JULIANA KELLY DA SILVA

"*IN VIVO* ANTIOXIDANT POTENTIAL OF PASSION FRUIT (*PASSIFLORA EDULIS*) PEEL"

"POTENCIAL ANTIOXIDANTE IN VIVO DA CASCA DO MARACUJÁ (PASSIFLORA EDULIS)"

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Orientador: Prof. Dr. Mário Roberto Maróstica Júnior

"POTENCIAL ANTIOXIDANTE IN VIVO DA CASCA DO MARACUJÁ (PASSIFLORA EDULIS)"

Master dissertation presented to the Food and Nutrition Postgraduation Programme of the School of Food Engineering of the University of Campinas to obtain the M.D. grade in Food and Nutrition in the Concentration Area of Experimental Nutrition applied to Food Technology.

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Martin Luther King

"Foi o tempo que dedicastes à tua rosa que a fez tão importante."

Antoine de Saint-Exupéry

SUMÁRIO

RESUMO GERAL

O maracujá (*Passiflora edulis*) é uma variedade cultivada no Brasil, destinada principalmente à indústria de suco, mas seu processamento gera muitos resíduos. Estes subprodutos podem ser fontes de compostos bioativos, atuando na prevenção e tratamento do estresse oxidativo e agravos à saúde. Neste sentido, o escopo deste trabalho foi avaliar a ação antioxidante da farinha da casca de *P. edulis* (PPF) em ratos saudáveis e com colite induzida. Também foi avaliada a ação da PPF na microbiota cecal e na produção de ácidos graxos de cadeia curta (AGCC) dos animais saudáveis. A caracterização química mostrou que a PPF possui aproximadamente 48 % de fibra insolúvel e 17 % de fibra solúvel. O teor de fenóis totais na PPF foi 2,53±0,03 mg GAE g^{-1}, de acordo com análise de Folin-Ciocateau.

Nos ensaios biológicos foram utilizados ratos *Wistar* machos (77 dias de idade). Para o preparo da dieta experimental (Peel) 50% do teor de celulose da dieta padrão foram substituídos por fibra de PPF. No primeiro ensaio, os animais foram divididos em 2 grupos: Peel e Control (n=4). Após 15 dias os animais foram anestesiados e sacrificados. O grupo Peel apresentou maior ingestão diária de dieta, sem alteração ponderal, porém mostrou menores níveis de albumina sérica e proteína total em comparação ao grupo Controle. Não houve diferenças no potencial antioxidante sérico de acordo com ensaio de FRAP e TBARS, mas o grupo Peel demonstrou menor capacidade antioxidante comparado ao grupo Controle, de acordo com a análise de ORAC. Nos rins dos animais do grupo Peel houve diminuição na peroxidação lipídica e atividade da enzima Superóxido Dismutase (SOD) em comparação aos animais Controle. Porém, no fígado dos animais que consumiram PFF, houve maior nível de TBARS e atividade de Glutationa Redutase (GR), e redução da atividade de Glutationa Peroxidase (GPx). O teor de AGCC foi maior no conteúdo cecal do

grupo Peel, sem alteração na microbiota ou pH fecal. Estes achados sugerem que a ingestão de PPF pode melhorar saúde intestinal ao promover aumento da produção AGCC, além de reduzir a peroxidação lipídica nos rins em ratos *Wistar*. Há ainda evidências que a PPF possa conter compostos termogênicos.

No segundo ensaio biológico, os animais foram divididos em 4 grupos: Casca Colite, Casca Controle, AIN Colite e AIN Controle (n=6). Após uma semana de adaptação à dieta, foi realizada injeção retal de ácido 2,4,6-trinitrobenzenosulfônico (TNBS) para indução da colite nos animais; no grupo Controle, o procedimento foi realizado utilizando solução salina 0,9 m/v em substituição ao TNBS. Os animais tratados mostraram melhora no potencial antioxidante sérico, segundo os ensaios de TBARS e ORAC, bem como modulação das enzimas antioxidantes SOD (cérebro e fígado), GR e GPx (fígado) em relação aos grupos AIN. Porém, nos grupos Peel houve elevação da peroxidação lipídica no fígado. Apesar da casca de maracujá ser boa fonte de fibras e compostos fenólicos, sendo uma alternativa de ingrediente para indústria de alimentos com ação antioxidante, além de amenizar a geração de resíduos, é preciso investigar melhor seus efeitos a nível hepático.

Palavras chave: Maracujá, *Passiflora edulis*, Antioxidantes, Fibras.

SUMMARY

Passion fruit (*Passiflora edulis*) is a broadly cultivated variety in Brazil and mainly used in the juice industry. Passion fruit processing results in several residues which may represent 50% fruit's gross weight. Fruit byproducts, as the peels, oftentimes have greater amount of bioactive compounds, including antioxidant substances, which could act in the prevention or treatment of the oxidative stress and pathologies. The aim of this study was to evaluable antioxidant action of *P. edulis* peel flour (PPF) in healthy rats, beyond its effect in rats with induced colitis. The determination of PPF proximate composition showed 48 % of insoluble fiber and 17 % of fiber soluble. Total phenolic was 2.53 ± 0.03 mg GAE g^{-1} by Folin-Ciocateau assay.

In the biological assays seventy seven-days-old male *Wistar* rats were used. The experimental diet was prepared with fifty percent of the cellulose content of standard diet replaced by fiber from PPF. In the first assay, the animals were divided in 2 groups: Peel and Control (n= 4). After 15 days of diet intake, the animals were anaesthetized and sacrificed. PPF group consumed greater amount of diet without alterations in body weight of animals, but serum levels of total albumin and protein were lower than Control group. The animals that received PPF in the diet showed no differences in serum antioxidant status (FRAP and TBARS assays), but they presented lower antioxidant potential according to ORAC assay. Animals from Peel group showed a reduction in kidneys lipid peroxidation and SOD activity compared to the control group. In addition, the livers of the animals fed with PPF showed high TBARS levels, increased GR activity and decreased GPx activity. The SCFA in the cecal content were greater in Peel group, without alteration in microbiota or fecal pH. These findings suggest that the PPF intake could improve bowel health by

increasing in SCFA production, further it reduced lipid peroxidation in kidneys. Some evidences show that *P. edulis* peel could contain thermogenic/ ergogenic compounds.

In the second biological assay, the animals were divided in 4 groups: Peel Colitis, Peel Sham, AIN Colitis, AIN Sham (*n*= 6). After one week of adaptation, the colitis induction was performed by rectal injection of TNBS. The animals that received PPF showed improvements in serum antioxidant potential (ORAC and TBARS assays), as well as modulation of antioxidant enzymes activities in brain (SOD) and liver (SOD, GR and GPx) relative to AIN groups. On the other hand, hepatic lipid peroxidation in Peel groups was increased. *P. edulis* peel is a rich source of fiber and phenolic compounds and could be an alternative ingredient to food industry which might avoid the oxidant action and to reduce the residue production. However, more investigations are necessaries to investigate possible hepatic effects.

Keywords: Passion fruit, *Passiflora edulis*, Antioxidant, Fiber.

INTRODUÇÃO GERAL

Atualmente, a busca por fontes naturais de compostos antioxidantes tem se fortalecido tendo em vista o combate ao estresse oxidativo (Ferreres et al., 2007), o qual ocorre diante do desequilíbrio entre os sistemas protetores e a produção de espécies reativas de oxigênio e nitrogênio (ERO/ERN) (Mccord, 2000). Os radicais livres podem danificar lipídeos, proteínas e DNA por ação oxidativa e levar a perda de função ou mesmo morte celular (Kaliora et al., 2006; Libby, 2007; Habib;Ibrahim, 2011). Este fenômeno tem sido associado a algumas doenças, inclusive às doenças inflamatórias intestinais (DII) (Libby, 2007; Rezaie et al., 2007; Durackova, 2010; Martinez et al., 2010).

As DII atingem milhões de pessoas no mundo com sintomas debilitantes que prejudicam tanto o desempenho como a qualidade de vida (Russel, 2000; Loftus et al., 2007). Com alterações que envolvem a imunidade da mucosa e a fisiologia gastrointestinal, possuem etiologia desconhecida e caracterizam-se por curso crônico e recorrente, com período de exacerbação dos sintomas (Cho;Abraham, 2007; Sands;Kaplan, 2007).

A retocolite ulcerativa inespecífica (RCUI) e a doença de Crohn (DC) representam os dois principais fenótipos das DII (Baumgart;Carding, 2007). A hipótese da patogênese destes distúrbios está associada à desregulação imune do trato gastrointestinal, uma vez que a doença é acompanhada por intensa infiltração de células inflamatórias na mucosa intestinal. Contudo, os mecanismos específicos dos danos celulares não estão completamente esclarecidos (Rezaie et al., 2007). O estresse oxidativo pode ser, não apenas um epifenômeno, mas um fator potencial para desencadear e propagar as DII (Rezaie et al.,

2007; Martinez et al., 2010). Supõe-se que o excesso de ERO/ ERN provoque a quebra de diversas linhas de defesa que compõem a barreira da mucosa (Martinez et al., 2010).

Todavia, o excesso de espécies reativas pode estar presente, também, em condições não patológicas (Durackova, 2010). E para evitar os efeitos destrutivos de ERO/ERN, os tecidos contam com a proteção dos sistemas de defesa antioxidante endógeno e exógeno (Rezaie et al., 2007).

Muitas vezes os componentes celulares não são protegidos totalmente por antioxidantes endógenos, sendo então imprescindível a obtenção de antioxidantes pela dieta. O consumo de frutas e hortaliças pode auxiliar na prevenção do estresse oxidativo, visto que estes alimentos são fontes destes compostos (Cerqueira et al., 2007; Habib;Ibrahim, 2011).

O maracujá representa uma boa fonte de componentes fitoquímicos com atividade antioxidante, tais como compostos fenólicos, glicosídeos e alcaloides (Tommonaro et al., 2007; Neuza Jorge, 2009; Zeraik;Yariwake, 2010). Algumas espécies do gênero *Passiflora*, tal como *P. edulis* são amplamente utilizadas para a produção doméstica e industrial de suco, tendo aproveitamento total da polpa, no entanto, as cascas que também são fontes de compostos bioativos, são descartadas como resíduo (Meletti;Maia, 1999; Pietta, 2000; Coleta et al., 2006; Ferreres et al., 2007; Tommonaro et al., 2007). O consumo da casca de *P. edulis* é uma alternativa para aumentar o consumo de antioxidantes e fibras, auxiliando no combate a algumas patologias, como as DII, além de amenizar a geração de remanescentes do processo industrial e seu impacto ambiental (Oliveira et al., 2002; De Oliveira et al., 2009).

Nesse sentido, o escopo do presente trabalho foi investigar a atividade antioxidante da farinha da casca de *P. edulis* em ratos *Wistar* em condições normais e, em situação de

maior estresse oxidativo durante a colite experimental induzida com ácido 2,4,6-trinitrobenzesulfônico (TNBS).

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CAPÍTULO 1 – Efeito dos compostos bioativos sobre estresse oxidativo e a inflamação intestinal: uma revisão

(Artigo em fase de preparação para envio à revista Alimentos e Nutrição)

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RESUMO

O estresse oxidativo ocorre com a alta produção de espécies reativas de oxigênio ou nitrogênio acima da capacidade de defesa antioxidante do organismo. Estudos têm relacionado este fenômeno a muitas patologias, inclusive a processos inflamatórios. Inflamação crônica e recorrente é característica das doenças inflamatórias intestinais (DII) as quais acometem milhões de pessoas no mundo. A etiologia e a perpetuação destas patologias ainda não são bem esclarecidas. Mas recentemente, investigações tem atribuído ao desequilíbrio oxidativo um papel importante no início e propagação das DII. Diante deste fato, antioxidantes presentes em alimentos podem contribuir para evitar o estresse oxidativo e, consequentemente, prevenir e amenizar agravos à saúde. Polifenóis são antioxidantes abundantes em alimentos de origem vegetal e com grande potencial para combater a geração de espécies reativas. Dentre as muitas fontes alimentares, estes compostos podem ser encontrados, até em maior concentração, em porções normalmente descartadas na produção agrícola e industrial. O maracujá (*Passiflora edulis*) é uma das fontes alimentares de compostos antioxidantes e sua casca é, também, fonte de fibras, solúveis e insolúveis. A ação das bactérias entéricas sobre as fibras solúveis pode levar a produção de ácidos graxos de cadeia curta. Aos quais, além do papel em manter a saúde intestinal, têm sido atribuído efeito anti-inflamatório. O objetivo deste trabalho foi efetuar uma revisão a respeito da atuação do estresse oxidativo na inflamação, principalmente a inflamação intestinal, destacando-se o papel protetor de compostos bioativos como aqueles presentes no maracujá e na sua casca.

Palavras chave: Estresse oxidativo, Inflamação, Alimento Funcional, Antioxidantes, Maracujá.

ABSTRACT

The oxidative stress occurs by highest production of oxygen or nitrogen reactive species apart from antioxidant defenses of organism. Reports have associated this phenomenon to some pathologies, including inflammatory processes. The inflammatory bowel diseases (IBD) affect millions of people worldwide. IBD are characterized by chronic and recurrent inflammation. The etiology and maintenance of these diseases are not well known. Recent investigations have been liked the oxidative misbalance with the start and propagation of IBD. Against this fact, antioxidants in foods may contribute to avoid the oxidative stress and, consequently, to prevent and to ease health problems. Polyphenols are abundant in vegetal foods and have strong potential to oppose the reactive species production. Among the many dietary sources, these compounds may be found in parts normally non-edible of fruits and vegetables. The passion fruit (*Passiflora edulis*) is an antioxidant source, and its peel is also fiber source, soluble and insoluble fibers. The fermentation of the fibers by enteric bacteria produces short-chain fat acids which are known to contribute to intestine health, reducing inflammation. The aim of this paper was elaborated a review about the action of oxidative stress on inflammation, mainly bowel inflammation, with highlight to the protector role of bioactive compounds, as those present on passion fruit and its peel.

Keywords: Oxidative stress, Inflammation, Functional Food, Antioxidants, Passion Fruit.

1. INTRODUÇÃO

O estresse oxidativo é causado pelo desbalanço entre os sistemas protetores e a produção de espécies reativas de oxigênio e nitrogênio (ERO/ERN) [50]. O excesso de espécies reativas pode danificar vários componentes celulares e provocar perda de função ou mesmo morte celular [35,45,28]. Atualmente algumas doenças, inclusive as doenças inflamatórias intestinais (DII), têm sido associadas a este desequilíbrio oxidativo [45,63,20,49].

As duas principais formas de DII são retocolite ulcerativa inespecífica (RCUI) e a doença de Crohn (DC), que têm em comum alterações que envolvem a mucosa com curso crônico e recorrente [12,66]. Os mecanismos que levam ao dano celular não são bem conhecidos [63]. Acredita-se que o estresse oxidativo, por meio do rompimento de linhas de defesa da mucosa, esteja envolvido no desencadeamento da DII bem como na sua propagação [63,49].

