 nm for real-time read-out and UV/VIS spectra, from 190 to 650 nm, were continuously collected. The MS were simultaneously acquired using ESI in the positive and negative ionization modes at an 80 V fragmentation voltage for the mass range of 100–1000 amu. A drying gas pressure of 35 psi, nebulizer gas pressure of 40 psi, drying gas temperature of 370 °C, capillary voltages of 3500 V for positive ionization and 3500 V for negative, and 600 V spray shield voltages were used. The HPLC system was coupled to the MS with a splitting of 50%.

## 2.2 *In vivo* experimental design

After approval by the institutional Ethics Committee in Animal Use (CEUA/UNICAMP, protocol #3157-1), 3-week-old weaned male Swiss mice (*Mus musculus*, $n = 40$) were acquired from the Multidisciplinary Center of Biological Investigation (CEMIB/UNICAMP), and cared for following the ethical guidelines of Brazilian Society of Animal Science Laboratory (SBCAL). Upon arrival, the mice were housed individually in separate cages at 22 ± 2°C, 50–60% relative humidity and a standard inverted 12/12 h dark/light cycle throughout the study. The animals were given free access to water and a

standard semi-purified diet for growth (AIN93-G) [24] for 4 weeks, when they achieved 37.35 ± 3.17 g (body weight mean ± SD). After that, the mice were randomized to receive four experimental diets based on a standard maintenance diet, AIN93-M [24] (Table A.1).

**Table A.1.** Composition of experimental diets (g $kg^{-1}$) by mouse groups.

| Ingredient | N | NM | H | HM |
|---|---|---|---|---|
| Casein (82.7% protein) | 143.80 | 143.80 | 143.80 | 143.80 |
| Corn starch | 463.24 | 445.58* | 262.92* | 245.26* |
| Maltodextrin | 154.18 | 148.31* | 87.51* | 81.63* |
| Sucrose | 99.47 | 95.68* | 56.46* | 52.67* |
| Soy oil | 40.00 | 40.00 | 40.00 | 40.00 |
| Lard | - | - | 310.00 | 310.00 |
| Cellulose | 50.00 | 37.33** | 50.00 | 37.33** |
| Mineral mix | 35.00 | 35.00 | 35.00 | 35.00 |
| Vitamin mix | 10.00 | 10.00 | 10.00 | 10.00 |
| L-Cystine | 1.80 | 1.80 | 1.80 | 1.80 |
| Choline bitartrate | 2.50 | 2.50 | 2.50 | 2.50 |
| *tert*-Butylhydroquinone | 0.008 | 0.008 | 0.008 | 0.008 |
| *M. jaboticaba* peel | - | 40.00 | - | 40.00 |
| Calories[a] | 4080.29 ± 6.06 | 4078.72 ± 12.23 | 5855.31 ± 0.509 | 5897.71 ± 37.35 |

* The carbohydrate ingredients were changed according to the addition of jaboticaba peel and/or lard content.
** The cellulose content was discounted according to the amount of insoluble fibers of jaboticaba peel (Table 1).
[a] Calorie values (kcal $kg^{-1}$) were determined using an Isoperibol Calorimeter 1261 instrument equipped with 1108 oxygen bomb (Parr Instrument Co, Moline, IL, USA) and expressed as means ± standard deviation.
N = group that received normal-fat diet.
NM = group that received normal-fat diet containing 4% *M. jaboticaba* peel.
H = high-fat diet group
HM = high-fat diet supplemented with 4% *M. jaboticaba* peel.

The mice were randomized into four groups ($n$ = 10) according to the respective diet (Table A.1): a normal-fat diet control group (N); a normal-fat diet containing 4% (w $w^{-1}$) MJP group (NM); a high-fat diet control group (H); and a high-fat diet containing 4% (w $w^{-1}$) MJP peel group (HM). The 4% MJP dose had been optimized in an earlier study on brain antioxidant status [18]. The MJP was mixed into the diets according to the proximate composition of MJP in order to match the same energy values as the respective control diet (Table A.1). The experimental diets were freely given to the mice for 10 weeks, after 1 week

of acclimatization. Thus, at the end of the experiment the animals were 18-weeks old. Weight gain and diet consumption were checked weekly. A sum of the weekly intake was calculated to express total food and calorie intake after 10 experimental weeks.

### 2.2.1. Morris Water Maze - Cognitive test

Spatial learning and memory in mice were assessed in the eighth week of treatment according to adaptations of the Morris water maze (MWM) test [25]. The maze consisted of a circular pool (120 cm diameter, 50 cm high) filled with water (25 ± 1 °C) that was made opaque with nontoxic grey paint. The pool was located in a 3 $m^2$ room with extra-maze visual cues on the walls, red-light lamp bulbs, a video camera positioned 2 m above the center and the experimenter. The pool was divided into four quadrants: three quadrants were used as release positions and the order was systematically varied throughout the experiment course. A circular plastic escape platform (9 cm diameter) was placed in one quadrant into the pool approximately 1 cm below the water surface. The animals were trained in the MWM over five consecutive daily sessions between 11:00 a.m. and 12:30 p.m. During the acquisition phase, each mouse participated in two trials per day with a 30 min interval in between. A trial was started when the animal was released from one of three randomly chosen start positions. After the mouse found and climbed onto the platform and was there for more than 10 s the escape latency, or the time taken to reach the platform, was recorded (maximum of 60 s). If the mouse had not climbed onto the platform in 60 s, the experimenter guided the animal by hand to the platform, an escape latency of 60 s was recorded and the mouse was kept there for 20 s. Twenty-four hours after the last acquisition session (day 6) a probe test was used to assess spatial retention of the location of the hidden platform. For this purpose, the platform was removed from the maze, and each mouse was allowed to swim freely to search for the platform for 60 s.

In the probe test, the circular area surrounding the location where the platform was hidden in the acquisition test was delimited three times larger and used as a counting zone to determine the number of times the platform place was crossed. The latency period for which the animals swam into the quadrant where the platform was previously hidden was also recorded to determine memory retention.

### 2.2.2 Sampling

After anesthesia (2:1 v $v^{-1}$ ketamine chloride (Dopalen®, 100 mg $kg^{-1}$), and xylazine chloride (Anasedan®, 10 mg $kg^{-1}$) (Fortvale, Valinhos, SP, Brazil)) blood was obtained from fasted and non-fasted mice by cardiac puncture at the end of the experiment. The blood samples were collected in tubes containing gel to optimize serum separation, and centrifuged at 2,000 g for 20 min and stored at -80 °C until analysis.

The brain of non-fasted mice was immediately removed after euthanasia by exsanguination, washed in a 0.9% saline solution, separated from the cerebellum, frozen in liquid nitrogen and kept at −80 °C.

### 2.2.3 Insulin resistance assessment

*Glucose tolerance test (GTT):* After 6 h fasting, a D-glucose solution (2 g $kg^{-1}$) was intraperitoneally (i.p.) injected into the mice and blood glucose was measured via the tail vein on fasting condition (baseline) and 30, 60, 90 and 120 min after the injection.

*Insulin tolerance test (ITT):* Under fasting conditions (6 h), a 0.9% saline solution containing 0.75 units $kg^{-1}$ of insulin (Novolin R, Novo Nordisk Bagsvaerd, Denmark) was i.p. injected and blood glucose was measured via the tail vein at baseline and 10, 20, 30, 45 and 60 min after the i.p. injection. The GTT and ITT were carried out in the ninth and tenth week

of treatment using a glucometer and respective test strips (FreeStyle Lite, Abbott, Alameda, CA, USA).

*Glycemia:* Fasting (6 h) blood glucose was measured via the tail vein on the last day of the experimentation period using the glucometer.

*Insulin:* Serum insulin of fasted mice (6 h) was assessed using a specific ELISA kit (Cat. #EZRMI-13K, Millipore, St. Charles, MO, USA).

### 2.2.4 Cytokine assessment

Fasting (6 h) serum adiponectin, leptin and TNF-α were assessed on the last day of the experiment using a multiplex kit (Merck Millipore, Darmstadt, Germany).

### 2.2.5 Antioxidant enzymes and lipid peroxidation analyses

Serum of non-fasted mice ($n$ = 5) collected as aforementioned was used for oxidative stress analysis. A piece of the frontal lobe of the brain was homogenized in phosphate buffer (PB), pH 7.4 (approximately 100 mg $mL^{-1}$). We used for antioxidant status analysis the same animals used for Western blotting analysis (Section 2.2.6). The measurement of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GRd) and superoxide dismutase (SOD) values in brain homogenates and serum was conducted as detailed in a previous study [18]. For catalase (CAT) activity, the method of Johansson was adapted [26]. Serum or brain homogenates were diluted in 25 mmol $L^{-1}$ PB, pH 7.5 (containing 1 mmol $L^{-1}$ EDTA and 0.1% BSA) and added to a 96-well microplate containing 100 mmol $L^{-1}$ PB (pH 7.0) and methanol. Briefly, 10.58 mmol $L^{-1}$ $H_2O_2$ was added to the microplate and allowed to react for 20 min in a microplate shaker in dark conditions. After that, 7.8 mol $L^{-1}$ KOH and 34.2 mmol $L^{-1}$ Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) solution were added to the microplate and allowed to

react for 10 min in a shaker; and then a 65.2 mmol $L^{-1}$ potassium periodate solution was added and mixed for 5 min before reading at 540 nm. Formaldehyde was used as the standard and results were expressed as nmol of formaldehyde formation per minute at 25 °C.

### 2.2.6 Western Blotting

The hippocampus dissection was performed by placing the frozen brain in a clean Petri dish in ice and covered with buffer. Using a scalpel, the hindbrain and olfactory bulb were removed. Next and carefully, the cerebral cortex was detached from the hippocampus using tweezers. Due to the presence of ventricles between the midbrain and neocortex the hippocampus could be easily separated [27]. The hippocampi ($n$ = 3–5) were immediately extracted in a lysis buffer (10 mmol $L^{-1}$ EDTA, 2 mmol $L^{-1}$ PMSF, 100 mmol $L^{-1}$ NaF, 10 mmol $L^{-1}$ sodium pyrophosphate, 10 mmol $L^{-1}$ $NaVO_4$, 10 μg $mL^{-1}$ aprotinin and 100 mmol $L^{-1}$ Tris, pH 7.4) and the protein concentration was assessed with the Bradford assay. An aliquot of protein (40 μg) was loaded and separated via SDS-PAGE. After electrotransferred onto nitrocellulose, the membranes were incubated with 5% BSA for 30 min to block non-specific antigenic sites, followed by washing with TBS-T (0.1% Tris-buffered saline with 0.05% Tween 20, pH 7.4). Subsequently, the membranes were incubated with the following primary antibodies: p-IRS-1 (Tyr 632)-R, IRS-1 (E-12), p-AKT 1/2/3 (Ser 473)-R, AKT 1/2/3 (H-136), p-GSK-3β (Ser 9), GSK3-β (H-76), p-tau (Thr 205), tau (TAU-5), TNF-α (M-18), IFN-γ (D-17) from Santa Cruz Biotechnology (Santa Cruz, CA, USA), NeuN (neuronal nuclear antigen) from Millipore (Billerica, MA, USA), and β-actin from Sigma-Aldrich. Then, the membranes were washed with TBS-T and incubated with appropriate secondary antibody. The proteins were visualized using an enhanced chemiluminescence substrate (Thermo Scientific, Waltham, MA, USA) and band densitometry was acquired using NIH Image J 1.45s software (Wayne Rasband, NIH, Bethesda, MD, USA). All proteins were

normalized to a β-actin internal control and the results were expressed as the ratio of p-protein to total protein for the phosphorylated proteins.

### 2.3 Statistics

Time × treatment data (weekly weight gain, GTT and ITT, MWM acquisition) were analyzed using two-way ANOVA followed by Bonferroni test ($p < 0.05$). The experimental groups were compared in pairs according to the fat content in the diets (N × H; N × NM and H × HM) using Student's *t*-test ($p < 0.05$). All tests were carried out using GraphPad Prism 5.0 software (GraphPad, Inc., La Jolla, CA, USA).

## 3 Results

### 3.1. Jaboticaba peel and chemical composition

The chemical composition of the powdered and dried jaboticaba peel is presented in Table 1. The dried powdered MJP showed higher amounts of dietary fiber in comparison to other studies in which the peel was lyophilized [22]. The content of soluble dietary fiber present in the MJP is worth mentioning since this is involved in the processes of fermentation by the intestinal microflora, and thus relevant for promoting intestinal and systemic health [28]. The presence of sugars in the material (Table 1) is also important, providing better palatability to the material.

The class of anthocyanins is strongly significant among the group of polyphenols and flavonoids from MJP [16]. Two important phenolic compounds were identified in MJP: cyanidin-3-*O*-glucoside and ellagic acid. These compounds contribute greatly to the MJP hydrophilic antioxidant capacity (Table 1) [20], as they have the ability to donate their electrons or atoms and stabilize free radicals.

Non-polar compounds like carotenoids are relevant for studies in the brain because of the lipophilicity of the blood–brain barrier [29-30]. The lipophilic fraction of the fruit peel

contributes to the total antioxidant capacity (Table 1), and the total carotenoid concentration in MJP is relevant to this characteristic. The lipophilic antioxidant capacity of MJP has been reported for the first time in this study.

**Table 1.** Nutrients, bioactive compounds and antioxidant capacity in jaboticaba peel.*

| Nutrient (g 100 $g^{-1}$) | |
|---|---|
| Moisture | 14.45 ± 0.44 |
| Ash | 3.01 ± 0.02 |
| Protein (N*6.25) | 6.88 ± 0.07 |
| Lipid | 2.98 ± 0.15 |
| Total dietary fiber | 33.77 ± 1.20 |
| Insoluble dietary fiber | 25.34 ± 0.33 |
| Soluble dietary fiber | 8.49 ± 0.48 |
| Total reducing sugar | 33.51 ± 1.43 |
| Soluble reducing sugar | 30.39 ± 1.94 |
| Bioactive compound (mg 100 $g^{-1}$) | |
| Total polyphenols | 3216.92 ± 124.24 |
| Total flavonoids | 642.99 ± 25.75 |
| Total anthocyanins | 541.49 ± 7.42 |
| Cyanidin-3-*O*-glucoside[a] | 1113.38 ± 19.37 |
| Ellagic acid[b] | 710.10 ± 33.77 |
| Total carotenoids | 2.87 ± 0.16 |
| Antioxidant capacity (µmol TE $g^{-1}$) | |
| Hydrophilic-ORAC | 575.78 ± 65.64[c] |
| Lipophilic-ORAC | 1.91 ± 0.09[d] |

a) $[M–H]^-$ 449; 287 *m/z;* b) $[M–H]^-$ 301; 257, 229 *m/z* ; c) y= 73.5x-7.18; $r^2$= 0.965; d) y=-0.0011x2+0.2851x+1.8609; $r^2$= 0.973.

*Values expressed as means ± SD. ORAC: oxygen radical absorbance capacity.

### 3.2 *In vivo* study

#### 3.2.1 Body weight and food intake parameters

The growth parameters indicate that the high-fat diet was able to promote higher body weight gain in the animals of H group ($p < 0.05$) from the third experimental week (Fig. 1a). Even if the weekly and total food intake (Fig. 1b and c) was higher in the N group in comparison to H ($p < 0.05$), the caloric intake was higher in the H group (Fig. d); however, a lower body weight was observed in the HM group from the fifth experimental week, when compared to H ($p < 0.05$) (Fig. 1a).

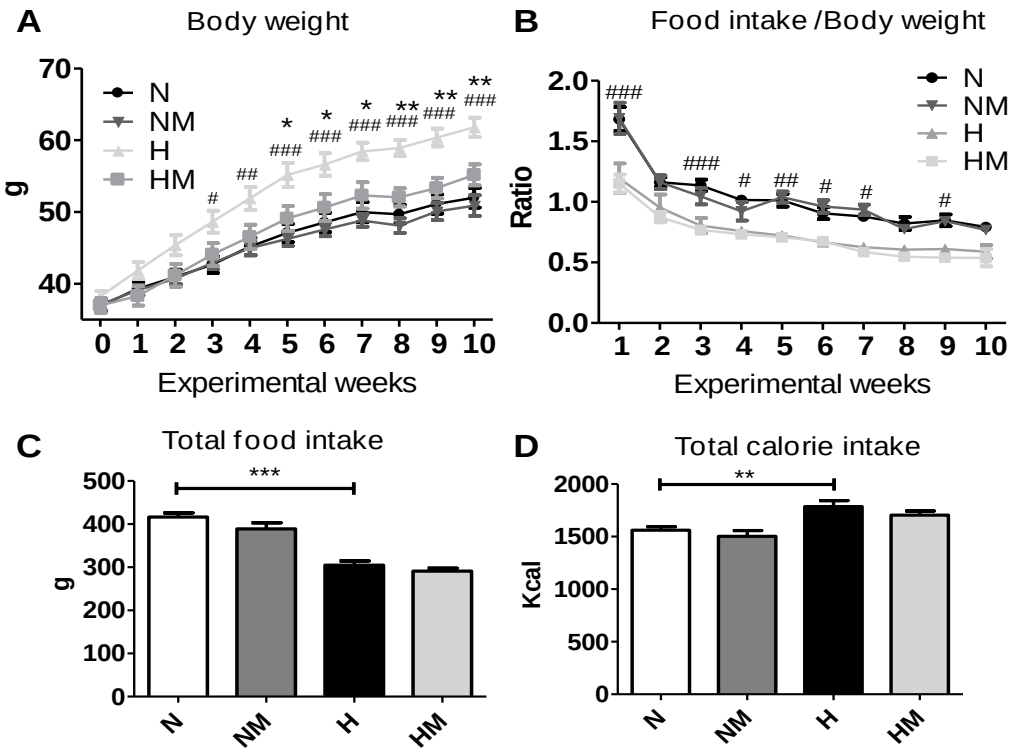



**Figure 1.** Body weight (a), total food intake/ body weight ratio (b), total food intake (c) and total calorie intake (d) of the mice groups. Total food and calorie intake were obtained summing the consumption of the diets during 10 experimental weeks. Values expressed as means ± standard error. Panel a-b: Statistical differences according to two-way ANOVA and Bonferroni tests: #indicates difference between N and H groups and *between H and HM (1 code = $p < 0.05$; 2 codes = $p < 0.01$; and 3 codes = $p < 0.001$). Panels c-d: Differences presented as **$p < 0.01$ and ***$p < 0.001$ according to Student's *t*-test between groups, $n = 10$.

### 3.2.2 Peripheral insulin resistance

The animals fed with the high-fat diet had a higher area under the curve (AUC) for glycemic response after injection of glucose or insulin in both the GTT and ITT ($p < 0.05$), respectively (Fig. 2a–d). However, the addition of MJP to the high-fat diet in HM was able to prevent peripheral insulin resistance in the animals, when compared to the H group ($p < 0.05$) (Fig. 2.a-f). The group HM showed lower glycemic response after glucose and insulin injection, being more sensitive to insulin stimulus than its control H ($p < 0.05$) (Fig. 2a–d).

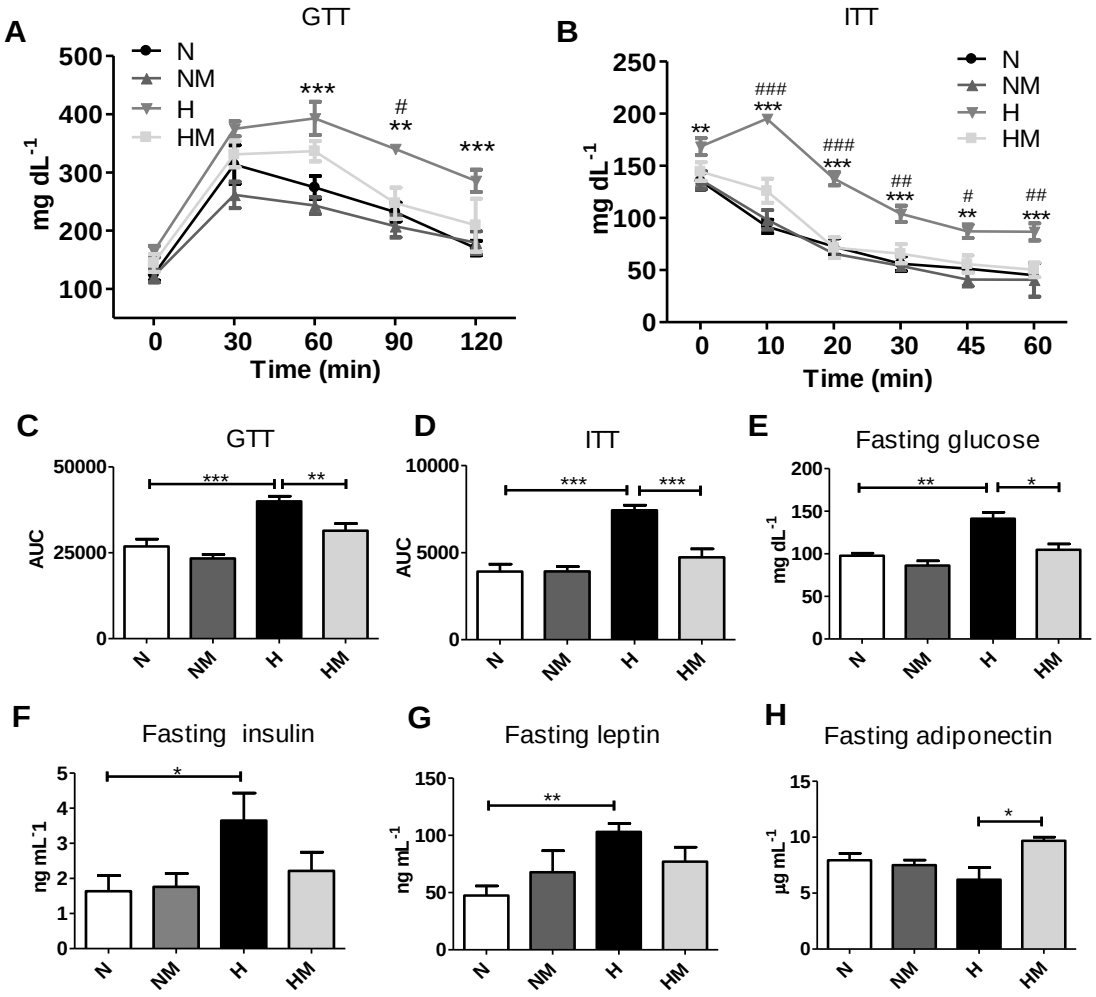



**Figure 2.** Indicators of peripheral insulin resistance, adipocytokines and inflammatory cytokines. a) Glycemic response in intraperitoneal glucose tolerance test (GTT); b) glycemic response in insulin tolerance test (ITT); c) AUC of GTT response; d) AUC of ITT response; e) fasting glucose; f) fasting insulin; g) fasting leptin; and h) fasting adiponectin. Values expressed as means ± standard error. Panels a and b: *Indicates statistical differences between N and H groups and #between H and HM according to two-way ANOVA followed by Bonferroni test (1 code = $p < 0.05$; 2 codes= $p < 0.01$ and 3 codes= $p < 0.001$). Panels c-h: Differences presented as *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ according to Student's *t*-test between groups; $n = 5$.

The fasting glucose and insulin of high-fat fed mice were increased in comparison to N ($p < 0.05$), although a difference between HM and H was observed only for the fasting glucose measurement (Fig. 2e–f).

The TNF-α circulating levels and fasting levels of leptin were higher in the H group when compared to N ($p < 0.05$). However, no changes were found for serum TNF-α (data not shown) or leptin (Fig. 2g) when the MJP-containing diet was given ($p > 0.05$). On the contrary, the HM diet was able to increase serum levels of adiponectin ($p < 0.05$) (Fig. 2h).

### 3.2.3 Serum and brain antioxidant defenses

The activity of the enzymes CAT and GPx in serum was higher in the NM group, when compared to N ($p < 0.05$) (Table 2). The GRd activity mean value in the serum of the HM group was higher than in the H group ($p < 0.01$). The other parameters in serum were not significantly different among the groups ($p > 0.05$) (Table 2).

**Table 2.** Lipid peroxidation and antioxidant enzyme activity in serum and brain.

| Marker | N | NM | H | HM |
|---|---|---|---|---|
| Serum (nmol $mL^{-1}$) | | | | |
| TBARS | 14.54 ± 0.96 | 14.47 ± 1.63 | 15.21 ± 2.04 | 11.31 ± 0.68 |
| SOD (U $mL^{-1}$) | 23.39 ± 1.45 | 26.87 ± 2.07 | 18.50 ± 2.65 | 23.67 ± 3.77 |
| CAT [a] | 13.00 ± 1.12 | 25.95 ± 1.94** | 17.18 ± 2.63 | 27.64 ± 3.63 |
| GPx [b] | 37.67 ± 8.97 | 183.23 ± 63.69* | 111.16 ± 29.70 | 187.26 ± 72.56 |
| GRd [b] | 138.03 ± 15.56 | 217.62 ± 43.12 | 132.10 ± 28.10 | 300.15 ± 54.29** |
| GSH | 145.52 ± 13.98 | 130.88 ± 10.89 | 100.41 ± 13.58 | 102.28 ± 16.61 |
| Brain frontal lobe (nmol $mg^{-1}$) | | | | |
| TBARS | 1.78 ± 0.07 | 1.88 ± 0.06 | 2.33 ± 0.09*** | 1.85 ± 0.10** |
| SOD (U $mg^{-1}$) | 0.969 ± 0.075 | 0.783 ± 0.048 | 0.475 ± 0.034*** | 0.810 ± 0.011*** |
| CAT [a] | 4.59 ± 0.12 | 4.50 ± 0.11 | 3.47 ± 0.23** | 4.78 ± 0.21*** |
| GPx [b] | 8.16 ± 0.33 | 8.59 ± 1.20 | 6.39 ± 0.41** | 8.22 ± 0.48* |
| GRd [b] | 20.61 ± 0.64 | 22.64 ± 0.74 | 19.12 ± 1.135 | 19.22 ± 0.58 |
| GSH | 18.22 ± 2.19 | 9.32 ± 1.59* | 5.37 ± 0.84*** | 19.03 ± 2.53*** |

a) nmol $min^{-1}$ formed formaldehyde.
b) nmol $min^{-1}$ consumed NADPH.
*Indicates significant differences between groups (N × H; N × NM and H × HM) according to Student *t*-test, *$p<0.05$; **$p<0.01$; and ***$p<0.001$; $n= 6$. Values expressed as means ± standard error.

According to the results shown in Table 2, the antioxidant defenses in the frontal lobe of the brain were weaken by the intake of the high-fat diet for 10 weeks. The activity of SOD, for example, was reduced to 50% and the GSH content to 70% in the H group ($p < 0.05$). In

contrast, there was a significant improvement in the activity of the antioxidant enzymes and GSH content in the brain of animals fed the high-fat diet containing 4% MJP (HM) in comparison to H. Such improvement was driven by the 20% decrease of TBARS, an increase of 70.5% in SOD activity, 37.75% in CAT activity, 28.64% in GPx activity and 254% in GSH content ($p < 0.05$). However, the GSH value in the NM group brain was decreased in relation to the N group, despite no difference in the lipid peroxidation parameter for this group (Table 2).

