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Tatiele Casagrande do Nascimento

AVALIAÇÃO DA INFLUÊNCIA DE BIOCOMPOSTOS MICROALGAIS NO *STATUS* ANTIOXIDANTE E PERFIL LIPÍDICO DE CAMUNDONGOS

Santa Maria, RS
2017

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Dissertação apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciência e Tecnologia dos Alimentos**.

Orientadora: Prof Dra. Leila Queiroz Zepka

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Aprovado em 06 de março de 2017:

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Santa Maria, RS
2017

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A persistência é o caminho do êxito.

(Charles Chaplin)

RESUMO

AVALIAÇÃO DA INFLUÊNCIA DE BIOCOMPOSTOS MICROALGAIS NO *STATUS* ANTIOXIDANTE E PERFIL LIPÍDICO DE CAMUNDONGOS

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A biomassa microalgal é constituída por inúmeros metabólitos de interesse, os quais podem desempenhar efeitos benéficos à saúde humana. Nesse sentido, o objetivo deste trabalho foi avaliar *in vivo* influência do consumo de biomassa de *Scenedesmus obliquus* CPCC05 no perfil lipídico e *status* antioxidante de camundongos. A biomassa microalgal foi investigada quanto a sua composição de acidos graxos, aminoácidos e carotenoides. Adicionalmente foi avaliado o potencial antioxidate do extrato de carotenoides. Utilizou-se no ensaio *in vivo* 18 camundongos machos, linhagem BALB/cAnUnib, os quais foram divididos em grupo controle e grupos tratados. A biomassa microalgal foi administrada via gavagem em uma suspenção de 0,15mL de água, nas concentrações de 400 ou 800mg.kg^{-1}bw.d^{-1} nos grupos tratados. Para fins de padronização o grupo controle recebeu apenas água via gavagem. Todos os grupos receberam dieta comercial (Nuvilab®) e água *ad libitum*. Após 32 dias, os animais foram eutanasiados e amostras de soro foram retiradas para análise de determinação de colesterol total, triglicerídeos, lipoproteina de alta e baixa densidade (HDL e LDL), e atividade de enzimas de função hepática, transaminase piruvica (TGP) e transaminase oxalacetica (TGO). Amostras de tecidos (coração, fígado, ríns e baço) foram recolhidos para determinação do potencial antioxidante (ORAC), conteúdo de especies reativas ao ácido tiobarbitúrio (TBARS), glutationa reduzida (GSH) e enzimas antioxidantes tais como a glutationa peroxidase (GPx), glutationa redutase (GR), superóxido dismutase (SOD) e catalase (CAT). A biomassa apresentou elevado conteúdo de biocompostos com influência positiva na saúde humana tais como ácidos graxos, aminoácidos e carotenoides com elevado potencial antioxidante. No estudo *in vivo*, verificou-se um aumento da HDL seguido da diminuição da LDL, independente da dose. No que se refere as enzimas de função hepática, a dose de 800mg.kg^{-1} foi a única não apresentou qualquer efeito adverso associado aos níveis de TGP e TGO. O sistema antioxidante endógeno (SOD, CAT, GPx, GR e GSH) apresentou-se em quase todos os tecidos, inalterado ou reduzido, exceto nos rins, uma vez que se observou um aumento na atividade GPx para dose de 400mg.kg^{-1} e da GSH para 800mg.kg^{-1}. Além disso, ambas as doses administradas diminuíram de forma significativa os níveis de TBARS em todos os tecidos. Observou-se ainda uma considerável correlação negativa entre TBARS e ORAC no coração, fígado e baço, exceto para os rins. Com base nos resultados obtidos, sugere-se que a biomassa de *Scenedesmus obliquus* CPCC05 é fonte potencial de inúmeros biocompostos capazes de modularem de forma positiva o perfil lipídico sérico de camundongos, bem como exercer efeito protetivo frente a peroxidação lipídica tecidual.

Palavras-chave: Microalga. Carotenoides. Aminoácidos. Ácidos graxos. Ensaio biológico. Potencial antioxidante. Enzimas antioxidantes. Peroxidação celular.

ABSTRACT

EVALUATION OF THE INFLUENCE OF MICROALGAL BIOCOMPOUNDS ON THE ANTIOXIDANT STATUS AND LIPID PROFILE IN MICE

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Microalgal biomass is constituted by numerous metabolites of interest, which may play beneficial effects on human health. In this sense, the aim of this work was to evaluate the influence of consumption of *Scenedesmus obliquus* CPCC05 biomass in lipid profile and antioxidant status of the mice. Microalgal biomass was investigated in relation to its composition of fatty acids, amino acids and carotenoids. Also, the antioxidant potential of the carotenoid extract was also evaluated. Was used in the in vivo test 18 male mice, lineage BALB / cAnUnib, which were divided into control and treated groups. The microalgal biomass was administered via gavage in a suspension of 0.15 mL of water, at concentrations of 400 or 800 $mg.kg^{-1}bw.d^{-1}$ in the treated groups. For standardization, the control group received only water by gavage. All groups received commercial diet (Nuvilab®) and water *ad libitum*. After 32 days, the animals were euthanized, and serum samples were taken for determination of total cholesterol, triglycerides, high and low-density lipoprotein (HDL and LDL), and activity of hepatic function enzymes, pyruvate transaminase (TGP), and Transaminase oxalacetic (TGO). Tissue samples (heart, liver, rins and spleen) were collected for determination of antioxidant potential (ORAC), thiobarbituric acid reactive species content (TBARS), reduced glutathione (GSH) activity and antioxidant enzymes such as glutathione peroxidase (GPx), Glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT). Biomass showed a high content of biocompounds with a positive influence on human health such as fatty acids, amino acids and carotenoids with high antioxidant potential. In the in vivo study, there was an increase in HDL followed by a decrease in LDL, regardless of dose. As regards liver enzymes, the dose of $800mg.kg^{-1}$ showed no adverse change associated with the levels of the TGP and TGO. The endogenous antioxidant system (SOD, CAT, GPx, GR, and GSH) showed in almost all tissues, unchanged or reduced, except in the kidneys, since an increase in GPx activity was observed for dose of $400mg.kg^{-1}$ and GSH to $800mg.kg^{-1}$. In addition, both doses administered significantly decreased TBARS levels in all tissues. There was also a considerable negative correlation between TBARS and ORAC in the heart, liver and spleen, except for kidneys. Based on the results, it is suggested that the biomass of *Scenedesmus obliquus* CPCC05 is a potential source of numerous biocompounds capable of modulating in a positive way the serum lipid profile of mice, as well as exerting a protective effect against tissue lipid peroxidation.

Keywords: Microalgae. Carotenoids. Amino acids. Fatty acids. Biological assay. Potential antioxidant. Antioxidant enzymes. Cell peroxidation.

LISTA DE ILUSTRAÇÕES

LISTA DE TABELAS

LISTA DE ABREVIATURAS

BCAA- Aminoácidos de cadeia ramificada (do inglês *Branched Chain Amino Acids*)

CAT - Catalase

CT - Colesterol Total

EROs - Espécies Reativas de Oxigênio

GPx - Glutationa Peroxidase

GR - Glutationa Redutase

GSH - Glutationa Reduzida

HDL - Lipoproteína de Alta Densidade (do inglês *High-Density Lipoprotein*)

LDC - Duplas Ligações Conjugadas

LDL - Lipoproteína de Baixa Densidade (do inglês *Low-Density Lipoprotein*)

MUFAs- Ácidos Graxos monoinsaturados (do inglês *Monounsaturated Fatty Acids*)

NADPH- Nicotinamida Adenina Dinucleotídeo Fosfato (do inglês *Nicotinamide Adenine Dinucleotide Phosphate*)

ORAC- Capacidade de absorção de radicais de oxigênio (do inglês *oxygen radical absorption capacity*)

PB - Tampão fosfato (do inglês *Phosphate buffer*)

PUFAs - Ácidos Graxos Poli-insaturados (do inglês *Polyunsaturated Fatty Acids*)

SFAs- Ácidos Graxos saturados (do inglês *Saturated Fatty Acids*)

SOD - Superóxido Dismutase

TAG - Triacilgliceróis

TBARS- Espécies Reativas ao Ácido Tiobarbitúrico (do inglês *thiobarbituric acid reactive species*)

TGO- Transaminase Oxalacética

TGP- Transaminase Pirúvica

VLDL - Lipoproteínas de Muito Baixa Densidade

SUMÁRIO

INTRODUÇÃO

Ao longo dos últimos anos o estresse oxidativo ocasionado pelo desequilíbrio entre a geração de espécies reativas de oxigênio (EROs) e o *status* antioxidante tem sido associado à inúmeras doenças de natureza crônico-degenerativas e processos de envelhecimento (BHAT et al., 2015; GARCÍA-BLANCO et al., 2017). Além disso, outras patologias tais como obesidade, diabetes *mellitus*, doenças cardiovasculares e hipertensão tem sido desencadeadas em função da quantidade e qualidade dos ácidos graxos ingeridos a partir da dieta (GOMEZ-GUTIERREZ et al., 2011; CONLON & BIRD, 2015, JIN et al., 2017). Em função disso, é crescente o interesse dos pesquisadores em identificar novos compostos ou fontes alimentares que apresentem potencialidades funcionais ou bioativas que vão além da nutrição básica.

Nesse contexto, as microalgas são um grupo de micro-organismos promissores, dos quais podem ser isolados uma gama de produtos naturais, inclusive bioativos. Elas podem ser incorporadas em uma infinidade de aplicações industriais, abrangendo alimentação humana, cosméticos, biocombustíveis, produtos químicos, entre outros (GOUVEIA et al., 2010; GAFFNEY, O'ROURKE & MURPHY, 2014). Adicionalmente, a biomassa microalgal apresenta elevado conteúdo de proteínas (JACOB-LOPES et al., 2006; BECKER, 2007), ácidos graxos poli-insaturados (PUFAs) (FRANCISCO et al., 2014; WANG, 2015), compostos bioativos, como carotenoides e clorofilas (ROGRIGUES et al., 2014), e em menor quantidade, compostos fenólicos (MAADANE et al., 2015), os quais podem aumentar o conteúdo nutricional de preparações de alimentos convencionais, além de afetar positivamente a saúde humana (SPOLAORE et al., 2006).

Dentre esses microrganismos promissores está a *Scendesmus obliquus,* uma microalga com característica esférica, unicelular e diâmetro aproximado de 5-10µm (BECKER, 2006). Pertence à classe das clorofíceas as quais são amplamente associadas a produção de biomassa e numerosos compostos celulares (JACOB-LOPES et al., 2006; QUEIROZ et al., 2007; JACOB-LOPES et al., 2007; JACOB-LOPES et al., 2008; ZEPKA et al., 2008; JACOB-LOPES et al., 2009; JACOB-LOPES et al., 2010; ZEPKA et al., 2010; QUEIROZ et al., 2011; RODRIGUES et al. 2015; SANTOS et al., 2016; MARONEZE et al, 2016).

