UNIVERSIDADE FEDERAL DO PARÁ

INSTITUTO DE TECNOLOGIA

PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIA E TECNOLOGIA DE ALIMENTOS

BIANCA SCOLARO

AÇÃO DE COMPOSTOS FENÓLICOS DO AÇAÍ SOBRE INFLAMAÇÃO E ESTRESSE OXIDATIVO PÓS-PRANDIAIS

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Dissertação de mestrado apresentada ao Programa de Pós-graduação em Ciência e Tecnologia de Alimentos da Universidade Federal do Pará, como requisito para obtenção do grau de Mestre em Ciência e Tecnologia de Alimentos. Área de Concentração: Compostos Bioativos.

Orientador: Prof. Dr. Yvan Larondelle
Co-orientador: Prof. Dr. Hervé Louis Ghislain Rogez

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AGRADECIMENTOS

Agradeço primeiramente ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ) pela bolsa de mestrado concedida durante a execução deste trabalho.

À Fundação de Amparo a Pesquisa do Estado do Pará (FAPESPA) e a *Commission Universitaire pour le Développement* (CUD) (Bélgica) pelo auxílio financeiro para realização do projeto.

Aos professores Dr. Yvan Larondelle e Dr. Hervé Rogez pela orientação, amizade, apoio e ensinamentos. Agradeço não apenas pela confiança para a realização deste estudo, mas ainda pela oportunidade de fazer parte desta equipe de pesquisa que ao longo de dois anos me proporcionou, além de conhecimento científico, vivência.

À Dra. Christelle André e toda equipe do laboratório *Biologie de la nutrition et toxicologie environnementale* (BNTE) da Universidade Católica de Louvain- Bélgica (UCL), pela gentileza, paciência e auxílio na realização das análises. Ainda à Carissa Michelle Goltara Bichara pelo acolhimento na Bélgica: intérprete, companheira de laboratório e amiga.

Ao Dr. Paulo Azevedo por conceder o espaço físico de um de seus laboratórios de análises clínicas para a realização do experimento.

Aos professores Dra. Renata Lopez e o Dr. Jofre Freitas por auxiliar o desenvolvimento do projeto, cedendo seus laboratórios para realização de análises.

A toda equipe da usina de alimentos que se mobilizou para que fosse possível a realização da intervenção humana, e aqueles que de algum modo me auxiliaram, seja "quebrando a cabeça" com minhas dúvidas ou para realização das análises, especialmente Ana Clara Vasconcelos, Ed Júnior, Luciana Lima e Rogério Vieira. Às amigas e colegas de mestrado Taiana Ladeira, Lara Lima, Ana Caroline Oliveira e Caroline Santos por todos os momentos, pensamentos e risadas divididas. Tenho certeza que isso nos deu força para superar os momentos difíceis.

Aos amigos Ana Kelly e Lucas Eduardo Araújo-Silva, nossa família em Belém, pela amizade, apoio e por tornar a vida mais divertida.

À minha família, em especial aos meus pais Antonio Scolaro Primo e Liane Ebeling Scolaro, que mesmo à distância me incentivaram diante das dificuldades e desafios.

Ao meu esposo, Gregory Thom e Silva por todo amor e cuidado, mas principalmente pela paciência, compreensão e por carinhosamente me fazer acreditar que era possível, mesmo naquelas vezes em que minha mente insistia em pensar o contrário.

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

Madre Teresa de Calcutá

RESUMO

A palmeira Açaí é largamente distribuída no estuário do Rio Amazonas, sendo a bebida açaí, ou suco de açaí, parte dos hábitos alimentares em algumas regiões do Brasil. Os polifenóis, especialmente antocianinas, são os principais compostos bioativos do açaí. Seus extratos têm exibido propriedades farmacológicas como atividade antiproliferativa, anti-inflamatórias, antioxidantes e cardio-protetoras, principalmente *in vitro.* Porém, dados sobre efeitos do açaí em humanos ainda são limitados. O estresse oxidativo pós-prandial é um estado caracterizado por uma maior susceptibilidade do organismo ao dano oxidativo, principalmente após o consumo de uma refeição rica em lipídeos e/ou carboidratos. Além disso, o cozimento de alimentos em altas temperaturas pode levar a geração de vários tipos de substâncias genotóxicas e pró-oxidativas como os Hidrocarbonetos Policíclicos Aromáticos (PAHs). Estes são contaminantes ambientais compostos de três ou mais anéis aromáticos fundidos, formados por pirólise e/ou combustão incompleta da matéria orgânica. Podem ser encontrados principalmente em carnes grelhadas diretamente sobre chamas e sobre altas temperaturas, como churrascos. O objetivo deste trabalho foi avaliar os efeitos da ingestão de carne grelhada (churrasco) sobre processos metabólicos, bem como o potencial de compostos fenólicos do açaí em atenuar ou impedir possíveis desordens metabólicas, quando associados à refeição. O estudo foi conduzido com a participação de 23 voluntários do sexo masculino, com idade entre 20 a 36 anos. Os voluntários foram divididos aleatoriamente em três grupos, e receberam as seguintes refeições-teste em três dias distintos: churrasco associado à ingestão de cápsulas de extrato de açaí rico em compostos fenólicos; churrasco associado à ingestão de cápsulas de placebo e carne cozida associada à ingestão de cápsulas de placebo. Foram coletadas amostras de sangue nos tempos 0 h (medida de base), 3 e 5 h após consumo das refeições teste. Amostras de urina foram coletadas nos tempos 0 h (medida de base), 4, 6 e 12 h pós o término do almoço. Foram coletadas duas amostras fecais: pré e pós-consumo das refeições-teste. PAHs foram determinados nas amostras de carnes por HPLC/FLD após extração líquida pressurizada e purificação por SPE. O churrasco apresentou quantidades significativamente maiores de PAHs comparado à carne cozida ($p<0,05$). A concentração de PAHs totais e benzo[*a*]pireno em churrasco e carne cozida foi 12.11 ± 4.30 e 1.05 ± 0.56 kg^{-1}, e 1.06 ± 0,38 e 0,05 ± 0,03 µg kg^{-1}, respectivamente. Mudanças sanguíneas de TBARS, CRP, ALP, GOT and GTP não foram estatisticamente diferentes entre as refeições. Atividade da glutationa peroxidase apresentou tendência à redução após consumo de churrasco, e de aumento após o consumo de compostos fenólicos. A excreção urinária de um biomarcador de exposição aos PAHs (1-hidroxipireno) foi significativamente maior nos tempos 6 e 12 h após o consumo de churrasco, comparado à ingestão de carne cozida. Excreção fecal de lipídeos não foi significativamente aumentada pela ingestão de polifenóis. A ingestão de churrasco representa um modo de exposição dietética aos PAHs. Embora os valores encontrados estejam de acordo com a legislação, a exposição recorrente pode levar a um comprometimento do estatus antioxidante do organismo.

Palavras-chave: polifenóis; açaí; hidrocarbonetos policíclicos aromáticos; churrasco.

ABSTRACT

Açai is palm tree widely distributed in the Amazon estuary, being the açai juice part of dietary habits in parts of Brazil. Polyphenols, most notably anthocyanins, are the predominant bioactive compounds in açai. Açai extracts have exhibited pharmacological properties including antiproliferative, anti-inflammatory, antioxidant, and cardio-protective activities, mostly in vitro. Nevertheless, there are still limited data on açai effects in humans. Postprandial oxidative stress is a state characterized by an increased susceptibility of the organism to oxidative damage, especially after consumption of a meal rich in lipids and/or carbohydrates. Furthermore, cooking food at high temperatures may generate genotoxic and pro-oxidative substances like Polycylic Aromatic Hydrocarbons (PAHs). These are environmental contaminants composed of three or more fused aromatic rings, formed by pyrolysis and/or incomplete combustion of organic matter. They can be found in meats grilled directly over flames and with high temperatures, like barbecues. The objective of this work was to evaluate the effects of barbecue intake on metabolic parameters and also the potential of phenolic compounds from açai to attenuate or prevent possible metabolic disorders, when associated to the meal. The study was conducted with the participation of 23 male volunteers, aged between 20-40 years. The volunteers were randomly divided into three groups, and received the following test-meals in three different days: barbecue associated to phenolic compounds from açai rich capsules intake; barbecue associated to placebo capsules intake; cooked meat associated to placebo capsules intake. Blood samples were collected at baseline (0 h), 3 and 5 hours after the test-meals intake. Urine samples were collected at baseline (0 h), 4, 6 and 12 hours after the meals. Volunteers collected two fecal samples: pre and post test-meals consumption. PAHs were determinated in meat samples by HPLC/FLD after pressurized liquid extraction and purification with SPE. Barbecue had significantly higher PAHs values than cooked meat ($p<0.05$). The mean concentration of total PAHs and benzo[a]pyrene (BaP), in barbecued meat and oven cooked meat (control) were 12.11 ± 4.30 and 1.05 ± 0.56 μg kg^{-1} and 1.06 ± 0,38 and 0,05 ± 0,03 μg kg^{-1}, respectively. Blood changes of TBARS, CRP, ALP, GOT and GTP were not significantly different between the test-meals. Glutathione peroxidase activity tended to decrease by barbecue, and to increase by phenolic compounds intake. The urinary excretion of PAH biomarker exposure (1-hydroxypyrene) was significantly higher at 6 and 12 hours after barbecue, compared to cooked meat intake. Fecal lipid was not significantly increased by polyphenols. Barbecue consumption represents a dietary exposure to PAHs. Even though PAH values in meats were in accordance to legislation, recurrent exposure may lead to antioxidant status impairment.

Key-words: polyphenols; açai; polycylic aromatic hydrocarbons; barbecued meat.

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CAPÍTULO 1: CONTEXTUALIZAÇÃO

1 INTRODUÇÃO

A evolução da dieta humana nos últimos anos tem afetado, de maneira adversa, vários parâmetros dietéticos relacionados com a saúde, incluindo carga glicêmica, composição de ácidos graxos, composição em macronutrientes e micronutrientes, carga ácido-básica, relação sódio/potássio, conteúdo de fibras, etc. (JEW; ABUMWEIS; JONES, 2009). Estas alterações têm provocado, entre outros, um maior desequilíbrio e dano oxidativo ao organismo.

Evidências de que estresse oxidativo e inflamação são características fundamentais no desenvolvimento de várias doenças crônicas, têm se tornado cada vez mais claras, especialmente naqueles agravos com origem metabólica (KHANSARI et al., 2009).

Alimentos de origem vegetal, especialmente frutas e verduras, têm sido consistentemente identificados em pesquisas epidemiológicas como componentes chave nos padrões alimentares que reduzem o risco para o desenvolvimento de doenças crônicas, incluindo doenças cardiovasculares ateroscleróticas, resistência à insulina, diabetes tipo II, síndrome metabólica e muitos tipos de câncer (KEY, 2011).

Essas fontes alimentares são ricas em compostos bioativos. Dentre estes, os compostos fenólicos são antioxidantes capazes de proteger o organismo dos radicais livres e do estresse oxidativo e possuem o potencial para modular uma série de outros processos biológicos, incluindo, agregação plaquetária, inflamação, iniciação e propagação de processos carcinogênicos (AGARWAL; SHARMA; AGARWAL, 2000; REIN et al., 2000; ZERN; FERNANDEZ, 2005). Esses compostos são fitoquímicos amplamente disseminados no reino vegetal, sendo o açaí uma importante fonte, como já foi demonstrado na literatura (PACHECO-PALENCIA et al., 2007; SCHAUSS et al., 2006). Os frutos da palmeira açaí (*Euterpe oleracea* Martius), nativa da América do Sul, recentemente tornaram-se populares como alimento funcional devido seu potencial antioxidante (UDANI et al., 2011).

Portanto, este trabalhado pretende avaliar os efeitos da ingestão de carne grelhada (churrasco) sobre processos metabólicos, visto seu alto consumo na sociedade brasileira, bem como o potencial de compostos fenólicos do açaí em atenuar ou impedir possíveis desordens metabólicas, quando associados à refeição.

Neste contexto, este trabalho tem como objetivo geral:

- Verificar a capacidade de redução e prevenção da inflamação e do estresse oxidativo pós-prandiais, ao se ingerir compostos fenólicos do açaí.

Os objetivos específicos do estudo englobam:

a) Verificar o caráter pró-inflamatório e pró-oxidativo de carnes grelhadas (churrasco);

b) Observar efeitos *in vivo* dos compostos fenólicos do açaí na prevenção da inflamação e redução do estresse oxidativo pós-prandias, decorrente da ingestão de carnes grelhadas;

c) Verificar efeitos *in vivo* dos compostos fenólicos do açaí sobre a inibição da lipase pancreática.

2 REVISÃO BIBLIOGRÁFICA

2.1 AÇAÍ

A palmeira *Euterpe oleracea* é conhecida no Brasil e na Região Amazônica como açaizeiro. Nativo do estuário do Rio Amazonas, o açaizeiro é encontrado nas matas de terra-firme, igapó e, sobretudo, nas áreas de várzea, onde cresce na forma de touceira. Esta é constituída por estipes, que frutificam na sua fase adulta, com a produção máxima ocorrendo entre 5 a 6 anos de idade (CAVALCANTE, 1976; ROGEZ, 2000).

A partir dos frutos maduros é preparada uma bebida chamada açaí ou suco de açaí. De acordo com Rogez (2000), o preparo desta bebida se faz tradicionalmente em duas etapas: na primeira, com o amolecimento dos frutos na água morna e na segunda, pelo despolpamento dos mesmos mediante máquinas convencionais, com a adição de água. Sorvetes, licores, doces, néctares e compotas também podem ser preparados a partir dos frutos do açaí. Mais recentemente, extrato de antocianinas ou corante tem sido separado de açaí clarificado (BICHARA; ROGEZ, 2011).

O açaí possui uma quantidade significante de lipídeos, variando de 40,7 a 60,4% da matéria seca (MS), e contém 6,7 a 10,5% de proteínas. O total de açúcares digestíveis é insignificante quando comparado a outras frutas tropicais, contribuindo com menos de 1% das recomendações energéticas diárias. Já a concentração de fibras dietéticas totais é elevada, variando de 20,9 a 21,8% da MS, o que faz das fibras o segundo constituinte mais prevalente do açaí (BICHARA; ROGEZ, 2011; ROGEZ, 2000;).

A quantidade total de α-tocoferol encontrado no açaí é alta, variando de 37 a 52 mg 100 g $^{-1}$ MS, o que torna o suco da fruta uma excelente fonte desta forma de vitamina E. Cada porção de açaí (200g) fornece mais de 100% do consumo recomendado pela FAO/WHO (2001), para homens e mulheres adultos. O açaí também tem um bom perfil de ácidos graxos (49,72% ácido oléico, 25,31% ácido palmítico, e 13,51% ácido linoléico) (BICHARA; ROGEZ, 2011).

A coloração violeta da bebida açaí deve-se a sua elevada concentração em pigmentos naturais chamados antocianinas. Estas pertencem à família dos flavonóides, e incluem cianidina-3-glicosídeo (C-3-G) e cianidina-3-rutinosídeo (C-3-R) (BICHARA; ROGEZ, 2011). Esses dois pigmentos são os principais compostos fenólicos do açaí, que atuam também como antioxidantes.

Estudos *in vitro* e *in vivo* já demonstraram efeitos funcionais do açaí ou seus componentes. Foram observados efeitos positivos sobre a redução da oxidabilidade do LDL-c e tendência ao aumento da capacidade antioxidante do plasma em humanos (SAMPAIO et al., 2006); aumento da capacidade antioxidante do plasma e inibição da peroxidação lipídica em humanos (JENSEN et al., 2008); efeitos anti-inflamatórios e potencial atero-protetor *in vitro* (KANG et al., 2011); atividade anti-inflamatória e antinociceptiva em ratos, devido à inibição de biossíntese de prostaglandinas (FAVACHO et al., 2011); redução dos níveis de marcadores de risco para síndrome metabólica em adultos com sobrepeso (UDANI et al., 2011); entre outros (BASTOS et al., 2007; COISSON et al., 2005; PACHECO-PALENCIA et al., 2008).