### 3.2.4 Hippocampal insulin resistance and inflammatory markers

Impairment in the studied insulin signaling cascade could be seen in the H group's hippocampus, since IRS and GSK3-β had low phosphorylation and tau was highly phosphorylated ($p < 0.05$) (Fig. 3a–c). On the other hand, the addition of MJP to the high-fat diet protected the hippocampus against the insulin resistance (Fig. 3.a) and memory impairment (Fig. 4), since there were significant effects in hippocampal IRS phosphorylation in Tyr 632 (+118.76%, Fig. 3a), GSK3-β phosphorylation in Ser 9 (+64.29%, Fig. 3b), and tau phosphorylation in Thr 205 (−37.37%, Fig. 3c) for the animals supplemented with HM diet in comparison to H ($p < 0.05$).Although the phosphorylation of IRS in the group NM showed lower values, there was no statistical difference in comparison to the N control ($p = 0.155$) according to the Student's *t*-test. No significant differences were found for phosphorylation of AKT in the hippocampus (data not shown), although the HM group trended toward a higher value in relation to the H group ($p = 0.10$).

Hippocampal levels of TNF-α and IFN-γ were over 100% lower in the animals fed the HM diet, when compared to the H group ($p < 0.05$) ), although no difference was found between the groups N and H (Fig. 3.d and e). These results which were inversely related to

higher NeuN values (59.07%) suggesting enhanced or at least maintained neuronal viability in the HM group (Fig 3f).

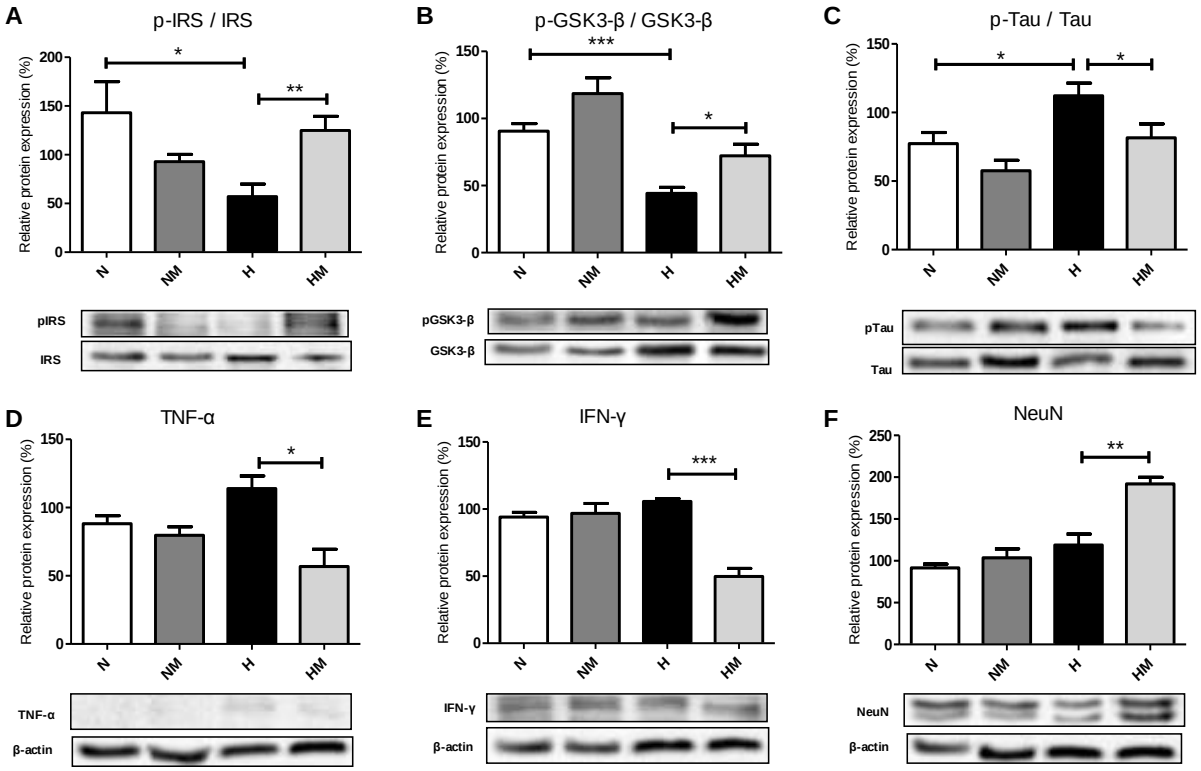



**Figure 3.** Hippocampal insulin signaling pathway (IRS-GSK3-β-tau) and inflammatory markers. Western blotting analyses of a) IRS phosphorylation; b) GSK3-β phosphorylation; c) Tau phosphorylation; d) TNF-α; e) IFN-γ; f) NeuN. The results are shown as a percentage of the control (N group as 100%); values expressed as means ± standard error. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ according to Student's *t*-test between groups; $n = 5$.

### 3.2.5 Learning and memory

The H group showed reduced learning process in acquisition sessions 4 and 5, since the escape latency at the MWM test was higher in this group when compared to the N group ($p < 0.05$), (Fig. 4a). For the probe trial, the difference between N and H was even more evident as can be seen for the time spent in the target quadrant (Fig 4b) and the number of times the place where the platform was hidden in the acquisition sessions was crossed (Fig. 4c), proving an impaired memory in the mice of H group. The HM group showed better performance than

H, noticed in the fourth session of memory acquisition and also in the probe test, as revealed by the time spent in the target quadrant and crossing the platform area (Fig. 4b and c). No differences were found for the NM treatment ($p > 0.05$).

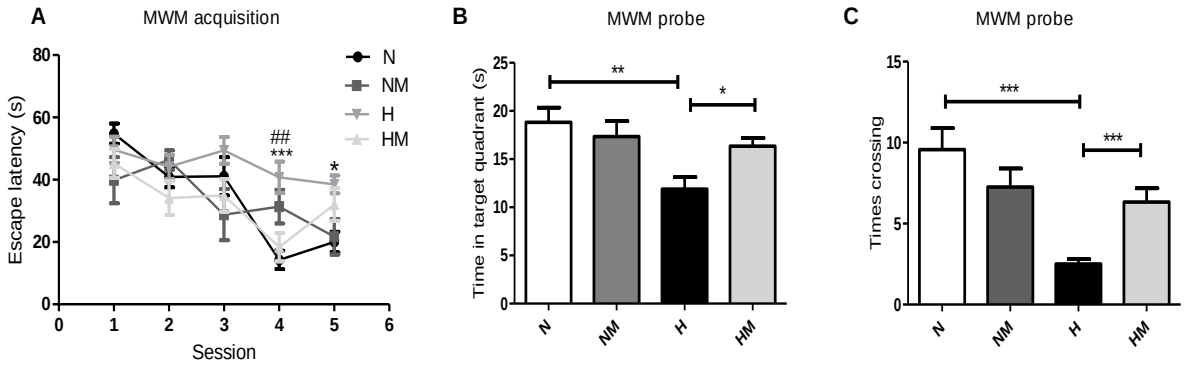



**Figure 4.** Learning and memory assessed by the Morris Water Maze (MWM) test. a) acquisition phase; b) MWM probe test as time spent in the target quadrant; c) MWM probe test as number of times crossing the position of the platform (counting zone). Panel a: *Indicates statistical differences between N × H groups and $^{\#}$H × HM according to two-way ANOVA and Bonferroni tests (1 code = $p < 0.05$; 2 codes = $p < 0.01$ and 3 codes= $p < 0.001$). Panels b and c: differences presented as *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ according to Student's *t*-test between groups; $n = 8$.

## 4 Discussion

We showed that obesity-related markers were minimized by the addition of 4% MJP powder in the high-fat diet-fed mice. The lower body weight of the animals supplemented with MJP could possibly be explained by the fecal excretion of triglycerides shown in a previous study [19]. The inhibition of digestive enzyme (e.g. pancreatic lipase) activity [31] has also been cited after administration of MJP in rodents. Moreover, anthocyanins present in MJP are linked to down-regulation of genes related to lipogenicity in the liver and adipose tissue of obese mice [32], which could be a hypothesis to explain the lower weight gain of HM animals (Fig. 1).

As well as the reduced body weight, the main findings of this study can be highlighted as reduced peripheral insulin resistance and decreased oxidative stress, corroborating earlier studies using different models [16-19, 31]. Moreover, this is the first report about how the intake of a berry peel can prevent the impairment in hippocampal function induced by a high-fat diet, investigating the IRS-GSK3-β-tau axis. Furthermore, the MWM test corroborates the results, since it shows an improvement in learning and spatial memory in the animals supplemented with the berry peel, proving its effect on counteracting the diet-induced cognitive impairment.

In relation to insulin resistance, impaired insulin signaling contributes to the activity GSK3-β, one of the kinases responsible for the phosphorylation of tau, which can lead to neuronal synaptic dysfunction and cognitive decline, features of AD [5-6, 9]. Our findings proved that the ingestion of the berry peel minimizes GSK3-β-mediated tau phosphorylation in the hippocampus (Fig. 3b) although some questions arise: Was the lower tau phosphorylation and cognitive enhancement shown by the HM animals related to direct action of MJP derivative compounds and metabolites on target neuron pathways, or was it an indirect effect of weight gain prevention? Or were both direct and indirect effects involved? When we look at the results observed for the NM group, we might conclude that the effects are mostly indirect, since the contrary would show MJP compounds boosting the effects on the hippocampus of this group. However, our results together with an earlier report [16] support the idea that both indirect and direct effects may happen simultaneously.

Studies have found that supplementation with a purified phenolic compound protects mice against tau phosphorylation and the cognitive decline promoted by streptozotocin-induced diabetes [33] or a high-fat diet [9]. In spite of that, when the non-diabetic [33] and the lean [9] groups were supplemented with the phenolic compound, no such effects were found, likewise in this study. In other words, phenolic compounds do not boost cognition and insulin

sensitivity, being effective only when the organism undergoes an insult, like saturated fatty acid overdose, as can be seen in our study and others [9, 33].

A recent review showed more understanding of how flavonoids can act direct and indirectly improving learning and memory [8]. They are able to adjust peripheral and cerebrovascular blood flow allowing better clearance of undesirable proteins, such as β-amyloid, thus improving the transit of oxygen and other compounds of interest , e.g. insulin or even phenolic metabolites, in the brain [8, 10, 34].

Cyanidin-3-*O*-glucoside, ellagic acid and carotenes, the main antioxidant compounds in MJP, are able to transit through the blood–brain barrier to be available in the brain parenchyma [29, 35-36], where they may play a direct role in modulating neuronal receptors, kinases, transcription factors, neurotrophins, synaptic plasticity and other enzymes or proteins linked to the antioxidant system and inflammatory and insulin cascades, as well as acting as reactive oxygen species scavengers [8, 10, 16, 18]. Our findings showed elevated activity of SOD, CAT and GPx antioxidant enzymes in the brain frontal lobe of the HM group in comparison to the H group, indicating lowered oxidative stress by activation of the antioxidant defense system.

Therefore, we concluded that MJP intake might prevent phosphorylation of tau induced by high-fat diet both indirectly, by attenuating body/fat weight and peripheral insulin resistance, and directly by modulating the insulin signal, inactivating GSK3-β. Furthermore, a previous study supports the idea of a direct effect of MJP intake on insulin sensitivity (IRS-AKT-FoxO-1 pathway in liver) without preventing weight gain [16].

In fact, the higher values of NeuN in the HM group may indicate increased neuronal viability and plasticity. Lower levels of TNF-α and IFN-γ in the hippocampus of HM also indicate a role of the HM diet in minimize inflammatory markers (Fig. 3d and e), although the fasting TNF-α levels in the serum did not change in the same group. The facilitated entrance

of antioxidant compounds present in MJP across the blood–brain barrier [29, 35-36] could be a clue to the lower hippocampal content of TNF-α and IFN-γ. In addition, circulating adiponectin levels were increased in the HM group, which might also have influenced the better cognitive performance of the HM group, in consequence of its role as an insulin sensitizer and tau phosphorylation blocker [9].

We concluded that the supplementation of a high-fat diet with MJP may play a role on controlling tau phosphorylation, insulin resistance and inflammatory markers in the hippocampus. Behavioral tests also revealed enhanced learning and memory of mice supplemented with the berry. Equally important, improvements in other studied parameters, e.g. peripheral insulin sensitivity and oxidative stress, showed that besides the direct action of derived bioactive compounds as reactive oxygen species scavengers and insulin cascade pathway stimulator, the indirect effects might also be the reason for better cognitive performance.

*We are grateful to FAPESP #2010/05262-0 CNPq (MRMJ for grant #300533/2013-6 and MACH #305099/2011-6) and CAPES for scholarship and financial support. We also thank Edy Souza de Brito for helpful collaboration.*

*The authors have declared no conflicts of interest.*

## 5 References

[1] Emmerzaal, T. L., Kiliaan, A. J., Gustafson, D. R., 2003-2013: A Decade of Body Mass Index, Alzheimer's Disease, and Dementia. *Journal of Alzheimer's Disease* 2015, *43*, 739-755.

[2] de la Monte, S. M., Wands, J. R., Alzheimer's disease is type 3 diabetes-evidence reviewed. *J Diabetes Sci Technol* 2008, *2*, 1101-1113.

[3] Savva , G. M., Wharton , S. B., Ince , P. G., Forster , G., *et al.*, Age, Neuropathology, and Dementia. *New England Journal of Medicine* 2009, *360*, 2302-2309.

[4] Castellani, R. J., Rolston, R. K., Smith, M. A., Alzheimer Disease. *Disease-a-month : DM* 2010, *56*, 484-546.

[5] Schubert, M., Brazil, D. P., Burks, D. J., Kushner, J. A., *et al.*, Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. *J Neurosci* 2003, *23*, 7084-7092.

[6] Schubert, M., Gautam, D., Surjo, D., Ueki, K., *et al.*, Role for neuronal insulin resistance in neurodegenerative diseases. *Proceedings of the National Academy of Sciences of the United States of America* 2004, *101*, 3100-3105.

[7] Spielman, L. J., Little, J. P., Klegeris, A., Inflammation and insulin/IGF-1 resistance as the possible link between obesity and neurodegeneration. *Journal of Neuroimmunology* 2014, *273*, 8-21.

[8] Rendeiro, C., Rhodes, J. S., Spencer, J. P. E., The mechanisms of action of flavonoids in the brain: Direct versus indirect effects. *Neurochemistry International* 2015, *89*, 126-139.

[9] Jeon, B. T., Jeong, E. A., Shin, H. J., Lee, Y., *et al.*, Resveratrol Attenuates Obesity-Associated Peripheral and Central Inflammation and Improves Memory Deficit in Mice Fed a High-Fat Diet. *Diabetes* 2012, *61*, 1444-1454.

[10] Rendeiro, C., Vauzour, D., Rattray, M., Waffo-Teguo, P., *et al.*, Dietary Levels of Pure Flavonoids Improve Spatial Memory Performance and Increase Hippocampal Brain-Derived Neurotrophic Factor. *Plos One* 2013, *8*.

[11] Casadesus, G., Shukitt-Hale, B., Stellwagen, H. M., Zhu, X., *et al.*, Modulation of Hippocampal Plasticity and Cognitive Behavior by Short-term Blueberry Supplementation in Aged Rats. *Nutritional Neuroscience* 2004, *7*, 309-316.

[12] Valente, T., Hidalgo, J., Bolea, I., Ramirez, B., *et al.*, A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain. *Journal of Alzheimer's Disease* 2009, *18*, 849-865.

[13] Williams, R. J., Spencer, J. P. E., Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. *Free Radical Bio Med* 2012, *52*, 35-45.

[14] Kaczmarczyk, M. M., Miller, M. J., Freund, G. G., The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism* 2012, *61*, 1058-1066.

[15] Nile, S. H., Park, S. W., Edible berries: Bioactive components and their effect on human health. *Nutrition* 2014, *30*, 134-144.

[16] Dragano, N. R. V., Marques, A. C., Cintra, D. E. C., Solon, C., *et al.*, Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. *British Journal of Nutrition* 2013, *110*, 447-455.

[17] Lenquiste, S. A., Batista, A. G., Marineli, R. D., Dragano, N. R. V., Marostica, M. R., Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. *Food Res Int* 2012, *49*, 153-160.

[18] Batista, A. G., Lenquiste, S. A., Cazarin, C. B. B., da Silva, J. K., *et al.*, Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *Journal of Functional Foods* 2014, *6*, 450-461.

[19] Batista, A. G., Lenquiste, S. A., Moldenhauer, C., Godoy, J. T., *et al.*, Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats. *Brazilian Journal of Nutrition* 2013, *26*, 571-581.

[20] Plaza, M., Batista, Â. G., Cazarin, C. B. B., Sandahl, M., *et al.*, Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. *Food Chem* 2016, *211*, 185-197.

[21] Batista, Â. G., da Silva, J. K., Betim Cazarin, C. B., Biasoto, A. C. T., *et al.*, Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves. *LWT - Food Science and Technology* 2017, *76, Part B*, 284-291.

[22] Leite-Legatti, A. V., Batista, A. G., Dragano, N. R. V., Marques, A. C., *et al.*, Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. *Food Res Int* 2012, *49*, 596-603.

[23] Lin, L.-Z., Harnly, J. M., A Screening Method for the Identification of Glycosylated Flavonoids and Other Phenolic Compounds Using a Standard Analytical Approach for All Plant Materials. *J Agr Food Chem* 2007, *55*, 1084-1096.

[24] Reeves, P. G., Nielsen, F. H., Fahey, G. C., Ain-93 Purified Diets for Laboratory Rodents - Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the Ain-76a Rodent Diet. *J Nutr* 1993, *123*, 1939-1951.

[25] Morris, R., Developments of a Water-Maze Procedure for Studying Spatial-Learning in the Rat. *J Neurosci Meth* 1984, *11*, 47-60.

[26] Johansson, L. H., Borg, L. A. H., A Spectrophotometric Method for Determination of Catalase Activity in Small Tissue Samples. *Anal Biochem* 1988, *174*, 331-336.

[27] Paxinos, G., Franklin, K. B., *The mouse brain in stereotaxic coordinates*, Gulf Professional Publishing 2004.

[28] Henningsson, A. M., Nyman, E. M. G. L., Bjorck, I. M. E., Short-chain fatty acid content in the hindgut of rats fed various composite foods and commercial dietary fibre fractions from similar sources. *J Sci Food Agr* 2002, *82*, 385-393.

[29] Sugiura, M., Ogawa, K., Yano, M., Comparison of bioavailability between β-cryptoxanthin and β-carotene and tissue distribution in its intact form in rats. *Bioscience, Biotechnology, and Biochemistry* 2014, *78*, 307-310.

[30] Alavijeh, M. S., Chishty, M., Qaiser, M. Z., Palmer, A. M., Drug Metabolism and Pharmacokinetics, the Blood-Brain Barrier, and Central Nervous System Drug Discovery. *NeuroRx* 2005, *2*, 554-571.

[31] Alezandro, M. R., Granato, D., Genovese, M. I., Jaboticaba (Myrciaria jaboticaba (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. *Food Res Int* 2013, *54*, 650-659.

[32] Tsuda, T., Horio, F., Uchida, K., Aoki, H., Osawa, T., Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J Nutr* 2003, *133*, 2125-2130.

[33] Thomas, J., Garg, M. L., Smith, D. W., Dietary resveratrol supplementation normalizes gene expression in the hippocampus of streptozotocin-induced diabetic C57Bl/6 mice. *Journal of Nutritional Biochemistry* 2014, *25*, 313-318.

[34] Correia, S. C., Santos, R. X., Carvalho, C., Cardoso, S., *et al.*, Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. *Brain Res* 2012, *1441*, 64-78.

[35] Yan, L., Yin, P., Ma, C., Liu, Y., Method Development and Validation for Pharmacokinetic and Tissue Distributions of Ellagic Acid Using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). *Molecules* 2014, *19*, 18923.

[36] Chen, T.-Y., Kritchevsky, J., Hargett, K., Feller, K., *et al.*, Plasma bioavailability and regional brain distribution of polyphenols from apple/grape seed and bilberry extracts in a young swine model. *Molecular Nutrition & Food Research* 2015, *59*, 2432-2447.

## CAPÍTULO 5

**Artigo Original: CASCA DE JABUTICABA AUMENTA A PRODUÇÃO DE ÁCIDOS GRAXOS DE CADEIA CURTA E PREVINE A ESTEATOSE HEPÁTICA EM CAMUNDONGOS ALIMENTADOS COM DIETA HIPERLIPÍDICA**

Ângela Giovana Batista, Juliana Kelly da Silva, Monique Culturato P. Mendonça, Edilene Siqueira Soares, Glaucia Carielo Lima, Stanislau Bogusz Junior, Maria Alice da Cruz-Höfling and Mário Roberto Maróstica Júnior. **Jaboticaba berry peel increases short chain fatty acids production and prevent hepatic steatosis in mice fed high-fat diet.** A ser submetido à revista internacional ***Nutrition,*** Elsevier, ISSN: 0899-9007.

Vide autorização CEUA no Anexo 2.

Research article

# Jaboticaba berry peel increases short chain fatty acids production and prevent hepatic steatosis in mice fed high-fat diet

Ângela Giovana Batista[1], Juliana Kelly da Silva[1], Monique Culturato P. Mendonça[2,3], Edilene Siqueira Soares[3], Glaucia Carielo Lima[1], Stanislau Bogusz Junior[4], Maria Alice da Cruz-Höfling[2,3] and Mário Roberto Maróstica Júnior[1,*]

[1]Faculty of Food Engineering, Department of Food and Nutrition, University of Campinas, Campinas, SP, Brazil

[2]Faculty of Medical Sciences, Department of Pharmacology, University of Campinas, Campinas, SP, Brazil

[3]Institute of Biology, Department of Biochemistry and Tissue Biology, University of Campinas, Campinas, SP, Brazil

[4]Institute of Chemistry, Department of Chemistry and Molecular Physics, University of São Paulo, São Carlos, SP, Brazil.

*Corresponding author:
Mário Roberto Maróstica Júnior
Contact details: Departamento de Alimentos e Nutrição. Rua Monteiro Lobato, 80. Cidade Universitária. Campinas, SP, Brasil. E-mail: mario@fea.unicamp.br

**Abbreviations:** **ALT** alanine transaminase; **ALP** alkaline phosphatase; **AST** aspartate aminotransferase; **EAT** epididymal adipose tissue; **GPx** glutathione peroxidase; **GRd** glutathione reductase; **GSH** reduced glutathione; **GTT** glucose tolerance test; **MJP** *Myrciaria jaboticaba* berry peel; **LPS** lipopolysaccharides; **MAT** mesenteric adipose tissue; **NAFLD** non-alcoholic fatty liver disease; **RAT** retroperitoneal adipose tissue; **SCFA** short chain fatty acids; **SOD** superoxide dismutase; **TBARS** thiobarbituric acid reactive substances.

**ABSTRACT**

Hepatic steatosis and intestinal health is closely related to obesity, low-grade inflammation and adipose tissue hipertrophy. Previous studies demonstrated that the treatment of obese animals with a diet containing *Myrciaria jaboticaba* berry peel (MJP) offers bioactive compounds that counteract obesity development and insulin resistance. In this study, a preventive model was proposed to check whether the MJP intake could avoid harmful effect caused by high-fat diet, such as adipose tissue inflammation, liver steatosis, dyslipidemia, oxidative state, and biomarkers of healthy gut. The intake of the high-fat diet supplemented with MJP (HM) down-regulated the IL-6 and TNF-α pro-inflammatory cytokines in adipose tissue, besides of preventing adipose tissue growth. In addition, the intake of the HM diet prevented weight gain, increased excretion of triglycerides, reduced hepatic steatotic areas and stimulated production of short chain fatty acids (butyrate and acetate) by the large intestinal microbiota. The lower activity of the enzymes alanine transaminase and aspartate aminotransferase associated to lower triglycerides content in the liver of the HM mice also indicated prevention of non-alcoholic fatty liver disease induced by high-fat diet consumption. Furthermore, hepatic mRNA PPAR-α levels were lower in non-fasted animals receiving the HM diet, indicating lower oxidation of fatty acids and lipotoxic metabolites by the liver. In conclusion, MJP intake induces higher production of short chain fatty acids in the gut, compounds associated to counteract obesity markers, as we found lower adipose tissue inflammation, lower weight gain, dyslipidemia and hepatic steatosis.

**Keywords:** *Plinia jaboticaba, Myrtacea,* obesity, metabolic syndrome, polyphenols.

## 1. Introduction

Adipose tissue dysfunction is associated to the development of obesity and non-alcoholic fatty liver disease (NAFLD), disorders of worldwide concern [1]. For example, the United States of America has the highest prevalence of obesity accounting to more than 30% of cases among adults [2] and 46% middle-aged adults with NAFLD [3]. In Brazil, the number of cases of obesity already reached 12% among adult men and 20% among adult women, accompanying the global rates [2].

Gut health may play a role on the start of obesity markers, such as insulin resistance, low-grade inflammation, increased visceral adiposity and NAFLD [4-6]. Studies have linked diets poor in fibers and rich in fat to dysbiosis, which is an imbalance in the intestinal microbiota provoked by the settlement of pathogenic microbiota [5,9-10]. Dysbiosis leads to local inflammation, damaging intestinal tight-junctions and encouraging increase of intestinal permeability [4, 7]. Thus, pathogenic bacteria and their products (LPS - lipopolysaccharides and bacterial DNA) increase in the circulation, which is enough to promote systemic inflammation, insulin resistance in tissues like adipose and liver [4, 6-7] and hepatic steatosis [5-6].

The low-grade inflammation installed in the adipose tissue markedly increases the macrophage infiltration and production of inflammatory cytokines, such as TNF-α, IL-6 and IL-1β [1]. The inflammation leads to insulin resistance in the adipose tissue, causing lipotoxicity and release of adipocytokines to the stream circulation [8]. The higher flux of inflammatory cytokines as well as fatty acids in the portal circulation is related to the development of hepatic insulin resistance and NAFLD that can progress to steatohepatitis [8].

Plant-based products, in special berries, have been used as natural supplements to minimize obesity, insulin resistance, cardiovascular diseases, inflammatory diseases and NAFLD [9-10]. Supplementing diets with berries provide dietary fibers, compounds reported

to improve gut-health by acting as local anti-inflammatory when fermented or metabolized by intestinal microbiota, producing short chain fatty acids (SCFA) [9-10]. Reaching the systemic circulation, the SCFA together with phenolic compounds of berries, as well as their metabolites, could also influence insulin sensitivity, inflammation and energy metabolism [9-10].