No que se refere aos ácidos graxos, a capacidade destes microrganismos em sintetizar estes compostos celulares, tais como ácido araquidônico, eicosapentaenoico, docosaexaenoico e outros da família ômega 3 e 6, confirma sua aplicabilidade, especialmente na indústria de alimentos funcionais (BECKER, 2004; PEREZ-GARCIA et al., 2011). De acordo com Colussi et al. (2017), a inclusão de ácidos graxos monoinsaturados (MUFAs) e PUFAs na dieta, reduz o colesterol total e a lipoproteína de baixa densidade (LDL), bem como impulsiona um aumento no conteúdo de lipoproteína de alta densidade (HDL). A incorporação de biomassa microalgal na alimentação humana poderia contribuir para a prevenção de doenças relacionadas ao baixo consumo de PUFAs, especialmente àqueles da família Ômega-3 (CHACON-LEE & GONZALEZ-MARINO, 2010).

Em adição, os aminoácidos de cadeia ramificada (BCAA - *branched chain amino acids*), que estão presentes na proteína microalgal, também têm apresentado importante papel na atividade anti-inflamatória e no equilíbrio do estresse oxidativo, entretanto o mecanismo de ação ainda é pouco conhecido (TAJIRI & SHIMIZU, 2013; LU et al., 2014; TANAKA et al., 2016). De acordo com Katayama e Mine (2007), a administração de BCAA aumentou expressão de algumas enzimas antioxidantes, inferindo maior efeito protetor.

Finalmente, a quantidade e a qualidade dos carotenoides encontrados na biomassa microalgal confirma o seu potencial de exploração para esses pigmentos (POWLS & BRITTON, 1976; PRASANNA et al., 2010; WALTER & STRACK, 2011; RODRIGUES et al., 2015). Pois em termos de saúde humana, a ingestão destes biocompostos tem sido amplamente associada a efeitos benéficos à saúde, que são atribuídos principalmente às suas propriedades antioxidantes. Em relação aos efeitos antioxidantes, estudos têm mostrado que estas estruturas moleculares podem reduzir o estresse oxidativo, atuando na regulação do crescimento celular, modulação da expressão gênica e resposta imune (STAHL & SIES, 2003).

Diante do exposto, acredita-se que a biomassa de microalgas pode desempenhar um papel importante a nível de saúde humana, principalmente na redução de EROs e modificação do perfil lipídico. Existe muito conhecimento acerca da identificação de biocompostos a partir da biomassa microalgal, no entanto verifica-se que ainda é um desafio científico entender a influência desses compostos na modulação de sistemas biológicos. Em face disso, o trabalho teve como objetivo

avaliar o impacto do consumo de biomassa de *Scenedesmus obliquus* CPCC05 no status antioxidante e perfil lipídico de camundongos.

OBJETIVOS

Objetivo geral

Considerando que a biomassa microalgal apresenta um elevado teor de biocompostos e que estes podem ser capazes de modular/interferir no perfil lipídico e *status* antioxidante em sistemas biológicos este trabalho teve como objetivo geral avaliar o impacto do consumo de biomassa microalgal no *status* antioxidante e perfil lipídico de camundongos saudáveis.

Objetivos específicos

Para atingir o objetivo geral foram estabelecidos os seguintes objetivos específicos:

Produzir biomassa microalgal de *Scenedesmus obliquus* (CPCC05).

Determinar o perfil de lipídios da biomassa de *Scenedesmus obliquus* (CPCC05).

Determinar o perfil de aminoácidos da biomassa de *Scenedesmus obliquus* (CPCC05).

Determinar o perfil de carotenoides da biomassa de *Scenedesmus obliquus* (CPCC05).

Determinar o potencial antioxidante dos extratos dos carotenoides microalgais *in vitro*.

Avaliar o perfil lipídico sérico de camundongos saudáveis após a administração diária de 400 e 800mg de biomassa microalgal por peso corpóreo.

Avaliar o *status* antioxidante tecidual de camundongos saudáveis após a administração diária de 400 e 800mg de biomassa microalgal por peso corpóreo.

CAPÍTULO 1

REVISÃO BIBLIOGRÁFICA

1. MICROALGAS

O termo microalgas não tem valor taxonômico e engloba microrganismos algais com clorofila e outros pigmentos fotossintéticos. Sua caracterização implica a consideração de uma série de critérios, tais como a natureza química dos produtos de reserva, a pigmentação, constituintes da parede celular e ainda critérios citológicos e morfológicos (CHACON-LEE & GONZALEZ-MARINO, 2010). No entanto, sob a denominação microalgas, estão incluídos organismos com dois tipos de estrutura celular: estrutura procariótica, com representantes nas divisões Cyanophyta e Prochlorophyta, e estrutura celular eucariótica, com representantes nas divisões Chlorophyta, Euglenophyta, Rhodophyta, Haptophyta, Heterokontophyta, Cryptophyta e Dinophyta (QIANG et al., 2008).

As microalgas são seres unicelulares (isolados ou coloniais) ou pluricelulares. Seus cloroplastos possuem clorofilas, carotenos e xantofilas. A reserva é representada por amido e as paredes celulares possuem celulose. Vivem em ambientes terrestres úmidos, na água doce ou no mar (RADMANN, et al. 2011).

No que se refere a microalga *Scenedesmus obliquus*, esta é uma espécie de água doce que apresenta característica esférica, unicelular com diâmetro aproximado de 5-10µm (BECKER, 2006) e pertence à classe das clorofíceas.

As clorofíceas estão entre as linhagens que vêm sendo utilizadas na produção de biomassa e compostos celulares (proteínas, lipídios, carboidratos, pigmentos e ácidos nucléicos), as quais podem ser amplamente aplicadas como insumos intermediários e produtos finais de processos relacionados à bioenergia, alimentação e farmacêuticos (JACOB-LOPES et al., 2006; QUEIROZ et al., 2007; JACOB-LOPES et al., 2007; JACOB-LOPES et al., 2008; ZEPKA et al., 2008; JACOB-LOPES et al., 2009; JACOB-LOPES et al., 2010; ZEPKA et al., 2010; QUEIROZ et al., 2011; RODRIGUES et al. 2015; SANTOS et al., 2016; MARONEZE et al, 2016).

Concomitantemente esses microrganismos oferecem uma alternativa às formas convencionais dos tratamentos de águas residuais, devido à sua alta eficiência na remoção de poluentes (QUEIROZ et al., 2007), bem como a possibilidade de valorização dos resíduos agroindustriais por biotransformação em produtos de valor agregado (QUEIROZ et al., 2002; JACOB-LOPES et al., 2007, RODRIGUES et al., 2014; SANTOS et al., 2016; FERNANDES et al., 2016). O modo mais comum de

cultivo de microalgas é o crescimento autotrófico, contudo, um número considerável destes microrganismos possui imensa versatilidade metabólica, sendo capazes de crescer de forma heterotrófica (WEN & CHEN, 2003). Em sistemas heterotróficos, a produção de biomassa ocorre por conversão de compostos orgânicos na ausência de energia luminosa. Por outro lado, em sistemas fotossintéticos, a biomassa é obtida por conversão de dióxido de carbono (CO_2) na presença de luz (QUEIROZ et al., 2007).

2. BIOCOMPOSTOS MICROALGAIS

As microalgas vêm sendo apontadas como um reservatório de metabólitos com potencial terapêutico e bioregulatório (ZEPKA et al., 2010; ADARME-VEGA et al., 2012; RAPOSO et al., 2013; RODRIGUES et al., 2015; GENG et al., 2016). Metabólitos com propriedades bioativas vem desempenhando papéis biológicos cada vez mais importantes baseados em suas propriedades antimicrobianas, antitumorais, antioxidantes e hipocolesterolêmica (AMBATI et al., 2014).

De acordo com Mao et al. (2017) esses potentes efeitos biológicos vem impulsionando a exploração/investigação de novas fontes potenciais como a biomassa de microalgas. Esses microrganismos tem se tornado o foco de exploração, pois sua biomassa é constituída de inúmeros compostos com atividades biológicas que podem apresentar elevada aplicabilidade terapêutica, entre estes compostos estão os ácidos graxos poli-insaturados, aminoácidos e carotenoides.

2.1. Ácidos graxos

Os lipídios algais são tipicamente compostos por glicerol, açúcares ou bases esterificadas ácidos graxos com número de carbonos variando de 12 a 22, e perfazem entre 1 a 40% do peso seco da biomassa (BECKER, 2004). As microalgas mostram-se como uma opção promissora para produção destes compostos a partir de organismos unicelulares (RATLEDGE, 2001; QUEIROZ et al., 2011).

Lipídios obtidos a partir destes micro-organismos atraem atenção em todo o mundo, tanto para aplicações de fins energéticos, quanto nutricionais ou terapêuticos (WIJFFELS & BARBOSA, 2010). A adequabilidade desses óleos para tais aplicações,

está relacionada as suas proporções relativas de ácidos graxos saturados (SFAs), MUFAs e PUFAs. Em linhas gerais, tornam-se promissores para produção de biocombustíveis o óleo que apresentar predominância de ácidos graxos saturados (KNOTHE, 2009).

Em termos nutricionais, o óleo de microalgas pode ser considerado fonte potencial PUFA's, tais como, os da família ômega-3 e 6 (UAUY et al., 2003). O foco de muitas pesquisas é tornar comercialmente viável a produção desses compostos (RATLEDGE & COHEN, 2008) visto que, em algumas espécies esses ácidos graxos podem representar cerca de 25 a 60% do total da fração lipídica (BECKER, 2004). Estudos têm demonstrado que estes compostos apresentam efeitos benéficos sobre o metabolismo lipídico, atuam na regulação dos níveis plasmáticos de colesterol total (CT), HDL e LDL, consequentemente reduzindo o desencadeamento de inúmeras patologias (FERNÁNDEZ et al., 2011; WILLET, 2012; SCHWINGSHACKL & HOFFMANN, 2012; COUCH et al., 2017).

2.2. Aminoácidos

Os aminoácidos são peptídeos que compõem as proteínas, os quais em função de seu teor, proporção, disponibilidade e variabilidade determinam a qualidade nutricional da proteína formada (BECKER, 2007).

Há alguns anos se tem movido esforços para explorar novas fontes alternativas de proteínas, principalmente em função das provisões feitas pela Organização das Nações Unidas (ONU/FAO) a cerca de uma futura escassez proteica (WU et al., 2014). Em função disso, passa ser uma alternativa atraente a proteína celular que compõem a biomassa de fungos, bactérias e microalgas, podendo chegar a 70% neste último grupo (BECKER, 2007).