A demanda global por açaí aumentou rapidamente nos últimos anos devido à publicidade e alegações funcionais de produtos contendo açaí, por parte da indústria de alimentos. Inicialmente o açaí era produzido e vendido apenas localmente na Região Amazônica, porém agora é vendido em muitos mercados internacionais, principalmente nos Estados Unidos, país que detém o maior mercado de alimentos e bebidas funcionais do mundo (BICHARA; ROGEZ, 2011; HEINRICH et al., 2011). Este fato, somado aos dados encontrados nos estudos científicos, como os citados anteriormente, tem promovido maior interesse da indústria na produção de extratos bioativos purificados de açaí.

2.2 LIPÍDEOS NA ALIMENTAÇÃO E IMPLICAÇÕES METABÓLICAS

2.2.1 Digestão, Absorção e Metabolismo

A digestão dos lipídeos começa na boca, pela ação da lipase lingual, porém de maneira reduzida. No estômago ocorre a secreção da lipase gástrica, mas sua contribuição para a digestão dos lipídeos é também pequena (JACKSON; McLAUGHLIN, 2006). A lipase gástrica hidrolisa parte dos triglicerídeos, especialmente os de cadeia curta em posição alfa, em ácidos graxos e glicerol (MAHAN; ESCOTT-STUMP, 2002).

A lipase pancreática é a principal enzima envolvida na digestão dos triglicerídeos, responsável por mais de 70% da hidrólise, embora as lipases lingual e gástrica tenham suas atividades prorrogadas no intestino delgado, agindo assim, sinergicamente (JACKSON; McLAUGHLIN, 2006).

Para serem absorvidos da parede intestinal, os triglicerídeos ingeridos precisam ser convertidos de partículas gordurosas macroscópicas insolúveis em micelas microscópicas

dispersas por intermédio dos sais biliares. Estes são sintetizados no fígado a partir do colesterol, estocados na vesícula biliar e, depois da ingestão de uma refeição gordurosa, liberados no intestino delgado (NELSON; COX, 2011).

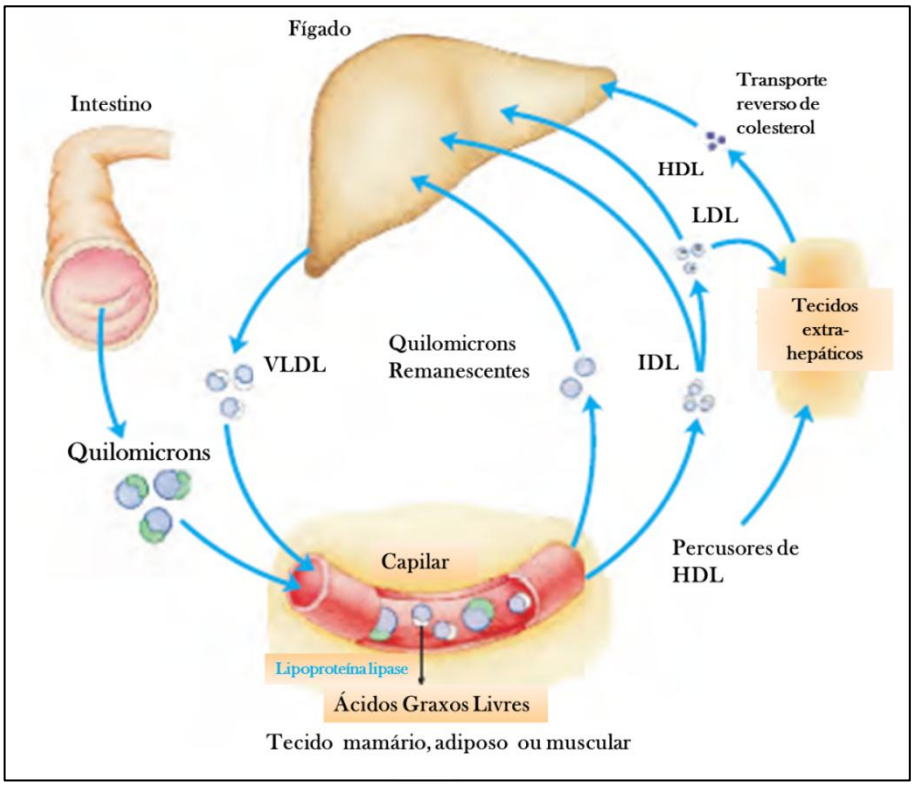
A formação de micelas aumenta a sensibilidade das moléculas lipídicas à ação da lípase pancreática, que hidrolisa os triglicerídeos em monoglicerídeos, diglicerídeos, ácidos graxos livres e glicerol. Estes produtos são difundidos para o interior das células epiteliais da mucosa intestinal, onde são novamente convertidos em triglicerídeos e agrupados com o colesterol da dieta e com proteínas específicas, formando agregados lipoproteicos chamados de quilomícrons (NELSON; COX, 2011).

Desta forma, os quilomícrons são responsáveis pelo transporte dos lipídeos absorvidos pelo intestino, originários da dieta e da circulação entero-hepática. Após refeição gordurosa, o pico de quilomícrons é alcançado, habitualmente entre 3 e 6 h, e após período de 12 h essas partículas não são mais detectáveis em pessoas normais (ISSA; DIAMENT; FORTI, 2005; SPOSITO et al., 2007).

Na captação dos lipídeos do intestino, os quilomícrons movem-se da mucosa intestinal para o sistema linfático, de onde saem para a corrente sanguínea e são transportados para os músculos e tecido adiposo. Nos capilares destes tecidos, a enzima extracelular lipase lipoprotéica (LLP) hidrolisa parte dos triglicerídeos em ácidos graxos e glicerol, que são captados pelas células alvo. Os quilomícrons ainda contendo triglicerídeos passam então a ser chamados de remanescentes e seguem para serem captados pelo fígado (NELSON; COX, 2011).

O transporte de lipídeos de origem hepática (Figura 1) ocorre por meio da VLDL (*very low density lipoprotein*), IDL (*intermediary density lipoprotein*) e LDL (*low density lipoprotein*). Os triglicerídeos das VLDLs, assim como os dos quilomícrons, são hidrolisados pela LLP e os ácidos graxos são liberados para os tecidos e metabolizados. As VLDLs, progressivamente depletadas de triglicerídeos, se transformam em remanescentes, também removidas pelo fígado por receptores específicos. Uma parte das VLDLs dá origem às IDLs, que são removidas rapidamente do plasma. O processo de catabolismo continua, envolvendo a ação da lipase hepática e resultando na LDL, que permanecem por longo tempo no plasma. Esta lipoproteína tem um conteúdo apenas residual de triglicerídeos e é composta principalmente de colesterol (SPOSITO et al., 2007).

Figura 1: Lipoproteínas e transporte lipídico



FONTE: NELSON; COX, 2011.

2.2.2 Lipídeos oxidados nos alimentos e patogenicidade

Quando expostos ao calor, ar, luz e agentes oxidantes, o colesterol e ácidos graxos poliinsaturados sofrem oxidação espontânea formando produtos oxidados. O processamento de alimentos, especialmente tratamentos térmicos e secagem, induzem a oxidação lipídica nos alimentos, incluindo derivados do leite, ovos e carnes (STAPRANS et al., 2005).

Usualmente as carnes devem sofrer um tratamento térmico antes de serem consumidas. O processo de cocção causa vários efeitos desejáveis na carne como aumento da digestibilidade e sabor, porém produz efeitos indesejáveis como perdas nutricionais e oxidação lipídica. Estes produtos da oxidação estão relacionados com patologias como aterosclerose, câncer, inflamação, etc. (BRONCANO, 2009).

A ingestão de lipídeos dietéticos oxidados está associada com o aparecimento de lipídeos oxidados nos quilomícrons. Staprans et al. (1994) demonstraram que os lipídeos oxidados provenientes da dieta são absorvidos pelo intestino delgado e são transportados pelos quilomícrons para a circulação, onde eles podem contribuir para o *pool* total de lipídeos

oxidados no organismo. Desta forma, podem produzir injúria celular e consequentemente respostas inflamatórias que indiretamente promovem oxidação em tecidos alvos, principalmente tecidos gastrointestinais (SIES; STAHL; SEVANIAN, 2005).

Considerando o metabolismo dos quilomícrons descrito anteriormente, pode-se afirmar que os lipídeos oxidados na dieta podem contribuir também para a formação de lipoproteínas oxidadas na circulação, as quais são potencialmente aterogênicas. Os triglicerídeos não são componentes das placas ateroscleróticas, mas admite-se que haja participação das lipoproteínas ricas em triglicérides na aterosclerose, de modo indireto. A participação indireta seria viabilizada pelo acúmulo de quilomícrons e consequente excesso das VLDLs, IDLs e LDLs. No estado pós-prandial, os quilomícrons e as VLDLs competem pela LLP, enzima que hidrolisa os triglicerídeos. Alguns estudos demonstram que os quilomícrons são o substrato preferencial da LLP, o que determinaria acúmulo de VLDL no estado pós-prandial (ISSA; DIAMENT; FORTI, 2005).

A formação da placa aterosclerótica inicia-se com a agressão ao endotélio vascular devido à elevação de lipoproteínas aterogênicas (LDL oxidada, IDL, VLDL, remanescentes de quilomícrons). Como consequência, a disfunção endotelial aumenta a permeabilidade da íntima, favorecendo a retenção das lipoproteínas no espaço subendotelial. Este depósito de lipoproteínas na parede arterial ocorre de maneira proporcional à concentração dessas lipoproteínas no plasma. Outra manifestação da disfunção endotelial é o surgimento de moléculas de adesão leucocitária na superfície endotelial, processo estimulado pela presença de LDL oxidada. As moléculas de adesão são responsáveis pela atração de monócitos e linfócitos para a parede arterial. Induzidos por proteínas quimiotáticas, os monócitos migram para o espaço subendotelial onde se diferenciam em macrófagos, que por sua vez captam as LDL oxidadas. Os macrófagos repletos de lípides são chamados células espumosas e são o principal componente das estrias gordurosas, lesões macroscópicas iniciais da aterosclerose (SPOSITO et al., 2007).

2.3 ESPÉCIES REATIVAS DE OXIGÊNIO E A DIETA

2.3.1 Generalidades

O termo “espécies reativas do oxigênio” (*Reactive Oxygen Species*) denomina o conjunto de espécies particularmente reduzidas formadas a partir do oxigênio molecular (O_2),

como o radical superóxido ($O_2^{\bullet -}$), hidroxila ($OH^{\bullet}$), alcoxila ($RO^{\bullet}$) e peroxila ($RO_2^{\bullet}$), além de espécies não radicalares derivadas do oxigênio, como o peróxido de hidrogênio (H_2O_2), o oxigênio singlete ($^1O_2$), o ácido hipocloroso (HOCl) e o ozônio. Eles são constantemente formados no corpo humano e implicam na patologia de numerosas doenças (PINCEMAIL et al., 2002).

O metabolismo celular de todos os organismos aeróbios é acompanhado pela formação de ROS. No entanto, é contrabalanceada pelo consumo de defesas antioxidantes não enzimáticas (α-tocoferol, carotenóides, ácido ascórbico, etc.) e pela atividade das enzimas antioxidantes. Quando esse balanço é rompido em favor dos agentes oxidantes, diz-se que a célula ou organismo se encontra sob estresse oxidativo (BELLÓ-KLEIN, 2002).

Com o intuito de evitar o desenvolvimento do estresse oxidativo, os níveis de ROS são mantidos sob rígido controle através da atividade enzimática de enzimas antioxidantes, como: superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GSH-Px) e redutase. Neste contexto, a SOD decompõe o ânion superóxido por uma sucessiva oxidação e redução do íon cobre, tendo como produto a formação de H_2O_2. Já a CAT reage com o H_2O_2 para formar água e O_2 e a GSH-Px reduz hidroperóxidos de ácidos graxos e H_2O_2 à custa de glutationa reduzida (GSH) (LLESUY, 2002).

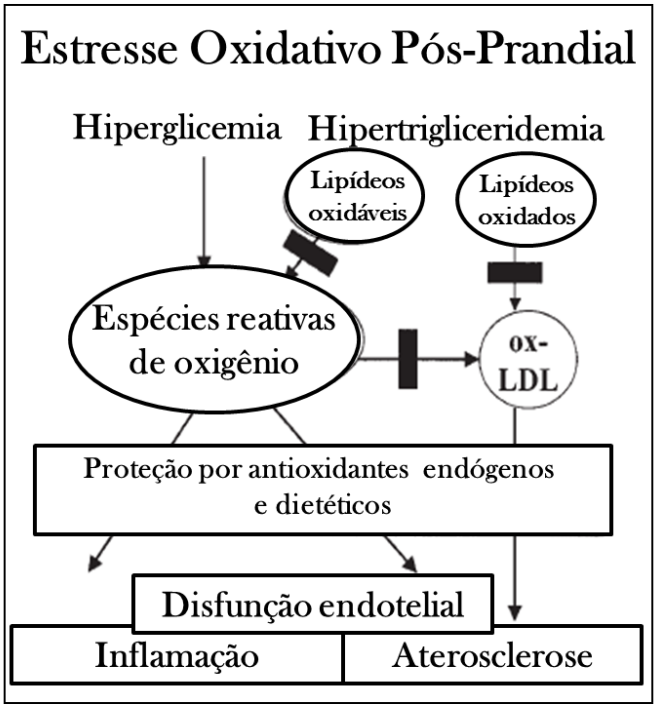
2.3.2 Estresse oxidativo pós-prandial

O estresse oxidativo pode ser definido como os efeitos adversos das reações oxidativas induzidas por espécies reativas de oxigênio dentro dos sistemas biológicos, ocasionando lesão em moléculas como o DNA, as proteínas, os carboidratos e os lipídeos. O componente lipídico da membrana celular é a primeira estrutura encontrada pelos radicais livres, fazendo da peroxidação lipídica a lesão mais frequentemente descrita como resultado da ação desses reativos tóxicos do oxigênio (LAMEU, 2005).

O estado pós-prandial é o estado que se segue após uma refeição. É caracterizado por um período dinâmico de tráfico metabólico, biossíntese e metabolismo oxidativo das substâncias absorvidas, como glicose, lipídeos, proteínas e outros componentes dietéticos. Desta forma, é naturalmente um estado pró-oxidante, uma vez que ROS são produzidas durante o metabolismo oxidativo dos nutrientes. Contudo, as ROS formadas neste estado são posteriormente neutralizadas por mecanismos antioxidantes endógenos (enzimas antioxidantes) ou antioxidantes dietéticos (BURTON-FREEMAN, 2010).

Apesar do controle antioxidante exercido pelo próprio organismo, os macronutrientes consumidos em uma refeição possuem a propriedade de alterar este balanço redox. Eles podem tanto ser alvos das modificações oxidativas, quanto podem estar presentes na dieta em uma forma pró-oxidante. Assim, o estresse oxidativo pós-prandial é caracterizado por uma maior susceptibilidade do organismo ao dano oxidativo, principalmente após o consumo de uma refeição rica em lipídeos e/ou carboidratos. A hiperlipidemia e a hiperglicemia têm sido associadas com as mudanças do estatus antioxidante e com o dano oxidativo aumentado das lipoproteínas, fato que está associado com o risco aumentado de desenvolvimento de aterosclerose e doenças relacionadas (Figura 2) (SIES; STAHL; SEVANIAN, 2005).

Figura 2: Estresse Oxidativo Pós-Prandial e sua relação com aterosclerose e inflamação



FONTE: adaptado de SIES; STAHL; SEVANIAN, 2005.

Uma refeição pode ainda fornecer nutrientes em uma forma pró-oxidante, os quais podem estar envolvidos no desencadeamento do estresse oxidativo pós-prandial, somando-se aos efeitos da hiperlipidemia e ou hiperglicemia induzidas após uma refeição. Lipídeos dietéticos oxidados parecem estar presentes nas lipoproteínas circulantes. Após a assimilação pelos quilomícrons e pelas VLDLs intestinais eles permanecem, pelo menos em parte, nas lipoproteínas remanescentes e no LDL. Assim, a dieta pode ser uma fonte direta de hidroperóxidos lipídicos nas lipoproteínas, que podem facilitar a peroxidação lipídica nestas (URSINI et al., 1998).