Improvement of peripheral insulin resistance is markedly an effect from jaboticaba berry peel (MJP) intake either in animal [11] or human studies [12], which also suggests beneficial effect on preventing NAFLD. Previous reports showed that MJP was able to reverse oxidative stress [13], transcription of inflammatory cytokines and insulin resistance in liver of obese animals [11]. In addition, ingestion of MJP was reported to treat dyslipidemias [14-15], diminishing circulating cholesterol [14] and triglycerides [16], serum saturated fatty acids [13], and increasing blood HDL-cholesterol in high-fat diets-fed animals [17].

Dietary fibers (e.g., pectin) and phenolic compounds, such as ellagic acid, condensed tannins, cyanidin 3-glucoside and delphinidin 3-glucoside, from jaboticaba berry, are the main bioactive compounds present in the fruit [12-13]. Although, ellagitannins were previously highlighted as the polyphenol with greatest contribution to the total antioxidant capacity of MJP and have important *in vivo* effects [12, 16].

Our previous study demonstrated that liver was one of the more benefited tissues from the intake of MJP after established obesity, improving biological antioxidant defense system, such as glutathiones and superoxide dismutase enzymes and reducing tissue lipid peroxidation [13]. In the present study we proposed a preventive model, in which mice received supplemented diets during 10 weeks, to check whether the MJP intake could protect adipose tissue, liver and large intestine against harmful effect of high-fat diet feeding.

## 2. Material and Methods

### 2.1. Jaboticaba chemical composition

Ripe jaboticaba fruits were harvested in October 2013 in Lagoa Branca, SP, Brazil. They were cleaned, peeled and dried in oven at 40 °C with forced air circulation for 96 h, and then grounded, homogenized and packed in plastic dark flasks protected from the light and frozen at -18 ± 5 °C until use.

The chemical characteristics of MJP are described in previous study [18]. Additionally, in this study we performed total and soluble pectic substances analyses. Pectin was extracted from sugar-free residues of ethanol extractions and determined by the uronic acid carbazole reaction by adding borate [19].

### 2.2. Animal care and diets

The animal study was carried out using 40 recently weaned (3-weeks old) male *Swiss* mice acquired from the Multidisciplinary Center of Biological Investigation (CEMIB/UNICAMP), and the ethical guidelines of Brazilian Society of Science of Laboratory Animals (SBCAL) was followed. The mice were housed separately in cages under 22 ± 2 °C, 50 - 60% relative humidity and a standard inverted 12/12 h of dark/light cycles. The animals were given free access to water and a semi-purified diet (AIN93-G) [20] during 4 weeks (35 g) and then randomized into four groups ($n$ = 10) according to the respective diets, formulated based on the AIN93-M (maintanance) diet [20]: a normal-fat diet control group (N); normal-fat diet supplemented with 4% (w/w) MJP group (NM); a high-fat diet control group (H); and a high-fat diet containing 4% (w/w) MJP group (HM).

After 1-week acclimatization, the animals were given free access to the diets during 10 weeks. The weight gain and diet consumption were determined weekly. In the present study we proposed to adjust the amount of cellulose in the NM and HM diets according to the

insoluble fiber content in MJP, since the AIN93-M diet [20] only offers cellulose as source of dietary fiber. The 4% MJP dose had been optimized in previous studies [11, 13]. The fat content in the high-fat diet offered 60% of total calories.This study was approved by the institutional Ethics Committee in Animal Use (CEUA UNICAMP, protocol #3157-1).

The powdered and semi-purified ingredients used to the standard diet (N) were: corn starch (46.32%); maltodextrin (15.4%); sucrose (9.95%) casein (14.38 %); cellulose (5.0%); soy oil (4.0%); mineral mix (3.5%), vitamin mix (1.0%); L-cystine (0.18%); choline substrate (0.25%); and *tert*-butylhydroquinone (0.0008%). The normal-fat diet containing MJP had its carbohydrates adjusted (corn starch (44.56%), maltodextrin (14.83%), sucrose (9.57%) and cellulose (3.73%), due to the addition of 4% MJP, containing 1.27% insoluble fiber. The high-fat group (H) was adjusted regarding corn starch (26.29%), maltodextrin (9.57%), and sucrose (5.65%) due to addition of 31% lard and for the HM diet: corn starch (24.53%), maltodextrin (8.16%), and sucrose (5.27%), and also cellulose (3.73%) were adjusted.

### 2.2.1. Experimental proceedings

After anesthesia with 2:1 v/v ketamine chloride (Dopalen®, 100 mg $kg^{-1}$), and xylazine chloride (Anasedan®, 10 mg $kg^{-1}$) (Fortvale, Valinhos, SP, Brazil), blood was collected by cardiac puncture in Vacuette® tubes containing clot activator (Greiner Bio-One GmbH, Kremsmünster, Austria). Liver, epididymal adipose tissue (EAT), retroperitoneal adipose tissue (RAT), mesenteric adipose tissue (MAT) and large intestine of non-fasted mice were rapidly dissect, washed in 0.9% saline solution, dried in appropriated tissue paper and weighed. Later, these tissues' weight were related to total body weight and expressed as percentage.

### 2.2.2. Lipid profile

*Serum measurements:* Six hours fasting total triglycerides, total cholesterol, LDL and HDL-cholesterol, alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were carried out in the serum of the animals ($n$ = 5 per group) using commercial kits (Wiener lab, Rosario, AR).

*Lipid contents:* Fresh liver from the 6 h-fasted mice and dried feces (collected in the last 7 days of experimentation period and dried at 60 $^{o}$C for 24 h) ($n$ = 5) were extracted using the Folch method [21] and the total triglycerides and cholesterol were analyzed using commercial kits (Wiener lab).

*Histological analysis of liver:* After anesthesia, the animals ($n$ = 3) were transcardially perfused with physiological saline, followed by 4% paraformaldehyde in 0.1 mol $L^{-1}$ phosphate buffered saline (PBS), pH 7.4. The liver was post-fixed in the same fixative solution overnight, dehydrated in an ethanol series, cleared in xylene, and processed for paraffin embedding (Paraplast Plus®, Sigma-Aldrich, St. Louis, MO, USA) . Slides containing paraffin sections (5 µm thickness) were deparaffinized, dehydrated and stained with hematoxylin-eosin (HE). The stained sections were examined by light microscopy on an Olympus BX51 photomicroscope (Olympus Corporation, Tokyo, Japan). The analyses were measured in a 20x objevtive lens in 12 fields of each animal. The images were processed to quantify the macro and microvesicular steatosis usingthe Image J Pro Plus software.

*SCFA analysis:* Fecal samples were collected directly from the large intestine of the non-fasted mice and stored in cryogenic tubes at -80 $^{o}$C. Later, samples ($n$ = 5) were extracted and analyzed as described in previous study [22], using a gas chromatograph equipment (Agilent model 7890, Palo Alto, CA, USA) coupled with flame ionization detector and capillary column Nukol™ (30 m x 0.25 mm x 0.25 µm, Supelko, Bellefonte, PA, USA). The chromatographic conditions were: injector and detector temperatures set at 200 °C and 240

°C, respectively. The injected volume was 1 μL (autosampler), splitless mode; carrier gas was hydrogen at 1.0 mL $min^{-1}$. The column oven was programmed as follows: held at 100 °C for 0.5 min, then heated to 8 °C $min^{-1}$ to 180 °C, held for 1 min, heated at 20 °C $min^{-1}$ to 200 °C and maintained for 1 min. For quantification standard curves of authentic standards acetate, propionate and butyrate were used (Sigma Aldrich, St. Louis, MO, USA).

*Serum measurement of LPS or endotoxin:* The concentration of endotoxin (LPS) was analyzed in non-fasted animal serum ($n$ = 5) using Limulus Amebocyte Lysate assay kit (Lonza Walkersville, MD, USA).

### 2.2.4. Antioxidant enzyme activities and lipid peroxidation analyses

Livers of non-fasted mice were washed, weighed, frozen instantaneously in liquid nitrogen, and kept at -80°C. Later, the tissue sample was homogenized in potassium phosphate buffer (PB) pH 7.4 (approximately 100 mg $mL^{-1}$).

*TBARS* (*Thiobarbituric Acid Reactive Substances*) *assay:* TBARS values in liver were determined using the method described by Ohkawa et al. [23], with adaptations. Briefly, liver PB-homogenates were mixed in 8.1% sodium dodecyl sulphate, and 0.53% TBA (2-thiobarbituric acid) solution in 20% acetic acid and 5% sodium hydroxide. After heating at 95°C for 60 min, samples were cooled in an ice bath for 10 min and then centrifuged at 10,000 g, 10 min, $4^{o}$C. The resulting MDA-TBA and related adducts were read at 532 nm using a 96-well microplate. A standard curve was prepared using the MDA standard (1,1,3,3-tetramethoxypropane, Sigma-Aldrich, St Louis MO, USA), and the results were expressed as nmol MDA.

*SOD* (*Superoxide dismutase activity*): Liver PB-homogenates were mixed with work solution (0.1 mmol $L^{-1}$ hypoxanthine, 0.07 U xanthine oxidase and 0.6 mmol $L^{-1}$ NTB in

phosphate buffer in 1: 1: 1 proportions) and read at 560 nm for 10 min. The SOD activity was expressed as U $mg^{-1}$ protein [24].

*GPx* (*Glutathione peroxidase activity*): Working reagent (10 mmol $L^{-1}$ reduced glutathione, 4 mmol $L^{-1}$ NADPH (β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate) and 1 U glutathione reductase) was added to liver PB-homogenates and mixed with 0.25 mmol $L^{-1}$ $H_2O_2$ [25]. The decrease in absorbance was monitored at 365 nm and the results were expressed as nmol NADPH consumed $min^{-1}$.

*GRd* (*Glutathione Reductase activity*): Glutathione activity was measured in liver PB-homogenates [26], following the decrease in absorbance at 340 nm induced by oxidized glutathione in the presence of NADPH in PB. The results were expressed as nmol NADPH consumed $min^{-1}$.

*CAT* (*Catalase activity*): For CAT activity, the method of Johansson was adapted [27]. Liver homogenates were diluted in 25 mmol $L^{-1}$ PB (pH 7.5, containing 1 mmol $L^{-1}$ EDTA and 0.1% BSA) and added to 96-well microplate containing 100 mmol $L^{-1}$ PB (pH 7.0) and methanol. Briefly, 10.58 mmol $L^{-1}$ $H_2O_2$ was added to the microplate and allowed to react for 20 min in a microplate shaker in dark conditions. After, 7.8 mol $L^{-1}$ KOH and 34.2 mmol $L^{-1}$ Purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole) solution were added to the microplate and allowed to react for 10 min in a shaker; and then a 65.2 mmol $L^{-1}$ potassium periodate solution were added and mixed during 5 min before it was read at 540 nm. Formaldehyde was used as standard and results were expressed as nmol of formaldehyde formation per minute at 25 $^{o}$C.

*Reduced thiol (GSH) contents:* The GSH level in liver was determined in liver PB homogenates precipitated with 0.75 mol $L^{-1}$ metaphosphoric acid [13]. The assay was based in the Ellman's reaction using DTNB (5′5′-dithio-bis-2-nitrobenzoic acid) [28] and the samples

were read at 412 nm. Using a GSH standard curve the concentration was expressed as nmol GSH.

The protein concentrations in liver homogenates were determined using the Bradford method [29].

### 2.2.5. RNA extraction and quantitative real-time PCR

Total RNA was extracted from liver and EAT of non-fasted mice ($n$ = 5) using a commercial acid phenol reagent, Trizol (Invitrogen, CA, USA). RNA integrity was confirmed by non-denaturing agarose gel electrophoresis. First strand complementary DNA was synthesized using SuperScript III RT and random hexamer primers, as described in the manufacturer's protocol (Invitrogen). Quantitative PCR was run to determine the expression of IL-1β (Mm00434228_m1; Applied Biosystems, CA, USA) and IL-6 (Mm00446190_m1; Applied Biosystems) and TNF-α (Mm00443258_m1) in the epididymal adipose tissue. In the liver, PPAR-α primer (Mm00440939_m1, Applied Biosystems) was used. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (#4352339E; Applied Biosystems). Real-time PCR analysis of gene expression was carried out in an ABI Prism 7500 sequence detection system (Applied Biosystems). Each PCR contained 25 ng of reverse-transcribed RNA and was run according to the manufacturer's recommendations using the TaqMan PCR master mix. Real-time data were analyzed using the Sequence Detector System 1.7 (Applied Biosystems). Results were expressed as relative transcript amounts.

## 2.3 Statistics

Results of mice study were expressed as means ± standard error of means (SEM). Data were analyzed using Student's $t$-test ($p < 0.05$) to compare the experimental groups in pairs

(N × H, N × NM and H × HM). All statistical analyses were done using GraphPad Prism 5.0 software (GraphPad, Inc. La Jolla, CA, USA).

## 3. Results

### 3.1. *Jaboticaba peel prevents weight gain and increases bioactive compounds in diets*

The fat content of diet (60% total calories) was able to promote body weight gain, as observed in H mice group ($p < 0.001$, Table1). On the other hand, the intake of MJP together with the high-fat diet prevented weight gain in HM mice group ($p < 0.001$), even that food and calorie intake were similar between H and HM groups (Table 1). The animals in the NM group did not lose weight ($p > 0.05$), which indicated no harms coming from the 4% MJP added to the normal-fat diet (Table 1).

The N and H diets had the same amount of cellulose. However, the total dietary fiber intake by the H group was lower than in N group, due to lower total food intake ($p < 0.001$). The NM and HM diets were supplemented with soluble fiber, like pectin and other bioactive compounds from MJP, such as cyanidin 3-glucoside and ellagic acid, among other phenolic compounds [12]. The lower ingestion of bioactive compounds in HM in relation to NM ($p < 0.05$) can be related with lower food intake of the group (Table 1).

### 3.2 *Jaboticaba peel intake prevents adipose tissue growth and inflammation*

Figure 1 shows representative histology images of the EAT. The high-fat diet-fed animals showed crown-like structures in some regions of the EAT. These structures were less frequent in NM and HM groups (Figure 1).

The transcription of inflammatory cytokines in the EAT was lower in the groups receiving MJP: The NM group had lower IL-1β than N, as well as the HM group showed lower IL-6 and TNF-α mRNA levels when compared to H ($p < 0.05$). However, no significant

differences were found between N and H for any studied cytokine ($p > 0.05$), but a trend towards higher TNF-α mRNA levels in H compared to N ($p = 0.067$) (Fig. 1).

**Table 1.** Body weight gain, bioactive compounds and dietary intake parameters of mice after 10 experimental weeks.

| Parameter | N | NM | H | HM |
|---|---|---|---|---|
| *Body weight* | | | | |
| Final body weight (g) | 51.99 ± 1.38 | 50.89 ± 1.45 | 61.80 ± 1.34*** | 55.17 ± 1.46** |
| Total weight gain (g) | 17.78 ± 0.95 | 15.26 ± 1.19 | 25.31 ± 1.42*** | 19.53 ± 1.20** |
| *Intake parameter* | | | | |
| *Total intake* | | | | |
| Food (g) | 416.5 ± 9.40 | 389.03 ± 14.01 | 304.8 ± 9.83*** | 293.89 ± 6.69 |
| Calorie (Kcal) | 1560.9 ± 32.48 | 1501.86 ± 54.91 | 1785.1 ± 57.53** | 1721.00 ± 39.16 |
| *Daily intake (mg /day)* | | | | |
| Total dietary fiber | 297.52 ± 6.71 | 282.32 ± 10.16 | 217.74±7.018*** | 211.23 ± 4.844 |
| Insoluble dietary fiber | 297.52 ± 6.71 | 265.10 ± 9.544* | 217.74±7.018*** | 200.26 ± 4.556 |
| Soluble dietary fiber | - | 22.01 ± 0.792 | - | 16.47 ± 0.378 |
| Total pectin [a] | - | 19.23 ± 0.692 | - | 14.39 ± 0.330 |
| Soluble fiber | - | 6.447 ± 0.2321 | - | 4.823 ± 0.110 |
| Cyanidin 3-glucoside | - | 2.518 ± 0.091 | - | 1.884 ± 0.043 |
| Ellagic acid | - | 1.134 ± 0.040 | - | 0.848 ± 0.020 |

[a] *M. jaboticaba* peel possessed 8.66 g $100g^{-1}$ of total pectin.
N= group that received normal-fat diet; NM= group that received normal-fat diet containing 4% *M. jaboticaba* peel; H= high-fat diet group; HM= high-fat diet with 4% of *M. jaboticaba* peel.
*Indicates significant differences between groups (N × H; N × NM and H × HM) according to Student's *t*-test, *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. Values expressed as means ± SEM, $n = 10$.

The high-fat diet increased fat mass in H-mice in comparison to N, as can be observed by heavier adipose tissue pads: EAT, RAT and MAT ($p < 0.05$) (Figure 1), as well as high total lipids in the carcass of the same animals ($p < 0.05$) (Table 2). Statistically significant lower values in the EAT and RAT tissues were found, when HM had 29.1 and 33.4% reduction in comparison to the H group, respectively ($p < 0.05$) (Figure 1). However, the group treated with the HM diet was not capable of preventing the growth of MAT ($p > 0.05$).

### 3.3 *Jaboticaba peel intake prevents non-alcoholic fatty liver disease*

The livers of animals receiving the high-fat diet were heavier in relation to the N group ($p < 0.05$). On the other hand, the group treated with the HM diet prevented liver

hypertrophy (Figure 2 .B), probably due to lower concentration of lipids as observed in the analysis of steatosis area ($p < 0.05$) (Figure 2 .C).

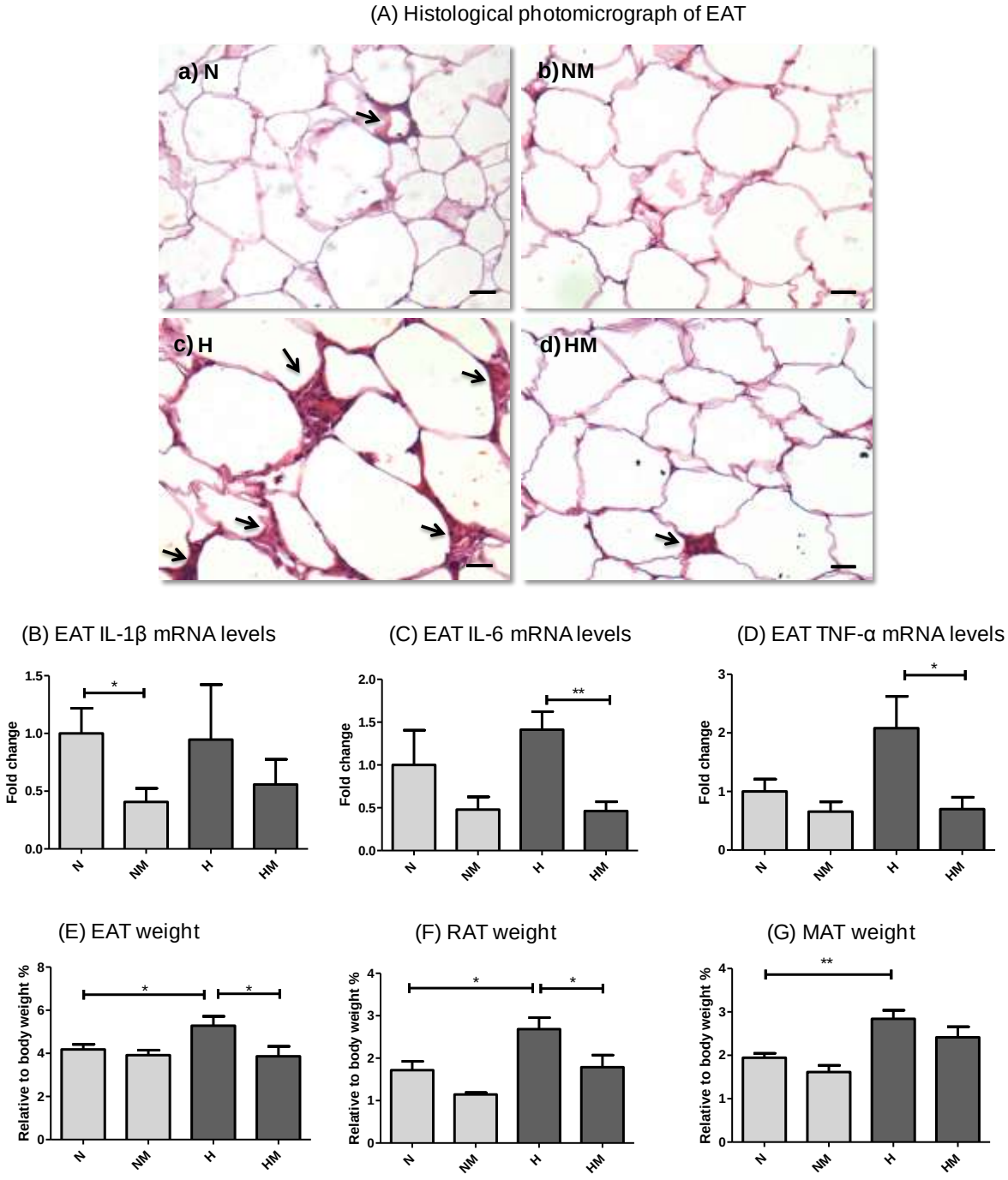



**Figure 1.** A) Panels of representative photomicrographs of the HE-stained epididymal adipose tissue (EAT) parenchyma. Bars a-d= 25 µm. Arrows indicate crown like structures. B) EAT mRNA levels of IL-1β, C) IL-6, and D) TNF-α; E) EAT weight; F) retroperitoneal adipose tissue (RAT) and G) Mesenteric adipose tissue (MAT) of mice after 10 weeks of experiment. *Indicates significant differences between groups according to Student's *t*-test, *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. Values expressed as means ± SEM, $n = 3$-5.

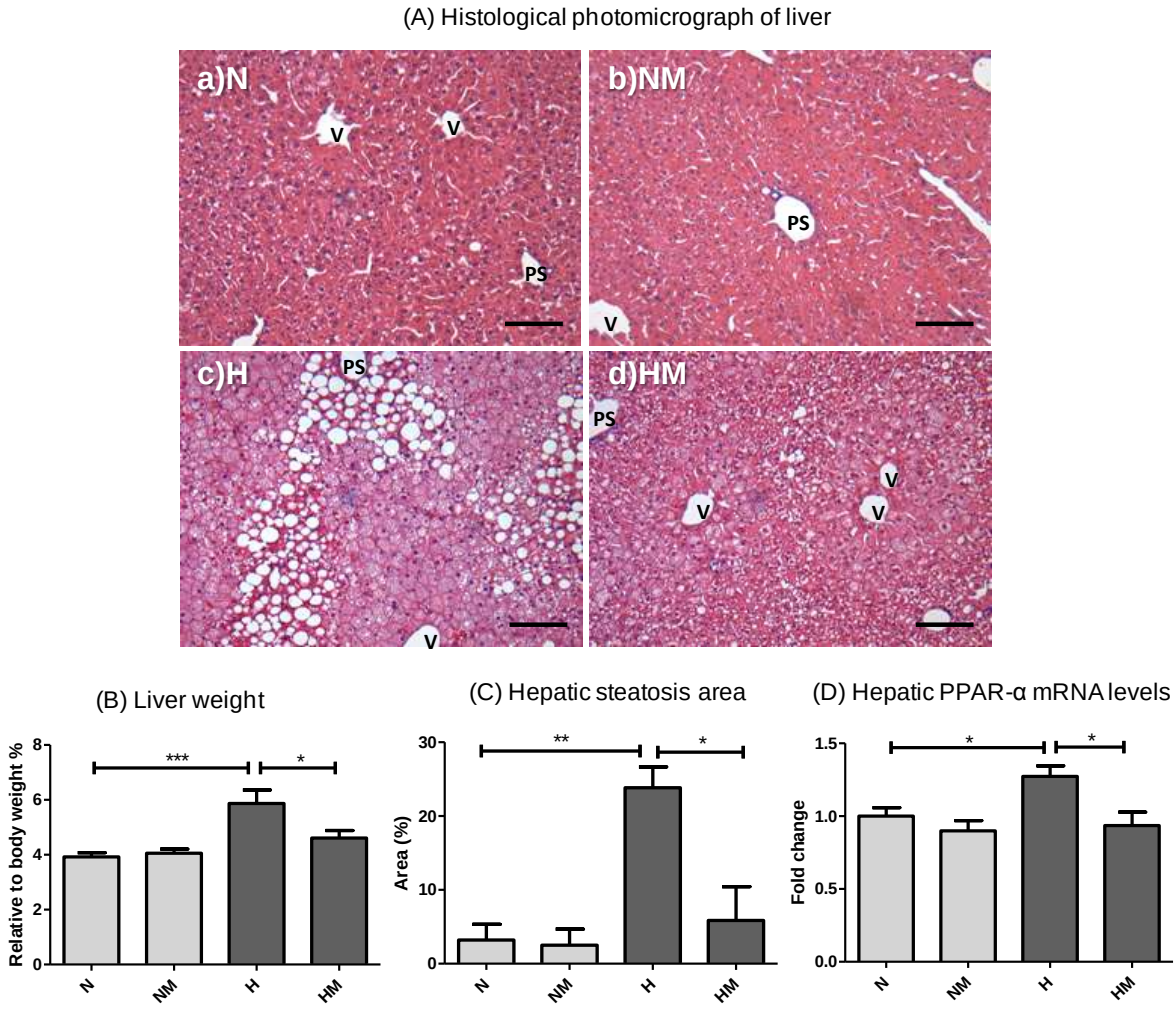



**Figure 2.** Photomicrographs of liver from mice in the different eperimental groups. (A) Representative photomicrographs of the hematoxylin and eosin-stained (H&E) hepatic parenchyma. Bars a-d= 100 µm. PS= portal space; V= centrilobular vein. (B) Liver weight; (c) Hepatic steatosis area (%) and (D) PPAR-α mRNA levels in liver of non-fasted mice. *Indicates significant differences between groups according to Student's *t*-test, *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. Values expressed as means ± SEM, $n$ = 3-5.

The photomicrographs of HE-stained livers showed a mix of large and small triglycerides droplets in the H group, characteristic of fatty liver disease (Fig. 2.A). Nevertheless, the areas of steatosis in the liver of the HM group were not as large as in H ($p < 0.05$).

Animals from H group showed higher expression of mRNA PPAR-α level ($p < 0.05$), demonstrating that obesity induces an oxidative state in liver even in feed condition (Figure 2.D). The mRNA levels of PPAR-α in the group HM was lower than H ($p< 0.05$), corroborating to the basal level shown in N (Figure 2).

The lipid profile of the carcass, serum, and liver indicates that the dietary fat in H was a harmful agent in comparison to N, capable of increasing body fat content (carcass),

total cholesterol and ALT enzyme activity in serum, and hepatic triglyceride content; as well as lowering HDL-cholesterol content in serum (Table 2). In contrast, the addition of the MJP to the high-fat diet was responsible for decreasing the fat content in carcass, the activities of ALT and AST, lipid and triglyceride contents in liver, and increasing serum HDL-cholesterol and fecal excretion of triglycerides, when compared to H ($p < 0.05$) (Table 2).