A produção de proteína unicelular, através da biomassa residual fornece uma fonte economicamente viável de proteína, para fins de utilização em ração animal, uma vez que muitas vezes atende aos requisitos nutricionais (VOLTOLINA et al., 2005). Além disso, algumas microalgas devido a qualidade de seu perfil aminoacídico, podem ser utilizadas como produtos nutracêuticos ou ser incluídas em preparações alimentícias como um composto funcional para prevenir algumas doenças e danos nas células ou tecidos (RAPOSO et al., 2013). O teor proteico associado ao balanço

de aminoácidos ratifica o elevado valor biológico destas proteínas (JACOB-LOPES et al., 2006; ZEPKA et al., 2010). De acordo com Becker (2004), o padrão de aminoácidos de quase todas as algas, se equipara a alguns alimentos convencionais e inclusive a padrão de referência recomendado pela Organização Mundial de Saúde (OMS).

Estudos relatam que BCAA incluindo a leucina, isoleucina e valina, além de exibirem importante papel na regulação nutricional apresentam outras funções marcantes no organismo. Tanaka et al. (2015) sugerem que estes aminoácidos podem equilibrar o estresse oxidativo que ocorre no pâncreas diabético e em algumas doenças do fígado, porém a relação entre esses aminoácidos e o estresse oxidativo ainda é pouco compreendida.

Estudos realizados por Becker (2007) demonstra que esses aminoácidos de cadeia ramificada estão presentes de forma significativa no perfil aminoácidico da grande maioria das microalgas.

2.3. Carotenoides

Os carotenoides são compostos bioativos responsáveis pela cor amarela, laranja ou vermelha dos alimentos; tal efeito decorre de sua estrutura básica que consiste em um esqueleto linear e simétrico com uma série de duplas ligações conjugadas (LDC), denominado cromóforo de absorção de luz (RODRIGUEZ-AMAYA, 2001).

Esses compostos são geralmente tetraterpênicos construídos por 8 unidades isoprenoides e podem ser classificados em dois grupos, com base nos grupamentos funcionais; xantofilas, contendo oxigênio, como grupo funcional, incluindo luteína e zeaxantina, e carotenos, que contêm apenas cadeia de hidrocarboneto progenitor sem qualquer grupo funcional, tais como α-caroteno, β-caroteno e licopeno (BRITTON, 2008). São pigmentos lipossolúveis produzidos como metabólitos secundários em frutas, vegetais, algas, fungos e algumas bactérias (ZAGHDOUDI et al., 2015).

Os principais carotenoides já relatados em microalgas são β-caroteno, zeaxantina, luteína, nostoxantina, oscilaxantinas, equinenona, mixoxantofilas e cantaxantina (POWLS & BRITTON, 1976; PRASANNA et al., 2010; WALTER & STRACK, 2011; RODRIGUES et al., 2015).

Essa classe de biocompostos possui elevado potencial antioxidante e esta propriedade está relacionada ao sistema de duplas ligações conjugadas presentes em sua estrutura (MERCADANTE, 2008), podendo ser influenciada pelo número de LDC, tipo de grupos terminais estruturais e oxigênio contendo substituintes (SAINI; NILE & PARK, 2015). Sugere-se uma maior atividade antioxidante para carotenoides microalgais em relação a fontes convencionais, devido à presença de carotenoides exclusivos, os quais apresentam efeito bato crômico, como é o caso da equinenona e cataxantina com cromóforo de 12 e 13 LDC respectivamente (ALBRECHT et al., 2000; KLASSEN & FOGHT, 2011), sabe-se que a extensão do cromóforo está intimamente relacionada ao aumento da atividade antioxidante.

Um dos mecanismos de desativação de espécies reativas é a eliminação do oxigênio singleto, que pode ocorrer de duas maneiras: o carotenoide reage com o mesmo, dando origem a produtos de oxidação; ou libera energia do estado excitado do oxigênio fazendo com que o mesmo volte a sua forma fundamental (KUSAMA et al, 2015). Além deste, outros mecanismos de desativação de radicais por carotenoides são conhecidos, tais como transferência de elétrons, captação do hidrogênio alílico e adição do radical ao sistema de duplas ligações (EL-AGAMEY, 2004).

Em termos de saúde humana, em decorrência do efeito protetor exercido nas células, estes compostos têm sido associados com a redução do risco de desenvolvimento de doenças crônicas tais como o câncer, doenças cardiovasculares, catarata e degeneração macular (RODRIGUEZ-AMAYA, 2015).

3. INFLUÊNCIA DE COMPOSTOS BIOATIVOS CONVENCIONAIS EM SISTEMAS BIOLÓGICOS

Compostos bioativos apresentam influências biológicas decorrida de efeitos proporcionados por sua estrutura química. São capazes de influenciar nas atividades celulares que modificam e reduzem o risco de diversas doenças crônicas-degenerativas associadas ao estresse oxidativo ou desregulação do metabolismo lipídico plasmático (ARON & KENNEDY, 2008; FERNÁNDEZ et al., 2011; WILLET, 2012; SCHWINGSHACKL & HOFFMANN, 2012; BHAT et al., 2015; MARTINEZ-FLORES, et al., 2015; GARCÍA-BLANCO et al., 2017; COUCH et al., 2017).

Em relação aos ácidos graxos poli-insaturados, estudos realizados por Takashima et al. (2016) demostraram que a administração de PUFAs, ômega-3, em camundongos atenuaram significativamente a desestabilização de placas ateroscleróticas, bem como suprimiram a expressão de RNAm de moléculas inflamatórias. Singer et al. (1990) demostraram que o óleo de linhaça rico, em ácido α-linolênico (ômega-3), foi capaz de reduzir significativamente a LDL, os triglicérides séricos bem como o nível de colesterol total e a relação LDL/HDL.

Colussi et al. (2015) observaram em pacientes com síndromes metabólicas que a redução da ingesta de ômega 3 ou 6 contribuiu para atenuação do risco cardiovascular, uma vez que reduziu os níveis plasmáticos da HDL. Relataram ainda uma correlação direta entre o consumo de PUFAs e o aumento da HDL. Além disso, propriedades antioxidantes de compostos ômega-3 através de óleo de peixe sob o endotélio de camundongos também foram relatadas, diminuiu-se a produção de ânions superóxido e danos lipo-oxidativos a proteínas, resultando numa progressão diminuída da aterosclerose (WANG et al., 2004).

Efeitos antioxidantes associados a peptídeos e aminoácidos proteicos também tem sido relatados (WU, CHEN, & SHIAU, 2003). Uma relação em função de estrutura pode ser usada para explicar as propriedades antioxidantes da maioria dos aminoácidos. Metionina e cisteína possuem um grupo tiol, que atua como precursor para síntese de GSH (importante antioxidante endógeno), tirosina e fenilalanina possuem grupos fenólicos que permitem a quelação de íons metálicos pró-oxidante, enquanto histidina tem um anel imidazol que mostrou-se responsável por certas propriedades antioxidantes envolvendo quelação de íons metálicos e capacidade de captura de radicais lipídicos (JE, PARK & KIM, 2005; AGYEI et al., 2015).

Em camundongos, dietas ricas em BCAA mostraram regular a expressão do principal regulador da biogênese mitocondrial (proliferador de peroxissoma (PPARγ)) e o sistema de defesa contra espécies reativas de oxigênio (D'ANTONA, et al., 2010). Além disso, Ichikawa et al. (2012) demonstraram em modelo de lesão hepática em ratos que os BCAA induzem a ativação de genes envolvidos em defesas antioxidantes e inibição da produção de EROs, bem como conduzem a expressão hepática de RNAm que codifica uma enzima envolvida na reparação de danos oxidativos ao DNA. Pesquisa realizada por Yeh and Yen (2006) demostrou ainda a influência de fenilalanina na regulação positiva da expressão de Nrf2 (regulador da expressão de

síntese enzimática), que ocasionou um aumento nos níveis de RNAm para transcrição das enzimas antioxidante SOD e GPx no fígado de ratos.

O estresse oxidativo leva a um dano celular que culmina no desenvolvimento de doenças degenerativas, nesse contexto a ação de carotenoides contra essas doenças tem sido amplamente associada a sua atividade antioxidante (KRINSKY, 2001; YOUNG & LOWE, 2001; STAHL & SIES, 2003; ASTLEY et al., 2004; KIOKIAS & GORDON, 2004). Além disso, a habilidade de promover um aumento na expressão do sistema endógeno de proteção anti-radical também tem sido considerada, resultados demonstrados por Serpeloni et al. (2014) sugerem que o tratamento com luteína pode melhorar a resposta antioxidante através de um aumento na expressão de GPx e níveis de GSH no fígado e rins de camundongos.

Osganian et al. (2003) demonstraram associação inversa, modesta, mas significativa entre os níveis de ingestão de β-caroteno e α-caroteno e o risco de doença arterial coronariana. Entre os vários radicais que são formados sob condições oxidativas no organismo, os carotenoides reagem eficientemente sob radicais peroxil, devido à sua lipofilicidade e propriedades específicas para eliminar esses radicais, Sies e Stahl (1995) sugeriram que os carotenoides desempenham um papel importante na proteção das membranas celulares e lipoproteínas contra o dano oxidativo.

4. INFLUÊNCIA DE BIOCOMPOSTOS MICROALGAIS EM SISTEMAS BIOLÓGICOS

Nos últimos anos, o uso de microalgas tem atraído interesse de pesquisadores em função do seu potencial produção de biomassa associado a valiosos biocompostos celulares, os quais podem ser amplamente aplicados como insumos intermediários e produtos finais de processos relacionados à bioenergia, alimentação e farmacêuticos (ZEPKA et al., 2008; JACOB-LOPES et al., 2010; ZEPKA et al., 2010; QUEIROZ et al., 2011; 2014; RODRIGUES et al. 2015; SANTOS et al., 2016; MARONEZE et al, 2016). Em linhas gerais a biomassa microalgal contêm 40-70% de proteínas, 12-30% de carboidratos, 4-20% de lipídios, 8-14% de caroteno e quantidades substanciais de vitaminas do complexo B, E, K e D (BECKER, 2007). A potencialidade bioativa exercida por alguns destes compostos torna passível sua aplicação para fins terapêuticos.

Em termos de nutrição humana a biomassa microalgal na sua forma integral é amplamente utilizada para fins de suplementação proteica. A *Spirulina* sp. é o gênero mais explorado por conter cerca de 50-70% de proteínas em sua biomassa, no entanto o gênero *Dunaliella* também tem apresentado potencial para fins de alimentação em função da sua produtividade proteica alcançada (PLAZA et al., 2008). Adicionalmente, o efeito anti-inflamatório de *Spirulina* sob artrite foi comprovado por Ramirez et al. (2002).

No que se refere a aplicação com finalidade terapêutica dos biocompostos microalgais, os PUFAs, especialmente ω-3 e ω-6, como ácido eicosapentaenóico (EPA), ácido docosahexaenóico (DHA) e ácido α-linolênico (ALA) que já foram encontrados em biomassa microalgal (BANDARRA et al., 2003) possuem efeitos positivos sobretudo sob progressão de doenças coronárias (MOZAFFARIAN et al., 2011). Poucos estudos relatam a inserção de PUFAs microalgais em sistemas biológicos, uma pesquisa clínica envolvendo pacientes hiperlipidêmicos que receberam suplementação de *Spirulina* em função de seu conteúdo de Y-linolênico (ω-6), demostrou reduções significativas nos níveis séricos de colesterol total, LDL e triglicérides, no entanto a HDL também mostrou-se reduzida (Samuels et al., 2002).