Nas sociedades ocidentais, a maior parte do dia é gasta no estado pós-prandial, já que grande parte da população realiza várias refeições ao dia. Considerando a baixa qualidade nutricional e o alto valor energético destas refeições, observa-se um exagerado e prolongado desbalanço metabólico, oxidativo e imunológico, representando uma oportunidade para insulto biológico ao organismo, que com o tempo pode superar a defesa endógena e sistemas de reparo, manifestando disfunção celular, doenças e por fim a morte (BURTON-FREEMAN, 2010).

O acúmulo de lipoproteínas ricas em triglicerídeos no estado pós-prandial ocorre devido uma menor taxa de remoção de quilomícrons remanescentes, e este estado hiperlipidêmico prolongado contribui para a injúria vascular e o desenvolvimento de aterosclerose. Em indivíduos que estão em um estado pós-prandial contínuo, níveis de quilomícrons remanescentes permanecem elevados na circulação indefinidamente (SIES; STAHL; SEVANIAN, 2005). Assim, pode-se afirmar que o estresse oxidativo pós-prandial é tipicamente acompanhado por inflamação e disfunção endotelial (BURTON-FREEMAN, 2010).

2.4 COMPOSTOS FENÓLICOS

2.4.1 Generalidades

Compostos fenólicos são moléculas que possuem um ou mais grupos hidroxila ligados diretamente a um anel aromático, neste caso um benzeno, e cuja estrutura básica é o fenol. São também chamados de polifenóis, compostos que possuem mais que um grupo hidroxila fenólico, ligado a um ou mais anéis benzênicos (VERMERRIS; NICHOLSON, 2006).

Os polifenóis são metabólitos secundários das plantas e estão geralmente envolvidos na defesa contra radiação ultravioleta e contra a agressão por patógenos. Podem ser classificados em diferentes grupos, em função do número de anéis fenólicos que contém e de acordo com os elementos estruturais que ligam estes anéis uns aos outros. Os principais grupos de polifenóis são flavonóides, ácidos fenólicos, estilbenos e lignanas. Os flavonóides podem ser divididos em subclasses, dentre as quais as mais representativas são: flavonas, flavanonas, flavonóis, flavanóis (também chamados flavan-3-óis) antocianidinas e isoflavonas (MANACH et al., 2004; WEICHSELBAUM; BUTTRIS, 2010).

Polifenóis são os antioxidantes mais abundantes presentes na dieta e podem promover benefícios à saúde através de vários mecanismos. O mecanismo de ação mais conhecido é com relação a propriedade antioxidante dos polifenóis e de modulação do estresse oxidativo biológico para prevenção do dano celular. Eles podem ainda atuar diretamente sobre as espécies reativas do oxigênio, capturando-as e tornando-as inertes (BURTON-FREEMAN, 2010).

Contudo, evidências de que polifenóis podem atuar como pró-oxidantes são também encontradas na literatura. Em algumas condições, como em altas doses ou na presença de íons metálicos, polifenóis podem apresentar atividade pró-oxidante. Flavonóides por exemplo, podem quelar íons metálicos, diminuindo a atividade pró-oxidantes destes. Assim, os polifenóis são capazes tanto de eliminar quanto de gerar ROS e podem exercer sua funcionalidade através da combinação de ambos os mecanismos, dependendo do ambiente (AHMED; AISSAT; DJEBLI, 2012).

Vários estudos observacionais já examinaram a associação entre o consumo de alimentos ricos em polifenóis (cebolas, maçãs, chá, cacau, vinho tinto, etc.) e a incidência de doenças crônicas. Esses estudos têm sugerido que o alto consumo de polifenóis dietéticos está associado com um menor risco de surgimento de doenças cardiovasculares, câncer e doenças neurodegenerativas (ARTS; HOLLMAN, 2005).

Contudo, uma limitação de muitos estudos é que se têm testado alimentos ricos em polifenóis (ao em vez de preparações puras de polifenóis) como frutas e vegetais, que contêm também outros componentes alimentares além de polifenóis. Isso significa que outras substâncias presentes no alimento podem contribuir para um resultado positivo encontrado nestes estudos, tornando difícil atribuir qualquer efeito encontrado especificamente aos polifenóis presentes (WEICHSELBAUM; BUTTRIS, 2010; WILLIAMSON; MANACH, 2005).

2.4.2 Atuação dos polifenóis sobre a inibição da lipase pancreática

A lipase pancreática é a principal enzima responsável pela absorção de lipídeos, hidrolisando triglicerídeos no trato gastrointestinal. Ela remove ácidos graxos da posição α e α’ dos triglicerídeos dietéticos, liberando monoglicerídeos, ácidos graxos saturados de cadeia longa e ácidos graxos poliinsaturados (BIRARI; BHUTANI, 2007).

Agentes inibidores dessa enzima, que ajudam a limitar a absorção intestinal de gorduras no estágio inicial da digestão, têm sido utilizados para o tratamento de hiperlipidemias e contra a obesidade. O fármaco Orlistat®, um dos medicamentos clinicamente aprovados para o tratamento da obesidade, age através da inibição da lipase pancreática. Porém, seu uso apresenta efeitos colaterais desagradáveis como fezes gordurosas e flatulência, o que incentivou a pesquisa para a busca de novos inibidores da lípase pancreática (BIRARI; BHUTANI, 2007; DE LA GARZA, 2011).

Os polifenóis têm recebido grande atenção, uma vez que apresentam uma afinidade por proteínas. Polifenóis possuem a propriedade de precipitar macromoléculas naturais (particularmente proteínas) em soluções, realizando ligações hidrofóbicas ou pontes de hidrogênio. Dessa forma os polifenóis apresentam habilidade de complexar enzimas, resultando inevitavelmente na alteração da configuração molecular da enzima, o que leva à perda da atividade catalítica (HE; LV; YAO, 2006).

Alguns estudos já demonstraram a atividade *in vitro* de componentes naturais sobre a inibição da lipase pancreática. Sugiyama et al. (2007) investigaram o efeito inibitório de um extrato de compostos fenólicos da maçã na atividade da lipase pancreática *in vitro* e na absorção de triglicerídeos em ratos e humanos. Os autores concluíram que o extrato de compostos fenólicos da maçã inibiu a lipase pancreática *in vitro* e previne contra a elevação dos níveis de triglicerídeos plasmáticos pós-prandial.

Polifenóis de certas frutas do tipo *berry* foram demonstrados como eficazes inibidores da lipase pancreática *in vitro.* Mcdougall et al. (2009) realizaram uma triagem de vários extratos ricos em polifenóis de frutas com ação inibitória sobre a lipase pancreática, que pudesse ser relevante ao combate da obesidade. Os extratos mais eficazes foram de framboesa, morango e amora silvestre.

Os flavonóides do trigo-sarraceno, conhecido como o único cereal rico em rutina e quercetina biologicamente ativas e ainda alimento básico em alguns países, também foram alvo de estudos. As interações entre lipase pancreática e três flavonóides (quercetina, isoquercetina e rutina) a partir de farelo de trigo-sarraceno foi estudada por Li et al. (2011), através de espectroscopia de fluorescência, cinética enzimática e comparação com o medicamento antiobesidade Orlistat®. Os resultados mostraram que rutina, isoquercetina e quercetina apresentaram capacidade inibitória satisfatória sobre a lipase pancreática de forma dose-dependente.

Contudo, há na literatura poucos estudos que demonstrem a inibição da lipase pancreática *in vivo* e em humanos, através do consumo de compostos fenólicos.

2.5 POTENCIAL CARCINOGÊNICO E PRÓ-INFLAMATÓRIO DE CARNES GRELHADAS

O cozimento de alimentos em altas temperaturas pode levar a geração de vários tipos de substâncias possivelmente inflamatórias. A exposição pode variar de acordo com os hábitos alimentares e as técnicas de processamento térmico aos quais os alimentos são submetidos (JÄGERSTAD; SKOG, 2005). Dentre estes compostos, podem-se citar os hidrocarbonetos policíclicos aromáticos (PAHs). Estes representam uma família de contaminantes ambientais que consistem de mais de 100 componentes lipofílicos, os quais são compostos de três ou mais anéis benzênicos fundidos sem grupos acíclicos, formados durante a combustão incompleta da matéria orgânica (RAMESH et al., 2004).

A dieta contribui com mais de 90% das exposições aos PAHs, da população em geral, em vários países. Podem ser encontrados principalmente em carnes grelhadas diretamente sobre chamas e sobre altas temperaturas. Este procedimento ocasiona o gotejamento de gorduras e leva a formação de chamas e fumaça contendo numerosos PAHs, que se aderem à superfície do alimento. Estudos têm demonstrado que a adoção de medidas que previnem o gotejamento de gordura derretida sobre a fonte de calor, como por exemplo, o cozimento em fornos ou utilização de grelhas elétricas, evita a presença de PAHs nas carnes (FARHADIAN et al., 2011; JÄGERSTAD; SKOG, 2005).

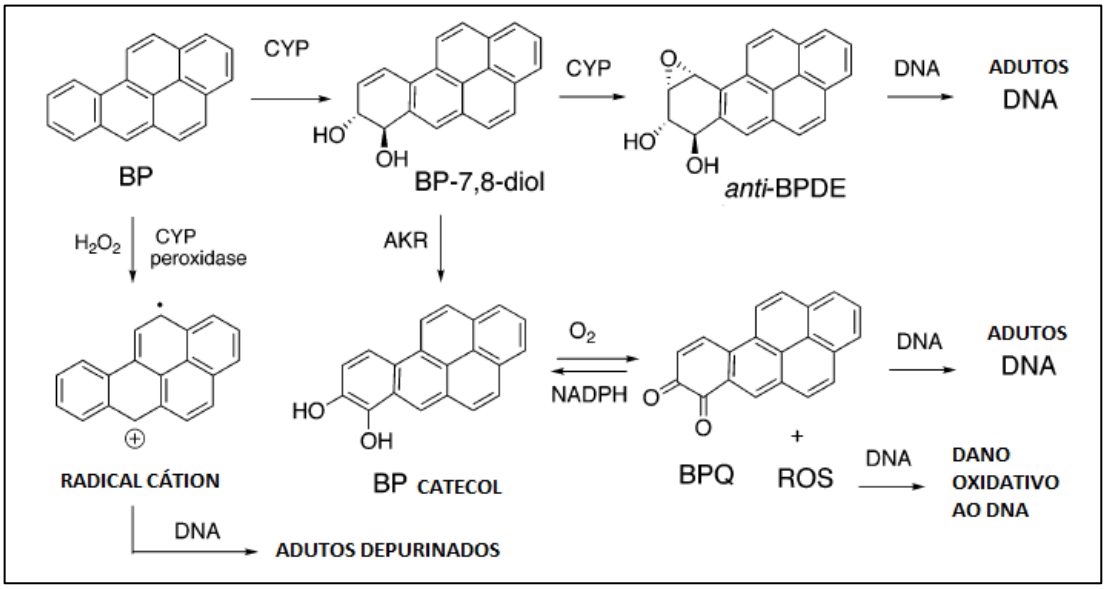
O benzo[*a*]pireno é o PAH presente na dieta mais bem descrito na literatura. Devido sua propriedade altamente lipofílica, é largamente absorvido pelos quilomícrons para o sistema linfático. Estudos *in vitro* e *in vivo* já propuseram que alimentos ricos em lipídeos podem facilitar e aumentar a transferência de benzo[*a*]pireno das partículas de alimentos para a parede intestinal (STAVRIC; KLASSEN, 1994).

Já foi observado que a ingestão de PAHs através do consumo de carnes grelhadas, pode causar a redução da defesa antioxidante enzimática, reduzindo a atividade da glutationa peroxidase, superóxido dismutase e catalase. Como resultado desses eventos metabólicos, as reações de formação de ROS são aceleradas e alterações importantes podem ocorrer nos níveis de produtos da peroxidação lipídica, redução de vitaminas antioxidantes do plasma e ainda produção de citocinas pró-inflamatórias (ELHASSANEEN, 2004).

PAHs sofrem transformação e ativação metabólica no organismo durante a detoxificação hepática (em menor extensão, nas próprias células epiteliais intestinais) resultando em produtos polares, que são destinados para a excreção, ou em metabólitos eletrofílicos reativos que podem formar adutos covalentes com o DNA. A formação química de adutos de DNA é considerada o evento iniciador no modelo de três etapas para a carcinogênese química (RAMESH et al., 2004).

A via de ativação mais estudada envolve a oxidação dos PAHs, mediada por citocromo P-450 (CYP), a qual produz metabólitos diol epóxido, como benzo[*a*]pireno anti-diol epoxido (anti-BPDE). O diol epóxido reage com o DNA para formar adutos que levam a mutação e indução de tumores. Outra via de ativação envolve a oxidação mediada por uma aldo-ceto redutase (AKR) de um metabólito dihidrodiol, para formar um catecol, que entra em um ciclo redox com a quinona correspondente (BPQ). Nesse processo, oxigênio é consumido e espécies reativas de oxigênio são geradas. Uma terceira via envolve a oxidação por citocromo peroxidase para gerar radicais cátions, que reagem com o DNA para formar adutos depurinados (Figura 3) (RAN et al., 2008).

Figura 3: Vias de ativação metabólica do benzo[*a*]pireno (BP).



FONTE: adaptado de RAN et al., 2008.

Os compostos fenólicos podem estar envolvidos com a redução da toxicidade e potencial carcinogênico dos PAHs. O mecanismo através do qual os polifenóis exercem esta proteção ainda não foi elucidado, mas parece envolver seu potencial antioxidante, a indução da expressão de enzimas de detoxificação ou ainda por estar relacionado com a redução da

absorção dos PAHs pelas células intestinais (EBERT; SEIDEL; LAMPEN, 2005; KIMA; KOOB; NOHA, 2012).

Estudo envolvendo ratos, cirurgicamente adaptados com cânulas linfáticas e biliares, demonstrou que um extrato de chá verde (29.2% de flavanóis) com dose equivalente a duas ou três porções por dia em humanos, diminui drasticamente a absorção intestinal de BP radioativo. A infusão duodenal de uma emulsão lipídica contendo ^{14}C-BP e extrato de chá verde reduziu a absorção intestinal em aproximadamente 50%, comparado ao controle (KIMA; KOOB; NOHA, 2012).

Pouco se sabe a respeito dos mecanismos responsáveis pela redução da absorção dos PAHs, porém evidencias indicam que os compostos fenólicos, além de inibirem a absorção de lipídeos, aumentam o efluxo apical de metabólitos de PAHs. Estes, uma vez formados no enterócito, podem ser transportados para fora da célula ao lúmen intestinal, via BCRP (proteína de resistência ao câncer de mama); proteína que pertence a família de transportadores ABC (ATP-binding cassette), e é expressa em vários tecidos, colón, intestino delgado, rins, fígado e placenta. A localização da BCRP na superfície apical do epitélio intestinal delgado permite o efluxo e eliminação de drogas e xenobióticos ingeridos. Vários pró-carcinogênicos dietéticos já foram identificados como substratos para BCRP (MALIEPAARD et al., 2001).

Fitoquímicos anti-carcinogênicos, como quercetina e curcumina, possuem a habilidade de indução da expressão de BCRP. Em estudo desenvolvido por Ebert et al. (2007), o pré-tratamento de uma cultura de células Caco-2 com quercetina aumentou o transporte de metabólitos de BP em direção à membrana apical das células.

3 PLANEJAMENTO DO ESTUDO

Este trabalho foi aprovado pelo Comitê de Ética em Pesquisa em Seres Humanos – CEP do Instituto de Ciências da Saúde da Universidade Federal do Pará, protocolo 0139.0.073.000-11 (ANEXO 1), segundo resolução 196/96 – do Conselho Nacional de Saúde do Ministério da Saúde CNS/MS. Os voluntários assinaram um Termo de Consentimento Livre e Esclarecido (APÊNDICE A), pelo qual foram informados de maneira clara e objetiva sobre o desenvolvimento do trabalho, assim como o livre arbítrio na participação da pesquisa.

Foi realizado um estudo clínico experimental randomizado, no período de novembro a dezembro de 2011, com delineamento do tipo *crossover*. A intervenção foi realizada nas dependências do Laboratório de Análises Clínicas Paulo C. Azevedo (Belém-PA), sendo este também responsável pelas análises bioquímicas. As demais análises laboratoriais foram realizadas nas dependências da UFPA, Universidade Estadual do Pará (UEPA) e da Universidade Católica de Louvain- Bélgica (UCL).