**Table 2.** Liver function test enzymes, lipid profile of carcass, serum, liver and feces of the experimental groups.

| Marker | N | NM | H | HM |
|---|---|---|---|---|
| *Carcass* | | | | |
| Total lipids % | 57.64 ± 1.51 | 58.64 ± 0.98 | 68.19 ± 1.32*** | 59.94 ± 1.67** |
| *Serum* | | | | |
| Triglycerides (mg $dL^{-1}$) | 43.45 ± 4.72 | 43.72 ± 5.56 | 33.26 ± 3.70 | 40.47 ± 7.33 |
| Cholesterol (mg $dL^{-1}$) | 203.91 ± 1.60 | 175.93 ± 4.26** | 215.91 ± 0.73** | 192.67 ± 14.60 |
| LDL-c (mg $dL^{-1}$) | 7.66 ± 3.26 | 3.33 ± 2.68 | 22.21 ± 6.22 | 11.54 ± 3.58 |
| HDL-c (mg $dL^{-1}$) | 158.49 ± 4.05 | 145.19 ± 4.14 | 125.97 ± 6.67** | 157.95 ± 12.06* |
| ALP (U $L^{-1}$) | 147.47 ± 3.17 | 112.82 ± 14.17*** | 220.45 ± 71.20 | 132.14 ± 19.22 |
| ALT (U $L^{-1}$) | 6.74 ± 0.49 | 3.19 ± 0.44 | 11.31 ± 1.62*** | 6.09 ± 0.48** |
| AST (U $L^{-1}$) | 13.34 ± 1.20 | 14.79 ± 3.01 | 14.60 ± 1.07 | 9.86 ± 1.35* |
| *Liver* | | | | |
| Total lipids % | 12.93 ± 1.78 | 13.15 ± 2.13 | 23.03 ± 2.75* | 15.25 ± 1.69* |
| Triglycerides (mg $g^{-1}$) | 65.59 ± 10.10 | 34.78 ± 5.2* | 115.50 ± 13.39* | 55.08 ± 4.40** |
| Cholesterol (mg $g^{-1}$) | 20.29 ± 2.5 | 11.09 ± 1.54* | 25.84 ± 5.87 | 16.54 ± 1.23 |
| *Feces* | | | | |
| Total lipids % | 2.18 ± 0.13 | 2.48 ± 0.15 | 7.39 ± 0.45*** | 4.33 ± 0.38*** |
| Triglycerides (mg $g^{-1}$) | 15.51 ± 0.61 | 15.04 ± 0.68 | 24.99 ± 0.76*** | 31.18 ± 1.11*** |
| Cholesterol (mg $g^{-1}$) | 7.14 ± 0.14 | 8.37 ± 0.42* | 13.88 ± 0.56*** | 15.94 ± 1.29 |

N= group that received normal-fat diet; NM= group that received normal-fat diet containing 4% *M. jaboticaba* peel; H= high-fat diet group; HM= high-fat diet with 4% *M. jaboticaba* peel. *Indicates significant differences between groups (N × H; N ×NM and H × HM) according to Student's *t*-test, *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. Values expressed as means ± SEM, $n = 5$.

In addition, the NM group showed lower total cholesterol in serum and liver than in the N group, corroborating higher excretion of cholesterol by the same group ($p < 0.05$) (Table 2). The levels of ALP activity and triglycerides in serum were reduced in NM in comparison to N ($p < 0.05$) (Table 2). No statistical differences were found for serum LDL cholesterol and triglycerides among the groups.

### 3.4 Jaboticaba peel improves hepatic antioxidant status

The highest lipid peroxidation, lower activity of SOD, CAT and contents of GSH in the mice's liver demonstrated that the high-fat diet induced deleterious effects on the antioxidant defense mechanism of the H-group. Although, the improvement noticed in the

animals fed MJP can be seen by higher activity of GPx and levels of GSH in NM, when compared to N; and the lower TBARS associated with higher SOD, CAT, GPx, and GSH values in HM in relation to H ($p < 0.05$), evidencing controlled oxidative stress (Table 3).

**Table 3.** Lipid peroxidation and antioxidant enzyme activities in liver of mice.

| Marker | N | NM | H | HM |
|---|---|---|---|---|
| TBARS (nmol $mg^{-1}$) | 0.393 ± 0.016 | 0.398 ± 0.016 | 0.559 ± 0.017*** | 0.463 ± 0.022* |
| SOD (U $mg^{-1}$) | 5.45 ± 0.54 | 7.08 ± 0.52 | 3.17 ± 0.35** | 6.09 ± 0.63** |
| CAT (nmol $mg^{-1}$)[a] | 751.08 ± 56.55 | 891.31 ± 36.91 | 543.73 ± 50.27* | 765.17 ± 52.37* |
| GPx (nmol $mg^{-1}$)[b] | 19.29 ± 1.86 | 61.07 ± 8.86*** | 17.36 ± 3.90 | 88.14 ± 6.80*** |
| GRd (nmol $mg^{-1}$)[b] | 9.09 ± 0.83 | 7.55 ± 0.78 | 6.93 ± 0.95 | 9.57 ± 1.08 |
| GSH (nmol $mg^{-1}$) | 109.2 ± 6.22 | 75.95 ± 5.42** | 57.18 ± 6.26*** | 111.2 ± 9.26*** |

[a]nmol $min^{-1}$ formed formaldehyde; [b]nmol $min^{-1}$ consumed NADPH. *Indicates significant differences between groups (N × H; N × NM and H × HM) according to Student's *t*-test, *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$. Values expressed as means ± SEM, $n = 5$.

### 3.5 Jaboticaba peel intake increases production of SCFA

The data concerning the weight percent of the large intestine showed that the high-fat diet caused intestinal atrophy (Figure 3). Such effects may reflect intestinal health, absorption of minerals and function of the intestinal microbiota. The higher endotoxin level in H also suggests intestinal permeability, which was not observed in N ($p < 0.05$). Although the MJP added to the diet does not seem to have an influence on the size of the intestinal tissue or in endotoxemia level, the production of SCFA may promote local beneficial effects, as illustrated in Figure 3.

The amounts of acetate, butyrate and consequently total SCFA were increased in the fecal content of HM animals when related to the findings in the H group ($p > 0.05$). The propionate and butyrate concentration decreased in the intestinal content of the H group, but not in HM. However, there was also a decrease in propionate content in animals of the NM group (Figure 3).

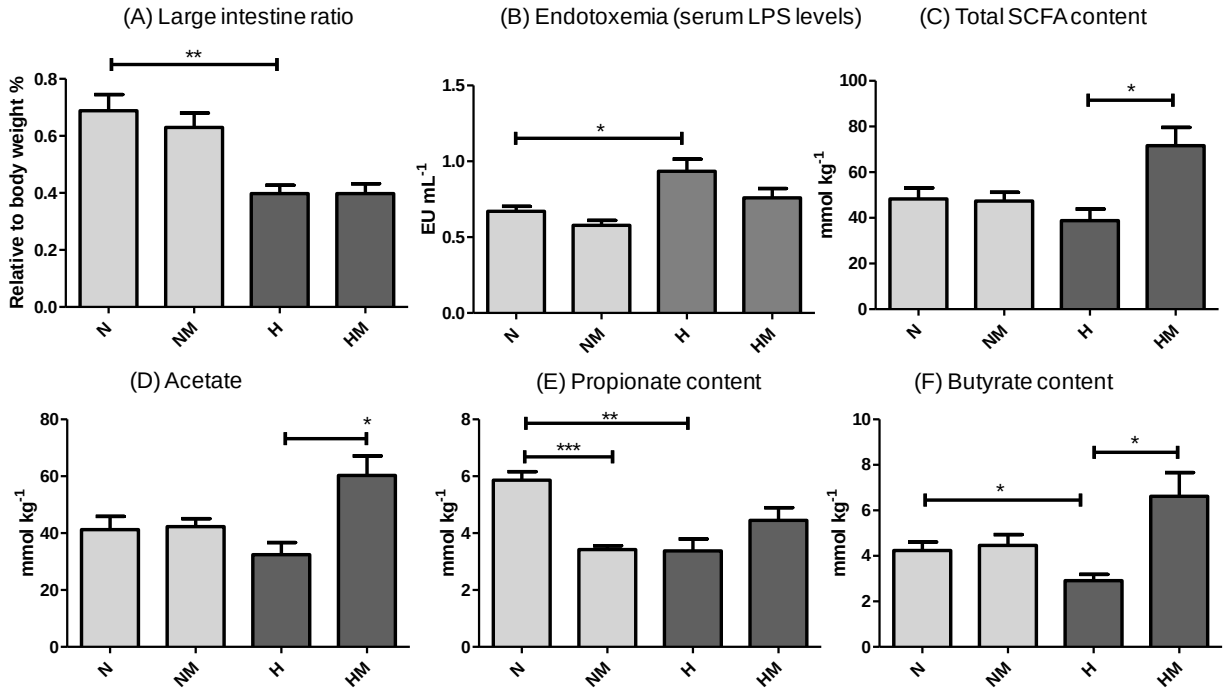



**Figure 3.** Large intestine weight, and short chain fatty acids production in large intestine of mice. (A) Large intestine weight related to body weight, (B) endotoxin serum levels, (C) total SCFA production, (D) acetate, (E) propionate and (F) butyrate content in fecal content in large intestine of the animals. Values expressed as means ± standard error. *Indicates significant differences between groups according to Student's *t*-test, *$p < 0.05$; **$p < 0.01$; and ***$p < 0.001$, $n$=5.

## 4. Discussion

We showed in this study that addition of jaboticaba berry peel to a high-fat diet promoted beneficial impacts on lowering weight gain, reducing fat amount in the animal carcass and fat mass, down-regulating inflammatory cytokines transcription by the epididymal adipose tissue and lower areas of hepatic steatosis.

The results showed that high-fat diet feeding reduced large intestinal size and increased endotoxemia in comparison to the N group, indicating an increased permeability of the intestine of the H group (Figure 3) [4]. Indeed, studies have demonstrated that obesity reduces the expression of genes coding for proteins of the intestinal tight-junctions inducing to a leaky gut, and so permeability for pathogens [4]. Leaky gut has also a connection with increased visceral adiposity, insulin resistance, hepatic steatosis and inflammation, shown in animal and human studies [5-6]. The addition of the MJP in the diet did not change serum

LPS levels in the animals, but higher production of SCFA could indicate healthier gut in comparison to the H animals [10, 30].

Dietary fibers escape digestion and absorption and stimulate SCFA production by large intestinal microbiota metabolism where they are considered healthy compounds [10, 30]. Jaboticaba berry is rich in dietary fibers [18] and pectin (Table 1), which corroborates improved acetate and butyrate production in the large intestine of HM animals. A study suggested that acetate and butyrate were associated with lower weight gain in high-fat feeding animals [31], which is in line with the findings in this report, since insttinal production of SCFA are related to the blood concentration. In particular, the higher levels of butyrate in the intestinal content of the HM group can be associated with gut health, since this compound influences enterocyte growth and differentiation, besides of exercing anti-inflammatory effects [30].

Very few studies supports the idea that propionate is related to lower cholesterol synthesis by the liver [32]. The NM group showed lower production of propionate (Fig. 3). However, higher fecal excretion of cholesterol was also observed in the same group, corroborating lower cholesterol levels in serum and liver of NM group (Table 2). In opposite way of pectin, the fermentation of cellulose increases the production of propionate, which partly explains the lower propionate levels found in the NM intestinal fecal bolus related to the N group [33]; however, inhibition of growth of propionate's producer bacteria may occur in the presence of certain (poly)phenols [10].

As well as dietary fibers, plant-derived (poly)phenols can improve gut health, since they are associated to growth of beneficial gut bacteria [9-10] and to anti-inflammatory effects; reported to be active in the treatment of intestinal bowel diseases, for example [10, 34]. By promoting intestinal health, MJP could have prevented the transcritption of pro-inflammatory markers in teh EAT and consequently prevented insulin resistance, as

demonstrated previously when mice consumed the same berry [11]. The findings of the present study showed that the EAT of animals in NM had lower expression of pro-inflammatory cytokines, corroborating this hypothesis.

Evidence rises that a dysfunctional adipose tissue may be the driver of NAFLD [1], as well as gut leakage of pathogenic antigens [5]. Indeed, the animals receiving H diet showed higher adiposity and markers of NAFLD, such as larger hepatic steatosis areas and higher activities of ALT and AST, but not the HM group. The HM diet may prevent NAFLD by increasing triglycerides fecal excretion and preserving healthy adipose tissue, as shown in this study. Other studies have shown that (poly)phenols from berries, such as jaboticaba berry, may inactivate digestive enzymes (e.g. lipase) and then could reduce the absorption of lipids and fat visceral accumulation [16, 35]. A previous study have also demonstrated lower activation of the transcription factor FOXO-1 in liver of mice fed MJP, which indicates lower production of lipogenic enzymes [11], and partly explains the healthier liver of the HM group. The same study has also shown lower expression of IL-6 and IL-1β in liver of animal treated with MJP after established obesity [11].

The triglycerides accumulation in the hepatocytes is a result of fatty acids uptake from diet or lipolysis and fat oxidation in the adipose tissue, increasing the sensitivity of the liver to secondary insults, such as oxidative stress [36]. The higher TBARS and lower GSH values in animals fed high-fat diet indicate oxidative state of their liver. Higher PPAR-α expression in this group during feed conditions also contribute to the oxidative state of fatty acids, since they are ligands for PPARs [37]. In this context, the large amounts of fatty acids from the diet and release from the adipose tissue can stimulate their own metabolism by activating PPAR-α in fatty liver, contributing to the production of reactive oxygen species and lipotoxic metabolites [37]. Additionally, dysfunctions on mitochondrial oxidation also leads to accumulation of fatty acids in the hepatocytes [36]. The livers of animals in the HM group

showed improved antioxidant defenses and lower PPAR-α expression, indicating prevention of hepatic oxidative stress induced by the high-fat diet.

In this context, we assume that MJP intake induces to higher production of SCFA in the large intestine, which in turn are active compounds against obesity-related injuries like insulin resistance [11], NAFLD and visceral adiposity.The supplementation of the high-fat diet with MJP prevented body and hepatic fat accumulation, as well as induced lower expression of pro-inflammatory markers in the epididymal adipose tissue.

**Funding:** This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (MRMJ for CNPq #300533/2013-6 and MACH for CNPq #305099/2011-6) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

## 5. References

[1] Stanton MC, Chen S-C, Jackson JV, Rojas-Triana A, Kinsley D, Cui L, et al. Inflammatory Signals shift from adipose to liver during high fat feeding and influence the development of steatohepatitis in mice. Journal of Inflammation (London, England). 2011;8:8-.

[2] Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet. 2014;384:766-81.

[3] Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis Among a Largely Middle-Aged Population Utilizing Ultrasound and Liver Biopsy: A Prospective Study. Gastroenterology. 2011;140:124-31.

[4] Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. Diabetes. 2008;57:1470-81.

[5] Gummesson A, Carlsson LMS, Storlien LH, Bäckhed F, Lundin P, Löfgren L, et al. Intestinal Permeability Is Associated With Visceral Adiposity in Healthy Women. Obesity. 2011;19:2280-2.

[6] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56:1761-72. (CANI et al., 2007)

[7] Winer Daniel A, Luck H, Tsai S, Winer S. The Intestinal Immune System in Obesity and Insulin Resistance. Cell Metabolism.2016; 23:413-26.

[8] Cusi K. Role of Obesity and Lipotoxicity in the Development of Nonalcoholic Steatohepatitis: Pathophysiology and Clinical Implications. Gastroenterology. 2012;142:711-25.e6.

[9] Anhê FF, Roy D, Pilon G, Dudonné S, Matamoros S, Varin TV, et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. Gut. 2015;64:872-83.

[10] Matziouridou C, Marungruang N, Nguyen TD, Nyman M, Fåk F. Lingonberries reduce atherosclerosis in Apoe-/- mice in association with altered gut microbiota composition and improved lipid profile. Molecular Nutrition & Food Research. 2016;60:1150-60.

[11] Dragano NRV, Marques AC, Cintra DEC, Solon C, Morari J, Leite-Legatti AV, et al. Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. British Journal of Nutrition. 2013;110:447-55.

[12] Plaza M, Batista ÂG, Cazarin CBB, Sandahl M, Turner C, Östman E, et al. Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. Food Chem. 2016;211:185-97.

[13] Batista AG, Lenquiste SA, Cazarin CBB, da Silva JK, Luiz-Ferreira A, Bogusz S, et al. Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. Journal of Functional Foods. 2014;6:450-61.

[14] Araújo CRR, Esteves EA, Dessimoni-Pinto NAV, Batista ÂG. Myrciaria cauliflora Peel Flour Had a Hypolipidemic Effect in Rats Fed a Moderately High-Fat Diet. J Med Food. 2013; 17:2 262 -267.

[15] Batista ÂG, Leite-Legatti AV, Lima MCG, Prado MA, Maróstica Júnior MR. Effects of jaboticaba (Myrciaria jaboticaba) peel on blood glucose and cholesterol levels in healthy rats. African Journal of Biotechnology. 2014;13:3805-11.

[16] Alezandro MR, Granato D, Genovese MI. Jaboticaba (Myrciaria jaboticaba (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. Food Res Int. 2013;54:650-9.

[17] Lenquiste SA, Batista AG, Marineli RD, Dragano NRV, Marostica MR. Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. Food Res Int. 2012;49:153-60.

[18] da Silva JK, Batista ÂG, Cazarin CBB, Dionísio AP, de Brito ES, Marques ATB, et al. Functional tea from a Brazilian berry: Overview of the bioactives compounds. LWT - Food Science and Technology.

[19] Bitter T, Muir HM. A modified uronic acid carbazole reaction. Anal Biochem. 1962;4:330-4.

[20] Reeves PG, Nielsen FH, Fahey GC. Ain-93 Purified Diets for Laboratory Rodents - Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the Ain-76a Rodent Diet. J Nutr. 1993;123:1939-51.

[21] Folch J, Less M, Sloane-Stanley GHA. Simple method for isolation and purification of total lipids from animals tissues. Journal of Biological Chemistry. 1957;226:407-11.

[22] Zhao GH, Nyman M, Jonsson JA. Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. Biomed Chromatogr. 2006;20:674-82.

[23] Ohkawa H, Ohishi N, Yagi K. Assay for Lipid Peroxides in Animal-Tissues by Thiobarbituric Acid Reaction. Anal Biochem. 1979;95:351-8.

[24] Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med. 1975;85:337-41.

[25] Flohe L, Gunzler WA. Assays of Glutathione-Peroxidase. Method Enzymol. 1984;105:114-21.

[26] Carlberg I, Mannervik B. Glutathione-Reductase. Method Enzymol. 1985;113:484-90.

[27] Johansson LH, Borg LAH. A Spectrophotometric Method for Determination of Catalase Activity in Small Tissue Samples. Anal Biochem. 1988;174:331-6.

[28] Ellman GL. Tissue Sulfhydryl Groups. Arch Biochem Biophys. 1959;82:70-7.

[29] Bradford MM. Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding. Anal Biochem. 1976;72:248-54.

[30] Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer RJ. Review article: the role of butyrate on colonic function. Aliment Pharm Therap. 2008;27:104-19.

[31] Lin HV, Frassetto A, Kowalik EJ, Nawrocki AR, Lu MFM, Kosinski JR, et al. Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. Plos One. 2012;7.

[32] Gunness P, Gidley MJ. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. Food & Function. 2010;1:149-55

[33] Henningsson AM, Nyman EMGL, Bjorck IME. Short-chain fatty acid content in the hindgut of rats fed various composite foods and commercial dietary fibre fractions from similar sources. J Sci Food Agr. 2002;82:385-93.

[34] Schroeder FA, Lin CL, Crusio WE, Akbarian S. Antidepressant-Like Effects of the Histone Deacetylase Inhibitor, Sodium Butyrate, in the Mouse. Biological Psychiatry. 2007;62:55-64.

[35] McDougall GJ, Kulkarni NN, Stewart D. Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. BioFactors. 2008;34:73-80.

[36] Tessari P, Coracina A, Cosma A, Tiengo A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutrition, Metabolism and Cardiovascular Diseases. 2009;19:291-302.

[37] Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature. 2000;405:421-4.

# DISCUSSÃO GERAL

O consumo regular de frutas e vegetais é recomendado por órgãos de saúde e a ingestão de frutas vermelhas agrega nutrientes e fitoquímicos com propriedades bioativas à dieta, trazendo benefícios para prevenção de várias doenças e desordens (WHO, 2003). As frutas vermelhas, como jabuticaba e jambo-vermelho, destacam-se pelo seu conteúdo de fibras dietéticas e antocianinas, pigmentos atrativos pela coloração que conferem aos vegetais. Em particular, comprovou-se neste trabalho que as farinhas da casca de jabuticaba e da fruta de jambo-vermelho impactaram de forma positiva na prevenção de doenças metabólicas como obesidade, diabetes do tipo 2 e doenças cardiovasculares, como discute-se a seguir.

## 1. CONSUMO DE FRUTAS VERMELHAS E A PREVENÇÃO DE MARCADORES DE DOENÇAS DE ORIGEM METABÓLICA

### 1.1 COMPOSTOS BIOATIVOS NA CASCA DE JABUTICABA E JAMBO-VERMELHO

A quantidade de fibras dietéticas presente na casca de jabuticaba e fruta do jambo-vermelho é semelhante, correspondendo à cerca de 34% da matéria seca. No entanto, o conteúdo de fibras solúveis é diferente, representando 8,5% na casca de jabuticaba e 5,6% na casca e polpa de jambo-vermelho. Os valores de fibras dietéticas encontradas no presente trabalho foram superiores aos encontrados em outros estudos, tanto para casca de jabuticaba (LEITE-LEGATTI et al., 2012), quanto para a fruta do jambo-vermelho (MAISUTHISAKUL et al., 2008).

A importância das fibras dietéticas em estudos envolvendo doenças metabólicas se dá pela diminuição ou retardo da absorção de outros nutrientes como triglicerídeos, colesterol, entre outros (JAKOBSDOTTIR; XU et al., 2013). A pectina presente tanto na casca de jabuticaba (8,66 g/100g), quanto no jambo-vermelho (7,55 g/100g) possui tais características, além de ser altamente fermentável pela microbiota intestinal, demonstrando forte impacto na produção intestinal de AGCC (JAKOBSDOTTIR; XU et al., 2013; TITGEMEYER et al., 1991).

A casca de jabuticaba também apresentou quantidades expressivas de carotenoides totais (2,87 mg/100g) e capacidade antioxidante lipofílica (L-ORAC: 1,91 µmol TE/g). A casca + polpa de jambo-vermelho apresentou menor conteúdo de carotenoides

(0,046 mg/100g), porém maior capacidade antioxidante lipofílica (L-ORAC: 10,50 µmol TE/g) indicando a presença de outros compostos antioxidantes de baixa polaridade. Os carotenoides foram investigados devido à sua importância como antioxidantes e uso na prevenção de doenças metabólicas e cognitivas (LI, Y. et al., 2015; SCHNORR et al., 2014; SLUIJS et al., 2015).

Os compostos fenólicos estão em maior abundância na casca de jabuticaba (3216,92 mg/100g) do que na fruta de jambo-vermelho (232.18 mg/100g). Dentre estes 541,49 mg/100g e 126,95 mg/100g eram antocianinas na casca de jabuticaba e na casca + polpa de jambo-vermelho, respectivamente. Entretanto, um estudo comparou as taxas de absorção das antocianinas de jabuticaba e jambo-vermelho e verificou que as antocianinas do jambo-vermelho mostrou maior bioacessibilidade após digestão gástrica (45%) e intestinal (15%) do que as antocianinas de jabuticaba (13% e 10%, respectivamente) (MARQUES PEIXOTO et al., 2016). Dessa forma, a biodisponibilidade destes compostos no trato intestinal tem maior importância do que a quantidade ingerida (WILLIAMSON; MANACH, 2005; YOUDIM et al., 2003).

Dada a importância dos açúcares para doenças metabólicas e neurodegenerativas (KOTHARI et al., 2017), é importante citar a quantidade de açúcares redutores no jambo-vermelho (49,55%) e na casca de jabuticaba (33,51%). Verificou-se no Capítulo 2 que o jambo-vermelho mostrou maiores quantidades de açúcares simples, concentradas principalmente na polpa, o que poderia ilustrar as diferenças encontradas em relação ao ensaio com a casca da jabuticaba, como discutidos à seguir.

### 1.2 GANHO DE PESO E BALANÇO DE GORDURAS

Em um trabalho recente, Heyman e colaboradores (2014) suplementaram 20% das dietas hiperlipídicas de camundongos com 8 diferentes frutas vermelhas de diferentes origens por 12 semanas e demonstraram que: uma delas aumentou o ganho de peso dos animais (açaí), 3 não alteraram o peso dos animais (amora-silvestre, ameixa-européia e empetrum-negro), e 4 foram capazes de reduzir o ganho de peso (framboesa, mirtilo, mirtilo-vermelho e groselha-negra) (HEYMAN et al., 2014). Este estudo demonstrou que cada *berry* possui diferentes focos de ação fisiológica, assim como observado no presente trabalho. A saber, a suplementação com 4% de casca da jabuticaba em dieta hiperlipídica, mas não com 5% do jambo-vermelho, foi capaz de prevenir o ganho de peso dos animais. Algumas hipóteses explicam estes achados como exposto na FIGURA 1.

Primeiramente, o Capitulo 5 mostrou que a excreção de triglicerídeos estava aumentada no grupo que recebeu a dieta HM (hiperlipídica + casca de jabuticaba). A maior excreção de triglicerídeos pode ser explicada pelo poder que polifenóis de frutas vermelhas tem em inibir a ação da enzima lipase pancreática no intestino (MCDOUGALL et al., 2008). De fato, Alezandro e colaboradores (2013) confirmaram em um estudo que o extrato de casca de jabuticaba mostrou ação inibitória da mesma enzima (ALEZANDRO et al., 2013). Desta forma, a ação de quebra de triglicerídeos prévia à sua absorção pode ter sido prejudicada pela inibição da lipase pelos polifenóis da casca de jabuticaba, corroborando menor acúmulo de gorduras após a absorção. Corroborando esta hipotese, os resultados de pesos dos tecidos adiposos epididimal, retroperitoneal e lipídeos na carcaça dos animais HM foram menores do que nos animais que consumiram dieta hiperlipídica somente.