Além da aplicação como importante fonte proteica vários estudos demonstraram que os peptídeos derivados da hidrólise enzimática de proteínas microalgais possuem propriedades antioxidantes e anti-hipertensivas que podem ser aplicadas para promover a saúde humana (EJIKE et al., 2017). A exemplo, Ko, Kim e Jeon (2012) demostraram que a hidrólise péptica de *Chlorella ellipsiodea* produziu um pentapeptídeo (Leu-Asn-Gly-Asp-Val-Trp) com potente capacidade de eliminação de radicais livres, este peptídeo promoveu a eliminação de radical peroxil intracelular em células de rim de macaco.

Chidambara-Murthy et al. (2005) demostraram que carotenoides microalgais causaram um aumento na atividade nas enzimas hepáticas antioxidantes CAT e GPx, bem como aumentos na atividade de SOD em ratos. Além disso, quando os ratos receberam 250 µg/kg de carotenoides de *Dunaliella salina,* os aumentos observados nos níveis destas enzimas foram superiores aos observados em ratos alimentados com carotenoide sintético. Além disso os carotenoides naturais demostraram efeito protetor frente a peroxidação lipídica induzida.

Em adição, o extrato total de carotenoides de *Dunaliella* foram fornecidos como suplemento dietético em modelo de ratos em estudo realizado por El-Baky et al., (2007). Os ratos alimentados com 0,5g e 1,0g de pigmento por kg de peso corporal demonstraram reduções de 29,6% e 40%, respectivamente, no colesterol total plasmático. Bem como reduções de 23,9% e 33,8% em comparação com animais alimentados com dietas padrão. Adicionalmente, os triglicérides (TG) provocaram reduções de 21,4% a 34,3% em ratos alimentados com carotenoides naturais em comparação com ratos alimentados com β-caroteno sintético.

5. MODULAÇÃO DE LIPÍDIOS SÉRICOS A PARTIR DA DIETA

Os perfis lipídicos circulantes, apesar de hereditários, podem ser modificáveis de acordo com a qualidade e a quantidade dos ácidos graxos ingeridos na dieta (ZHENG & QI, 2016). Segundo Zong et al. (2016), a ingestão de ácidos graxos saturados (SFA) tais como, láurico, mirístico e ácido palmítico em altas concentrações podem aumentar significativamente os níveis de triglicerídeos, lipoproteína de baixa densidade (LDL) e consequentemente o colesterol total, não apresentando qualquer melhoria sob os níveis de lipoproteínas de alta densidade (HDL). No entanto, a ingestão de PUFAs pode conduzir a uma redução ligeiramente maior do colesterol total do que os monoinsaturados, e ainda conduzir ao aumento da HDL (HODSON; SKEAFF & CHISHOLM, 2001). Adicionalmente, tem sido bem documentado que a ingestão elevada de carboidratos pode induzir um aumento dos triglicerídeos no sangue (PARKS, 2001).

As lipoproteínas assumem um papel importante no metabolismo, sendo as principais transportadoras de lipídios na corrente sanguínea. Os triacilgliceróis hidrofóbicos e ésteres de colesterol estão dentro do núcleo destas lipoproteínas, as quais são rodeadas por uma monocamada de fosfolípidio, que assume a função de solubilizar a partícula dentro do ambiente aquoso da corrente sanguínea (CALDER, 2016).

Os ácidos graxos dietéticos são transportados na corrente sanguínea sobretudo como triacilgliceróis dentro de quilomícrons, enquanto que as lipoproteínas de muito baixa densidade (VLDL) transportam triacilgliceróis de origem hepática (COLEMA & LEE, 2004). Através da ação enzimática a VLDL é desdobrada em ácidos

graxos, que serão utilizados como energia ou armazenados no tecido adiposo, e ainda em lipoproteínas de densidade intermediária (IDL) que posteriormente serão convertidas a LDLs (BUSNELLO, SANTOS & POTIN, 2016).

A HDL ao contrário da LDL, é uma mistura complexa constituída por um conteúdo proteico maior que o lipídico (LÜSCHER et al., 2014). Tem sido demonstrado em muitos estudos populacionais que a concentração de HDL está inversamente relacionada com o risco de ocorrências de evento cardiovascular (ROSENSON et al. 2013). A mais conhecida das funções potencialmente anti-aterogênicas das HDLs é a sua capacidade de promover o efluxo de colesterol a partir de células teciduais periféricas, incluindo a de macrófagos na parede arterial, o transportando até o fígado para ser excretado pela bile (ROSENSON et al. 2011).

O controle dos níveis lipídicos sanguíneos, é de extrema importância para a homeostase corporal, uma vez que um descontrole nos níveis de HDL e LDL por exemplo pode desencadear uma série de patologias, principalmente as de natureza cardiovascular (CUSI, 2012).

6. MECANISMOS DE DEFESA ANTIOXIDANTE ENDÓGENO

O excesso de espécies reativas de oxigênio representadas por moléculas oxigenadas de radicais livres tais como, superóxido (O_2^{-}), oxigênio singleto ($^1O_2$), hidroxila (OH^-) e não radicais livres como peróxido de hidrogênio (H_2O_2) ocasiona efeitos prejudiciais *in vivo*, tais como a peroxidação dos lipídios de membrana, agressão às proteínas teciduais, carboidratos e DNA (PISOSCHI & POP, 2015). Tal excesso sobrecarrega a capacidade de tamponamento antioxidante normal da célula e está situação definida como estresse oxidativo tem seu controle estabelecido por sistemas de defesa antioxidante (COSTANTINI & VERHULST, 2009).

Eliminar espécies reativas do organismo é função do sistema de defesa antioxidante endógeno, formado pelas enzimas glutationa peroxidase (GPx), glutationa redutase (GR), catalase (CAT), superóxido dismutase (SOD), e, também, complexos não enzimáticos que incluem a glutationa reduzida (GSH), peptídeos de histidina, proteínas ligadas ao ferro, entre outros (WANG, CHUN & SONG, 2013). Essas enzimas tornam-se importantes biomarcadores de danos oxidativos, pois suas atividades podem indicar o grau de oxidação celular (HO et al., 2013)

As enzimas SOD, CAT e GPx, agem por meio de mecanismos de prevenção, impedindo ou controlando a formação de radicais livres e espécies não-radicais, envolvidos com a iniciação das reações em cadeia que culminam com propagação e amplificação do processo e, consequentemente, com a ocorrência de danos oxidativos (BALABAN et al., 2005; WU et al., 2004; LU, 2009).

A defesa antioxidante de natureza endógena divide-se em três sistema: O primeiro é formado pela SOD, que catalisa a dismutação o ânion radicalar superóxido (O_2^-) em H_2O_2 e O_2. O segundo é formado pela CAT, que age sob a molécula de H_2O_2, transformando-a em O_2 e H_2O. O terceiro é composto pelo conjunto GSH, GPx e GR que também age na redução do H_2O_2, onde a GSH opera em ciclos entre sua forma reduzida e oxidada (MATES, 2000).

A eliminação do H_2O_2 celular, é de grande importância, uma vez que essa molécula, por meio das reações de Fenton, Haber-Weiss com a participação de metais ferro e cobre, culmina na geração do radical OH^-, contra o qual não existe sistema de defesa devido a sua meia-vida muito curta (RODRIGUEZ et al., 2004).

Além da atuação do sistema endógeno na eliminação de espécies reativas, o organismo também utiliza antioxidantes adquiridos na dieta, tais como, como α-tocoferol, β-caroteno, ácido ascórbico e compostos fenólicos (ZIECH et al., 2010), que tem sua ação em função de suas características estruturais específicas.

REFERÊNCIAS

ADARME-VEGA, C. T. et al. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. **Microbial Cell Factories**, v.96, n.11, p. 1–10, 2012.

AGYEI, D. et al. Antioxidative Peptides Derived from Food Proteins. **Free Radicals in Human Health and Disease.** Rani and U.C.S. Yadav (eds.), p. 417–430, 2015.

ALBRECHT, M. et al. Novel hydroxycarotenoids with improved antioxidative properties produced by gene combination in *Escherichia coli*. **Nature Biotechnology**, v.18, p.843–846, 2000.

AMBATI, R. et al. Astaxanthin, sources, extraction, stability, biological activities and its commercial applications–a review. **Marine Drugs**, v.12, p.128–152, 2014.

ARON, P.M.; KENNEDY, J.A. Flavan-3-ols nature, occurrence and biological activity. **Molecular Nutrition & Food Research**, v.52, p. 79–104, 2008.

ASTLEY, S. B. et al. DNA damage and susceptibility to oxidative damage in lymphocytes: effects of carotenoids in vitro and in vivo. **British Journal of Nutrition**, v.91, p.53–61, 2004.

BALABAN, R.S.; NEMOTO, S.; FINKEL, T. Mitochondria, oxidants, and aging. **Cell**, v.120, p.483–495, 2005.

BANDARRA, N.M. et al. Fatty acids, sterols and α-tocopherol in *Isochrysis galbana*.

Journal of Food Lipids, v.10, p.25–34, 2003.

BECKER, E.W. micro-algae as a source of protein. **Biotechnology Advances**, v.25, n 2, p. 207–210, 2007.

BECKER, W. **Microalgae in human and animal nutrition**. In: RICHMOND, A. (Ed). Handbook of microalgal culture: biotechnology and applied phycology. London: Blackwell Science, p.312–351, 2004.

BHAT, A. H. et al. Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; a mechanistic insight. **Biomedicine & Pharmacotherapy**, v.74, p. 101–110, 2015.

BRITTON, G. et al. (Eds.). (2008). **Carotenoids: Natural Functions**. Badel: Birkhäuser Verlag.

BUSNELLO, F. M.; SANTOS, Z. E. A. & PONTIN, B. **Lipids and Metabolic Syndrome**, Handbook of Lipids in Human Function: Fatty Acids, p. 543–553, 2016.

CALDER, P. C. **Fatty Acids: Metabolism**. Encyclopedia of Food and Health, p.632–644, 2016.

CHACON-LEE, T. L.; GONZALEZ-MARINO, G. E. Microalgae for “healthy” foods: possibilities and challenges. **Comprehensive Reviews in Food Science and Food Safety**, v. 9, n. 6, p. 655–675, 2010.

CHIDAMBARA-MURTHY, K. N. et al. *In vivo* antioxidant activity of carotenoids from *Dunaliella salina* — a green microalga. **Life Science**, v.76, p.1381–1390, 2005.

COLEMA, R. A., LEE, D. P. Enzymes of triacylglycerol synthesis and their regulation. **Progress in Lipid Research**, v.43, n.2, p.34–176, 2004.

COLUSSI, G. et al. Impact of omega-3 polyunsaturated fatty acids on vascular function and blood pressure: Relevance for cardiovascular outcomes. **Nutrition, Metabolism & Cardiovascular Diseases**, v.27, p.191–200, 2017.