O estudo foi conduzido com a participação de 23 voluntários do sexo masculino, com idade entre 20 a 36 anos, recrutados a partir de preenchimento de questionários na própria UFPA. Os critérios de inclusão foram: gozar de perfeita saúde mental; ser não fumante, alcoólatra ou praticante de atividades físicas extenuantes, como, maratonas e etc; não ter histórico de síndrome nefrótica, não ser portador de insuficiência renal crônica; não ser portador de diabetes melito; não ser portador de hipotireoidismo.

A intervenção experimental consistiu na ingestão de três refeições-teste (almoço) pelos voluntários, as quais foram consumidas em três sábados consecutivos. As refeições-teste foram constituídas por carnes grelhadas (churrasco) e carnes cozidas. Para tanto, foi realizado um delineamento do tipo *crossover,* no qual os voluntários foram divididos aleatoriamente em três grupos, e receberam as seguintes refeições-teste distribuídas nos dias mencionados: churrasco associado à ingestão de cápsulas de extrato de açaí rico em compostos fenólicos; churrasco associado à ingestão de cápsulas de placebo (talco farmacêutico); carne cozida associado à ingestão de cápsulas de placebo (Quadro 1).

Em cada refeição-teste foram preparadas as seguintes porções de carnes: 150g de cupim, 150g de picanha e duas unidades de linguiça de carne suína (60 g cada).

Os voluntários foram até o local da intervenção pela manhã, estando em jejum, quando foi realizada a primeira coleta de sangue, para avaliação de parâmetros bioquímicos; após, receberam café da manhã padronizado. Ao longo do dia receberam ainda a refeição-teste, lanche ao final do experimento e carne preparada e padronizada para o jantar (peito de frango)

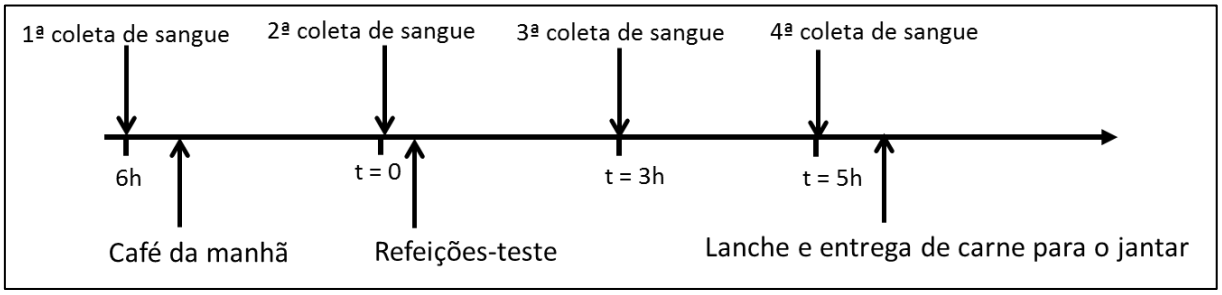
(Figura 4). O cardápio forneceu 3000 kcal, destas 25% a partir de proteínas, 46% de carboidratos e 29% de lipídeos (APÊNDICE B).

Quadro 1: Distribuição das refeições-teste com delineamento crossover.

Sábados	Grupo A	Grupo B	Grupo C
1	**Churrasco** + cápsulas **compostos fenólicos (CH+F)**	**Carne cozida** + cápsulas **placebo (CA+P)**	**Churrasco** + cápsulas **placebo (CH+P)**
2	**Carne cozida** + cápsulas **placebo (CA+P)**	**Churrasco** + cápsulas **placebo (CH+P)**	**Churrasco** + cápsulas **compostos fenólicos (CH+F)**
3	**Churrasco** + cápsulas **placebo (CH+P)**	**Churrasco** + cápsulas **compostos fenólicos (CH+F)**	**Carne cozida** + cápsulas **placebo (CA+P)**

A primeira coleta de sangue (jejum) foi realizada apenas no primeiro dia de experimento. Foram ainda coletadas amostras de sangue nos tempos 0 h (medida de base), 3 e 5 h após consumo das refeições teste nos três dias de intervenção. Amostras de urina foram coletadas nos tempos 0 h (medida de base), 4, 6 e 12 horas após o término do almoço. Foram coletadas duas amostras fecais: pré e pós-consumo das refeições-teste.

Figura 4: Planejamento dos dias de intervenção.



As cápsulas de extrato de açaí rico em compostos fenólicos, fornecidas aos voluntários, corresponderam à dose de 1g de compostos fenólicos e foram produzidas a partir de extrato aquoso liofilizado de açaí, pela empresa Amazon Dreams® (ANEXO 2).

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CAPÍTULO 2: FORMAÇÃO DE HIDROCARBONETOS POLICÍCLICOS AROMÁTICOS EM CARNES GRELHADAS COMO PRIMEIRO E SEGUNDO SERVIÇOS COMPARADOS À CARNE COZIDA

Artigo a ser submentido para a revista **Food and Chemical Toxicology.**

POLYCYCLIC AROMATIC HYDROCARBONS FORMATION IN MEATS GRILLED AS FIRST OR SECOND SERVICE OF BARBECUE OR OVEN COOKED MEAT

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds formed during incomplete combustion or pyrolysis of organic matter. Meat can be contaminated from grilling/barbecuing with intense heat over a direct flame which leads to the formation and adherence of PAHs to the meat surface. The objective of the present study was to evaluate 15 genotoxic PAHs in barbecued meat (*churrasco*) at two serving moments (grilled with the same charcoal) and compare to oven cooking method. The samples were extracted using pressurized liquid extraction followed by purification with SPE, and analyzed by HPLC/FLD. Second service barbecue had significantly higher total PAHs compared to first service. Both services showed significantly higher PAH levels than oven cooked meat.

Key-words: polycyclic aromatic hydrocarbons; food contamination; prolonged barbecuing; genotoxic PAHs.

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Abbreviations: ASE, accelerated solvent extractor; BaA, benz[a]anthracene; BaP, benzo[a]pyrene; BbFA, benzo[b]fluoranthene; BBQ, barbecue; BcFL, benzo[c]fluorene; BghiP, benzo[g,h,i]perylene; BjFA, benzo[j]fluoranthene; BkFA, benzo[k]fluoranthene; CHR, chrysene; DBaeP, dibenzo[a,e]pyrene; DBahA, dibenzo[a,h]anthracene; DiP D^{14}, dibenzo[*a*,*i*]pyrene-D_{14}; DBalP, dibenzo[a,l]pyrene; EU, European Union; IP, indenol[1,2,3-cd]pyrene; MCH, 5-methylchrysene; OCM, oven cooked meat; PAHs, polycyclic aromatic hydrocarbons; ΣPAH4, sum of BaA, BaP, BbF and CHR; SCF, Scientific Committee on Food; TEF, toxic equivalency factors.

Highlights

- Barbecue had higher PAH levels than oven cooked meat;
- Significantly higher PAH values were found in second service barbecue comparing to first service;
- Meat samples presented similar fat content, for all cooking methods.

1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds that are constituted by two or more fused aromatic rings. They are formed during the incomplete combustion or pyrolysis of organic matter. Humans can be exposed to PAHs through three main routes: inhalation, skin contact, and ingestion. While for non-smokers the major route of exposure is the last one, for smokers the contribution from smoking may be significant. Diet contributes to more than 90% of total PAH exposures of the general population in various countries. Food can be contaminated from environmental sources, industrial food processing and certain home cooking practices (EFSA, 2008; Purcaro et al., 2012).

PAHs generally occur in complex mixtures which may consist of hundreds of compounds. Among these compounds, fifteen were listed as a priority by the European Union (EU) because they show clear evidence of mutagenicity and genotoxicity in somatic cells, *in vivo* and in experimental animals, while other PAHs not considered as carcinogenic may act as synergists (European Commission, 2005). The addition of a 16th compound (benzo[*c*]fluorene,) also considered as genotoxic, was later recommended by EFSA (2008), being the term "15+1 EU priority PAHs" commonly used.

PAHs are chemically inert and hydrophobic. However, they undergo metabolic activation in mammalian cells to diol-epoxides that bind covalently to cellular macromolecules, including DNA, causing errors in DNA replication and mutations that initiate the carcinogenic process (Phillips, 1999; Purcaro et al., 2012). Due to the carcinogenic properties, the Scientific Committee on Food (SCF) (2002) recommended that the PAH contents in food should be "as low as reasonably achievable".

Cooking and food processing at high temperatures has been shown to generate various kinds of genotoxic substances that may significantly influence human health. The risk of exposure varies among individuals due to dietary habits and differences in cooking practices,

which often result from regional traditions (Ferguson, 2010; Jagerstad; Skog, 2005). Grilling/barbecuing meat, fish or other foods with intense heat over a direct flame results in pyrolysis of the fats and/or fat dripping on the hot fire, which leads to the formation of flames containing many PAHs adhering to the surface of the food. The more intense the heat, the more PAHs are present (Jagerstad and Skog, 2005). They are also formed in foods by smoke curing, broiling, roasting, frying, food processing or are added to foods in food additives such as smoking flavour agents (Chen; Lin, 1997; Kao et al., 2012; Simko, 2002).

Grilled foods have been reported to contain PAHs at levels varying from 0 to 130 µg kg^{-1} (Farhadian et al., 2010). Apart from analytical discrepancy, the differences in contamination levels may depend on: the time and temperature of processing (higher temperature and longer time increase the amount of PAHs); the distance from the heat source (the higher the distance, lower the contamination level in foods); the kind of process in particular, if food is directly in contact with the combustion products; the type of fuel used (e.g., burning of carbon produce less PAHs than wood), and the amount of fat in the processed food (fat is the major precursor of PAHs) (Alomirah et al., 2011; Purcaro et al., 2012). Additionally, cooking methods are not equivalent across countries and populations. In a few countries, barbecue is defined as cooking meat for a relatively short time over direct heat from burning charcoals or an open fire with high flames. Moreover, barbecuing may also be characterized by cooking meat or fish using low-level direct radiant heat from charcoals or embers, at lower temperatures and over longer cooking times. Therefore grilling conditions, as prolonged use of same charcoal during barbecuing, might result in different PAHs levels (Iwasaki et al., 2010).

The aim of the present study was to evaluate for the first time 15 genotoxic PAHs (14 + 1 EU priority) formations on barbecued meat (*churrasco*) at two serving moments and compare to an alternative cooking method.

2 MATERIALS AND METHODS

2.1 REAGENTS AND MATERIALS

Individual PAH (Table 2) standard solutions in acetonitrile (purity: 98.5–99.9 %) were purchased from Cluzeau Info Labo (Putteaux la Défense, France). The deuterated dibenzo[*a*,*i*]pyrene-D_{14} (DiP D^{14} in toluene, purity: 99.7 %), used as internal standard, was

purchased from LGC Promochem (France). Working standard solutions were prepared by dissolving the commercial solutions in acetonitrile and stored at 4°C in dark vials sealed with PTFE/silicone caps.

Florisil (100–200 mesh) was provided by Promochem. SPE Envi Chrom-P cartridges were provided by Sigma–Aldrich (St. Quentin Fallavier, France). Acetonitrile and methanol of GC-MS grade were supplied by Biosolve (Valkenswaard, The Netherlands). Dichloromethane and water (HPLC grade) were purchased from VWR (Leuven, Belgium). Cyclohexane and n-hexane in picograde quality were respectively purchased from Sigma-Aldrich (Bornem, Belgium) and Promochem (Wesel, Germany).

2.2. EQUIPMENTS

Standard laboratory apparatus was used throughout for the preparation of the samples and chemical solutions. Extraction and purification procedures were performed in glass materials. Samples were extracted by ASE 200® (Accelerated Solvent Extractor), from Dionex Corp. A TurboVap® II evaporator (Zymarck, Germany) was used for solvent evaporation.

HPLC analysis was carried out using a Model 600 E solvent delivery system, equipped with a Model 717 automatic injector, a Mistral^{TM} oven and both 996 PDA and 2475 Fluorescence detectors (all from WATERS). A C_{18} Pursuit 3 PAH (100 mm × 4.6 mm, 3 µm) equipped with a ChromGuard (10×3 mm) precolumn, both from VARIAN, were used to separate the PAHs.

2.3. SAMPLES

The meat samples used in this study were obtained from local butcheries in Belém-PA, Brazil, and submitted to two different cooking methods: barbecue and gas oven cooking. The samples were constituted by especial beef cuts of 150 grams each (one hump and one rumpsteak) and two pork sausages (60 grams each), representing a typical Brazilian barbecue meal (*churrasco*).

For barbecue preparation, a bed of traditional wood charcoal was prepared in an outdoor grill (garden-type). Samples were collected at two serving moments: the first grilled samples at the beginning of barbecuing, after the flames had diminish (1st service BBQ), and

the second (right after the first round of barbecue) when the samples were grilled without addition of new charcoal (2nd service BBQ). The steaks and sausages were barbecued over charcoal close to the heat source (15 cm) and were turned once during grilling at half the total cooking time, until well done.

For oven cooking process (OCM), the steaks and sausages were placed in an aluminum baking dish and cooked in a gas oven for 1 hour at 200°C, without toasting and with minimum browning on meat surface.

After cooking, the three kind of meat (one rumpsteak, one hump and two sausages) were randomly collected and mixed all together in a grinder, obtaining one representative sample. This procedure was done in triplicate for each cooking method or service. Samples were vacuum-packed and stored at -20°C until extraction and HPLC analysis. Moisture, ash, protein and fat content were determined by AOAC methods (AOAC, 2005).

2.4. SAMPLE EXTRACTION AND PURIFICATION

The extraction and clean up procedures were based on the method described by Veyrand et al. (2007). Briefly, ten grams of meat sample were first freeze-dried. The dry residue obtained was weighed in order to determine its residual water content. One gram of the dry residue was taken for the ASE extraction. The ASE cell was previously filled with 1.0 g of celite and 15.0 g of florisil, and extraction was performed with a mixture hexane/acetone (50:50, v:v). The extract obtained was evaporated to dryness and the dry residue dissolved in 5 mL of cyclohexane. Purification was performed using SPE (Envi-Chrom P SPE columns), and compounds were eluted using 12 mL of cyclohexane:ethyl acetate (40:60, v:v) as mobile phase. The eluate was evaporated to dryness and dissolved in 90 µL acetonitrile and 10 µL internal standard (DiP D^{14}).

2.5 ANALYSIS

An extract of 5 µL was injected on the HPLC column and separation was performed at 25°C using the gradient described in Table 1. Fluorescence detection conditions were previously optimized (Brasseur et al., 2007). Calibration curves for the 15 PAHs were injected for each series of sample extract. Results were corrected for the recovery, calculated from each PAH from the spiked sample (Table 2).

2.6 BENZO[*a*]PYRENE EQUIVALENT ESTIMATION (BaP_{eq})

This approach has been developed to enable a more accurate assessment of potential risk and possible effect of exposure to a complex mixture of PAHs using toxic equivalency factors based on BaP. Therefore, the toxic equivalency factors (TEF) has been used to simplify an interpretation of the real risk on human health. The BaP_{eq} was calculated as the sum of BaP equivalent value for individual PAHs. The individual BaP_{eq} value was calculated for each PAH from its concentration in the sample multiplied by its TEF as proposed earlier (Nisbet and LaGoy, 1992).

2.7 STATISTICS

The results were statistically analyzed by analysis of variance (ANOVA). The Tukey post hoc test was used for comparison of mean values, with differences being considered significant at $p< 0.05$. Statistical analyses were all performed with the software STATISTICA version 5.1.

3 RESULTS AND DISCUSSION

The recoveries of PAHs spiked in meat samples are indicated on the last column of Table 2. The recoveries of PAHs were in the range of 61.17–91.04 %, with a mean recovery of 77.5 %. These values are in accordance with performance criteria for methods of analysis for BaA, BaP, BbFA and CHR according to which the recovery for these compounds should be in the range 50–120 % for food samples (European Comission, 2011b).

PAH levels were determined in barbecued meat at two services of barbecuing and in oven cooked meat. Identification of PAHs was achieved on the basis of comparison of HPLC retention times of PAHs standards and those of spiked and unspiked meat samples. Typical HPLC chromatograms of PAH are presented in Figure 1.