Contudo, a produção de ERO/ERN não é fenômeno exclusivo de situações patológicas, ocorre também sob condições fisiológicas [20]. Tendo em vista a proteção contra os efeitos negativos da ação oxidativa destes compostos, o organismo possui sistemas de defesa antioxidante [63]. As defesas endógenas, formadas principalmente por enzimas antioxidantes, são muitas vezes incapazes de evitar totalmente os danos provocados por espécies reativas. Desta forma, antioxidantes de origem dietética podem complementar o aparato de defesa do organismo contra o estresse oxidativo [11,28].

Os polifenóis são compostos com forte potencial de combate aos radicais livres e estão presentes em muitas frutas e vegetais [3,64]. O maracujá representa uma boa fonte destes componentes fitoquímicos [75,56,79]. Mas, esta fruta não é consumida integralmente, a polpa é

utilizada produção de suco, levando a grande geração de resíduos, como as cascas, algumas propriedades funcionais desta porção já têm sido relatadas na literatura [51,62,14,23,75]. Da mesma forma que a porções não habitualmente consumidas de outros vegetais têm sido associados também efeitos benéficos à saúde [41,27,44,43].

O pericarpo do maracujá é, ainda, fonte de fibras que são substrato para a fermentação no colón e podem aumentar a produção de ácidos graxos de cadeia curta (AGCC) [52,7,74], os quais têm efeitos benéficos sobre o intestino e podem desempenhar ação anti-inflamatória [52,74,76].

2. ESPÉCIES REATIVAS DE OXIGÊNIO E NITROGÊNIO (ERO/ ERN) E O ESTRESSE OXIDATIVO

As ERO/ERN, tais como radical superóxido, peróxido de hidrogênio, radical hidroxil, oxigênio singlete, hidroperóxido de lipídeo, peroxinitrito, entre outros, são produtos do metabolismo normal do oxigênio/nitrogênio ou decorrentes de situações patológicas e fatores ambientais como tabagismo, radiação ultravioleta, poluição. Estas moléculas, popularmente conhecidas como radicais livres, são altamente reativas devido à presença de elétrons desemparelhados e capturam elétrons de outras substâncias para se neutralizarem [35,63,20,28]. No ataque inicial o radical livre se neutraliza, contudo, há formação de outra espécie reativa, resultando em reações em cadeia [35].

Muitas reações que envolvem espécies reativas são essenciais em mecanismos de defesas, como aquelas que envolvem neutrófilos e macrófagos, ou em várias reações bioquímicas, como por exemplo, reações de hidroxilação, carboxilação ou peroxidação [20]. O estresse oxidativo ocorre se houver um desbalanço entre pró e antioxidantes com

acúmulo de radicais livres [50]. O estresse oxidativo pode levar a danos aos constituintes essenciais das células como as membranas lipídicas, proteínas e DNA, o que resulta em perda de função e até mesmo morte celular [35,45,28]. Além disto, as ERO/ERN também podem promover a ativação de neutrófilos potencializando a produção de moléculas oxidativas, com consequente, disparo ou agravamento das repostas inflamatórias [45]. Todavia, o excesso de espécies reativas pode estar presente, também, em condições não patológicas [20].

3. INFLAMAÇÃO E ESTRESSE OXIDATIVO

A inflamação advém de condições pré-existentes (infecção ou injúria) ou de fatores genéticos, onde agentes quiomiotáxicos como prostaglandinas, leucotrienos, tromboxanos, produtos bacterianos e virais provocam a migração de células imunes dos vasos sanguíneos e a liberação de mediadores inflamatórios, seguida do recrutamento de células inflamatórias, produção de espécies reativas e citocinas pró-inflamatórias [60]. A produção de radicais livres acontece por meio da ação das células fagocitárias que reduzem O_2 a O_2^- no processo inflamatório, por ação da NADPH oxidase, com o objetivo de destruir agentes invasores [15]. No entanto, a inflamação prolongada pode causar distúrbios crônicos devido à produção contínua de moléculas reativas de oxigênio/nitrogênio [8].

O estresse oxidativo agudo bem como estresse oxidativo crônico têm sido relacionados a doenças inflamatórias, com a retocolite ulcerativa [11,45,63,49]. A superprodução de radicais livres durante episódios ativos de DII pode ultrapassar os mecanismos protetores e resultar em dano oxidativo a células e tecidos [61,8].

Os agravos oriundos do processo inflamatório crônico não se limitam ao tecido inflamado, uma vez que o excesso de mediadores inflamatórios e oxidantes atinge, também,

tecidos normais [45,60], provocando dano oxidativo sistêmico e refletindo em perda da seletividade da membrana celular, inibição das funções normais de órgãos e tecidos, e distúrbios sistêmicos [45]. Portanto, no curso da inflamação, o estresse oxidativo pode ser um potencial desencadeador e coadjuvante do processo, além de estar presente a nível local e sistêmico [45,63,60,49].

4. DOENÇAS INFLAMATÓRIAS INTESTINAIS (DII)

As doenças inflamatórias intestinais (DII) são distúrbios imunológicos crônicos intermitentes de etiologia desconhecida que afetam milhões de pessoas no mundo. Sua prevalência é maior em países desenvolvidos, como nos Estados Unidos e países do norte da Europa, onde 1,4 e 2,2 milhões de pessoas, respectivamente, são portadoras de DII. Porém, atualmente, os níveis nestas regiões estão se estabilizando, enquanto a incidência em regiões como sul e centro da Europa, Ásia, África e América do Sul, nas quais a prevalência hoje é baixa, há ascensão [72,47].

As principais formas de manifestação das DII são a retocolite ulcerativa inespecífica (RCUI) e a Doença de Crohn (DC) [65,4,46]. A inflamação crônica da mucosa intestinal, que resulta frequentemente em dor abdominal, febre e diarreia, é característica tanto da DC quanto da RCUI. A distinção entre as duas formas de inflamação intestinal ocorre em relação à localização, distribuição e acometimento do tecido, como apresentado na tabela 1 [12].

Tabela 1. Características diferenciais entre DC e a RCUI [12]

	DOENÇA DE CROHN	RETOCOLITE ULCERATIVA
Localização	Todo trato gastrointestinal	Cólon
Distribuição das lesões	Descontínua	Contínua
Tecido afetado	Transmural	Mucosa e submucosa
Anatomia patológica	Granulomas	Abscessos de criptas
	Agregados linfóides	Depleção da mucina
	Fibrose	Distorção glandular

As DII são consideradas doenças multifatoriais, pois, apesar da origem não estar clara, acredita-se que suscetibilidade genética, alterações do sistema imune, condições de higiene, composição da microbiota do hospedeiro e fatores ambientais como hábitos alimentares, tabagismos, estresse, podem contribuir para o desencadeamento desta patologia [17,48,78,38,16]. O processo inflamatório pode ser provocado por uma resposta imune desregulada às bactérias entéricas em indivíduos geneticamente predispostos, sob ação de fatores ambientais e imunológicos [55]. Há, ainda, evidências de que a DC e a RCUI estão associadas ao maior risco de câncer de colón [38].

5. BARREIRA DA MUCOSA E AS DII

A barreira do epitélio gastrointestinal é a primeira linha de defesa do sistema imunológico contra agentes agressores. Este efeito é assegurado por células caliciformes e células de Paneth que atuam na produção de muco e defensinas (ação antimicrobiana), respectivamente [21,55]. Faure, Moennoz *et al.* [21] demonstraram a associação entre a RCUI e a menor produção de mucina, com consequente aumento na exposição da mucosa a potenciais agentes agressores, resultados estes observados em ratos da linhagem Sprague-

Dawley após indução de RCUI por Dextran sulfato de sódio (DSS) 5%, no qual houve diminuição na expressão de RNAm de mucina 2.

O epitélio intestinal íntegro, portanto, separa as células da mucosa de componentes presentes no lúmen entérico e regula o tráfego normal de substâncias/ micro-organismos através destes compartimentos. Assim, uma disfunção da barreira intestinal pode gerar um elevado influxo e aumento da translocação bacteriana e de antígenos, o que promove a sobrecarga do sistema imune intestinal e pode induzir a resposta inflamatória [70] (Figura 1).

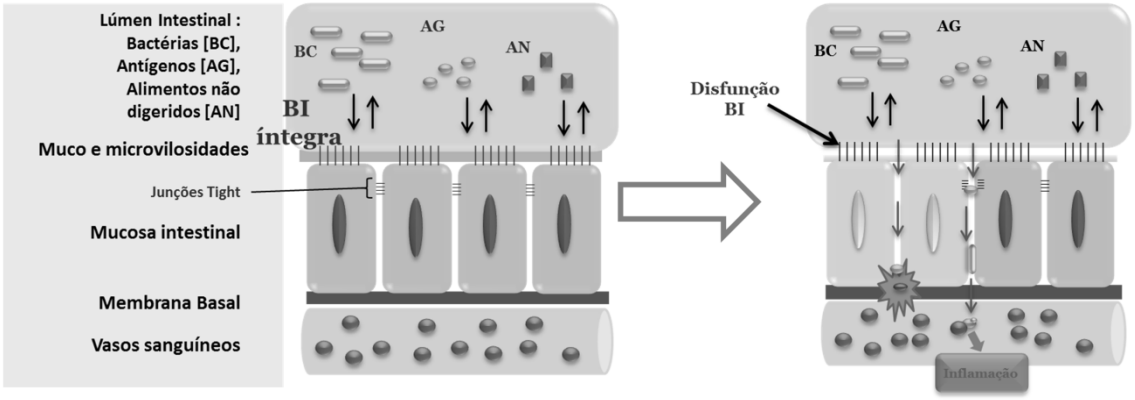

Figura 1 – Mucosa intestinal íntegra e com disfunção. Bactérias entéricas ou patógenas (BC); antígenos (AG); alimentos não digeridos (AN).

Na lâmina própria intestinal encontram-se células imunes às quais promovem o balanço entre tolerância à microbiota do trato gastrointestinal e defesa contra micro-organismos patógenos [55]. Neste sentido, o intestino está constantemente em um estado de "inflamação fisiológica" (processo controlado) diante da grande variedade de antígenos dietéticos e microbianos a qual é exposto diariamente [24,55].

De acordo com Sands & Kaplan [66], a mucosa intestinal possui, em estado normal, uma população residente de células com potencial inflamatório, e estas células estão na

interface entre o lúmen intestinal e a circulação sistêmica em prontidão para promover inflamação entérica e outros danos à mucosa. Quando ocorre uma incursão, a resposta inevitável é a inflamação e o que diferencia os indivíduos com DII de não portadores é a capacidade de regular negativamente esse estado inflamatório e manter uma condição de normal. Indivíduos suscetíveis a DII tendem a entrar em estado de inflamação crônica não controlada devido à incapacidade de regular negativamente este processo inflamatório [66]. Além do desiquilíbrio na resposta às bactérias comensais, a disbiose também tem sido associada ao desenvolvimento de DII, sendo marcada por um aumento da população de bactérias patógenas aderentes ao trato gastrointestinal em detrimento da colonização da microbiota residente [55].

Portanto, acredita-se que o estresse oxidativo pode ser, não apenas um epifenômeno, mas um fator potencial para desencadear e propagar as DII [63,49]. Supõe-se que o excesso de espécies reativas de oxigênio (ERO) provoque a quebra de diversas linhas de defesa que compõem a barreira da mucosa, representadas pelo muco que recobre os enterócitos, a membrana citoplasmática, o complexo de junções intercelulares e a membrana basal. Tais alterações podem permitir a translocação bacteriana para a submucosa, e, na tentativa de combater a invasão, células polimorfonucleadas (PMN), principalmente neutrófilos, migram para a região intestinal, iniciando a resposta inflamatória característica das doenças [49].

Logo, atualmente muitas hipóteses têm sido levantadas para tentar explicar as causas das DII, entre as quais a disfunção da barreira intestinal associada à intolerância da mucosa a substâncias patogênicas, fatores dietéticos e/ou aos componentes endógenos da microbiota compõem a vertente mais forte, todavia, os mecanismos que desencadeiam estes eventos permanecem incógnitos [73]. Apesar de não estar claramente estabelecido o

mecanismo, acredita-se que estresse oxidativo pode estar envolvido na patogênese e propagação das DII [45,63,60,49].

6. SISTEMAS DE DEFESA ANTIOXIDANTE

O sistema de defesa contra o estresse oxidativo pode ser endógeno ou exógeno. O sistema endógeno controla as concentrações de radicais livres por meio da ação das enzimas glutationa peroxidase (GPx), catalase (CAT), superóxido dismutase (SOD) e pelo sistema não enzimático formado principalmente por grupos tiol como a glutationa (GSH) [29]. Já o sistema exógeno constitui-se de substâncias antioxidantes alimentares (ácido ascórbico, tocoferol, carotenoides, compostos fenólicos e outras substâncias bioativas) [35,28].

Muitas vezes os componentes celulares não são protegidos totalmente pelos antioxidantes endógenos, sendo então imprescindível o fornecimento de antioxidantes exógenos ao organismo. O consumo de frutas e hortaliças pode auxiliar na prevenção do estresse oxidativo, visto que estes alimentos são fontes de antioxidantes [11,28].

Desta forma, o consumo de vegetais está associado ao menor risco de desenvolvimento de doenças crônicas, sendo que estudos mostram que os compostos fenólicos possuem atividade antioxidante, anti-inflamatória e neuroprotetora, sugerindo que eles podem contribuir para as propriedades de proteção à saúde de alimentos vegetais [32].

Antioxidantes são substâncias que, presentes em baixa concentração, inibem ou retardam a oxidação do substrato, prevenindo os danos causados por espécies reativas. Tradicionalmente, os antioxidantes são divididos em primários e secundários. Aqueles que inibem as fases de iniciação e propagação da formação de espécies reativas denominam-se antioxidantes primários. Os antioxidantes secundários ou preventivos reduzem a taxa de

oxidação, como por exemplo, através da quelação de metais. A ação antioxidante depende tanto da reatividade quanto da concentração da substância bioativa [3].

Nos últimos anos, os antioxidantes têm ganhado destaque por serem potenciais agentes profiláticos e/ou terapêuticos em muitas doenças [35]. Os principais mecanismos de ação destes compostos incluem captadores de radicais e supressores de estados excitados, ou seja, sistemas catalíticos que neutralizam ou eliminam ERO/ERN [3,60].

7. COMPOSTOS BIOATIVOS DIETÉTICOS

O consumo de compostos bioativos pela dieta é um fator protetor, adicional ao sistema antioxidante próprio do organismo, para manter o equilíbrio do estado redox da célula. Estes complexos mecanismos de proteção antioxidante interagem e atuam sinergicamente para neutralizar os radicais livres [35].

Os mecanismos de ação de alguns antioxidantes como o ácido ascórbico e α-tocoferol são bem conhecidos, assim como os carotenoides e polifenóis vêm sendo estudados para determinação de suas formas de atuação, absorção, biodisponibilidade e recomendações de consumo [11]. Há evidências de que os compostos fenólicos presentes nos cereais, frutas e vegetais sejam os principais fatores que contribuem para a baixa incidência de doenças crônicas e degenerativas encontradas em populações cujo consumo destes alimentos é elevado [64].