COLUSSI, G. The metabolic syndrome and the membrane content of polyunsaturated fatty acids in hypertensive patients. **Metabolic Syndrome and Related Disorders**, v.13, p.343–351, 2015.

CONLON, M. A.; BIRD, A. R. The Impact of Diet and Lifestyle on Gut Microbiota and Human Health. **Nutrients**, v.7, n.1, p.17–44, 2015.

COSTANTINI, D.; VERHULST, S. Does high antioxidant capacity indicate low oxidative stress? **Functional Ecology**, v. 23, p.506–509, 2009.

COUCH, S. C. et al. Associations between long chain polyunsaturated fatty acids and cardiovascular lipid risk factors in youth with type 1 diabetes: Search Nutrition Ancillary Study. **Journal of Diabetes and its Complications**, v.31, n.1, p.67–73, 2017.

CUSI, K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. **Gastroenterology**, v.142, n.6, p.711–725.

D’ANTONA, G. et al. Branched-chain amino acid supplementation promotes survival and supports cardiac and skeletal muscle mitochondrial biogenesis in middle-aged mice. **Cell Metabolism**, v.12, p.362–372, 2010.

DE CATERINA, A. Z. et al. Nutritional mechanisms that influence cardiovascular disease. **The American Journal of Clinical Nutrition**, v.83, p.421S–426S, 2006.

EJIKE, C. E. C. C. et al. Prospects of microalgae proteins in producing peptide-based functional foods for promoting cardiovascular health. **Trends in Food Science & Technology**, v.59, p.30–36, 2017.

EL–AGAMEY, A. et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. **Archives of Biochemistry and Biophysics**, v. 430, n. 1, p. 37–48, 2004.

EL-BAKY, H. H. A. et al. *Spirulina* species as a source of carotenoids and a-tocopherol and its anticarcinoma factors. **Biotechnology,** v.2, p.222–240, 2002.

FERNANDES, A.S. et al. Identification of chlorophyll molecules with peroxyl radical scavenger capacity in microalgae *Phormidium autumnale* using ultrasound-assisted

extraction. **Food Research International**, In Press, Corrected Proof, 2016. http://dx.doi.org/10.1016/j.foodres.2016.11.011

FERNÁNDEZ, L. C. SERRA, J. D. ÁLVAREZ, J. R. M. ALBERICH, R. S. JIMÉNEZ, F. P. Dietary fats and cardiovascular health. **Atención Primaria**, v.43, p.1–16, 2011.

FRANCISCO, E. C. FRANCO, T. T. WAGNER, R. JACOB-LOPES, E. Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria. **Bioprocess Biosystems Engineering**, v.37, n.8, p.1497-1505, 2014.

GAFFNEY, M., O'ROURKE, R., MURPHY, R. Manipulation of fatty acid and antioxidant profiles of the microalgae *Schizochytrium* sp. through flax seed oil supplementation. **Algal Research**, v. 6, p.195–200, 2014.

GARCÍA-BLANCO, A. et al. Potential oxidative stress biomarkers of mild cognitive impairment due to Alzheimer disease. **Journal of the Neurological Sciences**, v.373, n.15, p.295–302, 2017.

GENG, S. et al. Fatty acid and sterol composition reveal food selectivity of juvenile ark shell Tegillarca granosa Linnaeus after feeding with mixed microalgae Shasha Geng. **Aquaculture**, v.455, p.109–117, 2016.

GOMEZ-GUTIERREZ, C. et al. Marine Edible Algae as Disease Preventers. In: KIM, S-K. **Advances in Food and Nutrition Research**, v.64, p. 29–39, 2011.

GOUVEIA, L. et al. Microalgae-source of natural bioactive molecules as functional ingredients. **Functional Foods**. v. 7, p.21-37, 2010.

HO, E. et al. Biological markers of oxidative stress: Applications to cardiovascular research and practice. **Redox Biology**, v.1, n.1, p.483–491, 2013.

HODSON, L.; SKEAFF, C.M.; CHISHOLM, W-A.H. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. **European Journal of Clinical Nutrition**, v.55, p.908–915, 2001

ICHIKAWA, K. et al. Branched-chain amino acid-enriched nutrients stimulate antioxidant DNA repair in a rat model of liver injury induced by carbon tetrachloride. **Molecular Biology Reports**, v.39, p.10803–10810, 2012.

JACOB-LOPES, E. et al. Characteristics of thin-layer drying of the cyanobacterium Aphanothece microscopica Nägeli. Chemical **Engineering and Processing**, v. 46, p. 63–69, 2007.

JACOB-LOPES, E.; LACERDA, L. M. C. F.; FRANCO, T. T. Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor. **Biochemical Engineering Journal**, v.40, p. 27–34, 2008.

JACOB-LOPES, E.; QUEIROZ, M. I.; ZEPKA, L. Q.; NETTO, F. M. Protein characterisation of the *Aphanothece Microscopica Nägeli* cyanobacterium cultivated in parboiled rice effluent. **Food Science and Technology**, v. 26, p. 482–488, 2006.

JACOB-LOPES, E.; REVAH, S.; SHIRAI, K.; HERNANDEZ, S.; FRANCO, T. T. Development of operational strategies to remove carbon dioxide in photobioreactors. **Chemical Engineering Journal**, v. 153, p. 120–126, 2009.

JACOB-LOPES, E.; SCOPARO, C. H. G.; QUEIROZ, M. I.; FRANCO, T. T. Biotransformations of carbon dioxide in photobioreactors, **Energy Conversion and Management**, v. 51, p. 894–900, 2010.

JIN, M. et al. Regulation of growth, antioxidant capacity, fatty acid profiles, hematological characteristics and expression of lipid related genes by different dietary n-3 highly unsaturated fatty acids in juvenile black seabream (*Acanthopagrus schlegelii)*. **Aquaculture**, v.471, p.55–65, 2017.

KATAYAMA, S.; MINE, Y. Antioxidative activity of amino acids on tissue oxidative stress in human intestinal epithelial cell model. **Journal of Agricultural and Food Chemistry**, v.55, p. 8458–8464, 2007.

KIOKIAS, S.; GORDON, M. H. Antioxidant properties of carotenoids in vitro and in vivo. **Food Reviews International**, v.20, p.99–121, 2004.

KLASSEN, J. L.; FOGHT, J. M. Characterization of hymenobacter isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. **Extremophiles**, v.15, p.45-57, 2011.

KNOTHE, G. Improving biodiesel fuel properties by modifying fatty esters composition. **The Journal of Energy and Environmental Science**. v. 10, p. 1039-1054, 2009.

KO S.C.; KIM. D.; JEON, Y.J. Protective effect of a novel antioxidative peptide purified from a marine *Chlorella ellipsoidea* protein against free radical-induced oxidative stress. **Food and Chemical Toxicology**, v.50, p. 2294–2302, 2012.

KRINSKY, N. I. Carotenoids as antioxidants. Nutrition, v.17, p.815–817, 2001.

KUSAMA, Y., et al. Zeaxanthin and Echinenone Protect the Repair of Photosystem II from Inhibition by Singlet Oxygen in Synechocystis sp. PCC 6803. **Plant and Cell Physiology Advance Access**, v.1. p.1–36, 2015.

LU, M. et al. Branched-chain amino acids supplementation protects streptozotocin-induced insulin secretion and the correlated mechanism. **Biofactors**, v. 41, p. 127–133, 2014.

LÜSCHER, T. F. et al. High-density lipoprotein vascular protective effects, dysfunction, and potential as therapeutic. **Circulation Research**. v.114, p.171–182.

MAADANE, A et al. Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. **Journal of Biotechnology**, v.215, n.10 p.13–19, 2015.

MARONEZE, M. M. et al. The role of photoperiods on photobioreactors - A potential strategy to reduce costs. **Bioresource Technology**, v.219, p.493–499, 2016.

Martínez-Flores, H.E. et al., 2015. Physico-chemical parameters, bioactive compounds and microbial quality of thermo-sonicated carrot juice during storage. **Food Chemistry**, v.172, p.650–656, 2015.

MATES, J.M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. **Toxicology**, v.153, p.83–104, 2000.

MERCADANTE, A. Z. Food colorants – chemical and functional properties. In: SOCACIU, C. (Ed.) **Carotenoids in foods: sources and stability during processing and storage**. New York: CRC Press, 2008. p.213–235.

MOZAFFARIAN, D. et al. Components of a cardioprotective diet: new insights. **Circulation**, v.123, p.2870–2891, 2011.

NOEMAN, S. A., et al. Biochemical study of oxidative stress markers in the liver, kidney and heart of high fat diet induced obesity in rats. **Diabetology and Metabolic Syndrome**, v.3, n.1. p. 17, 2011.

OSGANIAN, S. K. et al. Dietary carotenoids and risk of coronary artery disease in women. **The American Journal of Clinical Nutrition**, v.77, n.6, p.1390-1399, 2003.

PEREZ-GARCIA, O. et al. Heterotrophic cultures of microalgae: metabolism and potential products. **Water Research**, v.45, n.1, p.11–36, 2011.

PISOSCHI, A. M.; POP, A. The role of antioxidants in the chemistry of oxidative stress: A review. **European Journal of Medicinal Chemistry**, v.97, n.5, p.55–74, 2015.

POWLS, R.; BRITTON, G. A. Carotenoprotein, containing violaxanthin, isolated from *Scenedesmus obliquus*. **Biochemical and Biophysical Research**, v.453, n.1, p.270–276, 1976.

PRASANNA, R. et al. Rediscovering cyanobacteria as valuable sources of bioactive compounds (review). **Applied Biochemistry and Microbiology**, v.46, n.2, p.119–134, 2010.

QIANG, H. et al. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. **The Plant Journal**, v.54, n.4, p.621–639, 2008.

QUEIROZ, M. I. et al. Single-cell oil production by cyanobacterium Aphanothece microscopica Nägeli cultivated heterotrophically in fish processing wastewater. **Applied Energy**, v.88, n.10, p.3438–3443, 2011.

QUEIROZ, M. I.; JACOB-LOPES, E.; ZEPKA, L. Q.; BASTOS, R. G.; GOLDBECK, R. The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. **Bioresource Technology**, v.98, p.2163–2169, 2007.

QUEIROZ, M.I. et al. Evaluación Del crecimiento de la *Aphanothece microscopica Nägeli* en las aguas residuales de la parbolizacion del arroz. **Revista información Tecnológica**, v.1, p.61–65, 2002.

RADMANN, E. M. et al. Isolation and application of SO_X and NO_X resistant microalgae in Biofixation of CO_2 from thermoelectricity plants. **Energy Conversion and Management**, v.52, p.3132–3136, 2011.

RAMIREZ, D. et al. Inhibitory effects of *Spirulina* in zymosan-induced arthritis in mice. **Mediators of Inflammation**, v.11, p.75–79, 2002.

RAPOSO, M. F. D. J. et al. Health applications of bioactive compounds from marine microalgae. **Life Sciences**, v.93, n.15, p.479–486, 2013.