To the best of our knowledge, this is the first study to compare the 14 +1 priority EU PAHs formations in two moments of homemade barbecuing and oven cooking meat. Individual PAH results are shown in Table 3. BcFL, BaA, and CHR were predominant in meat samples, whereas the lowest concentrations were those of DBalP, DBahA, and MCH. All PAH formation was significantly higher in BBQ meats comparing to OCM, except for

DBalP, DBahA and MCH. BBQ meat from the 1st service presented 8-fold ΣPAHs value from OCM samples; meanwhile for the 2nd service ΣPAHs were 12.5-fold higher than OCM samples. Perelló et al. (2009) investigated cooking-induced changes in the levels of 16 PAHs in various foodstuffs and found that fried and roasted chicken samples had highest total PAHs levels compared to grilled chicken samples, although the author added oil to roasting.

Barbecuing for longer time with the same charcoal significantly increased PAH formation. ΣPAHs was 9.48 ± 2.06 μg kg^{-1} of fresh weight in BBQ from the 1st service, while for the 2nd service this value was of 13.19 ± 4.85 μg kg^{-1} of fresh weight. Is has been reported that fat content of meat samples is an important factor for PAHs formation in grilled meat (Alomirah et al., 2011; Fretheim, 1983). As there was no difference in fat content between both services of barbecuing (table 4) the differences may be due to the major fat combustion, dropped during the grilling, contributing to a higher PAH formation. PAHs may accumulate in charcoal, being released later and then deposited on the surface of the later samples (Viegas et al., 2012).

Higher values of PAHs were found in commercial grilled and smoked food items commonly consumed in Arabian countries, where charcoal grilled lamb meat presented ΣPAHs of 241 μg kg^{-1} (mean of 6 samples), while smoked meat presented 76.3 μg kg^{-1} (mean of 4 samples) (Alomirah et al., 2011). The authors also observed that the process of electric grilling burgers over a hot surface produced an elevated PAHs concentration (mean 110 μg kg^{-1} for 3 samples). In addition, vertically roasting using gas flame presented mean ΣPAHs concentration of 27.0 μg kg^{-1} (mean of 6 samples). These values are much higher than those found in the present study as it tested the US EPA 16 priority PAHs (USEPA, 1986), which includes the eight high molecular weight PAHs from the EU list (BaA, CHR, BbFA, BkFA, BaP, IP, DBahA and BghiP) and also naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene. Also the samples tested by the cited authors presented heavily charred meat surface.

Chung et al. (2011) only analyzed 7 PAHs (CHR, BbFA, BkFA, BaP, DBahA, BghiP and IP). In charcoal-grilled meat total PAHs were 0.78 μg kg^{-1}, in charcoal-roasted and gas-roasted samples total PAHs were 0.03 μg kg^{-1}. Moreover, the authors found low means concentrations of BaP: 0.15 μg kg^{-1} in charcoal-grilled meat, 0.01 μg kg^{-1} in charcoal-roasted meat, and 0.003 μg kg^{-1} in gas-roasted meat, although the authors did not presented statistical comparison.

Concerning PAH4 sum, a better indicator of the occurrence and toxicity of the genotoxic and carcinogenic PAHs according to EFSA (2008), we found averages of 0.67 ± 0.19 µg kg^{-1} for OCM, 5.05 ± 1.06 µg kg^{-1} in BBQ meat from the 1st service and 6.97 ± 2.58 µg kg^{-1} for 2nd service. All values were significantly different from each other. These mean concentrations are below the EU maximum levels for the sum of BaA, BaP, BbFA and CHR, which is of 30 µg kg^{-1} for heat treated meat and heat treated meat products (European Commission, 2011a). Aaslyng et al. (2013) observed that the sum of PAH4 in 10 samples of homemade barbecue was highest in beef (average 17.3 µg kg^{-1}) compared with pork (average 2.6 µg kg^{-1}) and chicken (average 1.1 µg kg^{-1}).

BaP values found in the present study were significantly different between cooking methods: 0.05 ± 0.03 µg kg^{-1} for OCM, 0.75 ± 0.31 µg kg^{-1} for 1st service BBQ and 1.34 ± 0.62 µg kg^{-1} for 2nd service. These mean concentrations are below the EU maximum allowable limit for BaP which is of 5 µg kg^{-1} for heat treated meat and heat treated meat products (European Commission, 2011a). These results are similar to values found in a study realized by Farhadian et al. (2010), where charcoal grilled beef had 1.95 µg kg^{-1} of BaP. In the same study, authors found a BaP concentration of 0.37 µg kg^{-1} in meat grilled on a vertical rotisserie, with a vertical direct gas flame. Kazerouni et al. (2001) found that BaP was present in 200 different meat dishes from US, where the highest concentration (4.86 µg kg^{-1}) was in very well grilled/barbecued steak. BaP concentrations varied substantially in previously studies from 8.7 µg kg^{-1} in of Peking duck (Lin et al., 2011) to 6.04-3.07 µg kg^{-1} in smoked meat from different wood nature (Stumpe-Vīksna et al., 2008); both studies presented mean of three samples only.

Using the of benzo[a]pyrene equivalent values, it is possible to estimate the total toxicity of the samples. Σ BaP_{eq} were 0.12 ± 0.05 µg kg^{-1} for OCM, 1.18 ± 0.44 µg kg^{-1} for 1st service BBQ and 1.98 ± 0.92 µg kg^{-1} for 2nd service. BBQ meat presented a toxicity potential from 9 to 16 times higher than OCM samples. Our results were lower than those observed by Alomirah et al. (2011), where barbecued lamb meat presented Σ BaP_{eq} of 2.34 µg kg^{-1} and roasted lamb meat with gas flame had Σ BaP_{eq} of 1.41 µg kg^{-1} of fresh weight.

According to Table 4, all meat samples presented similar fat content, for all cooking methods. During barbecuing, dripping losses include moisture, fat, protein and ash (Murphy et al., 1975). Oven cooking avoids meat dripping, although losses occurred by exudation of moisture and fat, kept in the baking dish. The presence of moisture in the baking dish

prevented roasting of meat surface, leading to a lower formation of PAHs in oven cooked meat, when comparing to resembling methods.

Dost and Ideli (2012) compared the concentrations of nine PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[b]fluorene, BaA, BkFA and BaP) in raw and barbecued fish and meat. Barbecuing process increased the concentration (in the range of 2- to 8-fold) and caused the formation of PAHs in food samples, although the author's did not detected BaP formation in barbecued meat.

A few studies have suggested ways of reducing PAH formation during meat cooking process (Farhadian et al., 2012; Janoszka, 2011). In an effort of diminish PAH levels in charcoal grilled meat, two treatments, preheating (steam and microwave) and wrapping (aluminum foil or banana leaf) have been investigated. Using these pre-treatments before charcoal grilling resulted in reduced levels of carcinogenic PAHs in grilled meat samples (Farhadian et al., 2011). The addition of onion or garlic to pan-fried meat dishes was made in order to reduce PAH formation. Onion caused on average a decrease of 60% of the total content of PAHs in pan fried meat and of over 90% in its gravies, whereas garlic lowered the concentration of 54% in meat on average and from 13.5–79% in gravies (Janoszka, 2011). Similarly, Farhadian et al. (2012) suggests that the addition of acidic ingredients (especially lemon juice) in marinade can reduce the PAHs formation in grilled meat about o 70%.

It had also been observed that barbecuing with coconut shell charcoal may reduce PAHs formation in muscle foods, especially in fatty ones. This may be due to coconut charcoal being flameless and smokeless (as stated in label), justifying lower amounts of PAHs in samples grilled with this type of charcoal (Viegas et al., 2012).

In order to gather data on the actual consumption of PAHs from barbecued meat in Denmark, Aaslyng et al. (2013) collected barbecued meat samples prepared at home by participants, according to their own common practices. Values of PAHs from only 10 barbecue samples varied considerably: BaP ranged from 0-24 μg kg^{-1} and ΣPAH4 from 0.4–65 μg kg^{-1}. Means values were 6,3 μg kg^{-1} for BaP and 17,3 μg kg^{-1} for ΣPAH4, which differs from ours results. Additionally, Alomirah et al. (2011) found unmatching results when comparing similar meat dishes analyzed in previously studies. This suggests the influence of ethnic cooking practices on the PAHs levels in grilled foods. Moreover, the compounds studied vary a lot in the available reports, making it difficult to compare results.

4 CONCLUSIONS

Barbecuing lead the formation of significantly higher PAHs quantities, compared to oven cooking. In addition, barbecuing longer with the same charcoal increases PAHs contamination in meat. This may be significantly considering that a Brazilian typical barbecue takes place during many hours. Although, in further studies other collections should be done in advanced hours of barbecue, to estimate more proximate contamination and exposure. The PAHs values observed in the present study were different from previously reports leading the conclusion that meat cooking with same heat source are not always comparable due to discrepancy in geometry of the heating, apparatus and equipments utilized as well as texture and color of final meat surface; information's that are not often available in the reports. Once again, regional traditions have direct influence on levels of PAHs formation during meat cooking process, and further approaches should be realized to better know PAHs exposure in different cultures.

ACKNOWLEDGEMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Pará (FAPESPA) (Brazil) and Commission universitaire pour le Développement (CUD) (Belgium) for the financial support of this work.

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Table 1: Gradient used for the HPLC separation of PAHs.

Time (min)	Flow (mL min^{-1})	Water (%)	ACN (%)	Methanol (%)
0	1	15	30	55
2	1	15	30	55
20	1	0	100	0
25	1.5	0	100	0
40	1.5	0	100	0
45	1	15	30	55

Table 2: Parameters of the HPLC/FLD method for the quantification of 15 PAHs in meat.

Compound	Abrev.[1]	Retention time	LOQ (µg kg^{-1})[2]	Working range of the calibration curve (µg kg^{-1})[2]	Spiking level (µg kg^{-1})[2]	Recovery (%) n=4
Benzo[*b*]fluoranthene	BbFA	14.42	0.14	0.14 to 11	0.5	84.28 ± 5.5
Dibenzo[*a,l*]pyrene	DBalP	19.89	0.14	0.14 to 11	0.5	73.27 ± 5.5
Dibenzo[*a,h*]anthracene	DBahA	20.66	0.14	0.14 to 11	0.5	78.54 ± 9.1
Benzo[*g,h,i*]perylene	BghiP	21.55	0.14	0.14 to 11	0.5	75.81 ± 4.6
Dibenzo[*a,e*]pyrene	DBaeP	24.39	0.14	0.14 to 11	0.5	70.24 ± 8.3
Benzo[*j*]fluoranthene	BjFA	13.26	0.55	0.55 to 44	1	87.09 ± 7.5
Benzo[*c*]fluorene	BcFL	5.97	0.14	0.14 to 11	0.5	61.17 ± 14.1
Benz[*a*]anthracene	BaA	9.43	0.14	0.14 to 11	1	72.49 ± 11.4
Chrysene	CHR	10.60	0.14	0.14 to 11	0.5	91.04 ± 9.7
5-methylchrysene	MCH	11.64	0.14	0.14 to 11	0.5	81.12 ± 19.2
Benzo[*k*]fluoranthene	BkFA	16.20	0.14	0.14 to 11	0.5	79.81 ± 7.1
Benzo[*a*]pyrene	BaP	17.53	0.14	0.14 to 11	0.5	75.02 ± 6.5
Indenol[*1,2,3-cd*]pyrene	IP	22.67	0.55	0.55 to 44	1	77.40 ± 9.4

[1]Abbreviations according EFSA (2008); [2]of fresh weight. Dibenzo[*a,h*]pyrene (DBahP) and Dibenzo[*a,i*]pyrene (DBaiP) were not detected.

Table 3: Concentration[1] (μg kg^{-1} of fresh weight) of 14 + 1 EU priority PAHs in twelve barbecued meat and oven cooked meat.

PAH[2]	oven cooked meat	barbecued meat	
		1st service[3]	2nd service[4]
BbFA	0.07 ± 0.04[a]	0.85 ± 0.19[b]	1.27 ± 0.57[c]
DBalP	nd[a]	0.01 ± 0.03[a]	nd[a]
DBahA	0.01 ± 0.02[a]	0.05 ± 0.08[ab]	0.15 ± 0.20[b]
BghiP	0.05 ± 0.03[a]	0.53 ± 0.27[b]	1.00 ± 0.51[c]
DBaeP	0.02 ± 0.03[a]	0.26 ± 0.11[b]	0.55 ± 0.36[c]
BjFA	0.02 ± 0.03[a]	0.61 ± 0.30[b]	0.91 ± 0.55[b]
BcFL	0.13 ± 0.08[a]	2.01 ± 0.69[b]	2.36 ± 0.79[b]
BaA	0.47 ± 0.14[a]	1.81 ± 0.46[b]	2.19 ± 0.76[b]
CHR	0.07 ± 0.06[a]	1.64 ± 0.33[b]	2.17 ± 0.74[c]
MCH	0.05 ± 0.06[a]	0.09 ± 0.11[a]	0.17 ± 0.21[a]
BkFA	0.03 ± 0.04[a]	0.26 ± 0.09[b]	0.42 ± 0.21[c]
BaP	0.05 ± 0.03[a]	0.75 ± 0.31[b]	1.34 ± 0.62[c]
IP	0.07 ± 0.07[a]	0.59 ± 0.53[b]	0.65 ± 0.63[b]
Σ PAHs	1.06 ± 0.38[a]	9.48 ± 2.06[b]	13.19 ± 4.85[c]
Σ PAH4	0.67 ± 0.19[a]	5.05 ± 1.06[b]	6.97 ± 2.58[c]
Σ BaP_{eq}	0.12 ± 0.05[a]	1.18 ± 0.44[b]	1.98 ± 0.92[c]

[1] Means of triplicate determinations ± SD; [2] Full name and abbreviations of each PAH are reported on Table 2; nd, not detected; Σ PAHs: total PAHs sum; Σ PAH4: sum of BaA, BaP, BbFA and CHR; BaPeq: Benzo[a]pyrene equivalent; values labelled by different letters in the same line differ significantly at $p < 0.05$. *DiP and DhP were not detected. [3]1st Service: first meat samples barbecued; [4]2nd Service: second round of barbecued meat, without the addition of new charcoal.

Table 4: Meat composition[1] according to cooking method.

	Oven cooked meat	Barbecued meat	
		1st service[2]	2nd service[3]
Dry matter	53.08 ± 5.78[a]	53.10 ± 3.74[a]	50.91 ± 4.06[a]
Total Lipids	13.36 ± 2.71[a]	12.72 ± 3.04[a]	12.82 ± 2.40[a]
Crude protein	36.56 ± 4.57[a]	37.59 ± 2.51[a]	35.00 ± 2.89[a]
Ash	1.49 ± 0.26[a]	1.37 ± 0.31[a]	1.43 ± 0.33[a]

[1] Means of triplicate determinations ± SD, as a percentage of wet weight basis; values labelled by different letters in the same line differ significantly at $p < 0.05$. [2]1st Service: first meat samples barbecued; [3]2nd Service: second round of barbecued meat, without the addition of new charcoal.