Dentre os compostos bioativos naturais, os polifenóis são antioxidantes abundantes na dieta e estão entre as substâncias com atividades biológicas mais potentes e úteis terapeuticamente [3,60]. As substâncias fenólicas podem atuar como antioxidantes de diferentes maneiras: interromper as reações dos radicais livres pela doação de prótons H+, quebra de íons metálicos e sequestro de espécies reativas do oxigênio [62,2,30].

Os polifenóis são metabólitos secundários sintetizados por plantas que podem contribuir para pigmentação, atração de polinizadores e defesa dos vegetais, com ação antimicrobiana, antioxidante e proteção UV. Muitos destes compostos fenólicos são essenciais à vida das plantas, por exemplo, fornecendo defesa contra ataques microbianos e tornando o seu sabor desagradável para os predadores herbívoros [3]. Estes compostos têm em comum a presença de grupos hidroxilas nos anéis aromáticos e formam o grupo de fitoquímicos de maior ocorrência; seus representantes podem ser encontrados em alimentos e bebidas como chá, café, vinho, cereais, vegetais, legumes e frutas [34]. O consumo diário de compostos fenólicos pode atingir 1 g, o que é muito maior que o consumo de todos os outros fitoquímicos classificados como antioxidantes [3,59,60].

Khalatbary & Ahmadvand [39] demonstraram que um polifenol isolado foi capaz de reduzir a reposta inflamatória decorrente de um trauma induzido na medula espinhal em ratos (Spargue-Dawley), através da redução da expressão de mediadores inflamatórios. Karlsen *et al.* [37] em um estudo clínico com indivíduos com alto risco para doenças cardiovasculares verificaram que a ingestão de suco de mirtilo favoreceu redução significativa nas concentrações plasmáticas de citocinas pró-inflamatórias.

Ademais, efeito positivo no 'status' oxidativo e nos níveis de LDL-oxidada foi observado em pacientes em hemodiálise, após consumo de suco concentrado de uva vermelha (50 mL /dia, por duas semanas) [10]. A suplementação dietética com casca de jabuticaba, fruta rica em antocianinas, promoveu melhora no potencial antioxidante plasmático de ratos Spargue-Dawley [44]. Estes achados corroboram com a proposta de efeitos anti-inflamatórios e antioxidantes advindos de alimentos ricos compostos bioativos ou compostos fenólicos isolados [37].

8. SUBPRODUTOS DA PRODUÇÃO AGROINDUSTRIAL COMO FONTE DE COMPOSTOS BIOATIVOS

A maior parte da produção (96%) de frutas cítricas é destinada a indústria de suco nos países produtores [69]. Os remanescentes da produção de sucos cítricos, entre casca e sementes, podem chegar a 50% do peso da fruta integral e são potenciais fontes de compostos bioativos [6,69]. As partes frutas, vegetais e plantas medicinais remanescentes da agricultura e do processamento industrial podem conter grande quantidade de funcionais compostos [34].

Estudos têm demonstrado o poder antioxidante de alguns subprodutos como cascas de uvas, jaboticaba, *berries* e outros vegetais [41,27,44,43]; além de resíduos da produção de azeite e as folhas de oliva [31,40].

Partes normalmente não consumidas de muitas frutas apresentam maior teor de compostos fenólicos e atividade antioxidante, por exemplo, na polpa da maçã o conteúdo de fenóis totais foi 118 mg ácido gálico equivalente/ g matéria seca [67], enquanto a quantidade encontrada na casca foi quase 3 vezes superior [77]. A mesma tendência foi observada comparando-se a casca e sementes de tomate à sua polpa [26].

Desta maneira, a utilização de partes normalmente descartadas no processamento de frutas e vegetais pode ser otimizada com emprego na própria indústria de alimentos para evitar a alterações oxidativas em alguns produtos e agregar qualidade e valor nutricional aos alimentos, enquanto a contaminação ambiental também pode ser amenizada. Contudo, mais estudos são necessários para determinar as propriedades destes subprodutos e avaliar a viabilidade de sua utilização.

9. *PASSIFLORA EDULIS*

O gênero *Passiflora* compreende cerca de 450 espécies, popularmente denominadas maracujás, *Passiflora edulis* é a espécie mais conhecida e utilizada [14,5]. O maracujá-azedo ou amarelo (*P. edulis*) é o mais comercializado no Brasil devido à qualidade de seus frutos. Essa espécie é cultivada em quase todo o território nacional, destacando-se como principais produtores os Estados do Pará, Bahia, Sergipe, São Paulo e Minas Gerais que, juntos, são responsáveis pela liderança brasileira na produção mundial dessa fruta [51]. Todavia, a importância econômica do maracujá refere-se à produção de suco concentrado, onde há o aproveitamento total da polpa, porém as cascas são descartadas como resíduos [51,58,75].

O maracujá é uma boa fonte de componentes fitoquímicos com atividade antioxidante, tais como compostos fenólicos, glicosídeos e alcaloides [75,56,79]. Estudos revelam alto teor de flavonoides totais em *Passiflora ssp.*, contudo, a maioria dos compostos não foi ainda identificada. Atualmente, estudos têm determinado alguns compostos fenólicos no gênero *Passiflora* como C-glicosil devirados de apigenina e luteolina, como vitexina, isovitexina, orientina, schaftoside, 2″-O-rhamnoside and luteolin 7-O-(2-rhamnosylglucoside), os quais podem ter aplicações terapêuticas como antioxidante [53,14,22,75], mas a maioria dos estudos refere-se às folhas de *Passiflora*.

Além de fonte de polifenóis, o pericarpo do maracujá é rico em fibras solúveis e insolúveis que sob ação de bactérias entéricas, podem ser degradadas e originar AGCC como o propionato e o butirato [52,7,74]. O butirato além de representar fonte primária de energia para os colonócitos e atuar sobre a secreção de muco, fluxo vascular, permeabilidade da barreira, motilidade e absorção de água e eletrólitos [52,74]; assim como o propionato, apresenta capacidade anti-inflamatória ao inibir a ativação do fator nuclear κB

(NF-kB), por meio da inibição da via histona deacetilase e diminuição da expressão de citocinas pró-inflamatórias [68,18,25,54,76].

Outro papel dos AGCC no controle da inflamação refere-se à atividade de regulação da expressão de genes que codificam a via iNOS (óxido nítrico sintase indutível) exercida pelo NF-kB [13,25]. A produção elevada de óxido nítrico (NO), que ocorre durante a inflamação, associa-se ao estresse oxidativo e a sinalização para a apoptose celular, como observado em modelos experimentais e em humanos [1,9,25]. O NF-kB ativado promove a expressão de genes inflamatórios como TNF-α, óxido nítrico sintetase induzível (iNOS) e ciclooxigenase (COX) que, então, induzem a ativação de linfócitos, monócitos e células endoteliais [36]. Os AGCC, possivelmente, são uma alternativa para diminuir os níveis de NO e o estresse oxidativo em pacientes com DII, pois inibem a ativação do NF-kB e degradação de seu fator de inativação o IkBα por meio do impedimento da fosforilação da IkK, o fator de ativação e, consequentemente, reduzindo a expressão de iNOS e de outros mediadores inflamatórios [68,13,33].

Além do papel na homeostase intestinal [71], aos AGCC têm sido atribuída inibição da síntese de colesterol, prevenção de câncer e de outras patologias [42].

Desta forma, subprodutos do maracujá (*P. edulis*), como as cascas, podem ser uma alternativa para aumentar o consumo de antioxidantes e fibras, bem como auxiliar no combate a algumas patologias, como as DII, além de amenizar a produção de resíduo industrial e seu impacto ambiental [57,19].

10. CONCLUSÃO

O estresse oxidativo tem sido associado a doenças crônicas dentre elas as doenças inflamatórias intestinais. Acredita-se que o excesso de espécies reativas pode contribuir

para romper a defesas da mucosa intestinal e desencadear o processo inflamatório. Este também está atrelado à propagação da doença. Compostos de origem alimentar com ação antioxidante auxiliam a manter o equilíbrio oxidativo. O maracujá além de ser fonte de polifenóis, também pode contribuir para aumentar o aporte de fibras na dieta por meio do aproveitamento da casca. A fermentação intestinal das fibras presentes na casca do maracujá pode elevar a produção de ácidos graxos de cadeia curta, aos quais, além de promoção à saúde do ambiente intestinal, têm sido atribuída ação anti-inflamatória. Portanto, compostos bioativos, com efeito antioxidante, anti-inflamatório, dentre outros, podem ter benéfico efeito sobre o estresse oxidativo e o processo inflamatório, levando a prevenção ou amenização de patologias, como as DII.

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CAPÍTULO 2 - Passion fruit (*Passiflora edulis*) byproduct increases colonic production of short chain fatty acid and modulate antioxidant status of *Wistar* rats

(Artigo em fase de preparação para envio à revista Journal of Medicinal Food)

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ABSTRACT

Passion fruit (*Passiflora edulis*) peel is a byproduct of industrial processing of fruit, not widely explored; it contains fibers and other bioactive substances. The aim of this work was to investigate the antioxidant potential of *Passiflora edulis* peel and its effect on colonic production of short chain fatty acid (SCFA) in *Wistar* rats. Seventy seven-days-old male *Wistar* rats were divided in 2 groups: Peel and Control (n= 4). Both groups were fed with standard diet (AIN-93M), however fifty percent of the cellulose content in Peel group diet was replaced by fiber from *P. edulis* peel flour (PPF). After 15 days, the animals were anaesthetized and sacrificed. PPF group consumed greater amount of diet without alterations in body weight of animals, but serum levels of total albumin and protein were lower than Control group. The animals that received PPF in the diet showed no differences in serum antioxidant status (FRAP and TBARS assays) (P> 0.05), but they presented lower antioxidant potential according to ORAC assay (P< 0.05). In the kidneys of Peel group, it was observed a reduction in lipid peroxidation (by TBARS assay) (P< 0.05). In addition, the livers of the animals fed PPF, showed high TBARS levels, increased GR activity and decreased GPx and SOD activity. The SCFA in the cecal content were greater in Peel group (P< 0.05), without alteration in microbiota or their pH (P> 0.05). These findings strongly suggest that the *Passiflora* peel flour could improve bowel health by increasing in SCFA production. And, some evidences show that *P. edulis* peel could contain thermogenic/ ergogenic compounds.

Keywords: Antioxidant potential; Bioactive compounds; Dietary Fibers; Oxidative stress; *Passiflora edulis* peel; Short Chain Fat acid.

1. INTRODUCTION

Passion fruit (*Passiflora edulis*) is a native specie from Brazil explored in fruit culture [1, 2]. The country is the biggest World producer of this fruit, mainly of yellow passion fruit, and accounted for 85% worldwide marketed passion fruit in 2012 [3, 4].

Nearly 40% passion fruit production is directed to juice industry with use only of edible parts [2, 5]. The processing generates a large quantity of by-products (seeds and peels) that contain interesting substances that could be used for some applications, especially because it contains large amounts of fiber and phenolic compounds [6]. Powders of industrial residues of passion fruit showed five times more total phenolic compounds than the pulp of this fruit (103 ± 10.4 and 20 ± 2.6 mg GAE $100g^{-1}$, respectively). And the extract of this powder retarded significantly lipid peroxidation in a peroxyl radical-mediated lipid peroxidation membrane model [7].

The excess of reactive species might result in loss of function and even cellular death by to damage cell lipids, proteins and DNA [8-10]. The so-called oxidative stress has been linked to many diseases such as coronary heart disease, diabetes, obesity, and cancer [11]. Although, reactive species are also generated in physiologic metabolism, the exposition to environmental factors such as pollution, sunlight and cigarette smoke may be increase the production of these species [9, 12].

The organisms use endogenous and exogenous antioxidant defenses to protect against harms of oxygen and nitrogen reactive species. They are classified in enzyme system like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD); and non-enzyme system: thiol reduced (GSH), vitamins, minerals and

polyphenols[13]. Nevertheless, oftentimes endogenous antioxidants are not able to prevent oxidative damages, requiring the supply of exogenous scavengers, such that from diet [8, 14].

In this way, the interest in fruits has been increased mainly due to the presence of potential antioxidant substances in these foods, since their intake may contribute to avoid oxidative stress [8, 14-17]. Despite of edibles whole fruits and the vegetables, their non-edibles typically parts could also be a good alternative to add dietary bioactive compounds, as polyphenols [18].

Polyphenols are a group of secondary metabolites presents in fruits and vegetables with antioxidant action [19]. Currently, several phenolic compounds have been identified on *Passiflora* species, like C-glicosil derivatives apigenin and luteolin, as vitexin, isovitexin, orientin, schaftoside, 2″-O-rhamnoside and luteolin-7-O-(2-rhamnosylglucoside) [3, 20, 21]. Passiflora extracts have been showed therapeutic effect as immunomodulators, anticarcinogens and also antioxidants are often attributed to these compounds [3, 22, 23].

Passion fruit pericarp (albedo – white part and flavedo- yellow part), in addition to phenolic compounds, is rich in soluble and insoluble fibers with about 70% fibers[24]. Insoluble fiber is reported to reduce bowel transit time, while soluble fibers are involved in the reduction of both blood cholesterol and intestinal glucose absorption [25]. Passion fruit contains large amounts of pectin, a complex carbohydrate from plants with technological and physiological function [25]. It is the major substratum to fermentation by enteric bacteria and production of short chain fatty acids (SCFA): butyrate, propionate and acetate [26-28] which have positive action on health intestine [26, 28].

This work intended to investigate the *in vivo* antioxidant potential of *Passiflora edulis* peel and its effect on colonic microbiota and production of SCFA in *Wistar* rats.

2. MATERIAL AND METHODS

2.1. Chemicals

L-glutathione reduced, Glutathione reductase, glutathione oxidized form disodium salt, β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH), hypoxanthine, xanthine oxidase, nitrotetrazolium blue chloride (NTB), 5′5′-dithio-bis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX); 2,2′-azobis(2-methylpropionamidine) dihydrochloride (APPH), 2-thiobarbituric acid (TBA), SCFA standards (Acetic, propionic and butyric acids), L-cisteína and dicloxacillin were all obtained from Sigma–Aldrich (São Paulo – Brazil). Fluorescein sodium salt and metaphosphoric acid were purchased from Vetec Química Fina (Sao Paulo – Brazil). Malondialdehyde standard (MDA) was purchased from Chayman Chemical Company (#10009202). MRS agar and peptone water were purchased from Oxoid (UK).

2.2. *Passiflora edulis* peel flour (PPF)

Organic fruits of *Passiflora edulis* from the city of Torre de Pedra, São Paulo, Brazil, harvested in June 2010, were used to produce the peel flour. The fruits were cleaned and separated into pulp and peel (epicarp + mesocarp). Peels were cut in small pieces and dried in oven with air circulation at 50°C (Marconi, Piracicaba/SP - Brazil) until achieve about 10% moisture. Dried samples were grounded into a fine powder using a hammer mill (20 mesh). Water activity of PFF was evaluated in AquaLab equipment (Pullman, WA, USA) at 24.7 ºC. And PPF was stored in amber flask at room temperature (24 °C) to further analysis.

2.3. Fiber content in PPF

Total dietary fiber and insoluble fractions were determined in the peel powder by the enzymatic method [29]. Soluble fraction was calculated by the difference between total dietary and insoluble fiber.