RATLEDGE C.; COHEN. Fatty acid biosynthesis in microorganisms being used for single cell oil production. **Biochimie**, v. 86, n.11, p.807–815, 2004.

RATLEDGE, C. **Microorganisms as source of polyunsaturated fatty acids**. In: GUNSTONE, F.D. (Ed). Structured and modified lipids. New York: Marcel Dekker, 2001. p.351–399.

RODRIGUES, D. B. et al. Bioactive pigments from microalgae *Phormidium autumnale*. **Food Research International**, v.77, n.2, p.273–279, 2015.

RODRIGUES, D. B. et al. Production of carotenoids from microalgae cultivated using agroindustrial wastes. **Food Research International**, v.65, p.144–148, 2014.

RODRIGUEZ, C. et al. Regulation of antioxidant enzymes: a significant role for melatonin. **Journal of Pineal Research**, v.36, p.1–9, 2004.

RODRIGUEZ-AMAYA, D. B. **A guide to carotenoid analysis in foods**. Washington: ILSI Press, 2001. p.64.

RODRIGUEZ-AMAYA. Status of carotenoid analytical methods and in vitro assays for the assessment of food quality and health effects. **Food Chemistry and Biochemistry**, v.1, p.56-63, 2015.

ROSENSON, R. S. et al. Translation of High-Density Lipoprotein Function Into Clinical Practice. **Circulation**. v.128, p.1256–1267, 2013.

ROSENSON, R.S. et al. HDL Measures, Particle Heterogeneity, Proposed Nomenclature, and Relation to Atherosclerotic Cardiovascular Events. **Clinical Chemistry**, v.57, p.392–410, 2011.

SAINI, R.K.; NILE, S.H.; PARK, S.W. Carotenoids from Fruits and Vegetables: Chemistry, Analysis, Occurrence, Bioavailability and Biological Activities. **Food Research International**, v.76, p.735–750, 2015.

SAMUELS, R. et al. Hypocholesterolemic effect of *Spirulina* in patients with hyperlipidemic nephrotic syndrome. **Journal of Medicinal Food**, v.5, n.2. p.91–96, 2002.

SANTOS, A. B. et al. Biogeneration of volatile organic compounds produced by *Phormidium autumnale* in heterotrophic bioreactor. **Journal of Applied Phycology**, v.28, n.2, p.1561–1570, 2016.

SCHWINGSHACKL L.; HOFFMANN, G. et al. Monounsaturated Fatty Acids and Risk of Cardiovascular Disease: Synopsis of the Evidence Available from Systematic Reviews and Meta-Analyses. **Nutrients**, v.4, n.12, p.1989–2007, 2012.

SERPELONI, J. M. et al. Diet carotenoid lutein modulates the expression of genes related to oxygen transporters and decreases DNA damage and oxidative stress in mice. **Food and Chemical Toxicology**, v.70, p.205–213, 2014.

SIES, H.; STAHL, W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. **The American Journal of Clinical Nutrition**, v.62, p.1315–1321, 1995.

SINGER, P. et al. Effects of dietary oleic, linoleic and alpha-linolenic acids on blood pressure, serum lipids, lipoproteins and the formation of eicosanoid precursors in patients with mild essential hypertension. **Journal of Human Hypertension**, v.4, p.227–233, 1990.

SPIES, J. R. **Determination of tryptophan in proteins**. Analytical Chemistry, v.39, n.12, p.1412–1415, 1967.

SPOLAORE, P. et al. Commercial applications of microalgae. **Journal of Bioscience and Bioengineering**, v.101, n.2, p.87–96, 2006.

STAHL, W.; SIES, H. Antioxidant activity of carotenoids. **Molecular Aspects of Medicine**. v.24, n.6, p.345-51, 2003.

TAJIRI, K.; SHIMIZU Y. Branched-chain amino acids in liver diseases. **World Journal Gastroenterology**, v.19, n.43, p.7620–7629, 2013.

TAKASHIMA A. et al. Combination of n-3 polyunsaturated fatty acids reduces atherogenesis in apolipoprotein E-deficient mice by inhibiting macrophage activation. Atherosclerosis, v.254, p.142–150, 2016.

TANAKA, H. et al. Branched-chain amino acid-rich supplements containing microelements have antioxidant effects on *Nonalcoholic steatohepatitis* in mice. **Journal of Parenteral and Enteral Nutrition**, v.40, n.4, p.519–528, 2016.

UAUY, R. et al., Children term studies of DHA and ARA supplementation on neurodevelopment: results of randomized clinical trials. **Journal of Pediatrics**, v.143, p.17–25, 2003.

VAN BREEMEN, R.B.; DONG, L., PAJKOVIC, N.D. Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. **International Journal of Mass Spectrometry**, v.312, p.163–172, 2012.
VOLTOLINA, D.; GÓMEZ-VILLA, H.; CORREA, G. Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. **Bioresource technology**, v.96, n.3, p.359–362, 2005.

WALTER, M. H.; STRACK, D. Carotenoids and their cleavage products: biosynthesis and functions. **Natural Product Reports**, v.28, n.4, p. 663–692, 2011.

WANG, H. H. et al. Fish oil increases antioxidant enzyme activities in macrophages and reduces atherosclerotic lesions in apoE-knockout mice. **Cardiovascular Research**, v.61 p.169–176, 2004.

WANG, J. From microalgae oil to produce novel structured triacylglycerols enriched with unsaturated fatty acids. **Bioresource Technology**, v.184, n.1, p.405–414, 2015.

WANG, Y.; CHUN, O.K.; SONG, W.O. Plasma and dietary antioxidant status as cardiovascular disease risk factors: a review of human studies. **Nutrients**, v.5, n.8, p.2969–3004, 2013.

WEN, Z. Y.; CHEN, F. Heterotrophic production of eicosapentaenoic acid by microalgae. **Biotechnology Advances**, v.21, n.4, p.273–294, 2003.

WIJFFELS, R. H; BARBOSA, M. J. **An outlook on microalgal biofuels Science**, v.329, p.796–799, 2010.

WU, G. et al. Production and supply of high-quality food protein for human consumption: sustainability, challenge. Annals of the New York Academy of Sciences, v.1321, p.1–14. 2014.

WU, G.; FANG, Y.Z; YANG, S.; LUPTON, J.R.; TURNER, N.D. Glutathione metabolism and its implications for health. **Journal of Nutrition**, v.134, p.489–492, 2004.

Wu, H. C.; Chen, H. M.; Shiau, C.Y. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). **Food Research International**, v.36, p.949–95, 2003.

YEH, C.T.; Yen, G.C. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance–associated protein 3 mRNA expression. **Journal of Nutrition**, v.136, n.1, p.11–15, 2006.

ZAGHDOUDI, K. et al. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki L*.), peach (*Prunus persica L*.) and apricot (*Prunus armeniaca* L.). **Food Chemistry**, v.184, p.131–139, 2015.

ZEPKA, L. Q. et al. Nutritional evaluation of single-cell protein produced by *Aphanothece microscopica Nägeli*. **Bioresource Technology**, v.101, n.18, p.7107–7111, 2010.

ZEPKA, L. Q. et al. Production and biochemical profile of the microalgae Aphanothece microscopica Nägeli submitted to different drying conditions. **Chemical Engineering and Processing**, v. 47, n. 8, p. 1311–1316, 2008.

ZHENG, Y.; QI, L., Personalized Diet and Lifestyle Interventions on Lipids and Lipoproteins. **Fatty Acids, Handbook of Lipids in Human Function,** p.1–20, 2016.

ZIECH, D. et al. The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. **Chemico-Biological Interactions**, v.188, p. 334–339, 2010.

ZONG, G. et al. Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. **Research**, v.1, p.1–11, 2016.

CAPITULO 2

EVALUATION OF THE INFLUENCE OF MICROALGAL BIOCOMPOUNDS ON THE ANTIOXIDANT STATUS AND LIPID PROFILE IN MICE

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Evaluation of the influence of microalgal biocompounds on the antioxidant status and lipid profile in mice

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Abstract: The aim of this work was to evaluate the influence of consumption of *Scenedesmus obliquus* CPCC05 biomass in lipid profile and antioxidant status of the mice model. Microalgal biomass was investigated in relation to its composition of fatty acids, amino acids and carotenoids. Also, the antioxidant potential of the carotenoid extract was also evaluated. Was used in the biological assay, male mice, lineage BALB/cAnUnib. After 32 days, the animals were euthanized, and serum samples were taken for determination of total cholesterol, triglycerides, high and low-density lipoprotein (HDL and LDL), and activity of hepatic function enzymes, pyruvate transaminase (TGP), and transaminase oxalacetic (TGO). Tissue samples (heart, liver, kdneys and spleen) were collected for determination of antioxidant potential (ORAC), thiobarbituric acid reactive species content (TBARS), reduced glutathione (GSH) activity and antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT). Biomass showed a high content of biocompounds with a positive influence on human health, such as fatty acids (α-linolenic, linoleic and oleic acid), amino acids (histidine, methionine, cysteine, tyrosine, phenylalanine, lysine and leucine) and carotenoids (all-*trans*-β-carotene, all-*trans*-lutein, all-*trans*-equinenone and others) with high antioxidant potential. In the biological assay, there was an increase in HDL followed by a decrease in LDL, regardless of dose. As regards liver enzymes, the dose of 800mg.kg^{-1} showed no adverse change associated with the levels of the TGP and TGO. The endogenous antioxidant system (SOD, CAT, GPx, GR, and GSH) showed in almost all tissues, unchanged or reduced, except in the kidneys, since an increase in GPx activity was observed for dose of 400mg.kg^{-1} and GSH to 800mg.kg^{-1}. In addition, both doses administered significantly decreased TBARS levels in all tissues. There was also a significant negative correlation between TBARS and ORAC in the heart, liver and spleen. Based on the results, it is suggested that the biomass of *Scenedesmus obliquus* CPCC05 is a potential source of numerous biocompounds capable of modulating in a positive way the serum lipid profile of mice, as well as exerting a protective effect against tissue lipid peroxidation.

Keywords: Microalgae. Carotenoids. Amino acids. Fatty acids. Biological assay. Potential antioxidant. Antioxidant enzymes. Cell peroxidation.

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1. Introduction

Microalgae are acknowledged a very diverse source of biomolecules that demonstrate biological activities applied in the prevention, management and treatment of health conditions (Vaz et al., 2016). Among these metabolites are included the polyunsaturated fatty acids (Chacon-Lee; Gonzalez-Marino, 2010; Wang et al., 2015), amino acid (Jacob-Lopes et al., 2006; Becker, 2007) and carotenoids (Powls & Britton 1976; Prasanna et al., 2010; Walter & Strack, 2011; Rodrigues et al., 2014), that have a demonstrated influence on the reduction of human health risk factors.