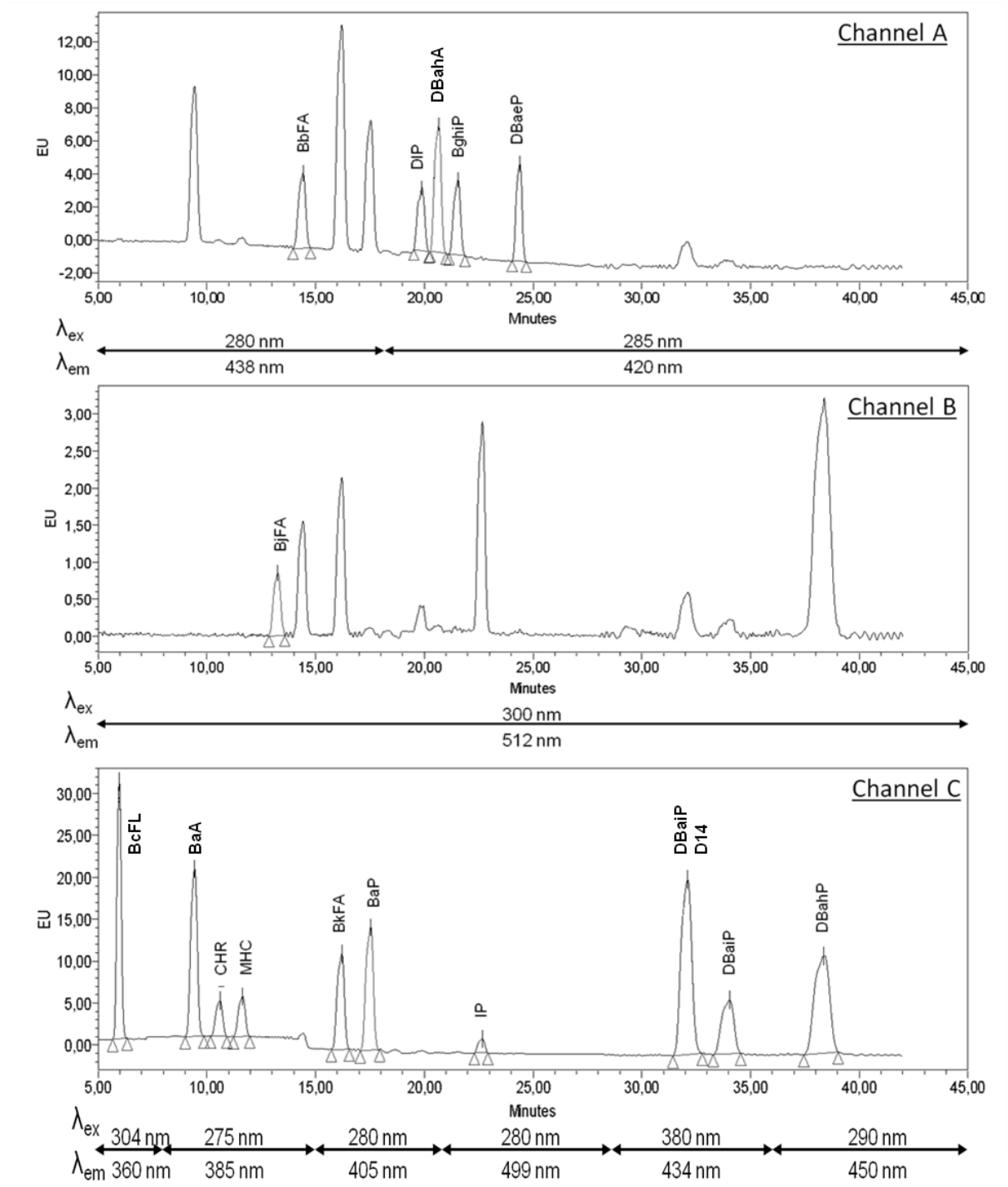

Figure 1: Chromatograms (HPLC/FLD) obtained for the 14+1 European Union priority PAHs acquired with the A, B and C channels of the fluorescence detector. The excitation (λ_{ex}) and emission (λ_{em}) wavelengths used for detection in each channel are indicated below the respective chromatogram. The PAHs detected in each channel are indicated on the respective chromatogram.

CAPÍTULO 3: ALTERAÇÕES MESTABÓLICAS PÓS-PRANDIAIS APÓS CONSUMO DE CHURRASCO E COMPOSTOS FENÓLICOS DO AÇAÍ

Artigo a ser submetido para a revista **Journal of Functional Foods.**

POSTPRANDIAL METABOLIC CHANGES INDUCED BY CONSUMPTION OF BARBECUE AND PHENOLIC COMPOUNDS FROM AÇAI

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are contaminants known to induce toxicity and oxidative stress in humans. Dietary exposure to PAHs occurs mostly due to food-processing techniques. On the other hand, phenolic compounds exhibit in vivo antioxidant capacities. The objective was to evaluate postprandial changes after barbecue intake associated or not to phenolic compounds from açai, and compare to a control meal. Twenty three healthy subjects were selected and randomly assigned to three different sequences of test-meals: barbecue with phenolic compounds; barbecue with placebo; and oven cooked meat with placebo. Blood changes of TBARS, CRP, ALP, GOT and GTP were not significantly different between the test-meals. GSH-Px activity tended to decrease by barbecue, and to increase by phenolic compounds intake. 1-hydroxypyrene urinary excretion was significantly higher after barbecue

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Abbreviations: 1-OHP, 1-hydroxypyrene; ALP, alkaline phosphatase; BaP, benzo[*a*]pyrene; BBQ, barbecue; BBQ+P, barbecue associated with placebo capsules; BBQ+PH, barbecue associated with phenolics from acai; BMI, body mass index; CRP, C-reactive protein; GOT , glutamic oxalacetic transaminase; GPT, glutamic pyruvic transaminase; GSH, glutathione; GSH-Px, glutathione peroxidase; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; OCM+P, oven cooked meat associated with placebo capsules; PAHs, polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; SOD; superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TC, total cholesterol; TG, triglycerides; TP, total phenolics.

intake. Fecal lipid was not significantly increased by polyphenols. Others parameters that could be affected by barbecue intake or attenuated by phenolic compounds should be investigated.

Key-words: dietary exposure, oxidative stress, antioxidants, test-meals.

Highlights

- Postprandial changes after test-meals;
- Barbecue intake tented to alter glutathione peroxidase activity;
- Phenolic compounds intake tented to increased glutathione peroxidase activity;
- Urinary 1-hydroxypyrene was significantly higher after barbecue intake.

1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a widespread environmental contaminants formed by pyrolysis or incomplete combustion of organic matter. PAH contamination of food arises from two sources, environmental (water and air pollution) and food-processing techniques, like barbecuing (Suzuki & Yoshinaga, 2007).

PAHs are highly liposoluble and well absorbed by the gastrointestinal tract. It has been demonstrated an association between dietary PAH and increased risks of esophagus, stomach, pancreatic cancer and colorectal adenoma. PAHs have also been reported to cause hemato, cardiological, renal, neuronal, immune, reproductive and developmental toxicities in animals and humans (Ramesh et al., 2004). The PAH toxicity occurs after their metabolic activation into electrophilic intermediates. These reactive intermediates are then able to covalently bind to DNA or engage in redox cycling. In turn, these mechanisms lead to an overproduction of reactive oxygen species (ROS), which causes oxidative stress and as a result, lipid peroxidation or DNA damage (Xue & Warshawsky, 2005). Data concerning plasma half-life of PAHs are limited; benzo[a]pyrene (BaP), one major carcinogenic PAH, was demonstrated to exibit half-life of 5.8 h in rats (Ramesh et al., 2001).

Ambient and occupational exposure to PAHs have been shown to increase oxidative stress biomarkers and to induce the overexpression of several pro-inflamatory factors, like interleukin-1β (IL-1β), interleukin-8 (IL-8), interferon-γ (IFNγ), and tumor necrosis

factor-α (TNFα) (Jeng et al., 2011; Khalil et al., 2010; Lai et al., 2012). Singh et al. (2008) demonstrated that blood PAH levels in Indian children are significantly correlated with oxidative stress and altered antioxidant status. Restaurant workers had a positive correlation between oxidative stress and urinary PAH metabolite levels and work hours per day (Pan et al., 2008).

Nevertheless, health outcomes from dietary exposure to these compounds have not been well investigated through controlled studies in humans. Only few studies demonstrated biological effects of PAHs related to barbecue (BBQ) consumption (Chien & Yeh, 2010a; Elhassaneen, 2004). Currently, there are no published data associating the phenolic compound intake with dietary PAHs.

Dietary polyphenols represent a group of secondary metabolites which widely occur in fruits, vegetables, wine, tea, extra virgin olive oil, chocolate and other cocoa products. They exhibit many biological functions mainly attributed to their intrinsic antioxidant capacity; they reach maximal concentration in plasma between 1.5 and 5.5 h, with half-lives that vary from 1 to 8 h (Manach et al., 2005). However, they may also offer an indirect protection by modulating cellular signaling processes such as glutathione (GSH) biosynthesis and inhibition of digestive enzymes (Han et al., 2007).

Polyphenols, mainly anthocyanins and other flavonoids, are the predominant bioactive compounds in açai. Açai is a palm tree widely distributed in the Amazon estuary, being the açai juice part of dietary habits in the Amazon floodplains part of Brazil. In the last decade it has been marketed as a dietary food supplement claiming to have superior health benefits, especially due to its antioxidant properties. Açai extracts have exhibited pharmacological properties including antiproliferative, anti-inflammatory, antioxidant, and cardio-protective activities, mostly in vitro. Nevertheless, there are still limited data on açai effects in humans (Heinrich et al., 2011).

The aim of the present study was to evaluate postprandial metabolic changes after barbecue intake associated or not to supplementation of phenolic compounds from açai, and also comparing to a control meal (oven cooked meat).

The PAH content of meats were determined by HPLC method previously described (chapter 2). The mean concentration of total PAHs and benzo[a]pyrene (BaP), one major carcinogenic PAH, in barbecued meat and oven cooked meat (control) were 12.11 ± 4.30 and 1.05 ± 0.56 µg/kg and 1.06 ± 0.38 and 0.05 ± 0.03 µg/kg, respectively. Mean portion size was 275.344 ± 20.575g which corresponded to 35.42 ± 8.43 g fat intake.

2 MATERIALS AND METHODS

2.1 CHEMICALS AND MATERIALS

White 96-well microplates were purchased from Greiner Bio-One (Frickenhausen, Germany). Aluminum sealing tapes for 96-well plates was from Nunc™ (Rochester, NY). 2-thiobarbituric acid (98%), trichloroacetic acid and malonaldehyde bis(dimethyl acetal) (99%) were obtained from Sigma (St. Louis, MO); L-GSH reduced, GSH reductase, β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (NADPH), 1-hydroxypyrene (purity 98%), β-glucuronidase (from *Helix pomatia)* were purchased from Sigma-Aldrich (São Paulo, Brazil). SPE Supelclean cartridges (C-18 ENVI, 500 mg and 3 ml) were purchased from Supelco (Sigma-Aldrich, São Paulo, Brazil). The methanol and chloroform was of HPLC grade (VETEC, Brazil). All the other reagents were of analytical grade. Açaí extract rich in total phenolic (TP) compound (450 mg gallic acid equivalents/g of powder; 160 mg of anthocyanins/g of powder) was kindly offered by Amazon Dreams (Belém, Brazil).

2.2 STUDY DESIGN

Twenty three male non-smoking healthy subjects, aged between 20 and 36 years were recruited. Subjects were asked to abstain from consuming polyphenol rich vegetables or fruits, and were provided with a list of others substances they could not consume 48 h before each trial e.g. tea-related products, vitamins, minerals, dietary or herbal supplements. Subjects refrained from strenuous exercise and alcohol the previous day. All subjects gave their consent and filled a medical history form, including weight and height informations. The study comprised three days of acute intervention, with a randomized crossover design. A one week washout period was used between each intervention. The subjects were randomly assigned to 3 different sequences of test-meals: Barbecue associated with phenolic compounds rich capsules (1 g phenolics), from açai fruit (BBQ + PH); Barbecue associated with placebo capsules (BBQ + P) and Oven cooked meat associated with placebo capsules (OCM + P). Before lunch, baseline blood samples were drawn. Subjects were allowed unlimited water intake and were provided with a low-fat snack and dinner. The daily menu provided 3000 kcal, 25% of these from protein, 46% carbohydrates and 29% lipids. The test-

meals lipids provided 76% of total lipids intake. The protocol was approved by the Human Research Ethics Committee of the Health Sciences Institute, Federal University of Pará (Belém, Brazil), under protocol number CAEE 0139.0.073.000-1.

2.3 BLOOD ASSAYS

In the first day of trial levels of glucose, total cholesterol (TC), HDL-cholesterol (HDL-c), LDL-cholesterol (LDL-c), triglycerides (TG) were measured in fasted blood samples for subject´s biochemical profile determination. Blood samples were also drawn at baseline (0 h), 3 and 5 h after the meal intake, for high-sensitivity C-reactive protein (CRP), alkaline phosphatase (ALP), glutamic pyruvic transaminase (GPT), and glutamic oxalacetic transaminase (GOT) serum determinations, measured by standard laboratory methods in a certified laboratory.

Plasma aliquots were obtained after centrifugation from heparinized blood collected at time 0 h, 3 h and 5 h after the intake of tested meals and used for thiobarbituric acid reactive substances (TBARS) determination. The method described by Yagi (1987) was modified for microplate measurements. Briefly, 30 μL of diluted plasma (in PBS pH 7.4) was incubated with 24 μL trichloroacetic acid (15%) and 48 μL thiobarbituric acid (0.67% in 0.3N NaOH). The microplate was covered with a aluminum lid and heated at 95 °C for 30 min. After cooling on ice, 100 μL butanol were added and the plate centrifuged at 200 *g* for 5 min (4 °C) (Jouan-BR4i-Vel, Leuven, Belgium). The fluorescence of the supernatant was measured at 515 nm ($\lambda_{excitation}$) and 555 nm ($\lambda_{emission}$) on a Fluoroskan Ascent (Labsystems, Helsinki, Finland). MDA, submitted to the same conditions, was used as a standard (0.65 μM to 10 μM).

After plasma aspiration from heparinized blood tubes, erythrocytes were washed three times with cold saline solution (0.153 mol/L sodium chloride) in order to remove white cells and plasma. Lysates were prepared by the addition of 1 mL of distilled water to 100 μL of washed erythrocytes and frozen at −80°C. GSH-Px activity was measured in the erythrocytes lysates by the method of Wendel (1981). NADPH disappearance was continuously monitored with a spectrophotometer (Molecular Devices, Spectramax Plus 384, Sunnyvale, CA, USA) at 340 nm for 4 min, in a medium containing 0.25 M potassium phosphate buffer/2.5 mM ethylenediaminetetraacetic acid pH 7.0, 2 mM GSH, 0.25 U/mL glutathione reductase, 0.25 M sodium azide, 0.24 M NADPH. Tert-butylhydroperoxide 70% was used as substrate. One

GSH-Px unit is defined as 1 mmol of NADPH consumed/minute and specific activity is reported as units/mg protein. Protein was measured by the method of Bradford (1976).

2.4 DETERMINATION OF 1-HYDROXYPYRENE (1-OHP) IN URINE

1-OHP was measured in samples from BBQ + P and OCM + P interventions only. The urine samples were collected by the volunteers in polyethylene bottles and brought to the laboratory. The volume of each sample was measured and then were divided into small volume aliquots of 20 mL to minimize the effect of freeze-thaw on the stability of specimens and stored at –20°C. The times point of collection were 0 h (baseline), 4, 6 and 12 h after the intake of the test meals. Creatinine concentration of each sample was determinate by a certified laboratory, in order to eliminate the difference of urinary concentration.

The procedure for analyzing urinary 1-OHP was based on published methods (Fan et al., 2006; Jongeneelen et al., 1987). Ten milliliters of urine were transferred to an erlenmeyer flask. The pH of the solution was adjusted to 5.0 with 0.2 M HCl, and 2.5 mL of 0.5 M sodium acetate buffer (pH 5.0) were added. After addition of 20 μL of β-glucuronidase the flask was covered with a tinfoil and then placed in a shaker overnight at 37°C to hydrolyze the conjugated PAH metabolites. The hydrolyzed urine samples were loaded in SPE cartridges after they had been pre-conditioned with 5 mL of methanol and 5 mL of water. The cartridge was sequentially washed with 10 mL of water and 30% methanol. After the cartridges were dried, the trapped metabolites were eluted with 4 mL of methanol using a Visiprep^{TM} SPE vacuum manifold system (Supleco, São Paulo, Brazil). The eluate was evaporated to dryness and re-dissolved in 1 mL of methanol. The solution was filtered through a 0.42 μm filter, and then stored at−20 ◦ C until HPLC/FLD analysis.

Analysis were performed with a Shimadzu HPLC System (LC 10-AD, Kyoto, Japan) equipped with two LC 10-AT VP solvent pump units, a SLC-10A system controller, a CTO-10AS VP column oven, a fluorescence detector (Shimadzu RF-10AXL, Japan) and an autoinjector model SIL 10AD. A Supelcosil LC- PAH analytical column (5 μm particle diameter, 15 cm x 4.6 mm) (Supelco, Brazil) was used for the chromatographic separation. The solvent gradient was as follows: 10 min of methanol-water (60:40); methanol-water (90:10) for 5 min and methanol-water (60:40) for 1 min. Flow rate was 1 mL/min and injection volume was 20 μL. The excitation (λ_{ex}) and emission (λ_{em}) wavelengths were 242

and 388 nm respectively. Calibration curve working range was 0.5 - 20 ng/mL and was prepared with 1-OHP stock solutions in methanol.