2.4. In vivo experimental design

The study was approved by the Institutional Animal Care and Use Committee (protocol #2385-1, CEUA, UNICAMP, Brazil). The animals were cared for in accordance with the institutional ethical guideline. Male *Wistar* rats were allocated under controlled conditions of temperature (22 °C ± 2), humidity (60 – 70 %), and a light-dark cycle (12/12 h). Seventy seven-days old rats were randomized into 2 groups (n= 4): Peel and Control. Both groups were fed with standard diet (AIN-93M) [30], however, it was replaced 50% cellulose by fiber from PPF in Peel group. Diets composition was presented in the Table 1. Body weight of animals was measured once week and diet intake was determined every two days. After 15 days of consumption the experimental diets, the animals were anaesthetized with ketamine and xylazine and killed by cardiac puncture exsanguination.

Table 1 - Formulations of experimental diets

	AIN-93M (AIN)	AIN-93M + PPF
	g kg^{-1} diet	
Corn starch	465.69	462.94**
Casein (≥85% protein)	140.00	133.58**
Dextrinized corn starch (90-94%)	155.00	155.00
Saccharose	100.00	100.00
Soybean oil	40.00	39.44**
Cellulose	50.00	25.00
Passiflora peel's flour	-	38.33*
Mix mineral (AIN-93M-MV)	35.00	32.28**
Mix vitaminic (AIN-93-Vx)	10.00	10.00
L-cystine	1.80	1.80
Choline bitartrate (41.1% choline)	2.50	2.50
Butylhydroquinone	0.01	0.01

AIN-93M refers to maintenance phase of growth [30].
* Amount to get 25g of *Passiflora* fiber.
** Values were corrected according content of these nutrients in the PPF.

2.5. Blood samples and carcasses

Blood samples were collected in appropriated tubes and centrifuged at 2000 g for 20 min. Serum was separated and stored at -80 °C until analyses. Whole brain, liver and kidneys were removed, weighed and frozen in liquid nitrogen, and then kept at -80 °C. Carcasses were weighed and stored at 4 °C or less.

2.6. Proximate composition of carcasses

The carcasses were weighed without some organs (liver, kidneys, brain, intestine, stomach, spleen and pancreas), freeze-dried and determined protein, moisture and ash

content using standard methods [31] as well as lipids which were determined by Soxhlet method, using petroleum ether as extractor [31].

2.7. Biochemical analyses

2.7.1. Serum level of albumin and total protein

Albumin (#03900) and total protein (#03800) were determined by commercial kit from LABORLAB (São Paulo, Brazil).

2.7.2. Antioxidant Potential in serum

Serum was treated with ethanol: ultrapure water and 0.75 mol L^{-1} metaphosphoric acid [32]**.** These extracts were used in ORAC [33, 34] and FRAP [35] assays, carried out as described below.

2.7.3. ORAC assay (Hydrophilic Oxygen Radical Absorbance Capacity)

In the dark, 20 µL of sample (prepared as described above), 120 µL of fluorescein in phosphate buffer - PB (pH 7.4), and 60 µL of AAPH were added in black microplates. The microplate reader was adjusted as previously described (fluorescent filters, excitation wavelength, 485 nm; emission wavelength, 520 nm) [33]. ORAC values were expressed in µmol trolox equivalent (TE) mL^{-1} serum by using the standard curves (2.5 - 50.0 µmol L^{-1} TE) [33, 34].

2.7.4. FRAP assay (Ferric Reducing Antioxidant Power)

The ferric reducing ability of the serum was determined by FRAP method [35], with adaptations. The FRAP reagent was prepared in the dark with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol TPTZ in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} $FeCl_3$. The

sample or standard solutions, ultrapure water and FRAP reagent were mixed and incubated in a water bath for 30 min at 37 ºC. After cooling to room temperature, the absorbance of the samples and standard were read at 595 nm. A trolox standard curve was prepared using concentrations raging 10 – 800 µmol L^{-1} TE. The results were expressed as µmol TE mL^{-1}.

2.7.5. Lipid peroxidation by Thiobarbituric Acid Reactive Substances (TBARS) assay

The TBARS determinations were done in liver, kidneys and serum according to Ohkawa, Ohishi, Yagi [36], with adaptations. The organ samples were macerated in liquid nitrogen. Samples (organs or serum) were mixed with 8.1% sodium dodecyl sulfate (SDS) and working reagent (2-thiobarbituric acid – TBA, 5% acetic acid and 20% sodium hydroxide). After heating at 95 °C for 60 min, the samples remained in ice bath for 10 min and centrifuged at 10,000 g for 10 min. The supernatant was read at 532 nm, using a clear 96-well microplate. Standard curve (0.625 – 50 nmol MDA mL^{-1}) was prepared using malondialdehyde standard (MDA). Results were expressed as nmol MDA mg^{-1} tissue (or nmol MDA mL^{-1} serum).

2.7.6. Enzyme and non-enzyme endogenous antioxidant system

2.7.6.1. Thiol groups content (GSH)

The GSH levels were determined in the phosphate buffer (PB) homogenates of liver and kidneys using Ellman's reagent (DTNB) [37], with modifications. GSH solution (2.5 – 500 nmol GSH mL^{-1}) was used as standard and absorbance was read at 412 nm. Reduced thiol contents were expressed as nmol GSH mg^{-1} protein. The protein concentration of tissues homogenates were done by Bradford method [38].

2.7.6.2. Glutathione Peroxidase activity (GPx)

The GPx activity was quantified in PB homogenates of liver. This assay is based on the oxidation of 10 mmol reduced glutathione by glutathione peroxidase coupled to the oxidation of 4 mmol NADPH by 1 U enzyme activity of GR in presence of 0.25 mmol L^{-1} H_2O_2. The rate of NADPH oxidation was monitored by the decrease in absorbance at 365 nm [39]. Results were expressed as nmol NADPH consumed min^{-1} mg^{-1} protein.

2.7.6.3. Glutathione Reductase activity (GR)

GR activity in liver was measured in PB homogenates, following the decrease in absorbance at 340 nm induced by 1 mmol L^{-1} oxidized glutathione in the presence of 0.1 mmol L^{-1} NADPH in phosphate buffer [40]. The results were expressed as nmol NADPH consumed min^{-1} mg^{-1} protein.

2.7.6.4. Superoxide Dismutase activity

The SOD activity was analyzed in liver and kidneys. One hundred microliters of appropriately diluted PB homogenates were added in 96-well microplate and 150 µL of the working solution (0.1 mmol hypoxanthine, 0.07 U xanthine oxidase, and 0.6 mmol NTB in PB in 1: 1: 1 proportions) and the kinetic reaction was monitored at 560 nm [41]. The area under the curve (AUC) was calculated and the SOD activity was expressed as U mg^{-1} protein.

2.7.7. Fecal pH

The feces samples were collected in cecum and diluted with deionized water (1 mg mL^{-1}) and homogenized and it was measured the fecal pH in a pH meter (Tecnal model TEC-5, Piracicaba,SP - Brazil) [42].

2.8. Cecal Microbiota (*Bifidobacterium, Lactobacillus, Enterobacteriaceae*, Total aerobics)

Feces samples were homogenized in peptone water (100 mg mL^{-1}) and then ten-fold serial dilutions were made in the same medium. Aliquots of 0.1 ml of the appropriate dilution were spread onto the MRS agar media for *Lactobacillus* count and supplemented MRS agar (0.5 mg L^{-1} dicloxacillin, 1 g L^{-1} LiCl and 0.5 g L^{-1} L-cysteine hydrochloride) for *Bifidobacterium.* Culture plates were incubated in anaerobic condition at 37°C for 24–48 h. Similarly, 1 mL of the diluted sample was spread onto specific count plates Petrifilm (3M®, São Paulo, MN) for *Enterobacteriaceae* and total aerobic. Plates were incubated at 37 °C for 24 – 48 h. After the incubation, the specific colonies grown on the selective culture media were counted and the number of viable microorganism g^{-1} feces (CFU g^{-1}) was calculated. The mean and standard error were calculated from the log 10 values of the CFU g^{-1}.

2.9. Short-chain fatty acids (SCFA)

Short chain fatty acids were analyzed by gas chromatography [43] using Agilent 6890N equipment with flame ionization detector (FID) and autosampler N10149 (Agilent, EUA). A 30 m × 0.25 mm i.d. × 0.25 µm Nukol™ capillary column (Supelco, Bellefonte, PA, US) was used. Chromatographic conditions were: injector and detector temperatures set at 250 ºC, injected volume 1 µL with split ratio set to 1:10; carrier gas was hydrogen at 1.0 mL min^{-1}. The column oven was programmed as follows: kept at 100 ºC for 0.5 min, then heated 8 ºC min^{-1} until 180 ºC, kept for 1 min, heated 20 ºC min^{-1} until 200 ºC and kept for 5 min.

2.10. Statistical analyses

Data were expressed as means ± standard error (SEM). The statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software. Analyzes were based on Student's t-test and the limit of significance was set at $P < 0.05$.

3. RESULTS

3.1. Dietary fibers content and water activity (aw) in PPF

Data indicate that PPF is a rich source of dietary fibers (65.22 ± 0.27 % total dietary fiber), with higher amount of insoluble fiber (48.12 ± 1.12 %) when compared to the soluble fiber content (17.11 ± 1.36 %). The water activity was low (0.43 ± 0.02) in PPF, therefore is a safe product related to microbial growth [44].

3.2. Nutritional parameters - Diet intake, body weight, organs and carcass weights, proximate composition of carcasses, serum albumin and total protein

Animal from Peel group showed food intake nearly 20% higher than the Control group's animals (25.93 ± 0.62; 20.25 ± 0.61 g day^{-1}, in Peel and Control group, respectively) during experimental period. However, there were no significant differences on body weight of rats, organ weights (liver, kidneys, whole brain) or carcass weight between groups ($P > 0.05$) (Table 2). Besides, the proximate composition of freeze-dried carcasses was similar in the two groups (Table 3). Relative to serum levels of albumin and total protein, the Peel group showed low levels of both ($P < 0.05$) (Figure 1).

Table 2 – Body weight (g), diet intake (g day^{-1}) and organ and carcass weights (g) of animals

Group	Peel	Control
Parameter		
Final Weight (g)	395.6 ± 7.81	386.6 ± 13.61
Diet intake (g dia-1)	25.93 ± 0.62 [a]	20.25 ± 0.61 [b]
Liver (g)	15.80 ± 0.64	13.70 ± 0.62
Kidneys (g)	2.72 ± 0.15	2.61 ± 0.18
Brain (g)	1.98 ± 0.01	2.07 ± 0.07
Carcass (g)	342.32 ± 9.72	328.73 ± 12.34

Peel group= animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control group = fed the AIN-93M. Data were expressed as mean ± SEM *(n*=4). Values (line) with different letters shows statistical difference significantly ($P < 0.05$) according with Student's t-test.

Table 3 - Proximate composition of freeze-dried carcasses

Group	Moisture	Ash	Proteins	Lipids	Carbohydrates**
	g/100 g				
Peel	2.41 ± 0.18 [a]	8.22 ± 0.43	49.13 ± 1.51	38.11 ± 1.36	2.13 ± 2.41
Control	1.49 ± 0.14 [b]	8.50 ± 0.15	49.45 ± 1.62	39.34 ± 2.11	1.22 ± 3.09

Peel group= animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control group = fed the AIN-93M. Data were expressed as mean ± SEM (*n*=4). Values (columns) with different letters shows statistical difference significantly ($P < 0.05$) according with Student's t-test.. * Carcass without stomach, intestine, pancreas, kidneys, liver, spleen, brain (They were taken out to subsequent analysis). ** Calculation per difference and standard deviation determined by means standard deviation of moisture, ash, proteins and lipids.

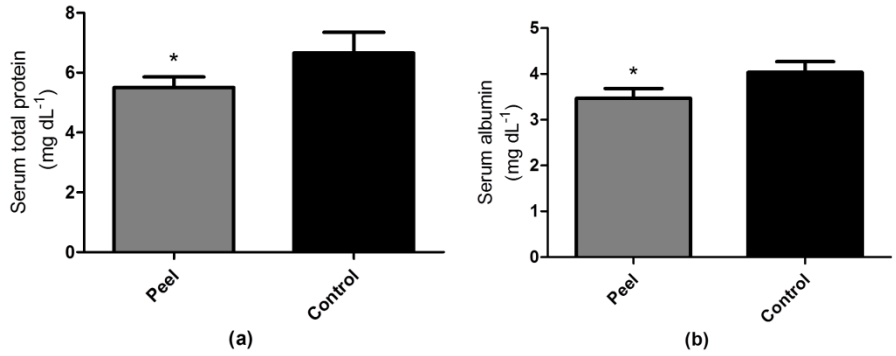

Figure 1 – Serum total protein (a) and serum albumin (b) (mg dL^{-1}). Peel = animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control = fed the AIN-93M. Data expressed as mean ± SEM (n= 4). *Indicates statistical differences from Control group according with Student's t-test ($P < 0.05$).

3.3. Antioxidant status in serum

The group that received experimental diet showed lower antioxidant potential in serum according to ORAC assay ($P< 0.05$) than Control group (Figure 2a), but this result were not confirmed by the others tests. According to FRAP assay there was no difference between the groups (Figure 2b) well as in lipid peroxidation level evaluated by TBARS assay (Figure 3a) ($P> 0.05$). In addition, Peel group presented tendency to decrease the lipid peroxidation levels in serum, when compared to Control group.

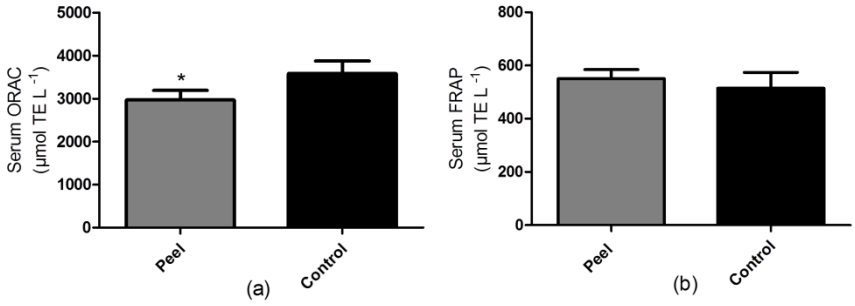

Figure 2 –Oxigen Radical Absorbance Capacity (ORAC) (a) and Ferric reducing power assay (b) and in serum (µmol TE L^{-1}). Peel = animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control = fed the AIN-93M. Data expressed as mean ± SEM (n=4). *Indicates statistical differences from Control group according with Student's t-test ($P < 0.05$).

3.4. Antioxidant status in tissues

The lipid peroxidation in liver was significantly ($P< 0.001$) higher in the experimental group compared to Control group (Figure 3b). Nevertheless, the opposite was observed on kidneys. The animals which received PPF in the diet showed 30% lower kidney TBARS than Control group (Figure 3c) ($P< 0.05$).