Photosynthetic organisms, such as microalgae are the primary natural producers of fatty acids, inclusive polyunsaturated (Adarme-Vega et al, 2012). Essential fatty acids, especially ω-3 and ω-6, are important for the integrity of tissues where they are incorporated linoleic and linolenic acids are essential nutrients for the synthesis of the cell membrane prostaglandins (Raposo et al., 2013). According to meta-analysis performed by Mensink et al. (2003), the inclusion of monounsaturated and polyunsaturated fatty acids in the diet, similarly improve blood lipid profiles, their associations with clinical cardiovascular events are less similar.

Besides, some research suggest that amino acids, which are present in the microalgal protein, have also show an important role in the anti-inflammatory activity and in the oxidative stress balance (Tajiri & Shimizu, 2013; Tanaka, et al., 2016). However, there is little information regarding the protective effects of amino acids against tissue oxidative stress. Researchers suggest that some amino acids modulate the cellular anti-oxidative systems and thereby suppress oxidative stress-induced inflammatory response (Son et al, 2002). Martínez-Tomé, García-Carmona and Murcia, (2001) showed which in vitro, branched chain amino acid, decreased lipid peroxidation of brain phospholipids. Katayama and Mine (2007) reported that the

isoleucine increased the gene expression of the enzymes antioxidants in human intestinal epithelial cells. Induction of antioxidants and detoxifying enzymes in the cells appears to be a promising strategy for protecting against oxidative tissue injury.

Moreover, from this group of promising microorganisms, a range of carotenoids, such as β-carotene, α-carotene, zeaxanthin, lutein, violaxanthin, echinenone and canthaxanthin can be isolated (Prasanna et al. 2010, Rodrigues et al., 2014, Rodrigues et al. 2015), some of these are produced specifically by microalgae. These pigments have been associated with the reduction of the risk of developing chronic diseases such as cancer, cardiovascular diseases, since, are efficient exogenous antioxidants scavenging singlet molecular oxygen and peroxyl radicals (Rodrigues-Amaya, 2010). In the human organism, complement the endogenous antioxidant defense system constituted for the by superoxide dismutase (SOD), catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Reduced glutathione (GSH) (Stahl & Sies, 2003).

In view of the above, it is believed that microalgal biomass can play an important role in human health mainly in the reduction of reactive oxygen species (EROS) and improvement of the lipid profile. Therefore, the objective of this work is to evaluate the antioxidant status and the lipid profile of mice after repeated doses microalgal biomass.

2. Material and Methods

2.1. Chemicals

Standards of FAMEs (fatty acid methyl esters), dl-2-aminobutyric acid, (6-hydroxy-2,5,7,8-etramethylchroman-2-carboxylic acid), fluorescein, AAPH (α,α′-azodiisobutyramidine dihydrochloride), were purchased from Sigma-Aldrich (St. Louis-MO, USA). All-*trans*-zeaxanthin, all-*trans*-violaxanthin, all-*trans*-lutein, all-*trans*-α-

carotene, and all-*trans*-β-carotene, trolox were purchased from Hoffmann-La Roche (Basel, Switzerland).

2.2. Microorganisms and culture media

Axenic cultures of *Scecnedesmus obliquus* CPCC05 were originally isolated from the Canadian Psychological Culture Collection University of Toronto, Canada. Stock cultures were propagated and maintained in solidified agar-agar (20g.L^{-1}) containing synthetic BG11 medium (Rippka et al., 1979). The incubation conditions used were 25ºC and intensidade luminosa constante de 1000Lux.

2.3. Microalgal biomass production

The biomass production was carried out in hybrid photobioreactor integrating two main units of reaction (Jacob-Lopes et al., 2014): in bubble column photobioreactor coupled to a lighting platform. The photobioreactor operating in intermittent regime, fed with 1.5L of BG11 medium (Rippka et al., 1979). The experimental conditions were as follows: initial concentration of inoculum of 100 mg.L^{-1}, temperature of 25ºC, aeration of 1 volume of air per volume of medium per minute, with injection of air enriched with 15.0% (v/v) of carbon dioxide. The ratio volume between the surrounding medium and dark/lighting platform area was 20% and the light intensity was constant 13000Lux.

The biomass was separated from the culture medium by centrifugation, the supernatant was discarded and the remaining biomass freezing at -18°C for 24 hours. After were lyophilized in Lyophilizer Liotop L101 for 24 hours under vacuum conditions: from 0.2 to 0.3 and μHg temperature of -37°C condenser, and then stored under refrigeration until the time of analysis.

2.4. Fatty acids profile

The method of Hartman and Lago (1973) was used to saponify and esterify the dried lipid extract in order to obtain the fatty acid methyl esters. Around 0.05 g of microalgal biomass was inserted into a flask tube and 4 mL of saponification reagent (KOH and NaOH 0.5 M in MeOH) followed by homogenization by vortex, heating for 5 min and cooling in an ice bath. 5 ml of esterification reagent were added, the tubes were shaken and put on again heating, 4 mL of saline solution and 5 mL of petroleum ether were added; the samples were subjected to homogenization by vortex for 30 seconds and subsequent centrifigação. The supernatant was transferred into a 1.5 mL vial for further chromatographic analysis.

The methylated samples were analyzed by an Agilent capillary gas chromatography system, Series 6850, FID detector, with an Agilent DB-23 capillary column (50% cyanopropyl-methylpolysiloxane; length 60m, internal diameter 0.25mm and 0.25µm film thickness). Flow rate was 1.0ml.min^{-1}, linear velocity of 24cm.s^{-1}, with a detector temperature of 280°C, injector temperature of 250°C, oven temperature: 110°C for 5min, 110-215°C (5°C.min^{-1}), 215°C for 24min, helium carrier gas; injected volume of 1.0µl.

The fatty acid methyl esters (FAMEs) were identified by comparison of the retention times with the authentic standards from FAME Mix-37 (P/N 47885-U, Sigma-Aldrich, St. Louis, USA) and quantified based on relative peak areas.

2.5. Amino acid profile

The amino acids totals, except tryptophan, were extracted according Hagen, Frost & Augustin (1989). The samples were submitted to acid hydrolysis, with HCl (6N) with phenol, for 24 hours, subjected to derivatization with phenylisothiocyanate (PITC).

Subsequent, the amino acids were analyzed by high performance liquid chromatography HPLC-UV, using a C18 column Luna C-18, 250 × 4.6mm (00G-4252-EQ; Phenomenex, Torrance, CA) at 50°C (White, Hart & Fry, 1986). The identification was undertaken by comparison with a standard mixture and quantification using dl-2-aminobutyric acid as an internal standard (Sigma-Aldrich Corp., St Louis, MO).

Tryptophan, which was obtained by enzymatic hydrolysis (Spies, 1967). 0.18 mg of tryptophan were weighed in a test tube with screw cap, add 0.4 mL of pronase solution, the volume was made up to 4.0 ml with sodium phosphate buffer pH 7.5. Doing so the ambient temperature readings for 6 hours. The white blank was made using only the pronase solution with the sodium phosphate buffer pH 7.5. The samples were incubated at 40 ° C for 24 hours in a water bath. After centrifugation at 3000 rpm for 10 minutes. 1 mL of the supernatant was removed and 9 mL of the DAB (4-dimethylamino-benzaldehyde) solution was added, homogenized and left to stand at room temperature. Subsequently 0.1 mL of sodium nitrite solution was added. After 30 minutes the spectrophotometer was read at 590 nm. The quantification of this amino acid was done via calibration curve with standard solution of tryptophan (100 $mg.L^{-1}$)

2.6. Carotenoids profile

The carotenoids were exhaustively extracted from the freeze-dried sample (0.2 ± 0.02 g) with ethyl acetate and methanol in a mortar with a pestle followed by centrifugation (Hitachi, Tokyo, Japan) for 7 min at 5500 rpm (Rodrigues et al., 2015).

The carotenoids were analyzed by HPLC (Shimadzu, Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), online degasser, and injection valve with a 20μL loop (Rheodyne, Rohnert Park-CA, USA). The equipment was connected in series to a PDA detector (model SPD-M20A) and a mass spectrometer with an ion-trap analyzer and atmospheric pressure chemical ionization (APCI) source (model

Esquire 4000, Bruker Daltonics, Bremem, Germany). The carotenoid separation was performed on a C30 YMC column (5µm, 250 × 4.6mm) (Waters, Wilmington-DE, USA). HPLC-PDA-MS/MS parameters were set as previously described by De Rosso & Mercadante (2007). The mobile phase consisted in a mixture of methanol and methyl tert-butyl ether (MTBE). A linear gradient was applied from 95:5 to 70:30 in 30 min, to 50:50 in 20 min. The flow rate was 0.9mL.min^{-1}.

The identification was performed according to the following combined information: elution order on C30 HPLC column, co-chromatography with authentic standards, UV-Visible spectrum (λ_{max}, spectral fine structure, peak *cis* intensity), and mass spectra characteristics (protonated molecule ($[M + H]^+$ and MS/MS fragments), compared with data available in the literature (Britton, 1995; De Rosso & Mercadante, 2007; Van Breemen et al., 2012; Zepka & Mercadante, 2009; Rodrigues et al., 2015).

The carotenoids were also quantified by HPLC-PDA, using five-point analytical curves of all-*trans*-violaxanthin, all-*trans*-zeaxanthin, all-*trans*-lutein, all-*trans*-α-carotene and all-*trans*-β-carotene. The *cis*-isomers were estimated using the curve of the corresponding all-*trans*-carotenoid. All other xanthophyll and carotene contents were estimated using the curve of all trans-lutein and all-trans-β-carotene, respectively.

2.6.1. Antioxidant capacity of carotenoid extracts

The antioxidant capacity of the extracts was carried out according the lipophilic ORAC method adapted (Ou, et al., 2013). The carotenoids extract was suspended in acetone. The test was carried out by adding 7% randomly methylated beta-cyclodextrin (RMCD) in 50% acetone solution diluted sample extracts or standard solutions, fluorescein and 2 AAPH diluted in buffer phosphate (PB) (pH 7.4), at proportion of 1:6:6, to black microplates. The fluorescence signal was registered every min until 160

min in Biotek microplate reader (Winooski, VT, USA) with Gen5™ 2.0 data analysis software. The fluorescence was monitored for the emission wavelength at 520nm with excitation at 485nm. The relative fluorescence versus time was recorded and the area under curve (AUC) of the sample and of the blank were calculated. The results were expressed in µmol trolox equivalent per dry weight microalgae biomass.

2.7. *In vivo* experimental design

The Institutional Animal Care and Use Committee (CEUA-UNICAMP, Campinas, Brazil) approved the study under protocol nº 4056-1. Male Balc/CanUnib mice (~25,1 ±2g) were obtained and maintained under controlled conditions of temperature (22–24°C), light–dark cycle (12/12h), and humidity (45-65%), there was free access to water and diet commercial (Nuvilab®). The animals were randomly distributed into 3 groups (n = 6), in which the microalgal biomass was administered by gavage in a suspension of 0.15mL of water at concentrations 400 and 800 $mg.kg^{-1}.bw.d^{-1}$. For all animals were handled in the same conditions the control group received only water by gavage. After 32 days, animals were submitted at 6h fasting, subsequently anesthetized with intraperitoneal injection of the ketamine and xylazine, and died by exsanguination by cardiac puncture.