2.5 FECAL ASSAYS

The subjects collected quantitatively two fecal samples at each intervention period: before the meal intake (baseline) and the first feces after the test meals consumption. Samples were weighted and frozen at -20°C before being freeze-dried and homogenized. Total fat content was quantified after extraction from aliquots of 3 grams with chloroform-methanol-water (1:2:0.8, v/v/v) (Bligh & Dyer, 1959; Li et al., 2010). The extraction tubes were agitated during 30 minutes and were added with chloroform-water (1:1, v/v). The solutions were filtered into a new tube to promote a better phase separation and centrifuged. The aqueous layer was removed and solvent layer was transferred into a clear tube added with anhydrous sodium sulfate and subsequently filtered. The final extract was evaporated using a centrivap concentrator (Labconco Corp., Kansas City, MO) and the fat content was determined by gravimetry. The TP content of feces was determined by the Folin–Ciocalteu colorimetric method (Singleton et al., 1999).

2.6 STATISTICS

Statistical analyses were performed with the software STATISTICA version 5.1. Baseline measurements were normalized to 100 %, and changes from baseline were calculated as percent change from baseline. All data are represented as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA with Tukey post hoc test for comparisons between different treatments, with differences being considered significant at $P< 0.05$. Student's *t* test was used when appropriate.

3 RESULTS AND DISCUSSION

3.1 SUBJECT CHARACTERISTICS

The mean serum glucose, TC, TG, HDL-c, LDL-c, measured in fasted blood samples for subject´s profile determination, and body mass index (kg/m^2) are listed in Table 1. Subjects presented no blood biochemical alterations.

3.2 CHANGES IN BLOOD PARAMETERS

Percent changes in blood parameters after intake of test-meals are shown in Table 2.

TBARS changes were not different between test-meals, and values tended to decrease in postprandial state. Although the mechanisms that lead to these results in our study is not clear, we suggest that fasting may slightly raised lipid peroxidation, compared to postprandial state (Sorensen et al., 2006).

GSH-Px activity in erythrocytes showed a tendency to increase (67 %) 3 h after BBQ+PH intake. Mean while, at the same time point of BBQ+P post-exposure, the activity decreased in 17 % ($P< 0.05$), although high SD values were found. Besides conventional antioxidant-reducing activities, polyphenols may offer an indirect protection by activating endogenous defense systems through cell signaling and gene expression, with the consequent modulation antioxidant enzymatic activities that drive the intracellular response against oxidative stress (Han et al., 2007; Masella et al., 2005).

A study developed with 12 volunteers who consumed 120 mL of a berry juice blend, which contained açai as the predominant ingredient, showed that serum antioxidant capacity was significant increased at 1 and 2 h post consumption, and the decrease in lipid peroxidation was significant 2 h after intake (Jensen et al., 2008). Similarly, Mertens-Talcott et al. (2008) observed that maximum time (t_{max}) of antioxidant capacity in plasma was 3 h after açai pulp intake.

The tendency of GSH-Px activity to decreased after BBQ+P exposure may be due to enzyme inhibition by superoxide radical (Blum & Fridovich, 1985; Pigeolet et al., 1990). GSH-Px in erythrocytes was shown to be inactivated by oxidative stress as result of selenocysteine residues modification at the active site, with enzymatic activity decrease after 3 h exposure to reactive oxygen species *in vitro* (Cho et al., 2010).

Available data suggest that a fat overload in a single meal could affect endogenous antioxidant system. Tsai et al. (2004) observed that plasma concentrations of GSH-Px decreased significantly from baseline at 2 h after intake of a high-fat meal (50 g of fat) and returned towards baseline levels at 4 h and 6 h post exposure. Considering the low response of GSH-Px at 3 h after OCM+P intake, and that the fat amount did not vary significantly between test-meals (data not shown) it is suggested that PAHs may be responsible for GSH-Px propensity to decreased at 3 h BBQ+P pos-exposure, although results were not statistic significant.

At 5 h post-exposure to BBQ+P, GSH-Px activity tended to increase (25 %) probably due to overexpression as a compensatory way, although it was similar to the values of other test-meals. The inactivation by superoxide was demonstrated to be reversible by glutatione, while sequential exposure to superoxide and hydroperoxides may cause irreversible inactivation (Blum & Fridovich, 1985).

In rats fed for eight weeks with carbonized meat (10 % added to animal feed) blood GSH-Px and superoxide dismutase (SOD) decreased and serum lipid peroxide increased significantly compared to control group. When treating animals with water or methanol leek extracts or even adding 2.5 % and 5 % leek powder to animal feed, GSH-Px was significantly increased while serum lipid peroxide decreased, compared to non-treated group (Hamedan & Anfenan, 2011).

The antioxidant defense system response after PAH oral exposure in humans was demonstrated before. Volunteers who consumed two charcoal-broiled beefburgers per day (mean weight 70 g each) over 28 consecutive days presented significantly lower GSH-Px, superoxide dismutase (SOD), and catalase activity in erythrocytes during the BBQ consumption period compared with background values (Elhassaneen, 2004). However, the present study is the first to evaluate the postprandial metabolic changes induced by barbecue intake and to indicate that concomitant consumption of phenolic compounds may acutely attenuate possible induced oxidative damages.

CRP is a major acute-phase inflammation protein. BaP was shown to increase serum CRP in rats after a single dose (Pappas et al., 2003). Moreover, CRP has been found to be associated with high levels of urinary monohydroxy PAH metabolites, suggesting that PAH exposure may induce inflammation in humans (Everett et al., 2010). In the present study hs-CRP increased 7.88 % and 10.53 % 3 h after BBQ+PH and BBQ+P intake, respectively, while minimum change was observed after OCM+P intake (not statistic significant). At time

point 5 h BBQ+PH post-exposure, CRP tended to increased at 13 %, suggesting that phenolics may have partially inhibited inflammation rise 3 h after intake, by quenching reactive oxygen species. Although, as the PAH values found in barbecued meat were considerably low (chapter 2), a higher dietary dose could significantly increase CRP values.

Despite anti-inflammatory allegation of açai, Udani et al. (2011) observed no change in CRP values in 10 overweight volunteers, given 100 g of açaí pulp twice daily for 1 month.

Hamedan & Anfenan (2011) also observed that consumption of carbonized meat in non-treated group of rats increased serum GOT compared to normal control group. Our findings show a GOT increase of 4 % at 5 h BBQ+P post-exposure, but it was not statistic different from values found after the other meals. ALP did not vary significantly between test-meals (5.4-6.12 %). We observed a significantly decrease in GTP after BBQ+PH (-9.47 %) compared to OCM+P (2.96 %).

Lima-oliveira et al. (2012) observed that ALP increases 2.7 e 1.1% at 1 and 4 h after a light meal consumption in 17 healthy volunteers. The authors consider that a clinically significant increase would be above 6.4 %. In the same study, it was shown that GOT and GTP increased 5.3 % and 14.3 % and 6 % e 17 % after 1 and 4 h post consumption, respectively. According to the authors, increases above 5.4 and 12 % for GOT and GTP respectively are clinically significant.

3.4 1-OHP IN URINE

Urinary monohydroxylated PAHs (OH-PAHs) are metabolites of PAHs and have been used as biomarkers of PAH exposure. 1-OHP is the most commonly used PAH biomarker and is well correlated with other metabolites, being a useful surrogate for representing PAH mixture exposure (Li et al., 2008; Suzuki & Yoshinaga, 2007).

Few studies have investigated urinary 1-OHP excretion after barbecue intake, and have been conducted with a small number of subjects (Buckley & Lioy, 1992; Chien & Yeh, 2010b; Li et al., 2008). The present study was the first known conducted to compare the 1-OHP excretion after barbecue and oven cooked meat consumption.

Urinary 1-OHP results are shown in Figure 1, and typical chromatograms in Figure 2. Creatinine adjusted concentrations (in ng of 1-OHP/mg of creatinine in urine) were calculated in order to take into account the urine dilution. Baseline concentrations were 0.09 ± 0.03 and 0.10 ± 0.05 ng/mg creatinine before BBQ + P and OCM + P interventions, respectively.

PAHs are ubiquitously present in the environment, and humans are exposed to background levels of PAHs continuously, mostly through inhalation of polluted air and cigarette smoke, and ingestion of contaminated foods. These factors, contribute to the large inter-individual variations of urinary 1-OHP background (Chien & Yeh, 2010b; Van Rooij et al., 1994; Viau et al., 2002).

Acute dietary exposure to a PAH rich meal led to a significantly higher excretion of 1-OHP. At 6 h and 12 h after BBQ+P intervention, 1-OHP concentration was 2 folds higher than those found after OCM+P intake ($P< 0.01$) and maximum values were 1.0 and 0.460 ng/mg creatinine, respectively. Total 1-OHP amount excreted was 77.26 ± 52.96 and 28.12 ± 19.62 ng at BBQ+P and OCM+P 6 h post exposure ($P< 0.001$), respectively.

PAHs are rapidlymetabolized and excreted. After dietary exposure, the 1-OHP mean half-lives was reported to be 3.9 −5.7 h, varying from 3.0 to 9.0 h (Chien & Yeh, 2010b; Li et al., 2012).

Li et al. (2012) observed that mean 1-OHP pre-exposure was 0.08 ng/mg creatinine, which corroborate with our findings. According to the authors at 3.5−8.5 h after the consumption of chicken barbecue (172 ± 33 g), the urinary 1-OHP mean maximum concentration was 10−87-fold higher (max conc./pre-exposure) among the 9 participants. Maximum mean concentration was 1.86 (1.57−2.95) ng/mg creatinine and the mean t_{max} was 5.5 ± 1.7 h. The maximum time excretion in the present study was at 6 h post exposure, although there is a lack of information in between time collections 6-12 h.

1-OHP excretion related to barbecue ingestion was also investigated by Chien & Yeh (2010b). The maximal post-consumption urinary 1-OHP concentration was 0.487 ng/mg creatinine among 9 volunteers, and the mean background was 0.033 ng/mg creatinine. 1-OHP excretion rate increased to maximum within a few hours after eating barbecued meat (4.0 ± 2.4 h) then decreased to the background level in less than 24 h. The authors related that some individuals presented lack of an obvious peak or plateau-shaped elimination curves.

3.5 FECAL ASSAYS

Fecal lipid determination has become an important diagnostic tool to quantify steatorrhea in subjects afflicted with pancreatitis, but also to monitor drug efficacy in obese patients treated for weight reduction with the inhibitor of gastrointestinal lipases Orlistat®. Quantitative analysis of total fecal fat is generally performed in individual using either

gravimetric or titrimetric methods after lipid extraction with organic solvents (Kunz et al., 2003). In the present study we evaluated fecal fat in samples from BBQ + P and BBQ + PH experiments, in order to investigate a possible lipase inhibition effect from açai phenolic compounds.

According to Table 3, fecal samples had a higher lipid concentration and lipid amount excreted after the intake of phenolic compound when comparing to placebo. It reinforces the tendency of lipase inhibition, although values were not statistically significant.

After a detailed evaluation, it is suggested that some volunteers did not collect all fecal volume excreted, observed by comparing fecal volume and dry matter from first and second samples (data not shown) for both test-meals. Thus, the lipid excreted might have been underestimated. Furthermore, subjects restricted intake of fibers during the interventions periods, as they consumed limited fruits and vegetables for 3 days, which may have slow down the gastrointestinal transit and resulted in incomplete evacuation of test-meal residues.

Changes in TP excretion are described in table 3. TP concentration in feces and amount excreted increased 72.38 ± 89.16 and 140.07 ± 230.14 %, respectively, after BBQ+PH intake. Surprisingly, part of phenolic compound maintained their antioxidant activity until the end of the gut, once 85 ± 56,97 mg of TP were present in the collected feces after BBQ+PH intake.

4 CONCLUSIONS

Barbecue intake tended to induced postprandial oxidative damage by decreasing GSH-Px activity, while phenolics compounds from açai associated to barbecue consumption tended to increased GSH-Px activity. There was no significant induction of inflammation or lipid peroxidation between test-meals. Urinary 1-OHP was significantly higher after barbecue intake compared to oven-cooked meat. Fecal fat excretion was not significantly affected by phenolic compounds ingestion. Further studies are needed to investigate chronic exposure for consecutive days or other postprandial parameters that could be affected by barbecue intake or attenuated by phenolic compounds.

ACKNOWLEDGEMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Pará (FAPESPA) (Brazil) and Commission universitaire pour le Développement (CUD) (Belgium) for the financial support of this work, and would particularly like to thank Luciana Lima, Ana Clara Vasconcelos, Edvaldo Pena e Joel Gonçalves de Souza for laboratory assistance.

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Table 1: Subjects characteristics profile.

Parameter	Mean (SD)
Glicose (mg/dL)	77.54 ± 6.25
TC (mg/dL)	155.09 ± 23.56
HDL-c (mg/dL)	42.00 ± 6.29
LDL-c (mg/dL)	91.63 ± 16.81
TG (mg/dL)	105.95 ± 61.72
BMI (kg/m^2)	24.51 ± 2.14

Characteristics for 23 male subjects at the screening. BMI: body mass index, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides.

Table 2: Percent change* of TBARS, GSH-Px, CRP, ALP, GOT and GTP measures in blood after test meals intake compared to baseline.

Measure	Time point collection	Test meals		
		BBQ + PH	BBQ + P	OCM + P
TBARS	t3h	-26.45 ± 22.8[a]	-25.17 ± 21.92[a]	-17.91 ± 15.59[a]
	t5h	-30.17 ± 18.87[a]	-22.49 ± 18.83[a]	-27.45 ± 20.07[a]
GSH-Px	t3h	67.82 ±124.58[a]	-17.71 ± 27.37[b]	2.54 ± 59.80[ab]
	t5h	32.83 ± 89.40[a]	24.99 ± 51.93[a]	30.97 ± 83.64[a]
CRP	t3h	7.88 ± 24.64[a]	10.53 ± 25.52[a]	0.24 ± 12.97[a]
	t5h	13.2 ± 36.25[a]	8.48 ± 19.88[a]	2.89 ± 19.01[a]
ALP	t5h	5.43 ± 7.27[a]	4.35 ± 8.47[a]	6.12 ± 11.11[a]
GOT	t5h	0.81 ± 15.33[a]	4.72 ± 15.61[a]	-2.32 ± 15.82[a]
GTP	t5h	-9.47 ± 15.61[a]	-0.82 ± 14.72[ab]	2.96 ± 15.97[b]

*Means (%) ± SD. BBQ+PH: barbecue + phenolics; BBQ+P: barbecue + placebo; OCM+P: oven cooked meat; TBARS: thiobarbituric acid reactive substances; GSH-Px, glutathione peroxidase; CRP: C-reactive protein; ALP: alkaline phosphatase; GOT:glutamic oxalacetic transaminase; GPT: glutamic pyruvic transaminase. Time point collections: 3 and 5h post-exposure. Values labelled by different letters in the same line differ significantly at $p < 0.05$.