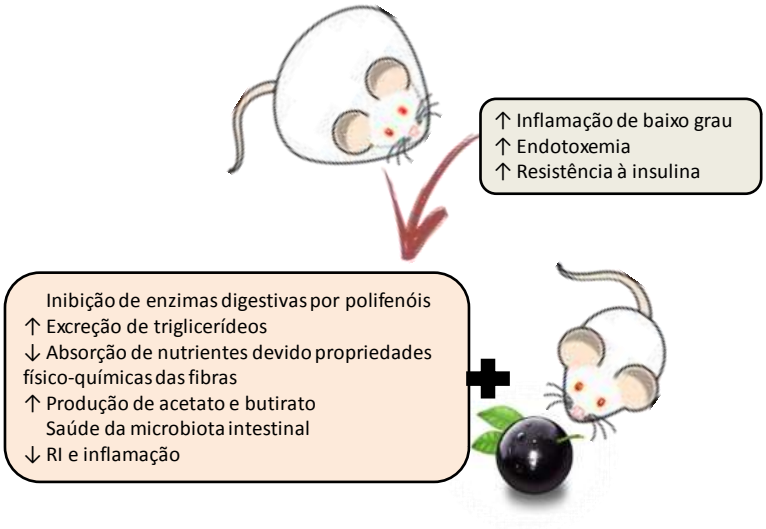



**FIGURA 1** - Efeitos da ingestão da casca de jabuticaba na prevenção do ganho de peso.

As fibras dietéticas são outros importantes componentes alimentares, responsáveis por maior saciedade e menor ingestão de dieta (ADAM et al., 2015). Apesar dos animais suplementados não terem diminuído a ingestão de dieta, acredita-se que as fibras da casca de jabuticaba funcionaram como barreira mecânica, diminuindo o sítio para absorção de nutrientes, ou até mesmo formaram complexos com estes, arrastando-os para as fezes (VILLANUEVA-SUÁREZ et al., 2016). Entre outras propriedades físico-químicas, as fibras dietéticas aceleram o transito intestinal e aumentam o bolo fecal (VILLANUEVA-SUÁREZ et al., 2016). Um trabalho anterior mostrou que a terapia de animais obesos com 4% de casca de jabuticaba na dieta hiperlipídica promoveu um maior incremento da excreção fecal (matéria seca) do que doses inferiores (BATISTA, A. G. et al., 2013). Desta forma

acreditamos que as fibras da casca de jabuticaba foram importantes para prevenção do ganho de peso, especialmente por conter um balanço fibras solúveis/ insolúveis que não havia nas dietas controle (celulose como fonte de fibra).

A fermentação das fibras dietéticas da casca de jabuticaba também contribuiu para maior produção de acetato e butirato no intestino grosso dos animais. Um estudo, que incorporou sais destes AGCC em dietas hiperlipídicas de camundongos, mostrou que os animais obtiveram menor ganho de peso, mais efetivamente os que receberam o butirato na dieta (LIN, H. V. et al., 2012). Os autores atribuem tais resultados a efeitos termogênicos e de estímulo de hormônios anorexigênicos pelos AGCC (LIN, H. V. et al., 2012).

Por fim, outros fatores como atenuação do estado inflamatório do tecido adiposo, como mostra o Capitulo 5, também explicariam a menor adiposidade e ganho de peso dos animais HM; uma vez que a inflamação predispõe ao aumento de gordura corporal como exposto no Capítulo 1.

### 1.3 RESISTÊNCIA À INSULINA E INFLAMAÇÃO

A resistência insulina medida pelos testes de GTT e ITT no Capitulo 4 corroboram dados de outro estudo que mostrou maior fosforilação de IR, IRS e AKT no tecido adiposo e fígado quando insulina exógena foi aplicada em camundongos tratados com casca de jabuticaba (DRAGANO et al., 2013). Estes autores obtiveram resultados semelhantes com o ITT, embora o GTT não tenha mostrado efeito benéfico da ingestão da casca de jabuticaba. Esta mesma tendência foi encontrada com a suplementação da casca de jambo-vermelho, uma vez que foram encontrados resultados benéficos da sua ingestão na glicemia de jejum e ITT, mas não para o GTT.

A suplementação da dieta hiperlipídica com casca de jabuticaba e casca+ polpa de jambo-vermelho propiciaram a diminuição da adiposidade visceral e da transcrição de citocinas inflamatórias, como IL-6 e TNF-α (Capítulos 3 e 4). A inflamação de baixo grau na obesidade culmina na resistência à insulina devido à fosforilação em tirosina do IRS (HOTAMISLIGIL, G. S., 2006; MILANSKI et al., 2012; POSEY et al., 2009). Desta forma, a diminuição da transcrição de mediadores inflamatórios em HM e HS, e a menor circulação dos mesmos favorecem a maior sensibilidade à insulina nestes grupos em relação ao seu controle. Ademais, Dragano et al. (2013) mostraram maior sensibilidade à insulina em camundongos tratados com 4% de casca de jabuticaba e dieta hiperlipídica, além de menor transcrição de IL-6 e IL-1β no fígado, corroborando o resultados do presente estudo.

Os resultados encontrados para os níveis circulantes de adiponectina no grupo de animais com dieta HM corrobora a menor resistência à insulina neste grupo, quando comparado ao grupo com dieta hiperlipídica somente, uma vez que a adiponectina é sensibilizadora da ação da insulina (TILG; MOSCHEN, 2006). Outro estudo que suplementou dieta hiperlipídica com açaí também mostrou um aumento tanto dos níveis de adiponectina circulantes quando a sua expressão (GUERRA et al., 2015). Estudos mostram que a antocianina C3G estimula a expressão de adiponectina nos adipócitos por meio de mecanismos de acetilação da FoxO1 (GUO; XIA et al., 2012; LIU et al., 2014), o que poderia explicar os resultados obtidos.

### 1.4 MEMÓRIA E SINALIZAÇÃO DA INSULINA NO HIPOCAMPO

O menor ganho de peso dos animais alimentados com a dieta hiperlipídica contendo a casca de jabuticaba, assim como a menor resistência à insulina periférica dos mesmo, podem ter sido fatores que indiretamente culminaram na menor fosforilação da tau no hipocampo mediada por GSK-3β (RENDEIRO, CATARINA et al., 2015). Estes animais também mostraram menores níveis de citocinas inflamatórias no hipocampo, o que pode ter contribuído para estes efeitos indiretos (FIGURA 2). Além disso, a ideia de efeito indireto corrobora o fato de que os animais que consumiram a dieta padrão ou normolipídica suplementada com a casca de jabuticaba não apresentaram efeitos significativos da suplementação no Capitulo 4. Outros autores também afirmaram que os compostos fenólicos mostram maior efetividade quando o organismo passa por injúrias (JEON et al., 2012; THOMAS et al., 2014), como neste caso aconteceu quando os animais receberam a dieta hiperlipídica (JEON et al., 2012) ou administração de streptozotocina (THOMAS et al., 2014), corroborando os resultados encontrados nos Capítulos 3 e 4.

No entanto, os efeitos da ingestão de jambo-vermelho sobre o ganho de massa gorda e resistência à insulina periférica, não foram significativos, e ainda assim constatou-se menor atividade da GSK-3β e menor fosforilação da tau no hipocampo dos animais que consumiram HS (dieta hiperlipídica suplementada com jambo-vermelho). Estes resultados corroboram a ideia de uma ação direta de compostos bioativos, como flavonoides e outros metabólitos bioativos da fruta, capazes de ultrapassar a barreira hematoencefálica e interferir na ação de kinases e da transcrição de genes de interesse (RENDEIRO, CATARINA et al., 2012; SPENCER, 2009; WILLIAMS, R. J.; SPENCER, 2012). Outros trabalhos apontam a ação direta de fenólicos como o resveratrol e outros compostos bioativos, como os ácidos

graxos ω-3 e a curcumina, minimizando tais marcadores de tauopatias (p. ex. DA) (JEON et al., 2012; MA et al., 2009). É importante salientar que, assim como em outros trabalhos (JEON et al., 2012; KOTHARI et al., 2017; MA et al., 2009), o presente estudo avaliou apenas um sítio de fosforilação da tau, o que não indicaria necessariamente a disfunção da molécula ou a sua hiperfosforilação (LEDREUX et al., 2016). Assim, outros sítios de fosforilação da tau devem ser investigados em um trabalho futuro, para reafirmar as conclusões de prevenção de fatores de risco da AD.

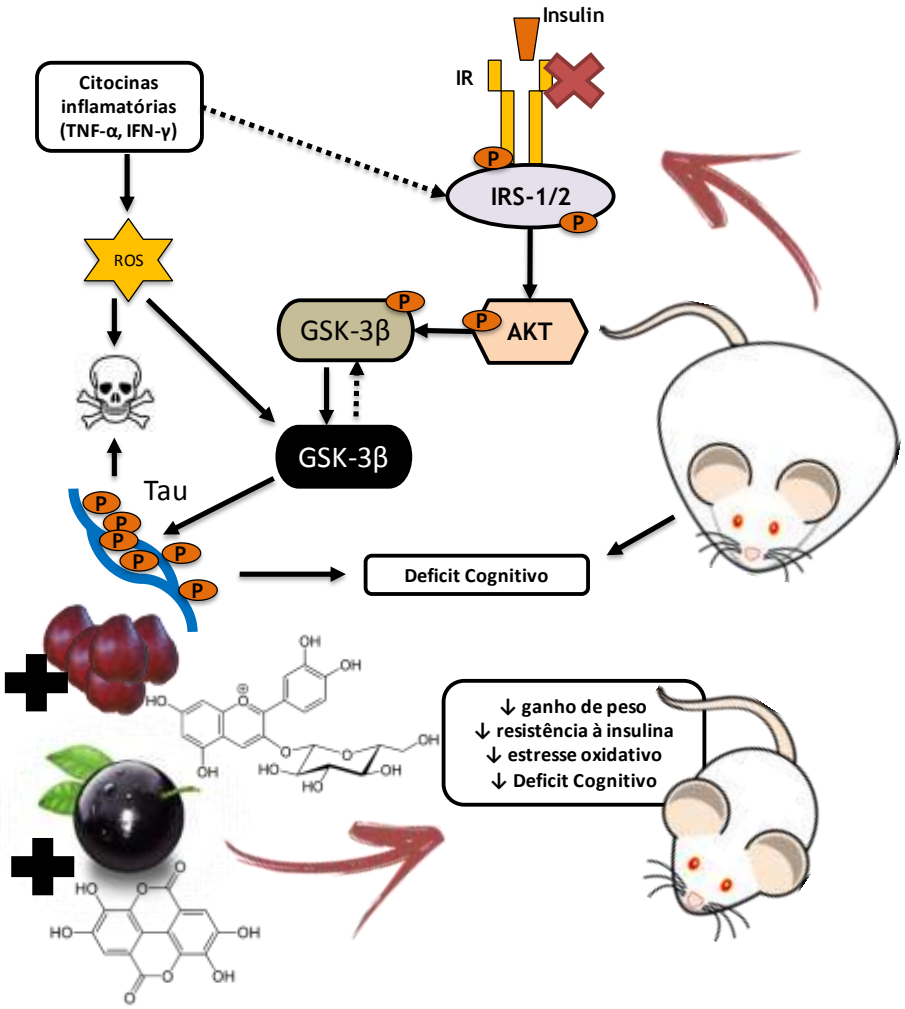



**FIGURA 2** - Efeitos da ingestão das casca+ polpa do jambo-vermelho e da casca de jabuticaba na prevenção da fosforilação da tau e déficits cognitivos.

Estudos mostraram que a fosforilação da tau além de ser regulada pela sinalização da insulina no hipocampo, também pode ser regulada por AMPK dependente da sinalização da adiponectina e leptina via fosforilação de GSK-3β (GRECO et al., 2009; JEON et al., 2012). Os achados do presente estudo mostraram aumento nos níveis circulantes de adiponectina nos animais HM (Capítulo 4), o que poderia ter contribuído para menor atividade/ maior fosforilação de GSK-3β.

Os animais que receberam a dieta hiperlipídica suplementada com casca de jabuticaba ou jambo-vermelho mostraram melhor aprendizado e memória em relação aos animais que receberam dieta hiperlipídica de acordo com o teste MWM. A maior sensibilidade ao sinal da insulina e a menor fosforilação da tau podem ter influenciado o melhor desempenho dos animais no teste de memória. No entanto, outras hipóteses existem, como: melhora do fluxo sanguíneo cerebrovascular, ativação da CREB e estimulo da neurogênese (RENDEIRO, CATARINA et al., 2015).

Animais alimentados com *berries* demonstraram melhor desempenho em atividades de acesso à memória espacial associado a estimulo para neurogênese no hipocampo (CASADESUS et al., 2004), fator que permite a consolidação da memória. Adicionalmente, a ativação de kinases que estimulam a transcrição de BDNF também pode contribuir para melhor memória espacial nos animais que consomem *berries*, assim como demontrado por alguns autores (CASADESUS et al., 2004; RENDEIRO, CATARINA et al., 2012; WILLIAMS, C. M. et al., 2008).

Existe também a evidência de que a administração de butirato (AGCC aumentado 127% em HM) está ligada à maior transcrição de neurotrofinas no córtex cerebral (SCHROEDER et al., 2007). Este dado apoia a ideia de comunicação entre o intestino e cérebro (chamado *gut-brain axis*), que neste caso também se aplicaria aos efeitos de uma microbiota saudável e produção de AGCC sobre melhoras na performance cognitiva (FORSYTHE et al., 2010; GOMEZ-PINILLA, 2008; STILLING; DINAN; CRYAN, 2014). Adicionalmente, a produção cecal de butirato reflete a concentrações deste AGCC na circulaçaõ sanguínea de animais (JAKOBSDOTTIR; JÄDERT et al., 2013), o que poderia facilitar a sua ação em ativar a transcrição de neurotrofinas no cérebro.

A expressão de NeuN também corrobora os resultados obtidos no hipocampo e comportamento cognitivo do ensaio com a casca de jabuticaba. Um estudo provou que a ingestão de dietas hiperlipídicas não altera os valores de NeuN no hipocampo de animais jovens (LEDREUX et al., 2016), assim como encontrado no presente trabalho. Mas, curiosamente, a suplementação da dieta hiperlipídica com casca de jabuticaba mostrou uma possível interação entre gorduras da dieta e compostos da fruta, favorecendo uma maior viabilidade neuronal (↑ NeuN). A porção apolar da casca de jabuticaba pode estar envolvida nestes mecanismos. A esclarecer, um estudo mostrou que a fração apolar da casca de jabuticaba foi mais eficaz em reduzir o crescimento de células tumorais em comparação com a fração polar, mostrando capacidade antioxidante lipofílica importante (LEITE-LEGATTI et

al., 2012). Assim acredita-se que a solubilidade dos compostos apolares da jabuticaba na dieta hiperlipídica, ou até mesmo a biotransformação dos polifenóis em moléculas mais apolares podem ter tido um papel importante no cérebro devido à sua maior biodisponibilidade (YOUDIM et al., 2003).

O estresse oxidativo no soro e lobo frontal do cérebro dos animais também demonstraram efeitos benéficos de ambas suplementações com casca de jabuticaba ou jambo-vermelho (Capitulos 3 e 4). O aumento do stress oxidativo e disfunção mitocondrial é uma característica em comum entre obesidade, diabetes do tipo 2 e AD, uma vez que as mitocôndrias coordenam o metabolismo energético e são tanto geradoras, quanto alvos da ação de ROS (CORREIA et al., 2012). A sinalização defeituosa da insulina torna os neurônios deficientes em energia e mais vulneráveis a insultos oxidantes, o que pode promover alterações estruturais e funcionais das mitocôndrias. A fosforilação da tau é facilitadora da disfunção mitocondrial e potencializa o estresse oxidativo, acelerando assim os mecanismos neurodegenerativos (CORREIA et al., 2012). Adicionalmente, a redução do estresse oxidativo dos animais suplementados com a casca de jabuticaba corroboram outros trabalhos usando o mesmo material (ALEZANDRO et al., 2013; BATISTA, A. G. et al., 2014). Foram demosntrados por eles: diminuição da peroxidação lipídica cerebral de forma dose-dependente, maior capacidade antioxidante do cérebro total (BATISTA, A. G. et al., 2014) e maior atividade de enzimas antioxidantes (ALEZANDRO et al., 2013).

Por fim, o teste MWM foi eficaz em mostrar as mudanças cognitivas provocadas pela ingestão de casca de jabuticaba ou jambo-vermelho, no que diz respeito à memória espacial e aprendizado. No entanto, para futuros estudos há que considerar-se o impacto metabólico do esforço físico (natação realizada no MWM) em minimizar marcadores envolvidos em estudos de obesidade e diabetes. Como alternativa ou adjuvante, sugere-se o uso de outros testes de memória associados nos quais um esforço mínimo seja induzido e não limitado às condições físicas, p. ex. o teste de reconhecimento de objetos que também acessa a memória espacial ligada ao hipocampo (LEGER et al., 2013).

### 1.5 ESTEATOSE HEPÁTICA

Baseando-se nos conteúdos de lipídeos total, triglicerídeos total e colesterol total, além da contagem de áreas de esteatose em cortes histológicos de fígado, e enzimas de prova hepática (ALT e AST), observou-se que a ingestão de casca de jabuticaba preveniu a NAFLD nos camundongos do grupo HM (Capitulo 5) (FIGURA 3). Por outro lado, a suplementação

com jambo-vermelho mostrou uma diminuição de 38% dos triglicerídeos hepáticos, no entanto o peso do fígado, lipídeos e colesterol totais hepáticos, e as enzimas de provas hepáticas, não se mostraram diferentes do grupo hiperlipídico (dados não divulgados).

A menor adiposidade visceral dos animais alimentados com a dieta HM pode ter sido o grande influenciador na redução da esteatose hepática encontrada nestes animais. Como visto no Capitulo 1 (Seção 1.1.3), menores níveis de inflamação, maior sensibilidade à insulina e menor estresse oxidativo nos animais suplementados com casca de jabuticaba tem papel importante na prevenção da NAFLD, mostrando a interligação de tais desordens metabólicas.

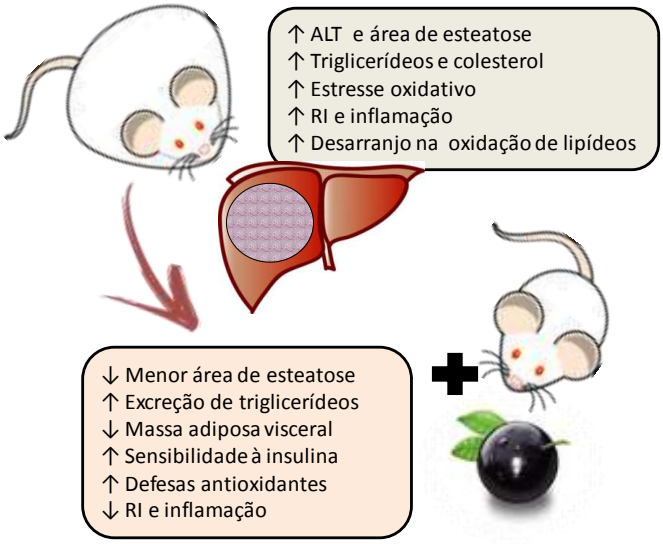



**FIGURA 3** - Efeitos da ingestão da casca de jabuticaba na prevenção da doença do fígado gordo não alcoólica.

Um trabalho recente (HEYMAN-LINDÉN et al., 2016) sugeriu que a ingestão de *berries* pode prevenir a NAFLD por diferentes mecanismos: absorção reduzida de lipídeos, redução da síntese hepática de lipídeos, regulação para menos de genes ligados à inflamação e à processos oxidativos. Comprovou-se no Capítulo 5, menor absorção de triglicerídeos pelos animais do grupo HM, assim como estresse oxidativo hepático reduzido nos animais deste grupo (↓peroxidação lipídica, ↑GSH, e ↑atividade de enzimas antioxidantes), demonstrando melhor defesa contra ROS no fígado. Todos estes fatores associados sugerem que a ingestão da casca de jabuticaba tem influência de forma indireta na diminuição da NAFLD. No entanto, um trabalho anterior comprovou que a suplementação com a casca de jabuticaba também interfere em vias de sinalização da insulina culminando na inativação de fatores de transcrição de genes para gliconeogênese e lipogênicos, independentemente da perda de peso

(DRAGANO et al., 2013). Dessa forma, acredita-se que compostos bioativos da casca de jabuticaba também possuam um papel direto na prevenção de NAFLD.

A produção de adiponectina nos adipócitos também é um fator protetor contra esteatose hepática (ZHOU et al., 2008), uma vez que os hepatócitos são sensíveis à sinalização do receptor de adiponectina que consequentemente ativam do receptor nuclear PPAR-α, principal regulador da oxidação de ácidos graxos. Um estudo demonstrou que animais tratados com açaí tiveram um aumento da expressão de receptores de adiponectina e de PPAR-α quando foram submetidos ao jejum de 12 h, sugerindo a ligação entre adiponectina e maior oxidação lipídica (GUERRA et al., 2015). No Capitulo 5, a menor expressão de PPAR-α no grupo HM em relação ao grupo que recebeu apenas dieta hiperlipídica foi relacionado à menor oxidação de lipídeos hepática, uma vez que os animais encontravam-se em estado alimentado. Desta forma conduziu-se à discussão em torno da menor formação de radicais livres oriundos da oxidação lipídica assim como menor formação de corpos cetônicos do grupo HM no estado alimentado. No entanto acredita-se que em jejum o perfil da expressão de PPAR-α apresentada pelos grupos experimentais seja invertida (KERSTEN et al., 2000) assim como o perfil encontrado por Guerra et al. (2015). Complementadando a idéia, um estudo mostrou que o tratamento com antocianinas esta ligado à maior ativação de PPAR-α no fígado, atuando como seu agonista (JIA et al., 2013). Assim, a menor esteatose hepática dos animais do grupo HM pode estar também relacionada à maior atividade de PPAR-α no jejum.

### 1.6 DISLIPIDEMIA

A casca de jabuticaba possui propriedade hipocolesterolêmica conhecida em animais saudáveis (BATISTA, Â. G. et al., 2014), pré-obesos(ARAÚJO et al., 2013), ou diabéticos (ALEZANDRO et al., 2013). Trabalhos anteriores mostraram que a terapia de animais obesos com 4% de casca de jabuticaba não foi eficaz em diminuir o colesterol sanguíneo, porém estes animais demonstraram maior nível de HDL-colesterol (LENQUISTE et al., 2012), assim como no presente estudo. No Capitulo 5 observou-se que os animais alimentados com dieta normolipídica contendo 4% de casca de jabuticaba (NM) mostraram menores níveis de colesterol no soro e fígado e maior nas fezes. Entretanto as diferenças encontradas para o colesterol sérico (-10%), hepático (-35%) e excreção fecal (+15%) no grupo HM em relação ao grupo hiperlipídico, não foram estatisticamente significativas.

Um possível mecanismo de ação para maior excreção de colesterol pelo grupo NM, pode está ligado à regulação do gene *Hmgcr* e *Cyp7a1* no fígado, responsáveis pela biosíntese de colesterol endógeno e produção de ácidos biliares, respectivamente, favorecendo maior excreção de colesterol (HEYMAN-LINDÉN et al., 2016). A ingestão de fibras aumenta a excreção de sais biliares nas fezes e diminui a reabsorção destes no intestino, o que requer o uso de colesterol do sangue ou síntese de novos sais biliares para repor o saldo negativo, diminuindo assim os níveis circulantes e hepáticos de colesterol (VILLANUEVA-SUÁREZ et al., 2016).

### 1.7 SAÚDE INTESTINAL

O Capitulo 5 mostra que a dieta hiperlipídica facilita a passagem de LPS de bactérias para circulação, sugerindo mudanças na permeabilidade intestinal (CANI et al., 2008). No entanto, mesmo que os níveis de LPS não tenham diminuído nos animais tratados com as frutas vermelhas, observou-se que ambas dietas normo e hiperlipídica suplementadas com jambo-vermelho (dados não divulgados) ou casca de jabuticaba promoveram maior produção de AGCC no intestino grosso dos animais. As dietas foram suplementadas com fibras solúveis oriundas das frutas, compostos aos quais se atribui alta capacidade de fermentação, principalmente à pectina presente nestas (JAKOBSDOTTIR; XU et al., 2013).

Todavia os níveis de propionato nos animais que receberam a dieta normolipídica contendo casca de jabuticaba foram diminuídos. A maior quantidade e a qualidade dos polifenóis na casca de jabuticaba pode ter selecionado e inibido o crescimento de bactérias produtoras de propionato especificamente, como as *Bacteroides* e *Clostridiales* (LOUIS et al., 2007); ou ter inibido as enzimas liberadas para fermentação pela microbiota (SCALBERT, 1991). O jambo-vermelho por conter uma quantidade inferior de polifenóis possui uma razão fibras dietéticas/polifenóis maior que na casca de jabuticaba, podendo assim ter sido mais eficaz em estimular o crescimento de bactérias produtoras deste AGCC. Os níveis de produção de AGCC estavam aumentados em ambos os tratamentos (dietas normo e hiperlipídica) com jambo-vermelho (dados não divulgados).

Outro fator que poderia ter influenciado este resultados se dá pela diferença da qualidade de fibras nas dietas N (somente celulose) e NM (celulose + fibras insolúveis e solúveis da casca de jabuticaba). Um trabalho mostrou que a celulose propicia o aumento da produção de propionato (HENNINGSSON et al., 2002), o que também poderia explicar a menor produção deste AGCC em NM.

O peso do intestino dos animais que receberam a dieta hiperlipídica suplementada com o jambo-vermelho (HS) também estava aumentado em relação ao seu controle (dados não divulgados), assim como reportado em outros trabalhos com suplementação com fibras solúveis (JAKOBSDOTTIR; XU et al., 2013). Entretanto o mesmo resultado não foi encontrado para a suplementação com a casca de jabuticaba. Um trabalho comparou a suplementação de dietas com 3 *berries* e verificou diferenças entre o efeito das mesmas no tamanho da parte cecal do intestino grosso, na produção de AGCC e na seleção da microbiota (JAKOBSDOTTIR; BLANCO et al., 2013). Os resultados mostrados por esses autores evidencia que a eficácia das *berries* em promover a saúde intestinal depende da sua composição em carboidratos fermentáveis, assim como da quantidade de antocianinas e flavonoides que as compõem (JAKOBSDOTTIR; BLANCO et al., 2013). Dessa forma, atribui-se os diferentes resultados obtidos entre suplementação com jambo-vermelho ou jabuticaba aos diferentes tipos de fibras, quantidade e tipo de compostos fenólicos.