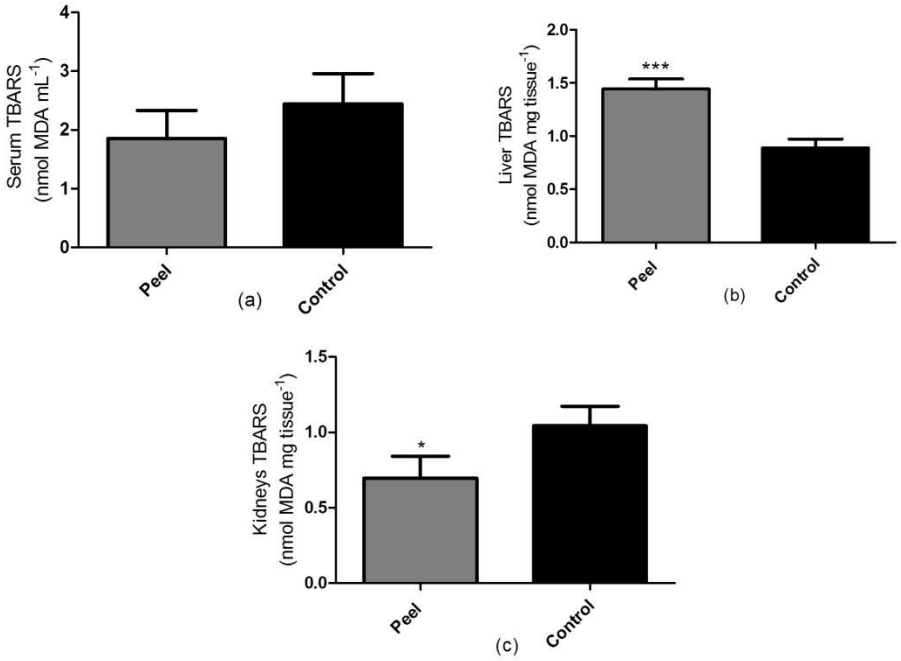

Figure 3 – Lipid peroxidation by TBARS assay in serum (a), liver (b), and kidneys (c) (nmol MDA mL^{-1} or nmol MDA mg $tissue^{-1}$). Peel = animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control = fed the AIN-93M. Data expressed as mean ± SEM (n=4). *Indicates statistical differences from Control group according with Student's t-test (1 code = $P < 0.05$; and 3= $P < 0.001$).

Regarding antioxidants defenses, the thiol group content (GSH) showed no differences between groups in the two sites analyzed ($P > 0.05$) (average 38.99 ± 5.79 and 37.69 ± 2.01 nmol mg^{-1} protein in liver and kidneys, respectively), SOD activity was 50% smaller in liver of Peel group compared to Control and there was no difference in kidneys (Figure 4a, b). The GPx activity was three times lower and GR activity was twice higher in Peel group compared to Control group (Figure 5a, b).

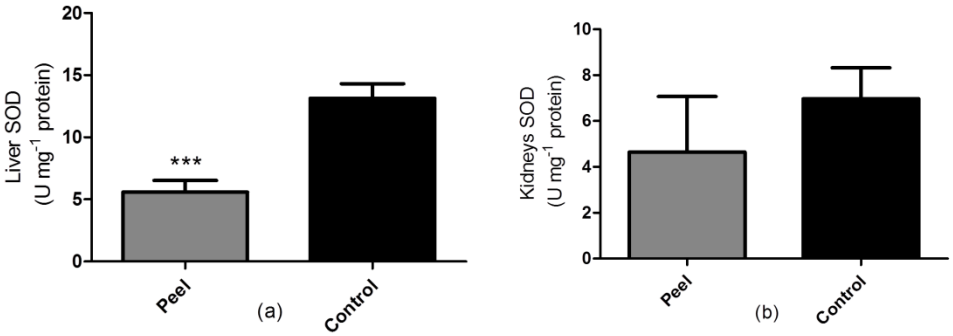

Figure 4 – Superoxide dismutase activity (SOD) in liver (a) and kidneys (b) (U mg^{-1} protein). Peel = animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control = fed the AIN-93M. Data expressed as mean ± SEM (*n*=4). ***Indicates statistical differences from Control group according with Student's t-test ($P < 0.001$).

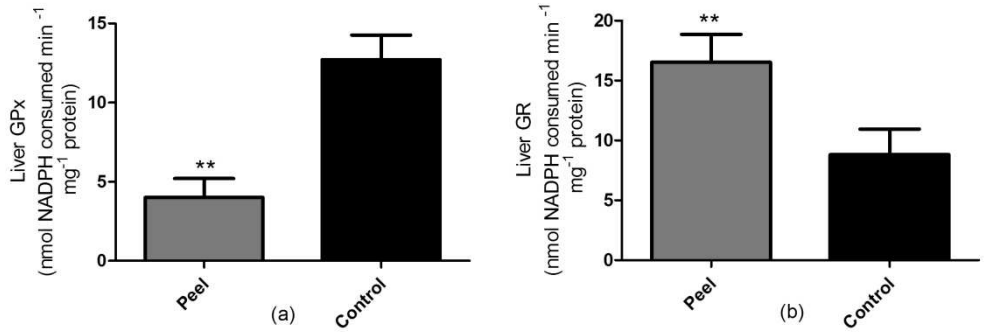

Figure 5 – Antioxidant enzymes activity: Glutathione peroxidase (GPx) (a) and Glutathione reductase (GR) (b) and (nmol NADPH consumed min^{-1} mg^{-1} protein) in liver. Peel = animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control = fed the AIN-93M. Data expressed in mean ± SEM (n=4). **Indicates statistical differences from Control group according with Student's t-test ($P < 0.01$).

3.5. Short chain fatty acids (SCFA) in feces, Cecal microbiota and feces pH

The group that consumed PPF presented larger SCFA concentrations in feces than Control group, especially acetic and butyric acids (75% and 66% higher, respectively) (Figure 6). However, there were no differences in feces pH (8.24 ± 0.20 Peel group and 8.53 ± 0.14 Control group) or on colonic bacteria (*Lactobacillus, Bifidobacterium*, *Enterobacteriaceae* and total aerobic) between the groups (Figure 6).

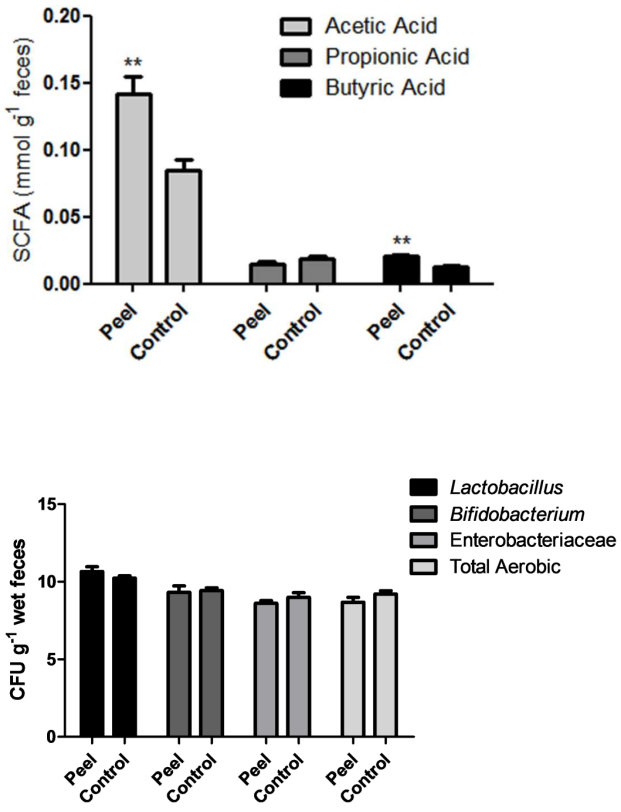

Figure 6 - Short-chain fatty acid (SCFA – acetic, propionic and butyric) and Microbiota of cecal contents (*Lactobacillus, Bifidobacterium, Enterobacteriaceae* and Total Aerobic bacteria). Peel = animals fed the AIN-93M with 50% fiber content replaced by fiber of PPF; Control = fed theAIN-93M. Data expressed as mean ± SEM (*n*=4). **Indicates statistical differences from Control group according with Student's t-test ($P < 0.01$).

4. DISCUSSION

The fruits intake has been considered important for health maintenance and prevention of chronic disease, as they are high sources of micronutrients, fiber and other bioactive compounds [45-47]. However, the fruit processing leads to huge amount of byproducts, which might contain many functional substances. In the production of passion fruit juice the residues volume may achieve above 50% peel plus seed [5, 8, 22, 48, 49].

Results showed that *P. edulis* peel contains twice the amount of insoluble dietary fiber and three times more soluble fiber than peel jaboticaba (*Myrciaria jaboticaba (Vell.) Berg*) [18]. Dietary fiber content was similar to mango peel [50] and to other study done with passion fruit peel [25]. The dietary fibers carry a blend of bioactive compounds and reports have shown that the intake of fiber is inversely related to some pathologies as obesity and type two diabetes [51]. Therefore, the *P. edulis* peel is a rich source of dietary fibers and could be as alternative food industry to increase the fiber content in products and bring to benefits to health. It is important to note that *P. edulis* peel has considerable pectin in its composition, which is a complex carbohydrate from plants with technological and physiological function. Kulkarni, Vijayanand [49] found about 14% pectin in passion fruit peel and it was high methoxyl type with good jellying capacity. This fiber fraction can then form viscous gels that turns slow gastric emptying and intestinal transit decreasing the velocity of absorption of carbohydrates and also in fat, besides it may occur inhibition of micelles formation [52].

There were no alterations in organs weight or proximate composition of carcasses. But total albumin and protein were lower in PPF group compared to Control group. Only serum total albumin values (3.47 ± 0.11 mg dL^{-1}) in PPF remained in accordance to the

reference to rodents (2.8 - 4.4 mg dL^{-1}) and serum protein (5.50 ± 0.18 mg dL^{-1}) was little below the reference (5.9 - 7.9 mg dL^{-1}) [53]. The reduction in serum albumin and total protein could be associated to soluble fiber content in PPF. This portion was not contained in the Control diet, and it could adsorb nutrients from diet, impeding their absorption [54]. This way, the PPF addition might have influenced the up-take of aminoacids and impaired the biological value of the proteins from diet [55]. The peels of the fruits are known to contain some compounds, such as protease inhibitors, lectins, tannins, oxalates and phytates that reduce nutrients bioavailability [52]. In *P. edulis* peel was found 17 mg tannin 100 g^{-1} fresh matter [52], which might was responsible for the protein quelation. And also, in the experimental diet the casein amount was reduced according to protein amount in PPF, to keep isocaloric and isoprotein the diets, but this could have decreased protein quality ingested by Peel group. Longer-term studies are needed to show the repercussions of prolonged intake of passion fruit peel.

Several bioactive compounds have been identified in the *Passiflora* genus and their antioxidant potential in pulp and leaves have been showed, but not in their peels [3, 20, 22, 56]. High amount of functional substances can be found on the peel since non-edible fruit parts have shown high polyphenol contents [57] and, oftentimes, fruit peels presented higher amounts of phenolics compared to their popular parts [58, 59].

Extract made with *Passiflora* endocarp plus seeds significantly retarded lipid peroxidation in a peroxyl radical-mediated lipid peroxidation membrane model [60]. And it was observed similar effect in serum of rats treated with *P. edulis* juice [61]. PPF intake was no capable to increase antioxidant power on serum according to FRAP assay. It was observed reduction on serum antioxidant potential by ORAC in the animals treated with peel. PPF intake also showed to reduce TBARS content in the kidneys compared to control.

The kidneys SOD activity in Peel group trended to decline, which demonstrated that compounds coming from the PPF addition in the diet could have antioxidant action on kidneys. But this was not observed in serum perhaps the period of treatment was short to have effects in this site.

Nevertheless literature data demonstrate that *Passiflora* peel is safe, toxicity test in liver showed unlike hepatoprotective effect [62] and a clinic study showed no negative effects from PPF intake (30 g day^{-1}) [63]. In this report, the consumption of PPF showed higher lipid peroxidation in liver than Control group. But, further detailed investigations should be made to explain these results in liver. Because they could be attributed to several factors like reduced antioxidant effects or lower ability to neutralize reactive specie.

In the same organ, high GR, low GR and SOD activities were observed. Some hypotheses are raised to explain these results. GSH values were similar between the groups, either in the liver or kidneys. High lipid peroxidation in liver could yield reactive species, overloading SOD and GPx, which could reduce their action, since these enzymes are responsible to control the hydrogen peroxide and lipid hydroperoxides incidence. The increase in GR activity could occur to recover reduced glutathione and to allow continuation of the glutathione cycle [64]. However analysis of variations in the enzymes or substrates of glutathione cycle are difficult because there are many interactions among these species [64].

The animals fed PPF showed higher diet intake, but no difference on final body weight, greater hepatic lipid peroxidation, increased SOD and GPx activities associated with a reduction on GR activity in liver. These are evidences that *P. edulis* peel could contain thermogenic/ ergogenic compounds. These composites stimulate energetic expenditure and contain no energy themselves. Natural herbal ingredients already are

source scientifically considered thermogenics, in the tea this effect is attributed to its flavonoids as the catechins and caffeine. Catechins up-regulate lipid-metabolizing enzymes and thereby stimulate fat oxidation, because avoid re-uptake of norepinephrine, and caffeine inactive hormone-sensitive lipase, promoting upper thermogenesis [65].

A report showed a preservation of lean body mass and lower adiposity in rats after viscous fiber intake, hydroxypropyl methylcellulose (HPMC), which could increase mitochondrial biogenesis and fatty acid oxidation in skeletal muscle [66]. In a citrus species of grapefruit, it was identified an ergogenic compound, the nootkatone (4,4a,5,6,7,8-hexahydro-6-isopropenyl-4,4a-dimethyl-2(3H)- naphthalenone), a kind of sesquiterpenoid present mainly in its peel. Reports with nootkatone in experimental and clinical studies showed body weight reduction and improvement in the insulin resistance in metabolic disorders [67]. Literature data also reports the use of extract of the fruit or peel from bitter orange (*Citrus aurantium, Rutaceae*) to weight management, with increased thermogenesis and lipolysis, as well as good sports performance products. The results are attributed to protoalkaloidal constituent, p-synephrine, which is considered safe to human nutrition [68].

In this way, the results, mainly about the liver, denote that more investigations are necessary. Some improvements could be higher study length, the usage of different *Passiflora* concentrations and more tests to evaluate hepatic and systemic effect of PPF intake.

Dietary fibers are fermented by anaerobic bacteria in the colon to produce SCFA. Butyrate is the preferred oxidative fuel of the colonocyte *in vitro*, and the provision of preferred intestinal fuels stimulating mucosal proliferation *in vivo*, besides it stimulates mucus secretion, vascular flow, barrier permeability, motility, water and electrolytes absorption [26, 28]. In this study an increasing in acetate and butyrate was observed in the

groups fed PPF, which could be promoted by soluble fiber content in *P. edulis* peel, a greater substratum to fermentation by enteric bacteria.

In addition SCFA have been shown anti-inflammatory capacity, like the inhibition of nuclear factor kβ (NF-kβ) activation by histone deacetylase inhibition, reducing pro-inflammatory cytokines expression in intestine and *in vitro*[69-71].