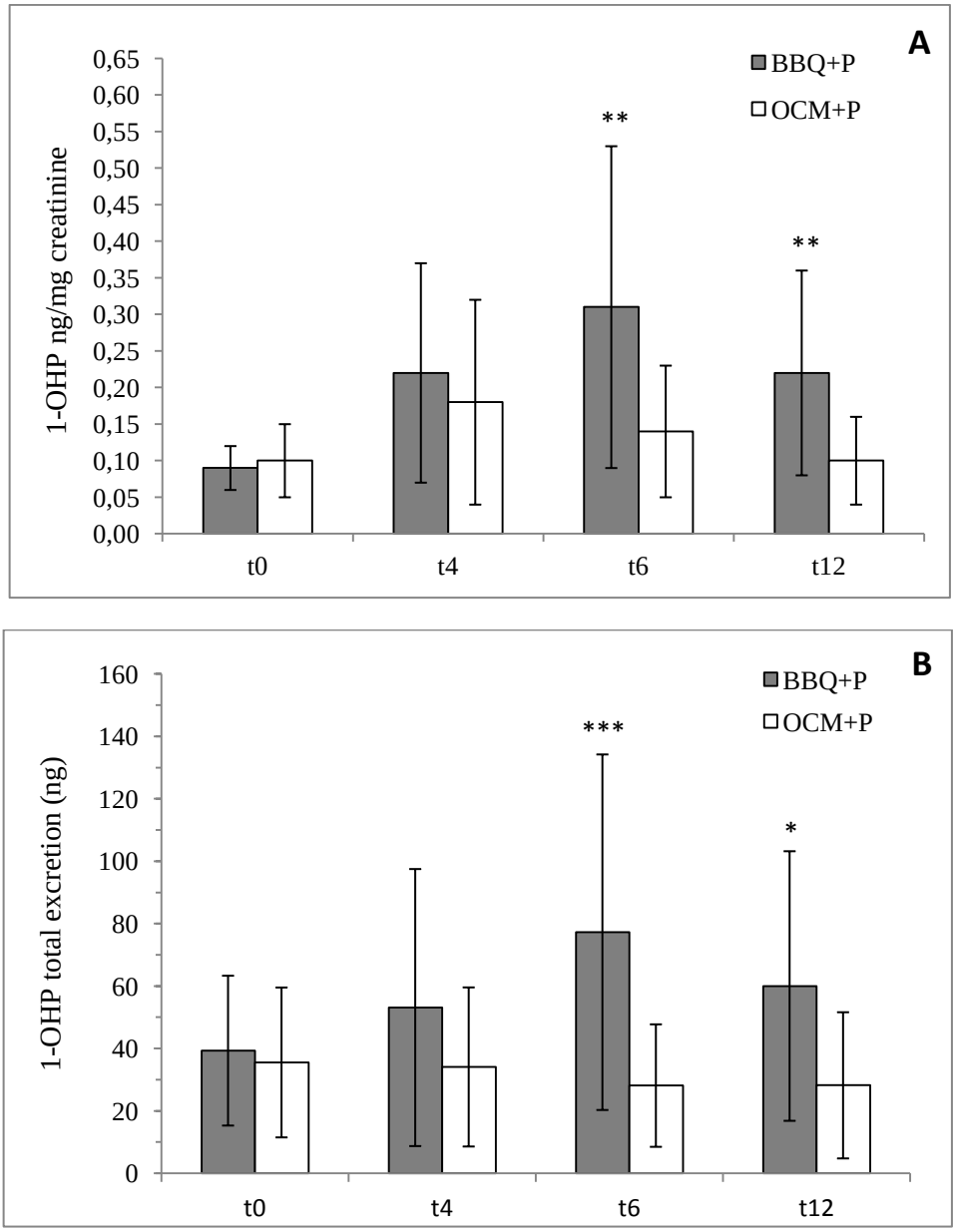

Figure 1: Urinary 1-OHP concentration (A) and total amount excreted (B) after barbecue (BBQ +P) and oven cooked meat (OCM+P) consumption at time collections 0h (baseline), 4h, 6h and 12h post-exposure (*P< 0.05; **P< 0.01; ***P< 0.001).

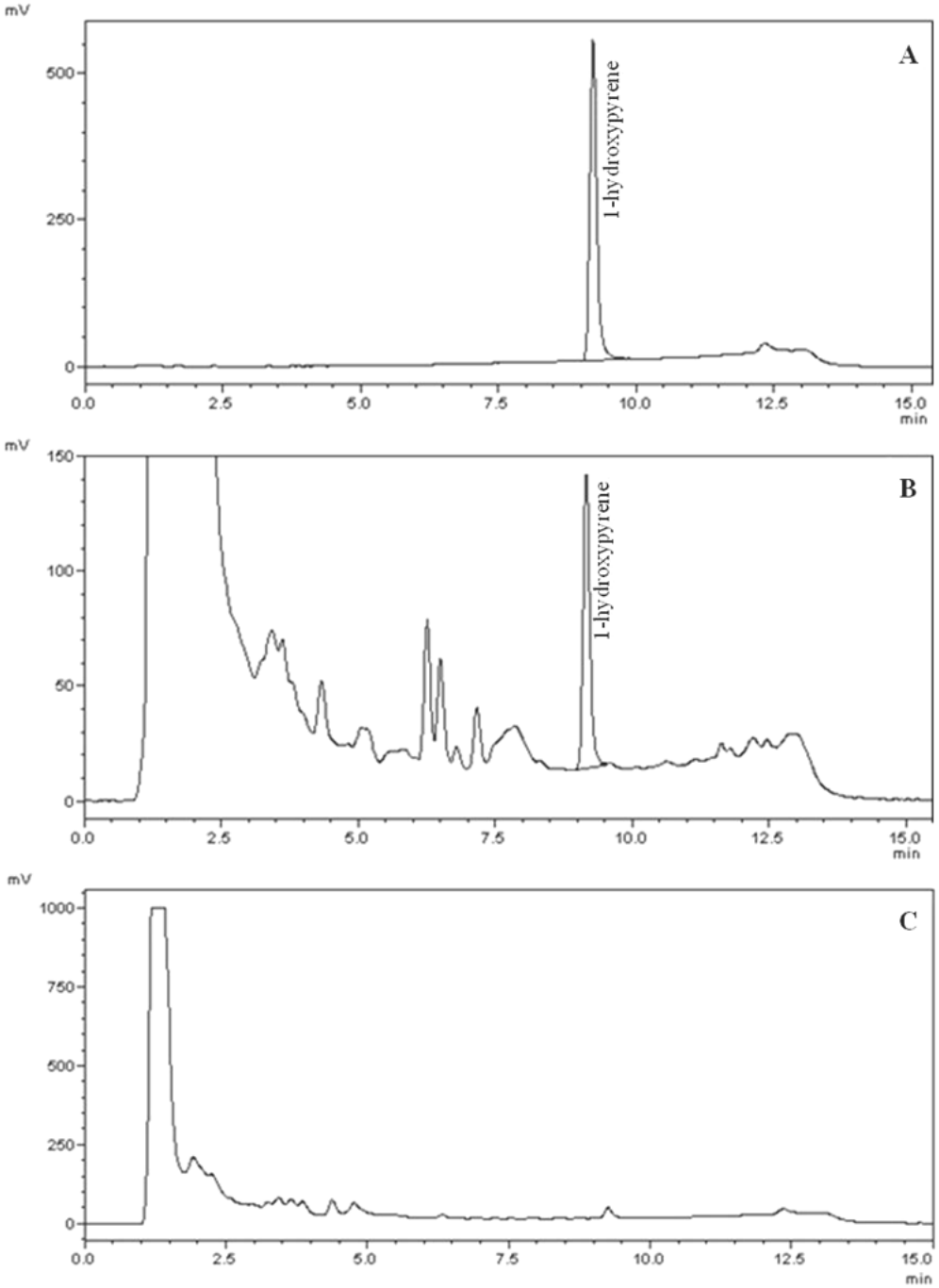

Figure 2: Chromatograms (HPLC/FLD) obtained for 1-hydroxypyrene in standard solution (A), exposed urine (B), and blank urine (C).

Table 3: Percent change[a] in fecal lipids and total phenolics after test-meals compared to baseline.

Measures	BBQ + PH	BBQ + P
Lipid concentration	11.38 ± 38.40	-0.91 ± 24.08
Lipid excreted	14.50 ± 75.52	-7.92 ± 40.01
TP concentration	72.38 ± 89.16*	3.01 ± 40.90
TP excreted	140.07 ± 230.14*	- 6.35 ± 43.25

[a]Means (%) ± SD. BBQ+PH: barbecue + phenolics; BBQ+P: barbecue + placebo. *significantly different at $P < 0.05$ (Student's t-test)

CONSIDERAÇÕES FINAIS

A ingestão de churrasco representa um modo de exposição dietética aos PAHs. Embora os valores encontrados nas carnes estejam de acordo com a legislação estipulada pela União Européia, a exposição recorrente pode levar a um comprometimento do estatus antioxidante do organismo. Deste modo, estudos futuros devem ser direcionados para avaliar a formação de PAHs em maior número de serviços de churrascos, assim como estimar a ingestão de PAHs considerando consumo de carne grelhada *ad libitum* e seus efeitos. Alterações de outros parâmetros metabólicos também podem ser investigados, como relação entre consumo de polifenóis e excreção de PAHs nas fezes, ou ainda diferentes tempos de coletas de amostras.

APÊNDICES

APÊNDICE A

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO
PROJETO: AÇÃO DE COMPOSTOS FENÓLICOS DO AÇAÍ SOBRE INFLAMAÇÃO E ESTRESSE OXIDATIVO PÓS PRANDIAIS

Os compostos fenólicos são compostos naturais encontrados na maioria dos vegetais e frutas, como o açaí, e atuam como antioxidantes protegendo o organismo. Porém, os alimentos em que são encontrados são também ricos em vários outros compostos, também benéficos à saúde, como por exemplo, as vitaminas. Por esse motivo este trabalho tem como objetivo avaliar a ação de compostos fenólicos isolados do açaí, sobre os efeitos que uma refeição rica em gorduras causa no organismo.

Esta pesquisa será dividida em quatro dias (em quatro finais de semana), sendo que haverá consumo churrasco em dois dias durante o almoço e consumo de carne cozida nos outros dias restantes, e sempre será acompanhado da ingestão de cápsulas. Estas irão conter compostos fenólicos isolados do açaí ou placebo. Todos os participantes receberão o placebo em determinado momento da pesquisa, assim como os compostos fenólicos. Os placebos têm a aparência, a textura e o gosto idênticos às cápsulas de açaí, porém não possuem os compostos fenólicos. Algumas vezes é denominado "comprimido de açúcar".

Ao longo do dia, serão realizadas coletas de sangue (por profissional experiente e com garantia de uso de material descartável, portanto não oferecendo risco aos participantes da pesquisa), além da verificação de peso dos voluntários. As coletas acontecerão nas dependências do Laboratório Dr. Paulo Azevedo, onde os voluntários deverão chegar em jejum de 12 horas, em horário que será previamente agendado. Serão fornecidos aos voluntários as seguintes refeições ao longo do dia: café da manhã, almoço e lanche. Amostras de urina e fezes também serão coletadas no dia do experimento, e por 2 dias após o experimento.

Os resultados das análises realizadas serão entregues aos participantes em seu local de trabalho ou em sua residência, tendo garantia de sigilo por parte do pesquisador. Os voluntários serão livres para participar desta pesquisa, assim como para retirar-se a qualquer momento, sem qualquer represália.

_____

Bianca Scolaro
Universidade Federal do Pará
Curso de Mestrado em Ciência e Tecnologia de Alimentos
Telefone para contato: 32129888/8230-9082
e-mail: bianca.scolaro@yahoo.com

Consentimento livre e esclarecido

Declaro que li as informações acima sobre a pesquisa, que me sinto perfeitamente esclarecido sobre o conteúdo da mesma, assim como seus riscos e benefícios. Declaro ainda que, por minha livre vontade, aceito participar da pesquisa cooperando com a coleta de material para exame.

Belém, ___/___/___

_____

Assinatura do sujeito da pesquisa ou do responsável

APÊNDICE B

CARDÁPIO

Café da manhã

Alimento	Medida Caseira	Quantidade
IOGURTE NATURAL	Unidade (120g)	1
PÃO FRANCÊS	Unid. M (50g)	2
QUEIJO TIPO PRATO	Fatia P (10g)	2

Almoço

Alimento	Medida Caseira	Quantidade
LINGÜIÇA PORCO - FRESCA	Unidade (60g)	2
CARNE BOVINA (CUPIM) - FRESCA	Gramas	150
PICANHA - FRESCA	Gramas	150
ARROZ BRANCO COZIDO	Esc. M. Cheia (85g)	2
FARINHA DE MANDIOCA TORRADA	Col. S. Rasa (10g)	4
Vinagrete:		
CEBOLA	Col. S. CH. Picada (16g)	1
CHEIRO VERDE	Col. S. CH. Picada (10g)	1
PEPINO	Col. S. CH. Picada (18g)	1
TOMATE	Col. S. CH. Cubos (15g)	1

Lanche da Tarde

Alimento	Medida Caseira	Quantidade
PÃO DE FORMA	Fatia (25g)	2
REQUEIJÃO LIGHT	Col. S. Cheia (30g)	1
PRESUNTO DE PERU	Fatia P (10g)	1
SUCO DE MARACUJÁ COM AÇÚCAR	Copo (200 ml)	1

Jantar

Alimento	Medida Caseira	Quantidade
FILÉ DE FRANGO COZIDO	Filé M (100g)	1

Obs.: Os voluntários foram orientados a consumir livremente com a carne qualquer fonte de carboidrato, desde que não fosse adicionada de uma fonte de lipídeos. Além disso, restringiu-se também o consumo de frutas e verduras.

ANEXOS

ANEXO 1

SERVIÇO PÚBLICO FEDERAL
UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS DA SAÚDE
COMITÊ DE ÉTICA EM PESQUISA EM SERES HUMANOS

Carta: 166/11 CEP-ICS/UFPA Belém, 26 de março de 2013.

Prof. Dr. Hervé Louis Ghislain Rogez

Senhor Pesquisador,

Temos a satisfação de informar que seu projeto de pesquisa **"AÇÃO DE COMPOSTOS FENÓLICOS DO AÇAÍ SOBRE INFLAMAÇÃO E ESTRESSE OXIDATIVO PÓS-PRANDIAIS" CAEE 0139.0.073.000-11** e parecer nº150/11 CEP-ICS/UFPA foi apreciado e aprovado pelo Comitê de Ética em Pesquisa em Seres Humano CEP-ICS/UFPA, do Instituto de Ciências da Saúde da Universidade Federal do Pará na reunião do dia 5 de outubro de 2011.

Atenciosamente,

Prof. Dr.Wallace Raimundo Araujo dos Santos.
Coordenador do CEP-ICS/UFPA

Comitê de Ética em Pesquisa em Seres (CEP-ICS/UFPA) - Complexo de Sala de Aula/ CCS - Sala 13 - Cidade Universitária Professor José da Silveira Netto, nº 01, Guamá - CEP: 66075-110 - Belém-Pará. Tel.: 3201-7735
e-mail: cepccs@ufpa.br/ Site: www.ufpa.br/ics

ANEXO 2

PRODUCT SPECIFICATION SHEET

AÇAÍ PURE ANTIOXIDANT

2011

OBTENTION MODE: Açaí Pure Antioxidant is obtained from fruits of Euterpe Oleracea through of the processes of aqueous mechanical extraction, concentration onto synthetic resins and freeze-drying.

Registered in the Brazilian Ministry of Agriculture, Livestock and Food Supply under N° 05979 00020-0

Amazon Dreams Indústria e Comércio S.A.
Rua Augusto Corrêa, 01, Guamá, Belém-PA
CEP: 66075-110
+55 91 3269 1001
+55 91 3201 7456
ivonete@amazondreams.com.br

AMAZONDREAMS

Tecnologia em Antioxidantes

REGISTERED IN THE FDA

PRODUCT CHARACTERISTICS

Botanical Name	*Euterpe Oleracea*
Plant Part	Whole fruit
Origin	Brazilian Amazon
Flavour	Characteristic
Colour	Dark purple colour
Appearance	Powder
Preservatives	None
Storage Temperature	room temperature
Availability	July – December

TECHNICAL INFORMATION

PPO (UI)	< 0.01	**Standard Plate Count**	< 1.0×10^3 CFU/ g
Anthocyanins (mg/100g)	> 16,000	**Yeast and Mould**	< 1.0×10^2 CFU/ g
Total Phenolics (mg/100g)	> 45,000	**Total Coliforms**	<3 MPN/ g
$ORAC_{FL}$ (µmol Eq. Trolox/100g)	> 360,000	**Coliforms at 45°C**	< 3MPN/ g
Moisture (%)	< 4.0	***E. Coli***	Absence / 25 g
		Salmonella sp.	Absence / 25 g

PACKAGING

Plastic buckets with inner bags 5-20 kg.

SHELF-LIFE

24 months from shipping date.

APPLICATIONS

Preparation of milk drinks, teas, fruit drinks, energy, nectars, ice cream, dairy desserts, natural dyes, cosmetics, etc.

Recommended in nutrition of diabetics.

Organic product registered by IMO (N° 27225).

Technical Leader: **Quaresma da Silva, Ivonete**