# CONCLUSÃO GERAL

A casca e polpa do jambo-vermelho possuem juntas compostos bioativos, à serem citados: fibras dietéticas solúveis e insolúveis, antocianinas, outros flavonoides, e compostos lipofílicos como carotenoides, que conferem à fruta importante capacidade antioxidante. A ingestão da casca e polpa do jambo-vermelho quando adicionada à dieta hiperlipídica não preveniu o ganho de peso corporal ou do fígado, mas diminuiu o peso da massa adiposa visceral, assim como reduziu a transcrição de citocinas inflamatórias como o IL-6 e o TNF-α. A glicemia de jejum e o ITT demonstraram maior sensibilidade periférica à ação da insulina dos animais HS, corroborando para maior fosforilação do receptor de insulina no hipocampo. Em consequência, a fosforilação das kinases AKT e GSK3-β estavam ativadas, impedindo a fosforilação da tau no sítio Thr 205 no hipocampo dos mesmos animais. Os animais alimentados com a casca + polpa do jambo-vermelho e dieta hiperlipídica mostraram melhor desempenho na aprendizagem e memória espacial quando testados no labirinto aquático.

Para a casca de jabuticaba, destacamos neste trabalho o teor de fibras dietéticas, principalmente pectina, compostos fenólicos como a cianidina 3-glicosídeo e ácido elágico, carotenoides totais, capacidade antioxidante hidrofílica e lipofílica. A bioatividade dos compostos da casca de jabuticaba ingeridos preveniu o ganho de peso corporal e da massa adiposa nos animais alimentados com dieta hiperlipídica. Assim como na suplementação com o jambo-vermelho, a suplementação com a casca de jabuticaba promoveu maior sensibilidade à ação da insulina periférica e hipocampal, prevenindo também a fosforilação da tau e déficit cognitivo induzido pela dieta hiperlipídica. Adicionalmente a ingestão da casca de jabuticaba associada à dieta hiperlipídica preveniu o estresse oxidativo no fígado, assim como o desenvolvimento de NAFLD, e inflamação do tecido adiposo. A maior produção de AGCC pelos mesmos animais indicaria que a ação bioativa da ingestão da casca de jabuticaba inicia-se no intestino, uma vez que estes estão envolvidos na prevenção do ganho de peso, resistência à insulina e até mesmo plasticidade neuronal.

De forma geral a suplementação da dieta hiperlipídica com ambas as frutas mostrou efeitos benéficos na prevenção de doenças metabólicas e déficits cognitivos, com destaque para a casca de jabuticaba. Mais investigações sobre o efeito da ingestão de jambo-vermelho sobre a saúde do fígado e outros tecidos são necessárias para elucidar o seu valor como alimento funcional.

## CONSIDERAÇÕES PARA FUTUROS TRABALHOS

Novos trabalhos que investiguem a interação dos compostos bioativos da casca de jabuticaba e fruta de jambo-vermelho com a gordura da dieta hiperlipídica poderiam responder algumas questões como: porque somente os grupos hiperlipidicos suplementados com as frutas mostraram benefícios mais pronuciados da suplementação. A investigação da composição de químicos bioativos de natureza apolar presentes nestas frutas também poderiam contribuir para compreensão destes achados.

A avaliação da diversidade da microbioata intestinal poderia explicar efeitos benéficos da ingestão de casca de jabuticaba e jambo-vermelho sobre a produção de ácidos graxos de cadeia curta, assim como a resposta inflamatória intestinal e sistêmica. Análises da expressão genética de *tight-junctions* e histologia de diversas partes do intestino, também corroborariam as conclusões sobre os efeitos das frutas estudadas sobre a saúde intestinal.

A investigação de outras vias de sinalização de proteínas no hipocampo como a do BDNF, também poderiam indicar justificativas para melhoras cognitivas nos animais que receberam a dieta hiperlipídica e suplementação com as frutas. Outros testes de comporatamento devem ser considerados para complementar os efeitos da ingestão das frutas sobre a memória.

## REFERÊNCIAS GERAL


ABE, L. T.; LAJOLO, F. M.; GENOVESE, M. I. Potential dietary sources of ellagic acid and other antioxidants among fruits consumed in Brazil: Jabuticaba (Myrciaria jaboticaba (Vell.) Berg). **Journal of the Science of Food and Agriculture,** v. 92, n. 8, p. 1679-1687, Jun 2012.

ADAM, C. L.; THOMSON, L. M.; WILLIAMS, P. A.; ROSS, A. W. Soluble Fermentable Dietary Fibre (Pectin) Decreases Caloric Intake, Adiposity and Lipidaemia in High-Fat Diet-Induced Obese Rats. **Plos One,** v. 10, n. 10, p. e0140392, 2015.

AL-MUAMMAR, M. N.; KHAN, F. Obesity: The preventive role of the pomegranate (Punica granatum). **Nutrition,** v. 28, n. 6, p. 595-604, 2012.

ALAVIJEH, M. S.; CHISHTY, M.; QAISER, M. Z.; PALMER, A. M. Drug Metabolism and Pharmacokinetics, the Blood-Brain Barrier, and Central Nervous System Drug Discovery. **NeuroRx,** v. 2, n. 4, p. 554-571, 2005.

ALEZANDRO, M. R.; GRANATO, D.; GENOVESE, M. I. Jaboticaba (Myrciaria jaboticaba (Vell.) Berg), a Brazilian grape-like fruit, improves plasma lipid profile in streptozotocin-mediated oxidative stress in diabetic rats. **Food Research International,** v. 54, n. 1, p. 650-659, 2013.

ALONSO, M.; VIANNA, M. R. M.; IZQUIERDO, I.; MEDINA, J. H. Signaling Mechanisms Mediating BDNF Modulation of Memory Formation In Vivo in the Hippocampus. **Cellular and Molecular Neurobiology,** v. 22, n. 5, p. 663-674, 2002.

ANDERSSON DUNSTAN, C.; NOREEN, Y.; SERRANO, G.; COX, P. A.; PERERA, P.; BOHLIN, L. Evaluation of some Samoan and Peruvian medicinal plants by prostaglandin biosynthesis and rat ear oedema assays. **Journal of Ethnopharmacology,** v. 57, n. 1, p. 35-56, 1997.

ANHÊ, F. F.; ROY, D.; PILON, G.; DUDONNÉ, S.; MATAMOROS, S.; VARIN, T. V. *et al.* A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. **Gut,** v. 64, n. 6, p. 872-883, June 1, 2015 2015.

AOAC. **Official methods of analysis of AOAC International**. Gaithersburg, Md.: AOAC International, 2002.

APPELDOORN, M. M.; VINCKEN, J.-P.; AURA, A.-M.; HOLLMAN, P. C. H.; GRUPPEN, H. Procyanidin Dimers Are Metabolized by Human Microbiota with 2-(3,4-Dihydroxyphenyl)acetic Acid and 5-(3,4-Dihydroxyphenyl)-γ-valerolactone as the Major Metabolites. **Journal of Agricultural and Food Chemistry,** v. 57, n. 3, p. 1084-1092, 2009/02/11 2009.

APPELDOORN, M. M.; VINCKEN, J.-P.; GRUPPEN, H.; HOLLMAN, P. C. H. Procyanidin Dimers A1, A2, and B2 Are Absorbed without Conjugation or Methylation from the Small Intestine of Rats. **The Journal of Nutrition,** v. 139, n. 8, p. 1469-1473, August 1, 2009 2009.

ARAÚJO, C. R.; ESTEVES, E. A.; DESSIMONI-PINTO, N. A.; BATISTA, Â. G. Myrciaria cauliflora peel flour had a hypolipidemic effect in rats fed a moderately high-fat diet. **Journal of Medicinal Food,** v. 17, n. 2, p. 262-267, 2013.

ARUMUGAM, B.; MANAHARAN, T.; HENG, C. K.; KUPPUSAMY, U. R.; PALANISAMY, U. D. Antioxidant and antiglycemic potentials of a standardized extract of Syzygium malaccense. **LWT - Food Science and Technology,** v. 59, n. 2, Part 1, p. 707-712, 2014.

ASQUIERI, E. R.; SILVA, A. G. D. M. E.; CÂNDIDO, M. A. Aguardente de jabuticaba obtida da casca e borra da fabricação de fermentado de jabuticaba. **Ciência e Tecnologia de Alimentos,** v. 29, p. 896-904, 2009.

AUGUSTA, I. M.; RESENDE, J. M.; BORGES, S. V.; MAIA, M. C., A.; ; COUTO, M. A. P. G. Caracterização física e química da casca e polpa de jambo vermelho (Syzygium malaccensis, (L.) Merryl & Perry). **Ciencia e Tecnologia de Alimentos,** v. 30, n. 4, p. 928-936, 2010.

BACH-FAIG, A.; BERRY, E. M.; LAIRON, D.; REGUANT, J.; TRICHOPOULOU, A.; DERNINI, S. *et al.* Mediterranean diet pyramid today. Science and cultural updates. **Public Health Nutrition,** v. 14, n. 12A, p. 2274-2284, 2011/12/13 2011.

BÄCKHED, F.; DING, H.; WANG, T.; HOOPER, L. V.; KOH, G. Y.; NAGY, A. *et al.* The gut microbiota as an environmental factor that regulates fat storage. **Proceedings of the National Academy of Sciences of the United States of America,** v. 101, n. 44, p. 15718-15723, November 2, 2004 2004.

BÄCKHED, F.; MANCHESTER, J. K.; SEMENKOVICH, C. F.; GORDON, J. I. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. **Proceedings of the National Academy of Sciences,** v. 104, n. 3, p. 979-984, January 16, 2007 2007.

BAIRY, K. L.; SHARMA, A.; SHALINI, A. **Evaluation of the Hypoglycemic, Hypolipidaemic and Hepatic Glycogen Raising Effects of Syzygium malaccense Upon Streptozotocin Induced Diabetic Rats**. 2005. ISBN 2320 –3358|escape}.

BATISTA, Â. G.; DA SILVA, J. K.; BETIM CAZARIN, C. B.; BIASOTO, A. C. T.; SAWAYA, A. C. H. F.; PRADO, M. A. *et al.* Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves. **LWT - Food Science and Technology,** v. 76, Part B, p. 284-291, 2017.

BATISTA, Â. G.; FERRARI, A. S.; DA CUNHA, D. C.; DA SILVA, J. K.; CAZARIN, C. B. B.; CORREA, L. C. *et al.* Polyphenols, antioxidants, and antimutagenic effects of Copaifera langsdorffii fruit. **Food Chemistry,** v. 197, Part B, p. 1153-1159, 2016.

BATISTA, Â. G.; LEITE-LEGATTI, A. V.; LIMA, M. C. G.; PRADO, M. A.; MARÓSTICA JÚNIOR, M. R. Effects of jaboticaba (Myrciaria jaboticaba) peel on blood glucose and cholesterol levels in healthy rats. **African Journal of Biotechnology,** v. 13, n. 37, p. 3805-3811, 2014.

BATISTA, A. G.; LENQUISTE, S. A.; CAZARIN, C. B. B.; DA SILVA, J. K.; LUIZ-FERREIRA, A.; BOGUSZ, S. *et al.* Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. **Journal of Functional Foods,** v. 6, p. 450-461, Jan 2014.

BATISTA, A. G.; LENQUISTE, S. A.; MOLDENHAUER, C.; GODOY, J. T.; REIS, S. M. P. M.; MARÓSTICA JÚNIOR, M. R. Jaboticaba (Myrciaria jaboticaba (Vell.) Berg.) peel improved triglycerides excretion and hepatic lipid peroxidation in high-fat-fed rats. **Brazilian Journal of Nutrition,** v. 26, n. 5, p. 571-581, 2013.

BELKACEMI, A.; DOGGUI, S.; DAO, L.; RAMASSAMY, C. Challenges associated with curcumin therapy in Alzheimer disease. **Expert Reviews in Molecular Medicine,** v. 13, Nov 4 2011.

BITTER, T.; MUIR, H. M. A modified uronic acid carbazole reaction. **Analytical Biochemistry,** v. 4, n. 4, p. 330-334, 1962/10/01 1962.

BLIGH, E. G.; DYER, W. J. A Rapid Method of Total Lipid Extraction and Purification. **Canadian Journal of Biochemistry and Physiology,** v. 37, n. 8, p. 911-917, 1959.

BRADFORD, M. M. Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding. **Analytical Biochemistry,** v. 72, n. 1-2, p. 248-254, 1976.

BRANDEIS, R.; BRANDYS, Y.; YEHUDA, S. The Use of the Morris Water Maze in the Study of Memory and Learning. **International Journal of Neuroscience,** v. 48, n. 1-2, p. 29-69, 1989/01/01 1989.

BRASIL. **Alimentos regionais brasileiros**. 2. ed. Brasília: Ministério da Saúde, 2015.

CANI, P. D.; AMAR, J.; IGLESIAS, M. A.; POGGI, M.; KNAUF, C.; BASTELICA, D. *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. **Diabetes,** v. 56, n. 7, p. 1761-1772, Jul 2007.

CANI, P. D.; BIBILONI, R.; KNAUF, C.; NEYRINCK, A. M.; NEYRINCK, A. M.; DELZENNE, N. M. *et al.* Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. **Diabetes,** v. 57, n. 6, p. 1470-1481, Jun 2008.

CARDOSO, R. L. **Estabilidade da cor de geléia de jambo (Eugenia malaccensis, l.) sem casca armazenada aos 25 ºC e 35 ºC na presença e ausência de luz** **Ciência e Agrotecnologia,** v. 32, n. 5, p. 1563-1567, 2008.

CARERI, M.; ELVIRI, L.; MANGIA, A.; MUSCI, M. Spectrophotometric and coulometric detection in the high-performance liquid chromatography of flavonoids and optimization of sample treatment for the determination of quercetin in orange juice. **Journal of Chromatography A,** v. 881, n. 1–2, p. 449-460, 2000.

CARLBERG, I.; MANNERVIK, B. Glutathione-Reductase. **Methods in Enzymology,** v. 113, p. 484-490, 1985.

CASADESUS, G.; SHUKITT-HALE, B.; STELLWAGEN, H. M.; ZHU, X.; LEE, H.-G.; SMITH, M. A. *et al.* Modulation of Hippocampal Plasticity and Cognitive Behavior by Short-term Blueberry Supplementation in Aged Rats. **Nutritional Neuroscience,** v. 7, n. 5-6, p. 309-316, 2004/10/01 2004.

CASTELLANI, R. J.; ROLSTON, R. K.; SMITH, M. A. Alzheimer Disease. **Disease-a-month : DM,** v. 56, n. 9, p. 484-546, 2010.

CERLETTI, C.; DE CURTIS, A.; BRACONE, F.; DIGESÙ, C.; MORGANTI, A. G.; IACOVIELLO, L. *et al.* Dietary anthocyanins and health: data from FLORA and ATHENA EU projects. **British Journal of Clinical Pharmacology**, p. n/a-n/a, 2016.

CHANDRA, V.; GANGULI, M.; PANDAV, R.; JOHNSTON, J.; BELLE, S.; DEKOSKY, S. T. Prevalence of Alzheimer's disease and other dementias in rural India - The Indo-US study. **Neurology,** v. 51, n. 4, p. 1000-1008, Oct 1998.

CHEN, T.-Y.; KRITCHEVSKY, J.; HARGETT, K.; FELLER, K.; KLOBUSNIK, R.; SONG, B. J. *et al.* Plasma bioavailability and regional brain distribution of polyphenols from apple/grape seed and bilberry extracts in a young swine model. **Molecular Nutrition & Food Research,** v. 59, n. 12, p. 2432-2447, 2015.

CHIVA-BLANCH, G.; ARRANZ, S.; LAMUELA-RAVENTOS, R. M.; ESTRUCH, R. Effects of Wine, Alcohol and Polyphenols on Cardiovascular Disease Risk Factors: Evidences from Human Studies. **Alcohol and Alcoholism,** v. 48, n. 3, p. 270-277, 2013.

CHO, B. O.; RYU, H. W.; JIN, C. H.; CHOI, D. S.; KANG, S. Y.; KIM, D. S. *et al.* Blackberry Extract Attenuates Oxidative Stress through Up-regulation of Nrf2-Dependent Antioxidant Enzymes in Carbon Tetrachloride-Treated Rats. **Journal of Agricultural and Food Chemistry,** v. 59, n. 21, p. 11442-11448, 2011/11/09 2011.

CLERICI, M. T. P. S.; CARVALHO-SILVA, L. B. Nutritional bioactive compounds and technological aspects of minor fruits grown in Brazil. **Food Research International,** v. 44, n. 7, p. 1658-1670, 2011.

CORREIA, S. C.; SANTOS, R. X.; CARVALHO, C.; CARDOSO, S.; CANDEIAS, E.; SANTOS, M. S. *et al.* Insulin signaling, glucose metabolism and mitochondria: Major players in Alzheimer's disease and diabetes interrelation. **Brain Research,** v. 1441, p. 64-78, Mar 2 2012.

CRESCENTE, M.; JESSEN, G.; MOMI, S.; HÖLTJE, H.-D.; GRESELE, P.; CERLETTI, C. *et al.* Interactions of gallic acid, resveratrol, quercetin and aspirin at the platelet cyclooxygenase-1 level - Functional and modelling studies. **Thrombosis and Haemostasis,** v. 102, n. 8, p. 336-346, 2009.

CROSS, D. A. E.; ALESSI, D. R.; COHEN, P.; ANDJELKOVICH, M.; HEMMINGS, B. A. Inhibition of Glycogen-Synthase Kinase-3 by Insulin-Mediated by Protein-Kinase-B. **Nature,** v. 378, n. 6559, p. 785-789, Dec 21 1995.

CUSI, K. Role of Obesity and Lipotoxicity in the Development of Nonalcoholic Steatohepatitis: Pathophysiology and Clinical Implications. **Gastroenterology,** v. 142, n. 4, p. 711-725.e6, 2012.

DA SILVA, J. K.; BATISTA, Â. G.; CAZARIN, C. B. B.; DIONÍSIO, A. P.; DE BRITO, E. S.; MARQUES, A. T. B. *et al.* Functional tea from a Brazilian berry: Overview of the bioactives compounds. **LWT - Food Science and Technology,** v. 76, Part B, p. 292-298, 2017.

DE LA MONTE, S. M.; WANDS, J. R. Alzheimer's disease is type 3 diabetes-evidence reviewed. **J Diabetes Sci Technol,** v. 2, n. 6, p. 1101-13, Nov 2008.

DÉCORDÉ, K.; TEISSÈDRE, P. L.; SUTRA, T.; VENTURA, E.; CRISTOL, J. P.; ROUANET, J. M. Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers. **Molecular Nutrition & Food Research,** v. 53, n. 5, p. 659-666, 2009.

DENEV, P. N.; KRATCHANOV, C. G.; CIZ, M.; LOJEK, A.; KRATCHANOVA, M. G. Bioavailability and Antioxidant Activity of Black Chokeberry (Aronia melanocarpa) Polyphenols: in vitro and in vivo Evidences and Possible Mechanisms of Action: A Review. **Comprehensive Reviews in Food Science and Food Safety,** v. 11, n. 5, p. 471-489, Aug 2012.

DESSIMONI-PINTO, N. A. V.; MOREIRA, W. A.; CARDOSO, L. D. M.; PANTOJA, L. A. Jaboticaba peel for jelly preparation: an alternative technology. **Ciência e Tecnologia de Alimentos,** v. 31, p. 864-869, 2011.

DRAGANO, N. R. V.; MARQUES, A. C.; CINTRA, D. E. C.; SOLON, C.; MORARI, J.; LEITE-LEGATTI, A. V. *et al.* Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. **British Journal of Nutrition,** v. 110, n. 3, p. 447-55, 2013.

DUDAI, Y. The Restless Engram: Consolidations Never End. **Annual Review of Neuroscience, Vol 35,** v. 35, p. 227-247, 2012.

DUFFY, K. B.; SPANGLER, E. L.; DEVAN, B. D.; GUO, Z. H.; BOWKER, J. L.; JANAS, A. M. *et al.* A blueberry-enriched diet provides cellular protection against oxidative stress and reduces a kainate-induced learning impairment in rats. **Neurobiology of Aging,** v. 29, n. 11, p. 1680-1689, Nov 2008.

ELLMAN, G. L. Tissue Sulfhydryl Groups. **Archives of Biochemistry and Biophysics,** v. 82, n. 1, p. 70-77, 1959.

EMMERZAAL, T. L.; KILIAAN, A. J.; GUSTAFSON, D. R. 2003-2013: A Decade of Body Mass Index, Alzheimer's Disease, and Dementia. **Journal of Alzheimer's Disease,** v. 43, n. 3, p. 739-755, 2015.

FALCÃO, M. A.; PARALUPPI, N. D.; CLEMENT, C. R. Fenologia e produtividade do jambo (*Syzigium malaccensis*) na Amazônia central. **Acta Amazônica,** v. 32, n. 1, p. 3-8, 2002.

FARIA, A.; CALHAU, C. The Bioactivity of Pomegranate: Impact on Health and Disease. **Critical Reviews in Food Science and Nutrition,** v. 51, n. 7, p. 626-634, 2011/08/01 2011.

FLOHE, L.; GUNZLER, W. A. Assays of Glutathione-Peroxidase. **Methods in Enzymology,** v. 105, p. 114-121, 1984.

FOLCH, J.; LESS, M.; SLOANE-STANLEY, G. H. A. Simple method for isolation and purification of total lipids from animals tissues. **Journal of Biological Chemistry,** v. 226, n. 1, p. 407-411, 1957.

FORSYTHE, P.; SUDO, N.; DINAN, T.; TAYLOR, V. H.; BIENENSTOCK, J. Mood and gut feelings. **Brain, Behavior, and Immunity,** v. 24, n. 1, p. 9-16, 2010.

FRANKLAND, P. W.; BONTEMPI, B. The organization of recent and remote memories. **Nat Rev Neurosci,** v. 6, n. 2, p. 119-130, 2005.

GACAR, N.; MUTLU, O.; UTKAN, T.; CELIKYURT, I. K.; GOCMEZ, S. S.; ULAK, G. Beneficial effects of resveratrol on scopolamine but not mecamylamine induced memory impairment in the passive avoidance and Morris water maze tests in rats. **Pharmacology Biochemistry and Behavior,** v. 99, n. 3, p. 316-323, Sep 2011.

GARDNER, D. S.; RHODES, P. Developmental Origins of Obesity: Programming of Food Intake or Physical Activity? **Early Nutrition Programming and Health Outcomes in Later Life: Obesity and Beyond,** v. 646, p. 83-93, 2009.

GHOSHAL, S.; WITTA, J.; ZHONG, J.; DE VILLIERS, W.; ECKHARDT, E. Chylomicrons promote intestinal absorption of lipopolysaccharides. **Journal of Lipid Research,** v. 50, n. 1, p. 90-97, January 1, 2009 2009.

GOMEZ-PINILLA, F. Brain foods: the effects of nutrients on brain function. **Nat Rev Neurosci,** v. 9, n. 7, p. 568-578, 2008.

GRECO, S. J.; SARKAR, S.; JOHNSTON, J. M.; TEZAPSIDIS, N. Leptin regulates tau phosphorylation and amyloid through AMPK in neuronal cells. **Biochemical and Biophysical Research Communications,** v. 380, n. 1, p. 98-104, 2009.

GUDALA, K.; BANSAL, D.; SCHIFANO, F.; BHANSALI, A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. **Journal of Diabetes Investigation,** v. 4, n. 6, p. 640-650, 2013.

GUERRA, J. F. D. C.; MACIEL, P. S.; DE ABREU, I. C. M. E.; PEREIRA, R. R.; SILVA, M.; CARDOSO, L. D. M. *et al.* Dietary açai attenuates hepatic steatosis via adiponectin-mediated effects on lipid metabolism in high-fat diet mice. **Journal of Functional Foods,** v. 14, p. 192-202, 2015.

GUMMESSON, A.; CARLSSON, L. M. S.; STORLIEN, L. H.; BÄCKHED, F.; LUNDIN, P.; LÖFGREN, L. *et al.* Intestinal Permeability Is Associated With Visceral Adiposity in Healthy Women. **Obesity,** v. 19, n. 11, p. 2280-2282, 2011.

GUNNESS, P.; GIDLEY, M. J. Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. **Food & Function,** v. 1, n. 2, p. 149-155, Nov 2010.

GUO, H. H.; GUO, J. B.; JIANG, X. W.; LI, Z.; LING, W. H. Cyanidin-3-O-beta-glucoside, a typical anthocyanin, exhibits antilipolytic effects in 3T3-L1 adipocytes during hyperglycemia: Involvement of FoxO1-mediated transcription of adipose triglyceride lipase. **Food and Chemical Toxicology,** v. 50, n. 9, p. 3040-3047, Sep 2012.

GUO, H. H.; LIU, G. L.; ZHONG, R. M.; WANG, Y.; WANG, D. A.; XIA, M. Cyanidin-3-O-beta-glucoside regulates fatty acid metabolism via an AMP-activated protein kinase-dependent signaling pathway in human HepG2 cells. **Lipids in Health and Disease,** v. 11, Jan 13 2012.

GUO, H. H.; XIA, M.; ZOU, T.; LING, W.; LIU, G. L.; ZHONG, R. M. *et al.* Cyanidin 3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. **Journal of Nutritional Biochemistry,** v. 23, p. 349–360, 2012.

HASLAM, E. Vegetable tannins – Lessons of a phytochemical lifetime. **Phytochemistry,** v. 68, n. 22–24, p. 2713-2721, 2007.

HENNINGSSON, A. M.; NYMAN, E. M. G. L.; BJORCK, I. M. E. Short-chain fatty acid content in the hindgut of rats fed various composite foods and commercial dietary fibre fractions from similar sources. **Journal of the Science of Food and Agriculture,** v. 82, n. 4, p. 385-393, Mar 2002.

HEYMAN-LINDÉN, L.; SEKI, Y.; STORM, P.; JONES, H. A.; CHARRON, M. J.; BERGER, K. *et al.* Berry intake changes hepatic gene expression and DNA methylation patterns associated with high-fat diet. **Journal of Nutritional Biochemistry,** v. 27, p. 79-95, 2016.

HEYMAN, L.; AXLING, U.; BLANCO, N.; STERNER, O.; HOLM, C.; BERGER, K. Evaluation of Beneficial Metabolic Effects of Berries in High-Fat Fed C57BL/6J Mice. **Journal of Nutrition and Metabolism,** v. 2014, p. 12, 2014.

HIGBY, W. K. A Simplified Method for Determination of Some Aspects of the Carotenoid Distribution in Natural and Carotene-Fortified Orange Juice. **Journal of Food Science,** v. 27, n. 1, p. 42-49, 1962.

HIMMLER, A.; DRECHSEL, D.; KIRSCHNER, M. W.; MARTIN, D. W. Tau Consists of a Set of Proteins with Repeated C-Terminal Microtubule-Binding Domains and Variable N-Terminal Domains. **Molecular and Cellular Biology,** v. 9, n. 4, p. 1381-1388, Apr 1989.

HOTAMISLIGIL, G. S. Inflammation and metabolic disorders. **Nature,** v. 444, n. 7121, p. 860-867, Dec 14 2006.

HOTAMISLIGIL, G. S.; SHARGILL, N. S.; SPIEGELMAN, B. M. Adipose Expression of Tumor Necrosis Factor- : Direct Role in Obesity-Linked Insulin Resistance. **SCIENCE-NEW YORK THEN WASHINGTON-,** v. 259, p. 87-87, 1993.