Other beneficial effect of carbohydrate fermentation, also linked to SCFA, is the enhance absorption of minerals, since the production of these may reduce gut pH, improving the minerals solubility [54]. But in the present work, despite the increase in SCFA of cecal contents, there was not alteration in feces pH.

Beyond role in intestinal homeostasis [54], the SCFA have been attributed to inhibit cholesterol synthesis, prevent cancer , exert anti-inflammatory effect and act in reducing in inflammatory bowel disease [51]. Therefore, the effect of PPF in fermentation and its consequences might be further investigated in future studies.

5. CONCLUSION

P. edulis peel is a good alternative to raise dietary content of fibers. The use of byproducts might minimize the generation of industrial process residuals and environment impact, as well as increasing industrial profitability. Besides, the results indicate that the *Passiflora* peel flour could improve bowel health by increasing in SCFA production and increase antioxidant potential in the kidneys**.** However, more investigations about possible thermogenic or negative effects on liver are necessary.

CONFLICT OF INTERESTS

The authors declare that they do not have any conflicts of interest.

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Capítulo 3 - Passion fruit (*Passiflora edulis*) peel: source of fibers, polyphenol compounds and its antioxidant effect in *Wistar* rats with induced colitis.

(Artigo em fase de preparação para envio à revista Nutrition)

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ABSTRACT

Passion fruit (*Passiflora edulis*), including its peel, is a rich source of bioactive compounds, as polyphenols. This study investigated proximate composition and phenolic content of *P. edulis* peel flour (PPF) and its *in vivo* antioxidant potential. Male *Wistar* rats were divided in 4 groups: Peel Colitis, Peel Sham, AIN Colitis, AIN Sham (*n*= 6). In the Peel groups' diet, 50 % of cellulose content on AIN diet was replaced by PPF fiber. After one week of adaptation, the colitis induction was performed by rectal injection of TNBS. The animals were anesthetized and sacrificed by exsanguination via cardiac puncture after 7 days of colitis induction. The PPF presented high content of fiber (65%) and phenolic compounds (2.53 ± 0.03 mg GAE g^{-1}). The animals that received PPF showed improvements in serum antioxidant potential (ORAC and TBARS assays) as well as modulation of antioxidant enzymes activities in brain (SOD) and liver (SOD, GR and GPx). Therefore, the introduction of PPF in food industry could contribute to increase amount of fiber and radical scavengers in theirs products. On the other hand, more studies are necessary to investigate PPF action in liver, since higher lipid peroxidation in this organ was observed in experimental animals.

Keywords: *Passiflora edulis* peel; Antioxidant potential; Oxidative stress; Bioactive compounds; Fiber.

1. INTRODUCTION

Inflammatory bowel diseases (IBD) affect millions of people worldwide and cause debilitating symptoms that harm both performance and quality of life [1, 2]. The IBDs involve alterations in mucosal immunity and gastrointestinal physiology. Its etiology is unknown and it is characterized by chronic and recurrent course, with periods of exacerbation of symptoms [3].

Ulcerative colitis (UC) and Crohn's disease (CD) represent two main phenotypes of IBD [4]. While CD is characterized by transmural inflammation (it could affect all gastrointestinal extension), UC develops in the colon and rectum region effecting only mucosa and submucosa [3]. Nevertheless the pathogenesis is not clear, these disorders are considered multifactorial illnesses and environment factors, genetic susceptibility, immune alterations and composition of microbiota may contribute to their trigger [5-7].

The intestinal mucosa separates luminal environment to the individual's tissue and it is first barrier of defense against aggressor agents. Mucosa dysfunctions may favors abnormal influx of lumen substances, as enteric bacteria and its antigens, which can overload the immune system and discharge inflammatory response [8].

Oxidative stress, acute or chronic, has been associated to inflammatory disease, such as ulcerative colitis [9-13]. It is defined as an imbalance between production of reactive oxygen species (ROS) and their elimination by protective mechanisms, known as antioxidative systems [11, 14].

Though specific mechanisms of cell damage are not completely understood [11], it is believed that oxidative stress could be not only an epiphenomenon, but a potential agent to initiate and propagate the IBD (Fig.1) [11-13]. The excess of ROS or nitrogen species

(RNS) downregulates mucosa defense (mucus, cytoplasmic membrane, intercellular junctions and basement membrane). Such disturbances may allow bacterial translocation to submucosa, and, in an attempt to combat the invasion, polymorphonuclear cells (PMNs), mostly neutrophils, migrate to intestinal region and set to inflammatory process [12]. Nevertheless, oxidative disturbance is found in local and systemic levels [11, 12]

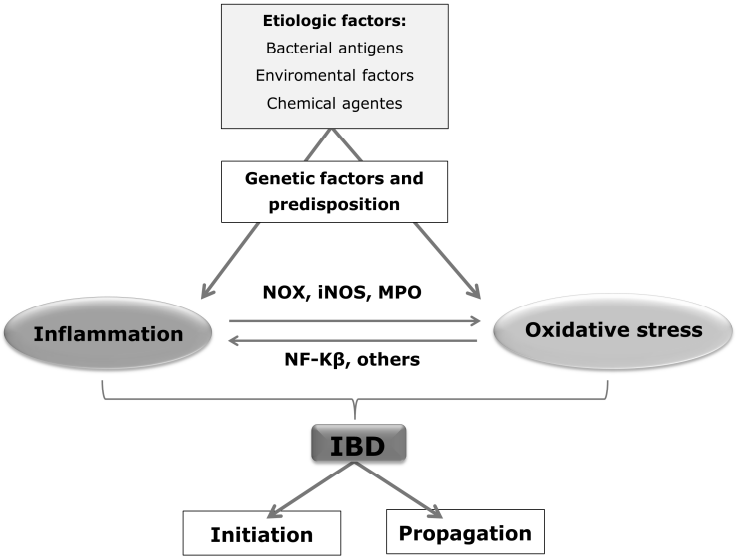

Fig.1 Scheme of the oxidative stress role in the IBD. Excess of reactive species by systems, such as NAPH oxidases (NOXs) and inducible nitric oxide synthase (iNOS), as well the release of myeloperoxidase (MPO) from inflammatory cells. On the other hand, oxidative stress is involved in the up regulation of inflammatory process by stimulating signaling pathways as NF-Kβ. Thus, both initial inflammation and oxidative stress may contribute to disparate or to keep IBD. Adapted of Zhu and Li [13].

To avoid the ROS/ RNS's destructive effects, tissues use antioxidant enzymatic systems, like catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), and non-enzymatic antioxidant systems, thiol reduced (GSH), vitamins, minerals, and polyphenols [11].

An overproduction of free radicals (like in inflammation processes) [15] leads to oxidative stress. In these situations, the supply of exogenous antiradicals to organism is important. Thus, fruits and vegetables consumption may support the prevention of oxidative stress, whereas these foods are sources of antioxidants [9, 16, 17].

Passion fruit (*Passiflora* edulis) has bioactive phytochemicals with antioxidant propriety, such as phenolic compounds (C-glicosil derivatives apigenin and luteolin, as vitexin, isovitexin, orientin, schaftoside, 2″-O-rhamnoside and luteolin 7-O-(2-rhamnosylglucoside)), glycosides and alkaloids [18-23]. Some species of *Passiflora* are largely used in industrial or domestic production of juice, with integral utilization of pulp; but peels and seeds, also rich in nutrients and bioactive compounds, are put away as residue [18, 24].

Thereby, consumption of *Passiflora*'s peel could be an alternative to increase dietary content of fiber and antioxidants and to aid in the prophylaxis and therapeutic of some pathologies as well as IBDs. Furthermore, its employment could minimize the generation of industrial process residuals and environment impact [24, 25].

There are few scientific studies about passion fruit peel. Our work aimed to investigate the systemic antioxidant potential of *P. edulis* peel's flour during a situation of stress, colitis induced in *Wistar* rats with 2,4,6-trinitrobenzene sulfonic acid (TNBS).

2. MATERIALS AND METHODS

2.1. Chemicals

L-glutathione reduced, Glutathione reductase, glutathione oxidized form disodium salt, β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH), hypoxanthine, xanthine oxidase, nitrotetrazolium blue chloride (NTB), 5′5′-

dithio-bis-2-nitrobenzoic acid (DTNB), albumin from bovine serum (BSA), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS); (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX); 2,2′-azobis(2-methylpropionamidine) dihydrochloride (APPH) and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) were used. Fluorescein sodium salt and metaphosphoric acid were purchased from Vetec Química Fina (São Paulo – Brazil). Malondialdehyde standard (MDA) was purchased from Chayman Chemical Company (#10009202).

2.2. *Passiflora edulis* peel's flour (PPF)

Organic crop of *Passiflora edulis* (passion fruit) from the city of Torre de Pedra/SP, harvested in June 2010, was used to produce the flour. The fruits were cleaned and separated into pulp and peel (flavedo/ epicarp plus albedo/ mesocarp). Peels were cut in small pieces and dried in oven with air circulation at 50°C (Marconi, Piracicaba/SP - Brazil). Dried samples were grounded into a fine powder using a hammer mill (20 mesh). PPF was stored in amber flask at room temperature (24 °C).

2.3. PPF Characterization

2.3.1. The proximate composition

Moisture, ash [26], lipids [27] and protein [26] contents were determined. Total dietary fiber and insoluble fractions were analyzed by enzymatic method [28] and soluble fraction was calculated by the difference between total dietary and insoluble fiber.

2.3.2. Total phenolic content

One gram of PPF was extracted with 25 mL of boil water. After 35 min. the extract was filtered under vacuum and it was stored under refrigeration (2 to 8 ºC) in amber glass bottles. The total phenolic content was determined according to Folin-Ciocalteu's method [29], with some modifications. In a vial, 50 µl of extract, 800 µL distilled water and 25 µl (0.25 N) Folin-Ciocalteu's reagent were mixed and incubated at room temperature for 3 min. Then, 100 µL sodium carbonate solution (1N) was added and further incubated for 2 h at room temperature. The absorbance was read at 725 nm in a microplate reader Synergy HT, Biotek (Winooski, USA) spectrophotometer with Gen5™ 2.0 data analysis software. Gallic acid was used in a standard curve and the results were expressed in terms of gallic acid equivalent (GAE g^{-1}).

2.3.3. Copper determination

Copper determination in PPF was accomplished by atomic absorption spectrometry (Analytik Jena – NOVA A300). Copper solution (100 µg mL^{-1}) was used as standard.

2.4. Experimental design

The study was approved by the Institutional Animal Care and Use Committee (protocol #2385-1, CEUA, UNICAMP, Brazil). The animals were cared for in accordance with the institutional ethical guideline. Male *Wistar* rats were allocated under controlled conditions of temperature (22 °C ± 2), humidity (60 – 70 %), and a light-dark cycle (12/12 h). Seventy seven-days old rats were randomized into 4 groups (n= 6): Peel Colitis, Peel Sham, AIN Colitis, AIN Sham. Peel group was fed with standard diet (AIN-93M) [30] with 50% fiber content replaced by fiber of PPF; AIN group was fed with standard diet. Colitis

was induced according to the protocol described by Morris & Beck [31]. Briefly, the rats were fasted for 12 h and sedated with halothane. After that, they received an injection (8cm from the anus using a teflon cannula 2mm extern diameter) of 4,6-trinitrobenzene sulfonic acid (TNBS) (10 mg animal^{-1}) solubilized in 0.25 mL ethanol 50% (v v^{-1}) to 8 cm from to anus . In Sham groups, an equal volume of saline solution (0.9% NaCl) was inserted using the same procedure. After one week, the animals were anaesthetized with ketamine and xylazine and died by exsanguination via cardiac puncture. Lesion score [32] was done to confirm and to evaluate the colitis in colon.

2.5. Biochemical analyses

All the absorbance and fluorescence readings from biochemical analyses were measured in a microplate reader Synergy HT, Biotek (Winooski, USA); with Gen5™ 2.0 data analysis software. Except for albumin and protein, which were determined using a spectrophotometer (Femto, 600 Plus, São Paulo, Brazil). All analyses were carried out in triplicate.

2.5.1. Blood samples and carcasses

Blood samples were collected in appropriated tubes and centrifuged at 2000 g for 20 min. Serum was separated and stored at -80 °C until analyses. Whole brain, liver and kidneys were removed, weighed and frozen in liquid nitrogen, and then kept at -80 °C. Carcasses were weighed and stored at 4 °C or less.

2.5.2. Serum level of albumin and total protein

Albumin (#03900) and total protein (#03800) were determined by commercial kit from LABORLAB (São Paulo, Brazil).

2.5.3. Proximate composition of carcasses

The carcasses without some organs (liver, kidneys, brain, intestine, stomach, spleen and pancreas) were separated and freeze-dried. The protein, moisture and ash contents were determined using standard methods [26] as well as lipids which were determined by Soxlet method [26].

2.5.4. Thiobarbituric Acid Reactive Substances (TBARS assay)

TBARS determinations were done in liver, kidneys and serum according to Ohkawa, Ohishi [33], with adaptations. Samples (organs or serum) were mixed with 8.1% sodium dodecyl sulfate (SDS) and working reagent (2-thiobarbituric acid – TBA, 5% acetic acid and 20% sodium hydroxide). After heating at 95 °C for 60 min, the samples remained in ice bath for 10 min and centrifuged at 10,000 g, 10 min. The supernatant was read at 532 nm, using a clear 96-well microplate. Standard curve (0.625 – 50 nmol MDA mL^{-1}) was obtained using malondialdehyde standard (MDA). Results were expressed in nmol MDA mg^{-1} tissue (or nmol MDA mL^{-1} serum).

2.5.5. Enzymatic and non-enzymatic endogenous antioxidant system in tissues

2.5.5.1. Thiol groups content (GSH)

GSH levels were determined in the phosphate buffer (PB) homogenates of liver, kidneys and brain using Ellman's reagent (DTNB) [34], with modifications. GSH solution

(2.5 – 500 nmol GSH mL^{-1}) was used as standard and absorbance was read at 412 nm. Reduced thiol contents were expressed in nmol GSH mg^{-1} protein. The protein concentration of tissues homogenates were done by Bradford method [35].

2.5.5.2. Superoxide Dismutase activity (SOD)

SOD activity was analyzed in liver, kidneys and brain. One hundred microliters of appropriately diluted PB homogenates were added in 96-well microplate and 150 µL of the working solution (0.1 mmol hypoxanthine, 0.07 U xanthine oxidase, and 0.6 mmol NTB in PB in 1: 1: 1 proportions) and the kinetic reaction was monitored at 560 nm [36]. The area under the curve (AUC) was calculated and the SOD activity was expressed as U mg^{-1} protein.

2.5.5.3. Glutathione Peroxidase activity (GPx)

GPx activity was quantified in PB homogenates of liver. This assay is based on the oxidation of 10 mmol reduced glutathione by glutathione peroxidase coupled to the oxidation of 4 mmol NADPH by 1 U enzymatic activity of GR in presence of 0.25 mmol H_2O_2. The rate of NADPH oxidation was monitored by the decrease in absorbance at 365 nm [37]. Results were expressed in nmol NADPH consumed min^{-1} mg^{-1} protein.

2.5.5.4. Glutathione Reductase activity (GR)

GR activity in liver was measured in PB homogenates, following the decrease in absorbance at 340 nm induced by 1 mmol oxidized glutathione in the presence of 0.1 mmol NADPH in phosphate buffer [38]. The results were expressed in nmol NADPH consumed min^{-1} mg^{-1} protein.

2.5.6. Antioxidant Potential in serum

2.5.6.1. Preparations of samples

Serum was treated with a solution of ethanol, ultrapure water and 0.75 mol L^{-1} metaphosphoric acid [39]. These extracts were used in ORAC [40, 41] and FRAP [42] assays, carried out as described below.

2.5.6.2. ORAC assay (Hydrophilic Oxygen Radical Absorbance Capacity)

In the dark, 20 μL of sample, 120 μL of fluorescein, and 60 μL of AAPH were added in black microplates. The microplate reader was adjusted as previously described (fluorescent filters, excitation wavelength, 485 nm; emission wavelength, 520 nm) [40]. ORAC values were expressed in μmol trolox equivalent (TE) mL^{-1} serum by using the standard curves (2.5 - 50.0 μM TE) [40, 41].

2.5.6.3. FRAP assay (Ferric Reducing Antioxidant Power)

The ferric reducing ability of tissues was determined by FRAP method [42], with adaptations. In the dark, FRAP reagent was made with 300 mmol L^{-1} acetate buffer (pH 3.6), 10 mmol TPTZ in a 40 mmol L^{-1} HCl solution and 20 mmol L^{-1} $FeCl_3$. Sample or standard solutions, ultrapure water and FRAP reagent were mixed and kept in a water bath for 30 min at 37 ºC. After cooling to room temperature, samples and standard were read at 595 nm. The trolox standard curve was made (10 – 800 μmol TE). Results expressed in μmol TE mL^{-1}.

2.6. Statistical analyses

Data were expressed as means ± standard error (SEM). The statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) software. Analyzes were based on Student's t-test and the limit of significance was set at $P < 0.05$.

3. RESULTS

3.1. PPF's proximate composition and phenol content

The results of protein, lipid, carbohydrate, ash, moisture and fiber (total, soluble and insoluble) are shown in Table 1. The total phenolic content in PPF (aqueous extract) was 2.53 ± 0.03 mg GAE g^{-1} and copper level was 5.1 ± 0.3 mg kg^{-1}.

Table 1 Proximate composition of passion fruit flour (g 100 g^{-1}) in dry matter.

Parameters	g 100 g-^{1}
Moisture	9.48±0.26
Ash	6.88±0.02
Lipid	0.31±0.01
Protein	3.94±0.18
Total dietary fiber	65.22±0.27
Insoluble dietary fiber	48.12±1.12
Soluble dietary fiber*	17.11±1.36
Carbohydrates**	79.39
Energy value***	3.76

*Soluble dietary fiber = total dietary fiber – insoluble dietary fiber
**Carbohydrates = 100 g – (moisture + ash + lipid + protein).
*** kcal g^{-1} of the peel

3.2. Lesion Score

Lesion scores were very similar among the experimental groups. PPF intake did not decrease pathologic process in the bowel but it showed other actions on systemic level as described below.

3.3. Weight gain and intake parameters

Peel and AIN Colitis groups consumed less diet than Peel and AIN Sham groups which was expected because the inflammation is a process that affect the appetite. PPF intake and inflammatory period also did not affect body composition whereas weight gain mean, organs and carcass weight did not differ among the groups (Table 2 and 3).

Table 2. Weight gain (g) and daily food intake (g)

Group	Weight gain (g)	Feed intake (g/dia)
Peel Colitis	302.17 ± 19.75	12.72 ± 0.69 [b]
Peel Sham	332.23 ± 12.06	22.79 ± 1.46 [a]
AIN Colitis	302.03 ± 8.67	13.79 ± 1.16 [b]
AIN Sham	323.65 ± 13.91	24.86 ± 1.02 [a]

Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. Data were analyzed by one-way ANOVA and Tukey test, and expressed in mean ± SEM. Values (columns) followed by different superscript are significantly different ($P < 0.05$).

Table 3. Organs and carcass weight (g) *

Groups	Subgroup	Liver	Kidneys	Brain	Carcass
Peel	Colitis	15.20 ± 0.73	2.62 ± 0.18	1.97 ± 0.04	307.97 ± 15.44
	Sham	16.07 ± 0.85	3.00 ± 0.15	1.99 ± 0.02	339.38 ± 8.67
AIN	Colitis	13.70 ± 0.58	2.77 ± 0.14	2.02 ± 0.05	300.76 ± 8.77
	Sham	14.44 ± 0.82	2.69 ± 0.07	1.84 ± 0.06	328.03 ± 11.98

Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. * Carcass without stomach, intestine, pancreas, kidneys, liver, spleen, brain (They were taken out to subsequent analysis). Data were analyzed by one-way ANOVA and Tukey test, and expressed in mean ± SEM. Values (columns) followed by different superscript are significantly different ($P < 0.05$).

3.4. Serum total protein and albumin

There were no significant differences in levels of total serum protein, however, Peel Sham group showed about 15% increase in total serum protein compared to AIN Sham groups (Fig. 2a). Unexpectedly, level of serum albumin was nearly 20% reduced in AIN Sham group relative to Peel Sham and even to AIN Colitis (Fig. 2b).

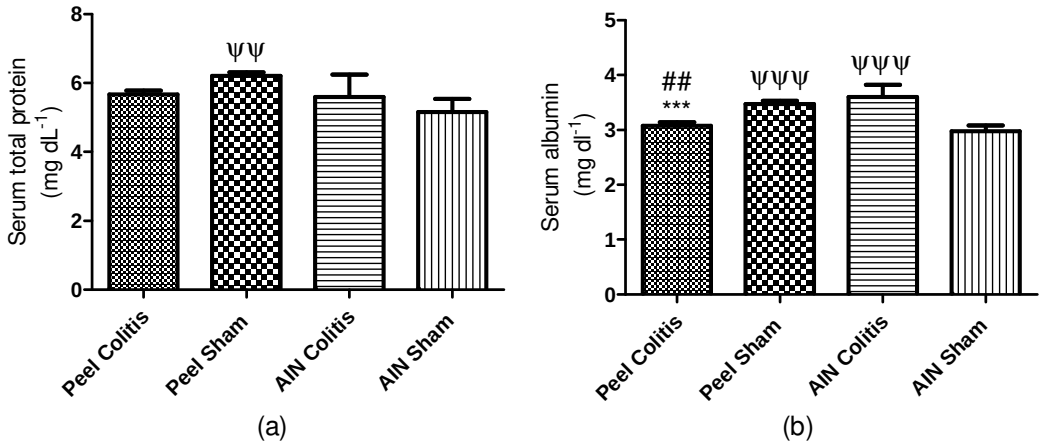

Fig.2 – Serum total protein (a) and serum albumin (b). Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. Data were analyzed by one-way ANOVA and Tukey test, and expressed in mean ± SEM. Symbols Indicates statistical differences from groups: *Peel Sham; $^{\#}$AIN Colitis; $^{\Psi}$AIN Sham (1 code= P< 0.05; 2 codes= P< 0.01; and 3= P< 0.001).

3.5. Proximate composition of freeze-dried carcass

The animals treated with PFF showed similar contents of carcass protein compared to control (there was a tendency in increasing protein contents in carcass of the Peel Colitis relative AIN Colitis, despite it do not differ statistically) (Table 4). But the carcass lipid content of Peel Colitis group was 35% lower than AIN Colitis (22.67 ± 0.33; 34.17 ± 3.50 g 100 g^{-1} respectively).

Table 4. Proximate composition of freeze-dried carcass

	Moisture	**Ashes**	**Proteins**	**Lipids**	**Carbohydrates ****
	g/100 g				
Peel Colitis	2.18 ± 0.31 ^{a}	10.00 ± 1.41	60.27 ± 4.20 ^{a}	22.67 ± 0.33 ^{c}	4.88 ±2.57
Peel Sham	2.18 ± 0.27 ^{a}	8.57 ± 1.12	50.43 ± 5.86 ^{b}	39.81 ± 5.04 ^{ab}	0.00 ± 4.41
AIN Colitis	1.51 ± 0.31 ^{b}	8.91 ± 0.59	53.49 ± 1.11 ^{ab}	34.17 ± 3.50 ^{b}	1.92 ± 2.15
AIN Sham	2.00 ± 0.18 ^{ab}	8.67 ± 1.37	49.72 ± 3.20 ^{b}	41.77 ± 2.17 ^{a}	0.00 ± 2.37

Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. * Carcass without stomach, intestine, pancreas, kidneys, liver, spleen, brain (They were taken out to subsequent analysis). Data were analyzed by one-way ANOVA and Tukey test, and expressed in mean ± SEM. Values (columns) followed by different superscript are significantly different ($P < 0.05$). **Calculation per difference and standard deviation determined by means standard deviation of moisture, ashes, proteins and lipids.

3.6. Serum antioxidant status

The experimental groups that received PPF (Colitis and Sham) showed a 50% increase in ORAC relative to AIN Sham (3872 ± 238.4;; 4381 ± 385.5; 2250 ± 137.2 µmol TE L^{-1} in Peel Colitis, Peel Sham and AIN Sham respectively) (Fig. 3a). Although, the results of FRAP were very similar among the groups (Fig. 3b). Corroborating ORAC results, the animals that received PFF showed lower serum lipid peroxidation, which might evidence antioxidant activity improvement (Fig. 3c). TBARS assay results revealed that lipid peroxidation was about 15% decreased in Peel Sham group in comparison with AIN Sham group (1.28 ± 0.10 nmol MDA ml^{-1} Peel Sham; 1.48 ± 0.12 10 nmol MDA ml^{-1} AIN Sham) ($P< 0.05$). A similar reduction was observed in Peel Colitis group compared to AIN

Colitis group, although there was not significant differences (P> 0.5) (1.47 ± 0.10 nmol MDA ml^{-1} Peel Colitis; 1.76 ± 0.09 nmol MDA ml^{-1} AIN Colitis).

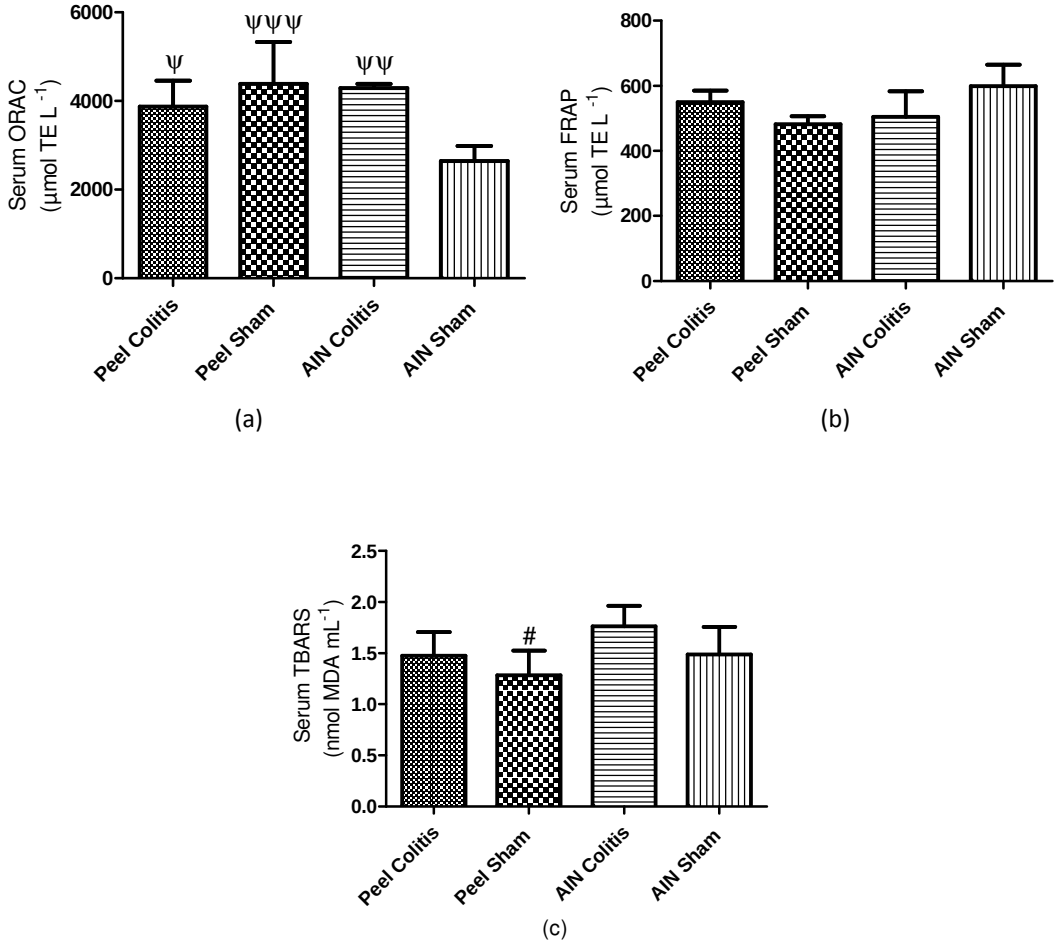

Fig. 3 – Ferric reducing power assay (FRAP) (a); Oxigen Radical Absorbance Capacity (ORAC) (b) and Lipid peroxidation by TBARS assay in serum. Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. Data were analyzed by one-way ANOVA and Tukey test, and expressed in mean ± SEM. Symbols Indicates statistical differences from groups: * Peel Sham; $^{\#}$ AIN Colitis; $^{\Psi}$ AIN Sham (1 code= P< 0.05; 2 codes= P< 0.01; and 3= P< 0.001).

3.7. Kidneys antioxidant status

The lipid peroxidation was higher in kidneys of the animals from Peel Colitis and AIN Colitis groups; and the PFF intake did not improve TBARS on kidneys ($P > 0.05$) (Fig. 4a). Additionally, there was no difference ($P > 0.05$) in GSH levels among groups (values ranged from 31.9 ± 2.24 to 35.7 ± 2.10 nmol mg^{-1} protein). SOD activity in this organ was not modulated by PFF intake (levels ranged from 6.72 ± 0.60 to 7.50 ± 0.70 U SOD mg^{-1} protein).

3.8. Brain antioxidant status

GSH content did not vary ($P > 0.05$) with the addition of PFF in the diet (values from 58.9 ± 2.62 to 63.2 ± 2.68 nmol GSH mg^{-1} protein); however, there was a lower SOD activity either in Peel Colitis ($P < 0.01$) and Peel Sham ($P < 0.001$) relative to AIN Colitis and Sham groups, respectively (Fig. 4 b).