JACOB, R. A. The Integrated Antioxidant System. **Nutrition Research,** v. 15, n. 5, p. 755-766, May 1995.

JAKOBSDOTTIR, G.; BLANCO, N.; XU, J.; AHRNÉ, S.; MOLIN, G.; STERNER, O. *et al.* Formation of short-chain fatty acids, excretion of anthocyanins, and microbial diversity in rats fed blackcurrants, blackberries, and raspberries. **Journal of Nutrition and Metabolism,** v. 2013, 2013.

JAKOBSDOTTIR, G.; JÄDERT, C.; HOLM, L.; NYMAN, M. E. Propionic and butyric acids, formed in the caecum of rats fed highly fermentable dietary fibre, are reflected in portal and aortic serum. **British Journal of Nutrition,** v. 110, n. 9, p. 1565-1572, 2013/11/14 2013.

JAKOBSDOTTIR, G.; XU, J.; MOLIN, G.; AHRNÉ, S.; NYMAN, M. High-Fat Diet Reduces the Formation of Butyrate, but Increases Succinate, Inflammation, Liver Fat and Cholesterol in Rats, while Dietary Fibre Counteracts These Effects. **Plos One,** v. 8, n. 11, p. e80476, 2013.

JENNINGS, A.; WELCH, A. A.; SPECTOR, T.; MACGREGOR, A.; CASSIDY, A. Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. **Journal of Nutrition,** v. 144, n. 2, p. 202-8, Feb 2014.

JEON, B. T.; JEONG, E. A.; SHIN, H. J.; LEE, Y.; LEE, D. H.; KIM, H. J. *et al.* Resveratrol Attenuates Obesity-Associated Peripheral and Central Inflammation and Improves Memory Deficit in Mice Fed a High-Fat Diet. **Diabetes,** v. 61, n. 6, p. 1444-1454, Jun 2012.

JIA, Y.; KIM, J.-Y.; JUN, H.-J.; KIM, S.-J.; LEE, J.-H.; HOANG, M. H. *et al.* Cyanidin is an agonistic ligand for peroxisome proliferator-activated receptor-alpha reducing hepatic lipid. **Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids,** v. 1831, n. 4, p. 698-708, 2013.

JOHANSSON, L. H.; BORG, L. A. H. A Spectrophotometric Method for Determination of Catalase Activity in Small Tissue Samples. **Analytical Biochemistry,** v. 174, n. 1, p. 331-336, Oct 1988.

JOHNSON, A. M. F.; OLEFSKY, J. M. The Origins and Drivers of Insulin Resistance. **Cell,** v. 152, n. 4, p. 673-684, Feb 14 2013.

JOLIVALT, C. G.; LEE, C. A.; BEISWENGER, K. K.; SMITH, J. L.; ORLOV, M.; TORRANCE, M. A. *et al.* Defective Insulin Signaling Pathway and Increased Glycogen Synthase Kinase-3 Activity in the Brain of Diabetic Mice: Parallels With Alzheimer's Disease and Correction by Insulin. **Journal of Neuroscience Research,** v. 86, n. 15, p. 3265-3274, Nov 15 2008.

KACZMARCZYK, M. M.; MILLER, M. J.; FREUND, G. G. The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. **Metabolism-Clinical and Experimental,** v. 61, n. 8, p. 1058-1066, 2012.

KAHN, S. E.; HULL, R. L.; UTZSCHNEIDER, K. M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. **Nature,** v. 444, n. 7121, p. 840-846, Dec 14 2006.

KALT, W.; FOOTE, K.; FILLMORE, S. A. E.; LYON, M.; VAN LUNEN, T. A.; MCRAE, K. B. Effect of blueberry feeding on plasma lipids in pigs. **British Journal of Nutrition,** v. 100, n. 1, p. 70-78, Jul 2008.

KANDEL, E. R. The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses. **Science,** v. 294, n. 5544, p. 1030-1038, 2001.

KANG, I.; KIM, Y.; TOMÁS-BARBERÁN, F. A.; ESPÍN, J. C.; CHUNG, S. Urolithin A, C, and D, but not iso-urolithin A and urolithin B, attenuate triglyceride accumulation in human cultures of adipocytes and hepatocytes. **Molecular Nutrition & Food Research,** v. 60, n. 5, p. 1129-1138, 2016.

KAPASAKALIDIS, P. G.; RASTALL, R. A.; GORDON, M. H. Extraction of Polyphenols from Processed Black Currant (Ribes nigrum L.) Residues. **Journal of Agricultural and Food Chemistry,** v. 54, n. 11, p. 4016-4021, 2006/05/01 2006.

KARIOTI, A.; SKALTSA, H.; GBOLADE, A. A. Analysis of the leaf Oil of Syzygium malaccense Merr. et Perry from Nigeria. **Journal of Essential Oil Research,** v. 19, n. 4, p. 313-315, 2007/07/01 2007.

KASTORINI, C.-M.; PANAGIOTAKOS, D. B.; CHRYSOHOOU, C.; GEORGOUSOPOULOU, E.; PITARAKI, E.; PUDDU, P. E. *et al*. Metabolic syndrome, adherence to the Mediterranean diet and 10-year cardiovascular disease incidence: The ATTICA study. **Atherosclerosis,** v. 246, p. 87-93, 2016.

KAWANO, Y.; COHEN, D. E. Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease. **Journal of Gastroenterology,** v. 48, n. 4, p. 434-441, 2013.

KERSTEN, S.; DESVERGNE, B.; WAHLI, W. Roles of PPARs in health and disease. **Nature,** v. 405, n. 6785, p. 421-424, 2000.

KHOO, H.; ISMAIL, A.; MOHD-ESA, N.; IDRIS, S. Carotenoid Content of Underutilized Tropical Fruits. **Plant Foods for Human Nutrition,** v. 63, n. 4, p. 170-175, 2008/12/01 2008.

KIM, H.; LEE, K.; KIM, D. J.; KIM, S. K.; AHN, C. W.; LIM, S.-K. *et al.* Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. **Archives of Internal Medicine,** v. 164, n. 19, p. 2169-2175, 2004.

KIMURA, I.; OZAWA, K.; INOUE, D.; IMAMURA, T.; KIMURA, K.; MAEDA, T. *et al.* The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. **Nature Communications,** v. 4, p. 1829, 2013.

KING, A. M. Y.; YOUNG, G. Characteristics and Occurrence of Phenolic Phytochemicals. **Journal of the American Dietetic Association,** v. 99, n. 2, p. 213-218, 1999.

KOGA, S.; KOJIMA, A.; KUWABARA, S.; YOSHIYAMA, Y. Immunohistochemical analysis of tau phosphorylation and astroglial activation with enhanced leptin receptor expression in diet-induced obesity mouse hippocampus. **Neuroscience Letters,** v. 571, p. 11-16, 2014.

KONG, J.-M.; CHIA, L.-S.; GOH, N.-K.; CHIA, T.-F.; BROUILLARD, R. Analysis and biological activities of anthocyanins. **Phytochemistry,** v. 64, n. 5, p. 923-933, 2003.

KOTHARI, V.; LUO, Y.; TORNABENE, T.; O'NEILL, A. M.; GREENE, M. W.; GEETHA, T. *et al.* High fat diet induces brain insulin resistance and cognitive impairment in mice. **Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease,** v. 1863, n. 2, p. 499-508, 2017.

LAKO, J.; TRENERRY, V. C.; WAHLQVIST, M.; WATTANAPENPAIBOON, N.; SOTHEESWARAN, S.; PREMIER, R. Phytochemical flavonols, carotenoids and the antioxidant

properties of a wide selection of Fijian fruit, vegetables and other readily available foods. **Food Chemistry,** v. 101, n. 4, p. 1727-1741, 2007.

LANDETE, J. M. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. **Food Research International,** v. 44, n. 5, p. 1150-1160, 2011.

LARROSA, M.; LUCERI, C.; VIVOLI, E.; PAGLIUCA, C.; LODOVICI, M.; MONETI, G. *et al.* Polyphenol metabolites from colonic microbiota exert anti-inflammatory activity on different inflammation models. **Molecular Nutrition & Food Research,** v. 53, n. 8, p. 1044-1054, 2009.

LASHERAS, C.; FERNANDEZ, S.; PATTERSON, A. M. Mediterranean diet and age with respect to overall survival in institutionalized, nonsmoking elderly people. **American Journal of Clinical Nutrition,** v. 71, n. 4, p. 987-992, Apr 2000.

LE CHATELIER, E.; NIELSEN, T.; QIN, J.; PRIFTI, E.; HILDEBRAND, F.; FALONY, G. *et al.* Richness of human gut microbiome correlates with metabolic markers. **Nature,** v. 500, n. 7464, p. 541-546, 2013.

LEDREUX, A.; WANG, X.; SCHULTZBERG, M.; GRANHOLM, A.-C.; FREEMAN, L. R. Detrimental effects of a high fat/high cholesterol diet on memory and hippocampal markers in aged rats. **Behavioural Brain Research,** v. 312, p. 294-304, 2016.

LEE, E. B. Obesity, leptin, and Alzheimer's disease. **Year in Diabetes and Obesity,** v. 1243, p. 15-29, 2011.

LEE, H. C.; JENNER, A. M.; LOW, C. S.; LEE, Y. K. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. **Research in Microbiology,** v. 157, n. 9, p. 876-884, 2006.

LEE, J.; RENNAKER, C.; WROLSTAD, R. E. Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods. **Food Chemistry,** v. 110, n. 3, p. 782-786, 2008.

LEE, J. S.; KIM, M. S. The role of GSK3 in glucose homeostasis and the development of insulin resistance. **Diabetes Research and Clinical Practice,** v. 77, p. S49-S57, Sep 2007.

LEE, S. G.; KIM, B.; YANG, Y.; PHAM, T. X.; PARK, Y.-K.; MANATOU, J. *et al.* Berry anthocyanins suppress the expression and secretion of proinflammatory mediators in macrophages by inhibiting nuclear translocation of NF-κB independent of NRF2-mediated mechanism. **The Journal of Nutritional Biochemistry,** v. 25, n. 4, p. 404-411, 2014.

LEGER, M.; QUIEDEVILLE, A.; BOUET, V.; HAELEWYN, B.; BOULOUARD, M.; SCHUMANN-BARD, P. *et al.* Object recognition test in mice. **Nat. Protocols,** v. 8, n. 12, p. 2531-2537, 2013.

LEITE-LEGATTI, A. V.; BATISTA, A. G.; DRAGANO, N. R. V.; MARQUES, A. C.; MALTA, L. G.; RICCIO, M. F. *et al.* Jaboticaba peel: Antioxidant compounds, antiproliferative and antimutagenic activities. **Food Research International,** v. 49, p. 596-603, 2012.

LEITE, A. V.; MALTA, L. G.; RICCIO, M. F.; EBERLIN, M. N.; PASTORE, G. M.; MAROSTICA, 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, n. 6, p. 2277-2283, Mar 23 2011.

LENQUISTE, S. A.; BATISTA, A. G.; MARINELI, R. D.; DRAGANO, N. R. V.; MAROSTICA, M. R. Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. **Food Research International,** v. 49, n. 1, p. 153-160, Nov 2012.

LETO, D.; SALTIEL, A. R. Regulation of glucose transport by insulin: traffic control of GLUT4. **Nature Reviews Molecular Cell Biology,** v. 13, n. 6, p. 383-396, Jun 2012.

LEY, R. E.; TURNBAUGH, P. J.; KLEIN, S.; GORDON, J. I. Microbial ecology: Human gut microbes associated with obesity. **Nature,** v. 444, n. 7122, p. 1022-1023, 2006.

LI, L.; WANG, L. Y.; WU, Z. Q.; YAO, L. J.; WU, Y. H.; HUANG, L. *et al.* Anthocyanin-rich fractions from red raspberries attenuate inflammation in both RAW264.7 macrophages and a mouse model of colitis. **Scientific Reports,** v. 4, n. 6234, Aug 29 2014.

LI, Y.; LIU, S.; MAN, Y.; LI, N.; ZHOU, Y. U. Effects of vitamins E and C combined with beta-carotene on cognitive function in the elderly. **Exp Ther Med,** v. 9, n. 4, p. 1489-1493, Apr 2015.

LIMA, A. D. B.; CORREA, A. D.; SACZK, A. A.; MARTINS, M. P.; CASTILHO, R. O. Anthocyanins, Pigment Stability and Antioxidant Activity in Jabuticaba [Myrciaria Cauliflora (Mart.) O. Berg]. **Revista Brasileira De Fruticultura,** v. 33, n. 3, p. 877-887, Sep 2011.

LIN, H. V.; FRASSETTO, A.; KOWALIK, E. J.; NAWROCKI, A. R.; LU, M. F. M.; KOSINSKI, J. R. *et al.* Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. **Plos One,** v. 7, n. 4, Apr 10 2012.

LIN, L.-Z.; HARNLY, J. M. A Screening Method for the Identification of Glycosylated Flavonoids and Other Phenolic Compounds Using a Standard Analytical Approach for All Plant Materials. **Journal of Agricultural and Food Chemistry,** v. 55, n. 4, p. 1084-1096, 2007/02/01 2007.

LIU, Y.; LI, D.; ZHANG, Y.; SUN, R.; XIA, M. Anthocyanin increases adiponectin secretion and protects against diabetes-related endothelial dysfunction. **American Journal of Physiology - Endocrinology And Metabolism,** v. 306, n. 8, p. E975-E988, 2014.

LOMONACO, R.; ORTIZ-LOPEZ, C.; ORSAK, B.; WEBB, A.; HARDIES, J.; DARLAND, C. *et al.* Effect of adipose tissue insulin resistance on metabolic parameters and liver histology in obese patients with nonalcoholic fatty liver disease. **Hepatology,** v. 55, n. 5, p. 1389-1397, 2012.

LOUIS, P.; SCOTT, K. P.; DUNCAN, S. H.; FLINT, H. J. Understanding the effects of diet on bacterial metabolism in the large intestine. **Journal of Applied Microbiology,** v. 102, n. 5, p. 1197-1208, 2007.

MA, Q.-L.; YANG, F.; ROSARIO, E. R.; UBEDA, O. J.; BEECH, W.; GANT, D. J. *et al.* β-Amyloid Oligomers Induce Phosphorylation of Tau and Inactivation of Insulin Receptor Substrate via c-Jun N-Terminal Kinase Signaling: Suppression by Omega-3 Fatty Acids and Curcumin. **The Journal of Neuroscience,** v. 29, n. 28, p. 9078-9089, 2009.

MAISUTHISAKUL, P.; PASUK, S.; RITTHIRUANGDEJ, P. Relationship between antioxidant properties and chemical composition of some Thai plants. **Journal of Food Composition and Analysis,** v. 21, n. 3, p. 229-240, May 2008.

MANACH, C.; SCALBERT, A.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Polyphenols: food sources and bioavailability. **The American Journal of Clinical Nutrition,** v. 79, n. 5, p. 727-747, May 1, 2004 2004.

MARQUES PEIXOTO, F.; FERNANDES, I.; GOUVÊA, A. C. M. S.; SANTIAGO, M. C. P. A.; GALHARDO BORGUINI, R.; MATEUS, N. *et al.* Simulation of in vitro digestion coupled to gastric and intestinal transport models to estimate absorption of anthocyanins from peel powder of jabuticaba, jamelão and jambo fruits. **Journal of Functional Foods,** v. 24, p. 373-381, 2016.

MARTIN, K. C.; BARAD, M.; KANDEL, E. R. Local protein synthesis and its role in synapse-specific plasticity. **Current Opinion in Neurobiology,** v. 10, n. 5, p. 587-592, 2000.

MASLOWSKI, K. M.; VIEIRA, A. T.; NG, A.; KRANICH, J.; SIERRO, F.; DI, Y. *et al.* Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. **Nature,** v. 461, n. 7268, p. 1282-1286, 2009.

MATTSON, M. P. Degenerative and protective signaling mechanisms in the neurofibrillary pathology of AD. **Neurobiology of Aging,** v. 16, n. 3, p. 447-457, 1995.

MATZIOURIDOU, C.; MARUNGRUANG, N.; NGUYEN, T. D.; NYMAN, M.; FÅK, F. Lingonberries reduce atherosclerosis in Apoe-/- mice in association with altered gut microbiota composition and improved lipid profile. **Molecular Nutrition & Food Research,** v. 60, n. 5, p. 1150-1160, 2016.

MCDOUGALL, G. J.; KULKARNI, N. N.; STEWART, D. Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. **BioFactors,** v. 34, n. 1, p. 73-80, 2008.

MCFARLAND, C. P.; GLISKY, E. L. Frontal lobe involvement in a task of time-based prospective memory. **Neuropsychologia,** v. 47, n. 7, p. 1660-1669, 2009.

MESSIER, C.; TEUTENBERG, K. The role of insulin, insulin growth factor, and insulin-degrating enzyme in brain and Alzheimer's disease. **Neural Plasticity,** v. 12, n. 4, p. 311-328, 2005.

MILALA, J.; KOSMALA, M.; SÓJKA, M.; KOŁODZIEJCZYK, K.; ZBRZEŹNIAK, M.; MARKOWSKI, J. Plum pomaces as a potential source of dietary fibre: composition and antioxidant properties. **Journal of Food Science and Technology,** v. 50, n. 5, p. 1012-1017, 2013.

MILANSKI, M.; ARRUDA, A. P.; COOPE, A.; IGNACIO-SOUZA, L. M.; NUNEZ, C. E.; ROMAN, E. A. *et al.* Inhibition of Hypothalamic Inflammation Reverses Diet-Induced Insulin Resistance in the Liver. **Diabetes,** v. 61, n. 6, p. 1455-1462, Jun 2012.

MOKHTAR, S. H.; BAKHURAYSAH, M. M.; CRAM, D. S.; PETRATOS, S. The Beta-Amyloid Protein of Alzheimer’s Disease: Communication Breakdown by Modifying the Neuronal Cytoskeleton. **International Journal of Alzheimer’s Disease,** v. 2013, p. 15, 2013.

MOLLER, D. E.; KAUFMAN, K. D. Metabolic syndrome: A clinical and molecular perspective. **Annual Review of Medicine,** v. 56, p. 45-+, 2005.

MONAGAS, M.; URPI-SARDA, M.; SANCHEZ-PATAN, F.; LLORACH, R.; GARRIDO, I.; GOMEZ-CORDOVES, C. *et al.* Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. **Food & Function,** v. 1, n. 3, p. 233-253, Dec 2010.

MORRIS, R. Developments of a Water-Maze Procedure for Studying Spatial-Learning in the Rat. **Journal of Neuroscience Methods,** v. 11, n. 1, p. 47-60, 1984.

MORTON, J. F. Malay apple. In: MORTON, J. F. (Ed.). **Fruits of warm climates**. Miami, FL: Creative Resources Systems, 1987. cap. 378–381, p.xii, 505 p., 24 p. of plates. ISBN 0961018410.

MYERS, M. G.; COWLEY, M. A.; MUNZBERG, H. Mechanisms of leptin action and leptin resistance. **Annual Review of Physiology,** v. 70, p. 537-556, 2008.

NAIR, M. P.; MAHAJAN, S.; REYNOLDS, J. L.; AALINKEEL, R.; NAIR, H.; SCHWARTZ, S. A. *et al.* The Flavonoid Quercetin Inhibits Proinflammatory Cytokine (Tumor Necrosis Factor Alpha) Gene Expression in Normal Peripheral Blood Mononuclear Cells via Modulation of the NF-κβ System. **Clinical and Vaccine Immunology,** v. 13, n. 3, p. 319-328, 2006.

NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. **Journal of Biological Chemistry,** v. 153, n. 2, p. 375-380, May 1944.

NG, M.; FLEMING, T.; ROBINSON, M.; THOMSON, B.; GRAETZ, N.; MARGONO, C. *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. **The Lancet,** v. 384, n. 9945, p. 766-781, 2014.

NILE, S. H.; PARK, S. W. Edible berries: Bioactive components and their effect on human health. **Nutrition,** v. 30, n. 2, p. 134-144, 2014.

NUNES, P. C.; AQUINO, J. D. S.; ROCKENBACH, I. I.; STAMFORD, T. L. M. Physico-Chemical Characterization, Bioactive Compounds and Antioxidant Activity of Malay Apple [Syzygium malaccense (L.) Merr. & L.M. Perry]. **Plos One,** v. 11, n. 6, p. e0158134, 2016.

OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for Lipid Peroxides in Animal-Tissues by Thiobarbituric Acid Reaction. **Analytical Biochemistry,** v. 95, n. 2, p. 351-358, 1979.

OU, K. Q.; GU, L. W. Absorption and metabolism of proanthocyanidins. **Journal of Functional Foods,** v. 7, p. 43-53, Mar 2014.

PALMA, M.; PINEIRO, Z.; BARROSO, C. G. In-line pressurized-fluid extraction-solid-phase extraction for determining phenolic compounds in grapes. **Journal of Chromatography A,** v. 968, n. 1-2, p. 1-6, Aug 30 2002.

PANAGIOTAKOS, D. B.; GEORGOUSOPOULOU, E. N.; PITSAVOS, C.; CHRYSOHOOU, C.; SKOUMAS, I.; PITARAKI, E. *et al.* Exploring the path of Mediterranean diet on 10-year incidence of cardiovascular disease: The ATTICA study (2002–2012). **Nutrition, Metabolism and Cardiovascular Diseases,** v. 25, n. 3, p. 327-335, 2015.

PANAGIOTAKOS, D. B.; PITSAVOS, C. H.; CHRYSOHOU, C.; SKOUMAS, J.; PAPADIMITRIOU, L.; STEFANADIS, C. *et al.* Status and management of hypertension in Greece: role of the adoption of a Mediterranean diet: the Attica study. **Journal of Hypertension,** v. 21, n. 8, p. 1483-1489, Aug 2003.

PAPANDREOU, M. A.; DIMAKOPOULOU, A.; LINARDAKI, Z. I.; CORDOPATIS, P.; KLIMIS-ZACAS, D.; MARGARITY, M. *et al.* Effect of a polyphenol-rich wild blueberry extract on cognitive performance of mice, brain antioxidant markers and acetylcholinesterase activity. **Behavioural Brain Research,** v. 198, n. 2, p. 352-358, Mar 17 2009.

PAREDES-LÓPEZ, O.; CERVANTES-CEJA, M.; VIGNA-PÉREZ, M.; HERNÁNDEZ-PÉREZ, T. Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life—A Review. **Plant Foods for Human Nutrition,** v. 65, n. 3, p. 299-308, 2010/09/01 2010.

PAXINOS, G.; FRANKLIN, K. B. **The mouse brain in stereotaxic coordinates**. Gulf Professional Publishing, 2004. ISBN 012547640X.

PINO, J. A.; MARBOT, R.; ROSADO, A.; VAZQUEZ, C. Volatile constituents of Malay rose apple [Syzygium malaccense, (L.) Merr. & Perry]. **Flavour and Fragrance Journal,** v. 19, n. 1, p. 32-35, Jan-Feb 2004.

PLAGEMANN, I., KRINGS, I., BERGER, R. G., & MARÓSTICA JÚNIOR., M. R. Volatile constituents of jabuticaba (Myrciaria jaboticaba (Vell.) O. Berg) fruits. **Journal of Essential Oil Research,** v. 24, n. 1, p. 45-51, 2012.

PLAZA, M.; BATISTA, Â. G.; CAZARIN, C. B. B.; SANDAHL, M.; TURNER, C.; ÖSTMAN, E. *et al.* Characterization of antioxidant polyphenols from Myrciaria jaboticaba peel and their effects on glucose metabolism and antioxidant status: A pilot clinical study. **Food Chemistry,** v. 211, p. 185-197, 2016.

POLI, G.; ALBANO, E.; DIANZANI, M. U. The role of lipid peroxidation in liver damage. **Chemistry and Physics of Lipids,** v. 45, n. 2, p. 117-142, 1987/11/01 1987.

POSEY, K. A.; CLEGG, D. J.; PRINTZ, R. L.; BYUN, J.; MORTON, G. J.; VIVEKANANDAN-GIRI, A. *et al.* Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. **American Journal of Physiology - Endocrinology And Metabolism,** v. 296, n. 5, p. E1003-E1012, 2009.

POULOSE, S. M.; FISHER, D. R.; LARSON, J.; BIELINSKI, D. F.; RIMANDO, A. M.; CAREY, A. N. *et al.* Anthocyanin-rich Açai (Euterpe oleracea Mart.) Fruit Pulp Fractions Attenuate Inflammatory Stress Signaling in Mouse Brain BV-2 Microglial Cells. **Journal of Agricultural and Food Chemistry,** v. 60, n. 4, p. 1084-1093, 2012/02/01 2012.

PRIOR, R. L. Fruits and vegetables in the prevention of cellular oxidative damage. **American Journal of Clinical Nutrition,** v. 78, n. 3, p. 570s-578s, Sep 2003.

PRIOR, R. L.; HOANG, H.; GU, L. W.; WU, X. L.; BACCHIOCCA, M.; HOWARD, L. *et al.* Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. **Journal of Agricultural and Food Chemistry,** v. 51, n. 11, p. 3273-3279, May 21 2003.

PROFENNO, L. A.; FARAONE, S. V. Diabetes and overweight associate with non-APOE4 genotype in an alzheimer's disease population. **American Journal of Medical Genetics Part B: Neuropsychiatric Genetics,** v. 147B, n. 6, p. 822-829, 2008.

PROFENNO, L. A.; PORSTEINSSON, A. P.; FARAONE, S. V. Meta-Analysis of Alzheimer's Disease Risk with Obesity, Diabetes, and Related Disorders. **Biological Psychiatry,** v. 67, n. 6, p. 505-512, 2010.

PYRZYNSKA, K.; BIESAGA, M. Analysis of phenolic acids and flavonoids in honey. **TrAC Trends in Analytical Chemistry,** v. 28, n. 7, p. 893-902, 2009.

REEVES, P. G.; NIELSEN, F. H.; FAHEY, G. C. Ain-93 Purified Diets for Laboratory Rodents - Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the Ain-76a Rodent Diet. **Journal of Nutrition,** v. 123, n. 11, p. 1939-1951, Nov 1993.

REITZ, C.; BRAYNE, C.; MAYEUX, R. Epidemiology of Alzheimer disease. **Nat Rev Neurol,** v. 7, n. 3, p. 137-152, 2011.

REITZ, C.; MAYEUX, R. Alzheimer disease: Epidemiology, diagnostic criteria, risk factors and biomarkers. **Biochemical Pharmacology,** v. 88, n. 4, p. 640-651, 2014.

RENAUD, S.; DE LORGERIL, M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. **The Lancet,** v. 339, n. 8808, p. 1523-1526, 1992.

RENDEIRO, C.; RHODES, J. S.; SPENCER, J. P. E. The mechanisms of action of flavonoids in the brain: Direct versus indirect effects. **Neurochemistry International,** v. 89, p. 126-139, 2015.