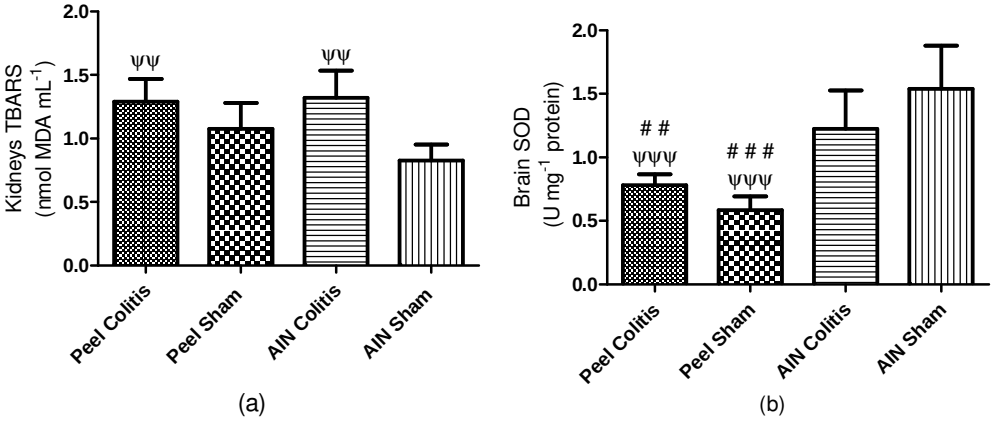

Fig. 4 – Lipid peroxidation by TBARS assay in kidneys (a) and SOD activity in brain (b). Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. Data were analyzed by one-way ANOVA and Tukey test, and

expressed in mean ± SEM. Symbols Indicates statistical differences from groups: * Peel Sham; $^{\#}$ AIN Colitis; $^{\Psi}$ AIN Sham (1 code= P< 0.05; 2 codes= P< 0.01; and 3= P< 0.001).

3.9. Liver antioxidant status

SOD activity was similar amongst AIN and Peel colitis groups (P>0.05) (Fig. 5a). Likewise that in other organs, the experimental groups showed similar GSH levels in the liver (P>0.05) (values ranged from 35.03 ± 1.61 to 40.04 ± 3.86 nmol GSH mg^{-1} protein). Although, there was a significant rise in the GR activity in Peel Colitis group (P<0.01) and a similar tendency for Peel Sham compared to AIN colitis and AIN sham groups (Fig. 5b). Moreover, GPx activity was lower in Peel Colitis group (P<0.01) compared to the other groups. The GPx levels of Peel Sham group were lower than AIN Colitis and AIN Sham groups (Fig. 5c), but the difference is not statistically significant. TBARS assay indicated a higher lipid peroxidation in liver of animals fed with PPF which may demonstrate elevated oxidative stress (Fig. 5d).

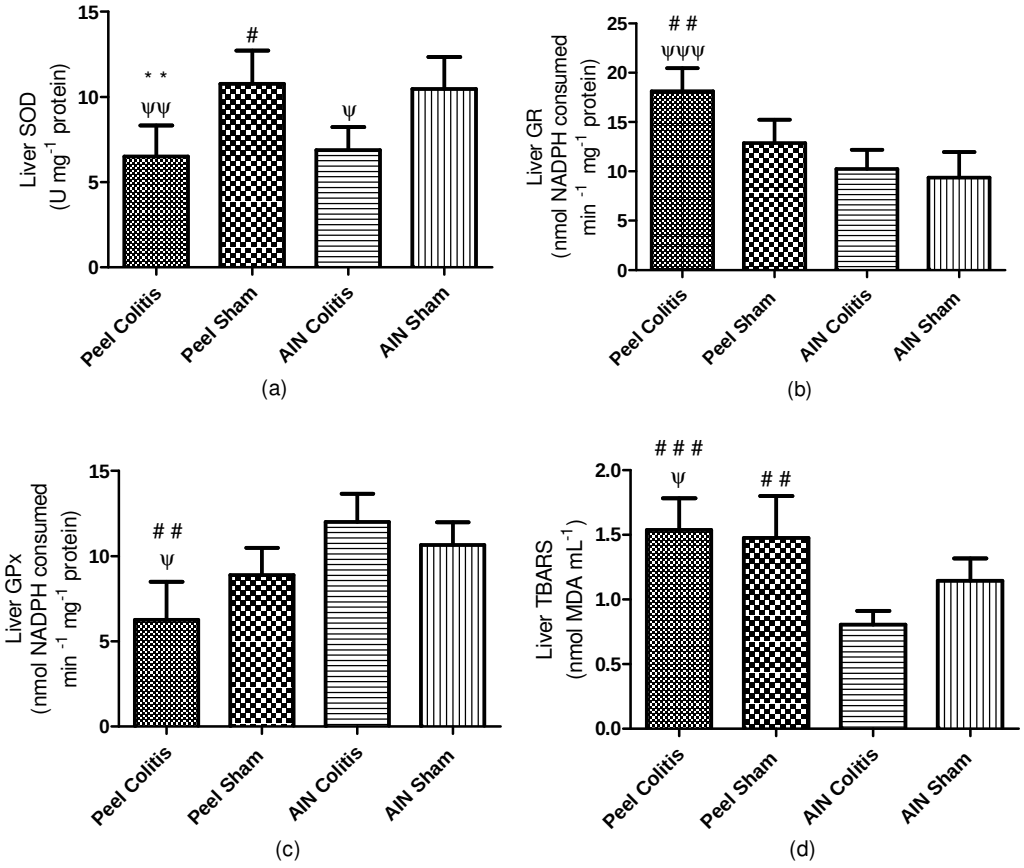

Fig. 5 – Antioxidant enzymes: Superoxide dismutase activity (SOD) (a); Glutathione reductase (GR) (b); Glutathione peroxidase (GPx) (c) Lipid peroxidation by TBARS assay (d) in liver. Peel = animals fed AIN-93M with 50% fiber content replaced by fiber of PPF; AIN = animals fed AIN-93M; Colitis = animals with induced colitis; Sham = healthy animals without induced colitis. Data were analyzed by one-way ANOVA and Tukey test, and expressed in mean ± SEM. Symbols Indicates statistical differences from groups: * Peel Sham; $^{\#}$ AIN Colitis; $^{\Psi}$ AIN Sham (1 code= P< 0.05; 2 codes= P< 0.01; and 3= P< 0.001).

4. DISCUSSION

The search for sources of bioactive compounds has been strengthened due their effects in the combating to chronic diseases, including inflammatory bowel disease [10-12, 14, 21]. Fruits consumption can lead to several benefits because they are rich in fibers,

vitamins, minerals and phytochemicals, which may exhibit biological activity, as antioxidant action [43].

PPF has higher content of total dietary fiber (TDF) than the peel of some banana varieties (*Musa ssp.)* (30- 50 %) [44, 45], freeze-dried jaboticaba (Myrciaria jaboticaba Vell Berg) peel (25%) [46], similar content compared to mango peel [47, 48], as well as another study with *P.edulis* peel [49]. These data indicate that PPF is a rich source of dietary fibers, with higher amount of insoluble fiber (48%) than soluble fiber (17%). A study determined the amount of soluble and insoluble fiber in 70 foods, including cereal grains, fruits and raw vegetables (edible parts). The authors reported a maximum content of insoluble and soluble fiber of 11.8 and 4.5 % respectively. Therefore, PPF could be an alternative ingredient in food industries to increase the amount of fibers in food products. Fibers consumption has proved to exert health benefits, combating obesity, cardiovascular disease, type II diabetes [50] and IBD as well [51].

Polyphenol content in PPF was higher than Brazilian guava, cupuaçu, pear apple, mountain papaya, other exotic fruits [52] and Citrus species peel [53] . Non-edible fruit parts have been shown high polyphenol content [54] and, oftentimes, fruit peels have been even found to contain higher amounts of phenolics than the edible parts [53, 55]. This could be explained because phenolic compounds may act as protective agents against UV lights, pathogens and predators in fruits and vegetables. Fruit parts that receive more UV light may contain higher concentrations of phenolic compounds [55]. These phytochemicals could play a role as antioxidants and anti-inflammatory agents [53, 55]. Phenolic compounds could play a role in signaling pathways, reducing the expression of proinflammatory units, as MPO, COX-2, IL-1β, TNF-α, iNOS [56], and, consequently, also it to decrease the production of reactive species by these molecules.

Thus, PPF consumption could increase the antioxidant and fiber intake, preventing diseases, as IBD. Furthermore, the use of residues could reduce the environment impact generated by industrial residues [57, 58].

In general, inflammatory processes reduces the appetite and its mechanisms are not clear yet [59], though the cytokines are major factors have been linked to this [59, 60]. This is consistent with our results since after the colitis induction there was decrease in dietary intake in all groups. Nevertheless, the addition of PFF in diet was well accepted by the rats, once the consumption did not differ among Peel and AIN groups. The colitis period was too short (7 days) to induce differences in body composition parameters (weight gain and organs weight). In the other hand, total serum protein was similar in Colitis Peel and Sham groups. This result is important because in IBD, nutritional status may be significantly compromised. Eight five percent of individuals with colitis reported protein-energetic deficiency, and inadequate intake is one cause [5]. The reduced serum albumin in AIN Sham group was not expected because the animals were not exposed to TNBS.

Diet added with PFF appears to exert positive effect during bowel inflammation. Its consumption promoted reduced fat tissue and preservation of lean mass according to proximate composition of carcass. Inflammatory process leads to lean tissue catabolism assigned by increased production and release of cytokines, which favors catabolism and suppress anabolism [61, 62]. Therefore, PFF could contribute to avoid protein deficiency, common in IBD, and improve nutritional state and response to treatment.

P. edulis peel intake was able to improve the antioxidant potential and to avoid lipid peroxidation in the rats' serum, in both colitis and control group. GSH content was not altered in the tissues, but SOD was reduced in brain and GPx action decreased in liver. These results indicate that the PPF intake probably avoids the formation of ROS and

consequently reduces antioxidant enzymes activity during induced inflammatory process. The SOD may have a decreased action by performance of the non-enzymatic antioxidants. Probably this occur due the antioxidants capacity to chelate metals ions and to prevent lipid peroxidation leading to an increasing on H_2O_2 and O_2^- concentrations as a consequence of reduced SOD and GPx activities [63]. These positive effects were not observed after treatment with grape fiber in rats, which did not alter plasma antioxidant activity [64]. In other study, *P. edulis* juice intake also led to reduction in thiobarbituric acid–reactive substances concentration in the serum of treated group [65]. Likewise the berries (jaboticaba, blackberries and blueberries) administration has shown to increase serum antioxidant activity [39, 66, 67]. *P. edulis* peel intake demonstrated positive effect against oxidative stress, mainly in serum. And thus, as others products rich in antioxidant compounds, it might play a role in the prevention of diseases and complications that have been associated with excess of oxidative species [14], including IBD, but also many other pathologies and their aspects. Polyphenols of *Rhizophora mangle* bark had gastric antiulcer action which was attributed to antioxidant effect [68].

In addition, Peel group showed an increase on hepatic lipid peroxidation which could be an indicative of peel's oxidative effect. To investigate this result, cooper content of PFF was determined because there may be cooper excess in passion fruit peel, due to products used in agricultural production. Cooper toxicity primarily affects the liver, because it is the first site of deposition this metal after it enters the blood and it is strongly linked to oxidative stress [69]. Bordeaux mixture is a fungicide that can be used in the agriculture to protect against fungi, but increased use of this mixture could lead to environment contamination and toxicity to consumers [70]. Although copper level in peel has been higher than data of literature for passion fruit pulp (1.2 mg kg^{-1}) [71], it did not

exceed the allowed limits [72]. This hypothesis was raised since the Cu toxicity primarily affects the liver, because it is the first site of Cu deposition after it enters the blood and it is strongly linked to oxidative stress [69]. The literature data suggest that *Passiflora* gender is safe and not hepatotoxic [73], however, more investigations, as cytotoxicity assay, are necessary to determinate if *Passiflora* peel have negative action to this organ.

5. CONCLUSION

The *Passiflora edulis* peel' flour (PPF) is a potent source of antioxidants. The PPF added to the diet reduced oxidative stress *in vivo*, mainly in serum. The use of *P. edulis* peel as an industrial food product could contribute to prevent the oxidative stress. Further investigations are necessary to elucidate the possible effects of PPF in liver.

CONFLICT OF INTERESTS

The authors declare that they do not have any conflicts of interest.

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CONCLUSÃO GERAL

A casca de *Passiflora edulis* apresenta teor de compostos fenólicos superior ao encontrado em outras cascas de frutas, e alta quantidade de fibras, tanto solúveis quanto insolúveis, com 65 % de fibra total. A ação *in vivo* avaliada em ratos saudáveis mostrou que a ingestão farinha da casca de *P. edulis* (PPF) promoveu melhora no perfil antioxidante nos rins, o mesmo não foi observado no fígado e soro. Porém, os resultados, principalmente a nível hepático, sugerem que a casca de maracujá pode ter exercido efeito termogênico/ ergogênico, o que explica o aumento no 'status' oxidativo no fígado. Além disso, houve efeito positivo do tratamento sobre a produção de ácidos graxos de cadeia curta (AGCC) no colón, embora a contagem de bactérias nas fezes e pH fecal não ter sofrido alteração, talvez o período experimental tenha sido insuficiente para desempenhar efeito no número de bactérias entéricas.

Verificou-se que a ingestão de PPF melhorou o potencial antioxidante (ORAC) e reduziu a peroxidação lipídica no soro tanto dos animais saudáveis quanto daqueles que sofreram a indução da colite com TNBS. As ações das enzimas antioxidantes, também, foram alteradas pela ingestão de PPF, assim como observado no tratamento em animais saudáveis, a nível hepático houve atividade aumentada de GR e redução na atividade de GPx e SOD, sendo que a diminuição da ação de SOD também ocorreu nos rins. Contudo, ocorreu elevação na peroxidação lipídica hepática entre os animais controle ou com colite que ingeriram PPF, o que mostra aumento no estado oxidativo deste órgão sem ligação com a inflamação.

Os resultados dos estudos realizados demonstram que a casca de maracujá é fonte de fibras e compostos fenólicos, com potencial para melhorar a capacidade antioxidante

sérica e renal durante condições patológicas ou saudáveis. Além disso, os resultados apontam que a casca do maracujá poderia contribuir para a manutenção da saúde intestinal com o aumento da produção de AGCC nos animais que sofreram indução da colite com TNBS, o que poderia auxiliar na proteção contra o desenvolvimento de doenças intestinais. Portanto, o uso da casca é uma alternativa de ingrediente para indústria de alimentos, com efeito positivo sobre a geração de resíduos advindos da produção de suco e compossíveis benefícios à saúde dos indivíduos. Contudo, são necessárias novas pesquisas que avaliem uma possível ação hepática da PPF, e determinar se o consumo da casca traz benefícios e é segura a nível sistêmico.

Anexo 1 - Certificado de aprovação dos ensaios biológicos pela Comissão de Ética no Uso de Animais (CEUA) da Unicamp

UNICAMP

CEUA/Unicamp

Comissão de Ética no Uso de Animais
CEUA/Unicamp

CERTIFICADO

Certificamos que o projeto **"Avaliação da atividade antioxidante da casca de *Passiflora edulis* em ratos com colite ulcerativa"** (protocolo nº **2385-1**), sob a responsabilidade de **Prof. Dr. Mário Roberto Maróstica Junior / Juliana Kelly da Silva**, está de acordo com os **Princípios Éticos na Experimentação Animal** adotados pela **Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)** e com a legislação vigente, **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, e o **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**.

O projeto foi aprovado pela Comissão de Ética no Uso de Animais da Universidade Estadual de Campinas - CEUA/UNICAMP - em 04 de abril de 2011.

Campinas, 04 de abril de 2011.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

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