RENDEIRO, C.; VAUZOUR, D.; KEAN, R. J.; BUTLER, L. T.; RATTRAY, M.; SPENCER, J. P. E. *et al.* Blueberry supplementation induces spatial memory improvements and region-specific regulation of hippocampal BDNF mRNA expression in young rats. **Psychopharmacology,** v. 223, n. 3, p. 319-330, 2012.

RENDEIRO, C.; VAUZOUR, D.; RATTRAY, M.; WAFFO-TEGUO, P.; MERILLON, J. M.; BUTLER, L. T. *et al.* Dietary Levels of Pure Flavonoids Improve Spatial Memory Performance and Increase Hippocampal Brain-Derived Neurotrophic Factor. **Plos One,** v. 8, n. 5, May 28 2013.

REYNERTSON, K. A.; WALLACE, A. M.; ADACHI, S.; GIL, R. R.; YANG, H.; BASILE, M. J. *et al.* Bioactive depsides and anthocyanins from jaboticaba (Myrciaria cauliflora). **J Nat Prod,** v. 69, n. 8, p. 1228-30, Aug 2006.

REYNERTSON, K. A.; YANG, H.; JIANG, B.; BASILE, M. J.; KENNELLY, E. J. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. **Food Chemistry,** v. 109, n. 4, p. 883-890, Aug 15 2008.

ROLLS, A.; SHECHTER, R.; LONDON, A.; ZIV, Y.; RONEN, A.; LEVY, R. *et al.* Toll-like receptors modulate adult hippocampal neurogenesis. **Nat Cell Biol,** v. 9, n. 9, p. 1081-1088, 2007.

SAVITHA, R. C.; PADMAVATHY, S.; SUNDHARARAJAN, A. Invitro antioxidant activities on leaf extracts of syzygium malaccense (L.) merr and perry. **Anc Sci Life,** v. 30, n. 4, p. 110-3, Apr 2011.

SAVVA , G. M.; WHARTON , S. B.; INCE , P. G.; FORSTER , G.; MATTHEWS , F. E.; BRAYNE , C. Age, Neuropathology, and Dementia. **New England Journal of Medicine,** v. 360, n. 22, p. 2302-2309, 2009.

SCALBERT, A. Antimicrobial properties of tannins. **Phytochemistry,** v. 30, n. 12, p. 3875-3883, 1991.

SCARMEAS, N.; STERN, Y.; TANG, M.-X.; MAYEUX, R.; LUCHSINGER, J. A. Mediterranean diet and risk for Alzheimer's disease. **Annals of Neurology,** v. 59, n. 6, p. 912-921, 2006.

SCHNORR, C.; MORRONE, M.; SIMÕES-PIRES, A.; BITTENCOURT, L.; ZEIDÁN-CHULIÁ, F.; MOREIRA, J. Supplementation of Adult Rats with Moderate Amounts of β-Carotene Modulates the Redox Status in Plasma without Exerting Pro-Oxidant Effects in the Brain: A Safer Alternative to Food Fortification with Vitamin A? **Nutrients,** v. 6, n. 12, p. 5572, 2014.

SCHROEDER, F. A.; LIN, C. L.; CRUSIO, W. E.; AKBARIAN, S. Antidepressant-Like Effects of the Histone Deacetylase Inhibitor, Sodium Butyrate, in the Mouse. **Biological Psychiatry,** v. 62, n. 1, p. 55-64, 2007.

SCHUBERT, M.; BRAZIL, D. P.; BURKS, D. J.; KUSHNER, J. A.; YE, J.; FLINT, C. L. *et al.* Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. **Journal of Neuroscience,** v. 23, n. 18, p. 7084-7092, Aug 6 2003.

SCHUBERT, M.; GAUTAM, D.; SURJO, D.; UEKI, K.; BAUDLER, S.; SCHUBERT, D. *et al.* Role for neuronal insulin resistance in neurodegenerative diseases. **Proceedings of the National Academy of Sciences of the United States of America,** v. 101, n. 9, p. 3100-3105, March 2, 2004 2004.

SHAW, J. E.; SICREE, R. A.; ZIMMET, P. Z. Global estimates of the prevalence of diabetes for 2010 and 2030. **Diabetes Research and Clinical Practice,** v. 87, n. 1, p. 4-14, Jan 2010.

SHIMIZU, M. Multifunctions of dietary polyphenols in the regulation of intestinal inflammation. **Journal of Food and Drug Analysis,** v. 25, n. 1, p. 93-99, 2017.

SHUKITT-HALE, B.; BIELINSKI, D. F.; LAU, F. C.; WILLIS, L. M.; CAREY, A. N.; JOSEPH, J. A. The beneficial effects of berries on cognition, motor behaviour and neuronal function in ageing. **British Journal of Nutrition,** v. 114, n. 10, p. 1542-1549, 2015.

SLUIJS, I.; CADIER, E.; BEULENS, J. W. J.; VAN DER A, D. L.; SPIJKERMAN, A. M. W.; VAN DER SCHOUW, Y. T. Dietary intake of carotenoids and risk of type 2 diabetes. **Nutrition, Metabolism and Cardiovascular Diseases,** v. 25, n. 4, p. 376-381, 2015.

SOFI, F.; CESARI, F.; ABBATE, R.; GENSINI, G. F.; CASINI, A. Adherence to Mediterranean diet and health status: meta-analysis. **British Medical Journal,** v. 337, n. 7671, Sep 20 2008.

SOONG, Y.-Y.; BARLOW, P. J. Antioxidant activity and phenolic content of selected fruit seeds. **Food Chemistry,** v. 88, n. 3, p. 411-417, 2004.

SOUZA, A. D. M.; PEREIRA, R. A.; YOKOO, E. M.; LEVY, R. B.; SICHIERI, R. Alimentos mais consumidos no Brasil: Inquérito Nacional de Alimentação 2008-2009. **Revista de Saúde Pública,** v. 47, p. 190s-199s, 2013.

SPENCER, J. P. E. The impact of flavonoids on memory: physiological and molecular considerations. **Chemical Society Reviews,** v. 38, n. 4, p. 1152-1161, 2009.

SPIELMAN, L. J.; LITTLE, J. P.; KLEGERIS, A. Inflammation and insulin/IGF-1 resistance as the possible link between obesity and neurodegeneration. **Journal of Neuroimmunology,** v. 273, n. 1–2, p. 8-21, 2014.

STANTON, M. C.; CHEN, S.-C.; JACKSON, J. V.; ROJAS-TRIANA, A.; KINSLEY, D.; CUI, L. *et al.* Inflammatory Signals shift from adipose to liver during high fat feeding and influence the development of steatohepatitis in mice. **Journal of Inflammation (London, England),** v. 8, p. 8-8, 2011.

STILLING, R. M.; DINAN, T. G.; CRYAN, J. F. Microbial genes, brain & behaviour – epigenetic regulation of the gut–brain axis. **Genes, Brain and Behavior,** v. 13, n. 1, p. 69-86, 2014.

STYSKAL, J.; VAN REMMEN, H.; RICHARDSON, A.; SALMON, A. B. Oxidative stress and diabetes: What can we learn about insulin resistance from antioxidant mutant mouse models? **Free Radical Biology and Medicine,** v. 52, n. 1, p. 46-58, 2012.

SUGANAMI, T.; TANIMOTO-KOYAMA, K.; NISHIDA, J.; ITOH, M.; YUAN, X.; MIZUARAI, S. *et al.* Role of the Toll-like Receptor 4/NF-κB Pathway in Saturated Fatty Acid–Induced Inflammatory

Changes in the Interaction Between Adipocytes and Macrophages. **Arteriosclerosis, Thrombosis, and Vascular Biology,** v. 27, n. 1, p. 84-91, 2007.

SUGIURA, M.; OGAWA, K.; YANO, M. Comparison of bioavailability between β-cryptoxanthin and β-carotene and tissue distribution in its intact form in rats. **Bioscience, Biotechnology, and Biochemistry,** v. 78, n. 2, p. 307-310, 2014/02/01 2014.

TAKAHASHI, H.; KATO, M.; HAYASHI, M.; OKUBO, Y.; TAKANO, A.; ITO, H. *et al.* Memory and frontal lobe functions; possible relations with dopamine D2 receptors in the hippocampus. **NeuroImage,** v. 34, n. 4, p. 1643-1649, 2007.

TESSARI, P.; CORACINA, A.; COSMA, A.; TIENGO, A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. **Nutrition, Metabolism and Cardiovascular Diseases,** v. 19, n. 4, p. 291-302, 2009.

THOMAS, J.; GARG, M. L.; SMITH, D. W. Dietary resveratrol supplementation normalizes gene expression in the hippocampus of streptozotocin-induced diabetic C57Bl/6 mice. **Journal of Nutritional Biochemistry,** v. 25, n. 3, p. 313-318, 2014.

TILG, H.; MOSCHEN, A. R. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. **Nature Reviews Immunology,** v. 6, n. 10, p. 772-783, Oct 2006.

TITGEMEYER, E. C.; BOURQUIN, L. D.; FAHEY, G. C.; GARLEB, K. A. Fermentability of various fiber sources by human fecal bacteria in vitro. **The American Journal of Clinical Nutrition,** v. 53, n. 6, p. 1418-1424, 1991.

TREMAROLI, V.; BACKHED, F. Functional interactions between the gut microbiota and host metabolism. **Nature,** v. 489, n. 7415, p. 242-249, 2012.

TRICHOPOULOU, A.; COSTACOU, T.; BAMIA, C.; TRICHOPOULOS, D. Adherence to a Mediterranean diet and survival in a Greek population. **New England Journal of Medicine,** v. 348, n. 26, p. 2599-2608, Jun 26 2003.

TRICHOPOULOU, A.; KYROZIS, A.; ROSSI, M.; KATSOULIS, M.; TRICHOPOULOS, D.; LA VECCHIA, C. *et al.* Mediterranean diet and cognitive decline over time in an elderly Mediterranean population. **European Journal of Nutrition,** v. 54, n. 8, p. 1311-1321, 2015.

TRICHOPOULOU, A.; LAGIOU, P.; KUPER, H.; TRICHOPOULOS, D. Cancer and Mediterranean Dietary Traditions. **Cancer Epidemiology Biomarkers & Prevention,** v. 9, n. 9, p. 869-873, 2000.

_____. Cancer and Mediterranean dietary traditions. **Cancer Epidemiology Biomarkers & Prevention,** v. 9, n. 9, p. 869-873, Sep 2000.

TSUDA, T. Regulation of adipocyte function by anthocyanins; Possibility of preventing the metabolic syndrome. **Journal of Agricultural and Food Chemistry,** v. 56, n. 3, p. 642-646, Feb 13 2008.

TSUDA, T.; HORIO, F.; UCHIDA, K.; AOKI, H.; OSAWA, T. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. **Journal of Nutrition,** v. 133, n. 7, p. 2125-2130, Jul 2003.

TURNBAUGH, P. J.; LEY, R. E.; MAHOWALD, M. A.; MAGRINI, V.; MARDIS, E. R.; GORDON, J. I. An obesity-associated gut microbiome with increased capacity for energy harvest. **Nature,** v. 444, n. 7122, p. 1027-131, 2006.

UYSAL, K. T.; WIESBROCK, S. M.; MARINO, M. W.; HOTAMISLIGIL, G. S. Protection from obesity-induced insulin resistance in mice lacking TNF-[alpha] function. **Nature,** v. 389, n. 6651, p. 610-614, 1997.

VALENTE, T.; HIDALGO, J.; BOLEA, I.; RAMIREZ, B.; ANGLÉS, N.; REGUANT, J. *et al.* A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain. **Journal of Alzheimer's Disease,** v. 18, n. 4, p. 849-865, 2009.

VALKO, M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M. T. D.; MAZUR, M.; TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. **The International Journal of Biochemistry & Cell Biology,** v. 39, n. 1, p. 44-84, 2007.

VARGHA-KHADEM, F.; GADIAN, D. G.; WATKINS, K. E.; CONNELLY, A.; VAN PAESSCHEN, W.; MISHKIN, M. Differential Effects of Early Hippocampal Pathology on Episodic and Semantic Memory. **Science,** v. 277, n. 5324, p. 376-380, 1997.

VAS, C. J.; PINTO, C.; PANIKKER, D.; NORONHA, S.; DESHPANDE, N.; KULKARNI, L. *et al.* Prevalence of dementia in an urban Indian population. **International Psychogeriatrics,** v. 13, n. 4, p. 439-450, Dec 2001.

VEBERIC, R.; SLATNAR, A.; BIZJAK, J.; STAMPAR, F.; MIKULIC-PETKOVSEK, M. Anthocyanin composition of different wild and cultivated berry species. **LWT - Food Science and Technology,** v. 60, n. 1, p. 509-517, 2015.

VILLANUEVA-SUÁREZ, M.-J.; PÉREZ-CÓZAR, M.-L.; MATEOS-APARICIO, I.; REDONDO-CUENCA, A. Potential fat-lowering and prebiotic effects of enzymatically treated okara in high-cholesterol-fed Wistar rats. **International Journal of Food Sciences and Nutrition,** v. 67, n. 7, p. 828-833, 2016/10/02 2016.

WELLBURN, A. R. The Spectral Determination of Chlorophyll-a and Chlorophhyll-B, as Well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. **Journal of Plant Physiology,** v. 144, n. 3, p. 307-313, Sep 1994.

WHO. **Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation**. Geneva: World Health Organization 2003.

_____. **World health statistics 2012**. Geneva: WHO Press**:** 176 p. 2012.

WILLIAMS, C. D.; STENGEL, J.; ASIKE, M. I.; TORRES, D. M.; SHAW, J.; CONTRERAS, M. *et al.* Prevalence of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis Among a Largely Middle-Aged Population Utilizing Ultrasound and Liver Biopsy: A Prospective Study. **Gastroenterology,** v. 140, n. 1, p. 124-131, 2011.

WILLIAMS, C. M.; EL MOHSEN, M. A.; VAUZOUR, D.; RENDEIRO, C.; BUTLER, L. T.; ELLIS, J. A. *et al.* Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. **Free Radical Biology and Medicine,** v. 45, n. 3, p. 295-305, 2008.

WILLIAMS, R. J.; SPENCER, J. P. E. Flavonoids, cognition, and dementia: Actions, mechanisms, and potential therapeutic utility for Alzheimer disease. **Free Radical Biology and Medicine,** v. 52, n. 1, p. 35-45, Jan 1 2012.

WILLIAMSON, G.; MANACH, C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. **The American Journal of Clinical Nutrition,** v. 81, n. 1, p. 243S-255S, 2005.

WINER, DANIEL A.; LUCK, H.; TSAI, S.; WINER, S. The Intestinal Immune System in Obesity and Insulin Resistance. **Cell Metabolism,** v. 23, n. 3, p. 413-426, 2016.

WINTERBOURN, C. C.; HAWKINS, R. E.; BRIAN, M.; CARRELL, R. W. The estimation of red cell superoxide dismutase activity. **Journal of Laboratory and Clinical Medicine,** v. 85, n. 2, p. 337-41, Feb 1975.

WROLSTAD, R. E., Ed. **Color and pigment analysis in fruit products**. Corvallis: Oregon Agricultural Experimental Station, p.17ed. 1976.

YAN, L.; YIN, P.; MA, C.; LIU, Y. Method Development and Validation for Pharmacokinetic and Tissue Distributions of Ellagic Acid Using Ultrahigh Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MS/MS). **Molecules,** v. 19, n. 11, p. 18923, 2014.

YANG, B.; KORTESNIEMI, M.; LIU, P.; KARONEN, M.; SALMINEN, J.-P. Analysis of Hydrolyzable Tannins and Other Phenolic Compounds in Emblic Leafflower (Phyllanthus emblica L.) Fruits by High Performance Liquid Chromatography–Electrospray Ionization Mass Spectrometry. **Journal of Agricultural and Food Chemistry,** v. 60, n. 35, p. 8672-8683, 2012/09/05 2012.

YOUDIM, K. A.; DOBBIE, M. S.; KUHNLE, G.; PROTEGGENTE, A. R.; ABBOTT, N. J.; RICE-EVANS, C. Interaction between flavonoids and the blood–brain barrier: in vitro studies. **Journal of Neurochemistry,** v. 85, n. 1, p. 180-192, 2003.

ZHAO, C.; DENG, W.; GAGE, F. H. Mechanisms and Functional Implications of Adult Neurogenesis. **Cell,** v. 132, n. 4, p. 645-660, 2008.

ZHAO, G. H.; NYMAN, M.; JONSSON, J. A. Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. **Biomedical Chromatography,** v. 20, n. 8, p. 674-682, Aug 2006.

ZHAO, W. Q.; ALKON, D. L. Role of insulin and insulin receptor in learning and memory. **Molecular and Cellular Endocrinology,** v. 177, n. 1-2, p. 125-134, May 25 2001.

ZHISHEN, J.; MENGCHENG, T.; JIANMING, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. **Food Chemistry,** v. 64, n. 4, p. 555-559, Mar 1999.

ZHOU, M.; XU, A.; TAM, P. K. H.; LAM, K. S. L.; CHAN, L.; HOO, R. L. C. *et al.* Mitochondrial dysfunction contributes to the increased vulnerabilities of adiponectin knockout mice to liver injury. **Hepatology,** v. 48, n. 4, p. 1087-1096, 2008.

## ANEXO 1

Autorização da Elsevier para reprodução do artigo do capítulo 2 publicado na revista LWT.

### ELSEVIER LICENSE TERMS AND CONDITIONS

Feb 19, 2017

This Agreement between Ângela Batista ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

| | |
|---|---|
| License Number | 4050880406045 |
| License date | Feb 16, 2017 |
| Licensed Content Publisher | Elsevier |
| Licensed Content Publication | LWT - Food Science and Technology |
| Licensed Content Title | Red-jambo (Syzygium malaccense): Bioactive compounds in fruits and leaves |
| Licensed Content Author | Ângela Giovana Batista,Juliana Kelly da Silva,Cinthia B. Betim Cazarin,Aline Camarão Telles Biasoto,Alexandra Christine Helena Frankland Sawaya,Marcelo Alexandre Prado,Mário Roberto Maróstica Júnior |
| Licensed Content Date | March 2017 |
| Licensed Content Volume | 76 |
| Licensed Content Issue | n/a |
| Licensed Content Pages | 8 |
| Start Page | 284 |
| End Page | 291 |
| Type of Use | reuse in a thesis/dissertation |
| Portion | full article |
| Format | both print and electronic |
| Are you the author of this Elsevier article? | Yes |
| Will you be translating? | No |
| Order reference number | |
| Title of your thesis/dissertation | BERRY FRUITS: PREVENTION OF METABOLIC DISEASES AND COGNITIVE IMPAIRMENT |
| Expected completion date | Apr 2017 |
| Estimated size (number of pages) | 172 |
| Elsevier VAT number | GB 494 6272 12 |
| Requestor Location | Ângela Batista<br>Monteiro Lobato street, 80<br><br>Campinas, São Paulo 13083-862<br>Brazil<br>Attn: Ângela Batista |
| Total | **0.00 USD** |
| Terms and Conditions | |

**ANEXO 2**

Autorização CEUA

UNICAMP

CEUA/Unicamp

**Comissão de Ética no Uso de Animais**
**CEUA/Unicamp**

**CERTIFICADO**

Certificamos que o projeto **"PODER ANTIOXIDANTE E BIOATIVIDADE DE FITOQUÍMICOS DE FRUTAS VERMELHAS NO COMBATE A DOENÇAS CORRELATAS À OBESIDADE UTILIZANDO CAMUNDONGOS SWISS"** (protocolo nº **3157-1**), sob a responsabilidade de **Prof. Dr. Mário Roberto Maróstica Júnior / Ângela Giovana Batista**, está de acordo com os **Princípios Éticos na Experimentação Animal** adotados pela **Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)** e com a legislação vigente, **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, e o **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**.

O projeto foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP - em **16 de setembro de 2013**.

Campinas, 16 de setembro de 2013.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

## ANEXO 3

06-Mar-2017

Dear Miss Batista:

Thank you for submitting your revised manuscript (# mnfr.201600952.R1) entitled "Jaboticaba berry peel intake prevents insulin resistance-induced tau phosphorylation in mice" to Molecular Nutrition & Food Research. Your manuscript has been reviewed and the comments of the referee(s) are included at the end of this email.

A further revised version of your manuscript that takes into account the comments of the referees will be reconsidered for publication.
Please note that submitting a revision of your manuscript does not guarantee eventual acceptance, and that your revision may be subject to re-review by the referees before a decision is rendered.
As before, you can upload your revised manuscript using this direct link:
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc.manuscriptcentral.com/mnf?URL_MASK=857231635bd34ecdb44a44e625e07ddd

Alternatively, log in to https://mc.manuscriptcentral.com/mnf and enter your Author Center, where you will find your manuscript listed under "Manuscripts with Decisions." Please click "Create a Revision".

Please note that there is a TIME LIMIT OF TWO MONTHS for uploading the revised paper.
[...]
Once again, thank you for submitting your work to Molecular Nutrition & Food Research. I look forward to receiving your revision.

Sincerely,

Dr. Christine Mayer
Deputy Editor, Molecular Nutrition & Food Research

Prof. Hans-Ulrich Humpf
Editor-in-Chief, Molecular Nutrition & Food Research

## ANEXO 4

Paracer do Conselho de Gestão do Patrimônio Genético a respeito dos prazos para cadastro e sobre a não disponibilização do cadastro on line:

**CGEN** <cgen@mma.gov.br>

Para
Angela Giovana Batista
08/10/16 às 2:30 PM

Sra. Ângela,

Este é o entendimento que deve prevalecer, com base no que estabelece o art. 118 do Decreto nº 8.772, de 2016. Ressalto, entretanto, a atenção necessária a ser dada quanto aos prazos e procedimentos estabelecidos nos §§ 1º e 2º do art. 118, conforme transcrito abaixo, a fim de evitar qualquer sanção administrativa estabelecida na Lei nº 13.123, de 2015 e regulamentada no Decreto nº 8.772, de 2016.

Art. 118. O usuário que requereu qualquer direito de propriedade intelectual, explorou economicamente produto acabado ou material reprodutivo, ou divulgou resultados, finais ou parciais, em meios científicos ou de comunicação, entre 17 de novembro de 2015 e a data de disponibilização do cadastro, deverá cadastrar as atividades de que trata o art 12 da Lei nº 13.123, de 2015 e notificar o produto acabado ou o material reprodutivo desenvolvido em decorrência do acesso.

§ 1º O prazo para o cadastramento ou notificação de que trata o caput será de 1 (um) ano, contado da data da disponibilização do cadastro pelo CGen.

§ 2º Realizado o cadastramento ou notificação tempestivamente, o usuário não estará sujeito a sanção administrativa.

Atenciosamente,

Conselho de Gestão do Patrimônio Genético
Secretaria de Biodiversidade e Florestas
Ministério do Meio Ambiente

Em 06/07/2016 22:18, Angela Giovana Batista escreveu:

Prezados,

Entendo pelo exposto no último e-mail, que estou autorizada a fazer a divulgação dos meus resultados (sobe a forma de artigos cientificos, tese, etc) mesmo sem ter feito o cadastro no sistema, uma vez que este não se encontra disponível.

A interpretação procede?

Obrigada,

Ângela

**----------**

**-Ângela Giovana Batista-**
PhD student in Food and Nutrition - UNICAMP.
Campinas, SP Brazil.
a114718@unicamp.br
+55 19 3521 4069 (Lab)
---

Em Quinta-feira, 30 de Junho de 2016 17:00, CGEN <cgen@mma.gov.br> escreveu:

Sra. Ângela,

A partir da entrada em vigor da Lei nº 13.123, de 2015, que revoga a Medida Provisória nº 2.186-16, de 2001, foram estabelecidas novas regras para acesso ao patrimônio genético, acesso ao conhecimento tradicional associado e repartição de benefícios. As pesquisas com patrimônio genético brasileiro e conhecimento tradicional associado, assim como o desenvolvimento de produtos com nossa biodiversidade, não necessitam de autorização prévia para o seu desenvolvimento, sendo necessário apenas um registro das atividades de acesso no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado - SisGen, conforme previsto no art. 22 do Decreto nº 8.772, de 2016.

Será necessário obter autorização prévia somente nos casos de acesso em áreas indispensáveis à segurança nacional, em águas jurisdicionais brasileiras, na plataforma continental e na zona econômica exclusiva, conforme previsto no art. 13 da Lei nº 13.123, de 2015 e regulamentado pelos arts. 27 e 28 do Decreto nº 8.772, de 2016.

O cadastramento deverá ser realizado previamente à remessa, ou ao requerimento de qualquer direito de propriedade intelectual, ou à comercialização do produto intermediário, ou à divulgação dos resultados, finais ou parciais, em meios científicos ou de comunicação, ou à notificação de produto acabado ou material reprodutivo desenvolvido em decorrência do acesso (conforme o § 2º do art. 12 da Lei nº 13.123, de 2015).

As informações a serem cadastradas e procedimentos para acesso ao patrimônio genético e ao conhecimento tradicional associado constam das Seções I e II do Capítulo IV do Decreto nº 8.772, de 2016.

O SisGen, previsto no art. 20 do Decreto nº 8.772, de 2016, ainda não está disponível ao público, pois este Decreto estabeleceu diversos procedimentos relativos aos cadastros e funcionamento do SisGen que dependem da implementação do Plenário e demais

estruturas do novo Conselho de Gestão do Patrimônio Genético - CGen. Frente ao exposto, informo que o Ministério do Meio Ambiente está trabalhando na implementação do Plenário do novo CGen para que este entre em funcionamento o mais breve possível.

Ressalta-se, porém, que as atividades de acesso podem ser iniciadas mesmo sem a disponibilização do cadastro, desde que seja obtido previamente ao acesso o consentimento prévio informado da população indígena, comunidade tradicional ou agricultor tradicional detentor do conhecimento tradicional a ser acessado, conforme o art. 9º da Lei nº 13.123, de 2015 e o Capítulo III do Decreto nº 8.772, de 2016.

Atenciosamente,

Departamento do Patrimônio Genético

Em 28/06/2016 14:13, Angela Giovana Batista escreveu:

Prezados,

Tenho um trabalho concluído com uma planta nativa, que está em vias de ser submetido para publicação. Existe uma certa urgência com a publicação devido a normas acadêmicas do meu programa de doutorado.

Gostaria de saber se o formulário para cumprir a lei da Biodiversidade está disponível ou se há algum processo que viabilize a submissão do meu trabalho neste momento.

Obrigada

Angela

**----------**
**-Ângela Giovana Batista-**
PhD student in Food and Nutrition - UNICAMP.
Campinas, SP Brazil.
a114718@unicamp.br
+55 19 3521 4069 (Lab)
----------

--

Departamento do Patrimônio Genético
Secretaria de Biodiversidade e Florestas
Ministério do Meio Ambiente
Fone: (61) 2028-2182
http://www.mma.gov.br/patrimonio-genetico/