2.7.1. Samples

Blood samples were collected in appropriate tubes centrifuged at 6.500 rpm for 15min and serum was separated. The whole heart, kidneys, liver and spleen were removed, weighted and freezing in liquid nitrogen. Were prepared tissue homogenates in 100mg wet tissue per 1mL of phosphate buffer (PB) in pH 7.4. All samples were stored at −80°C until further analyses. Protein content were determined in all tissue

homogenates by Bradford method (Bradford, 1976). The results were expressed relative to the protein concentrations.

2.7.2 Serum lipid profile.

The lipid profile in vivo was realized in the serum, were determinate the total cholesterol (CT), triglyceride (TRG) and high-density lipoprotein (HDL) levels, through commercial kits Labtest®, besides the low-density lipoprotein level (LDL) was estimated by difference using the Friedewald equation (Friedewald, Levy & Fredrickson, 1972). The results were expressed in $mg^{-1}.dL^{-1}$.

2.7.3 Serum transaminases

In the serum also were determinate, hepatic function enzymes, oxaloacetic and pyruvic transaminase through the commercial kits Labtest®. The results were expressed $U.dL^{-1}$.

2.7.4 Lipid peroxidation in the tissues

2.7.4.1 TBARS (thiobarbituric acid reactive substances)

The TBARS assay was realized according to Ohkawa, Ohishi, & Yagi (1979). Twenty-five microliters of tissue homogenate PB were mixed with 8.1% sodium dodecyl sulphate (SDS) plus working reagent (TBA, 20% acetic acid, and 5% sodium hydroxide). After heating at 100°C for 60min, the samples were cooled in an ice bath for 10min and then centrifuged at 4,000rpm, 10min and 4°C. The supernatant was read at 532nm using a 96-well microplate. A standard curve was prepared using the malondialdehyde (MDA) (125µM). The results were expressed as nmol MDA equivalents.mg^{-1} protein.

2.7.5 Antioxidant capacity in tissues

The Free radical scavenging capacity was carried out according the ORAC method (Ou, et al., 2013). The test was carried out by adding diluted tissue homogenates or standard solutions, fluorescein and AAPH diluted in PB (pH 7.4), at proportion of 1:6:6, to black microplates. The fluorescence signal was monitored for the emission wavelength at 520nm with excitation at 485nm, registered every min until 160min. The relative fluorescence versus time was recorded and the area under curve (AUC) of the sample and of the blank were calculated. The results were expressed in µmol trolox equivalent.mg^{-1} protein.

2.7.6. Enzymatic and non-enzymatic endogenous antioxidant systems in the tissues

2.7.6.1 Superoxide dismutase activity (SOD)

The SOD activity was measured according with (Winterbourn, Hawkins, Brian, & Carrell, 1975). One hundred microliters of appropriately diluted samples were added to a 96-well microplate. One hundred and fifty microliters of a previously prepared solution (0.1mmol.L^{-1} hypoxanthine, 0.07U xanthine oxidase and 0.6mmol.L^{-1} NTB in phosphate buffer in 1:1:1 proportions) were added just before the readings. The reading was taken at 560nm and the reaction monitored for 10 min. The SOD activity expressed as U.mg^{-1} protein.

2.7.6.2 Catalase (CAT)

The CAT activity was measured according with Aebi (1984), with adaptations. The tissue homogenates, PB (50mM, pH 7.0) and hydrogen peroxide (H_2O_2) (0.5M) were mixed in a 1:18:1. Reading was performed in 96-wells for reading the special ultra violet range. The activity was determinate using the rate constant of the enzyme (k), the logarithm of the ratio of the change in absorbance and the correction factor for

the optical path. One catalase unit decomposes 1µmol de H_2O_2 per mg de protein per min. The result was expressed in µmol H_2O_2.mg^{-1} protein.min^{-1}.

2.7.6.3 Glutathione peroxidase (GPx)

The GPx activity in the tissues was quantified in PB homogenates by the method described in Flohe & Gunzler (1984). The decrease in absorbance was monitored at 365nm after induction by 0.25mmol.L^{-1} H_2O_2 in the presence of 10mmol.L^{-1} reduced glutathione, 4mmol.L^{-1} NADPH (β-nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate) and 1U GR enzyme. The results were expressed as nmol NADPH consumed.min^{-1}.mg^{-1} protein.

2.7.6.4 Glutathione reductase (GR)

The GR activity was measured in PB homogenates according with Carlberg & Mannervik (1985), following the decrease in absorbance at 340nm induced by oxidized glutathione in the presence of NADPH. The results were expressed as nmol NADPH consumed.min^{-1}.mg^{-1} protein.

2.7.6.5 Glutathione reduced (GSH)

The GSH levels in the tissues were determined according Faure & Lafond (1995), by Ellman's reaction using DTNB (5′5′-dithio-bis-2-nitrobenzoic acid) (Ellman, 1959). The intensity of the yellow colour was read at 412nm and GSH was used as the external standard. The GSH concentrations were expressed as nmol GSH.mg^{-1} protein.

2.8. Statistical analyses

The statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) software. Analyses were based on a one-way

analysis of variance (ANOVA) followed by a Tukey test and the limit of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Lipid profile

The Table 1 shows the fatty acid profile of *Scenedesmus obliquus* CPCC05 microalgae. Fourteen fatty acids were identified; among them the majority was the palmitic acid (272.7± 0.5 $mg.g^{-1}$) follow to α-linolenic acid (179.0 ± 0.0 $mg.g^{-1}$), linoleic acid (174.7 ± 0.4 $mg.g^{-1}$) and oleic acid (137.3 ± 0.1 $mg.g^{-1}$). The biomass presented highest fraction of PUFA (384.6 ± 0.5 $mg.g^{-1}$), followed by SFA (345.8 ± 0.5 $mg.g^{-1}$) and MUFA (269.6 ± 0.1 $mg.g^{-1}$).

Table 1. Fatty acid profile of the *Scenedesmus obliquus* CPCC05 biomass obtained by GC-FID.

Fatty acid	Chemical structure	($mg.g^{-1}$)[a]
capric (C10:0)	$C_{10}H_{20}O_2$	12.7 ± 0.1
lauric (C12:0)	$C_{12}H_{24}O_2$	4.9 ± 0.0
meristic (C14:0)	$C_{14}H_{28}O_2$	6.5 ± 0.0
pentadecylic (C15:0)	$C_{15}H_{30}O_2$	2.1 ± 0.0
palmitic (C16:0)	$C_{16}H_{32}O_2$	272.7± 0.5
palmitoleic (C16:1)	$C_{16}H_{30}O_2$	130.2 ± 0.1
margaric (C17:0)	$C_{17}H_{34}O_2$	4.5 ± 0.0
stearic (C18:0)	$C_{18}H_{36}O_2$	23.8 ± 0.0
oleic (C18:1n9)	$C_{18}H_{34}O_2$	137.3 ± 0.1
linoleic (C18:2n6)	$C_{18}H_{32}O_2$	174.7 ± 0.4
linolenic (C18:2n3)	$C_{18}H_{30}O_2$	179.0 ± 0.0
stearidonic (C18:4n3)	$C_{18}H_{28}O_2$	27.8 ± 0.0
behenic (C22:0)	$C_{22}H_{44}O_2$	4.3 ± 0.0
lignoceric (C24:0)	$C_{24}H_{48}O_2$	11.8 ± 0.0

SFA Σ	345.8 ± 0.5
MUFA Σ	269.6 ± 0.1
PUFA Σ	384.6 ± 0.5

SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; a: Relative area of peak .

According with Tabeli et al. (2013) the fraction unsaturated fatty acids of the oil extracted from microalgae biomass is mainly composed of palmitoleic, oleic, linoleic and linolenic acids, this information corroborates with the results obtained in this study. The content of PUFAs from *Scenedesmus obliquus* CPCC05 in the present study was about 1.7, 7.7, 1.4 and 1.6-fold higher when compared to that found for *Botryococcus* sp., *Dunaliella salina*, *Chlorella* sp. and *Chlorococcum* sp, respectively (Sahua et al., 2013). Conforming to Uauy et al. (2003), PUFA's such as those of the omega-3 family found in microalgal biomass, are capable of raising HDL and reducing LDL formation. Such information confirms the results obtained herein for these lipoproteins.

3.2. Amino acid profile

In according show Table 2, eighteen amino acids were determined; the glutamine (62.5 ± 0.1 $mg.g^{-1}$) was the predominant. Furthermore, nine essential amino acids were determined, leucine (44.8 ± 0.3 $mg.g^{-1}$), valine (32.6 ±1.3 $mg.g^{-1}$) lysine (31.3 ± 0.3 $mg.g^{-1}$), threonine (29.5 ± 0.2 $mg.g^{-1}$), phenylalanine (26.0 ± 0.4 $mg.g^{-1}$), isoleucine (22.6 ± 1.5 $mg.g^{-1}$), tyrosine (20.4 ± 0.8 $mg.g^{-1}$), methionine (9.0 ± 0.2 $mg.g^{-1}$) and tryptophan (4.0 ± 0.2 $mg.g^{-1}$). However, these amino acids are in concentrations below that of the FAO / WHO standard (Pires et al., 2006).

Table 2. Amino acid profile of the *Scenedesmus obliquus* CPCC05 biomass obtained by HPLC-UV.

Amino acid	Chemical structure	(mg.g^{-1})[a]
asparagine	$C_4H_8N_2O_3$	48.1 ± 0.4
glutamine	$C_5H_{10}N_2O_3$	62.5 ± 0.1
serine	$C_3H_7NO_3$	25.7 ± 0.3
glycine	$C_2H_5NO_2$	27.8 ± 0.4
histidine	$C_6H_9N_3O_2$	7.9 ± 2.5
arginine	$C_6H_{14}N_4O_2$	38.0 ± 0.3
threonine	$C_4H_9NO_3$	29.5 ± 0.2
alanine	$C_3H_7NO_2$	44.6 ± 0.9
proline	$C_5H_9NO_2$	23.2 ± 0.7
valine	$C_5H_{11}NO_2$	32.6 ± 1.3
methionine	$C_5H_{11}NO_2S$	9.0 ± 0.2
cysteine	$C_3H_7NO_2S$	3.9 ± 1.7
isoleucine	$C_6H_{13}NO_2$	22.6 ± 1.5
leucin	$C_6H_{13}NO_2$	44.8 ± 0.3
tyrosine	$C_9H_{11}NO_3$	20.4 ± 0.8
phenylalanine	$C_9H_{11}NO_2$	26.0 ± 0.4
lysine	$C_6H_{14}N_2O_2$	31.3 ± 0.3
tryptophan	$C_{11}H_{12}N_2O_2$	4.0 ± 0.2
Total amino acids		501.8

a: Amino acid content per gram of protein.

The essentials amino acids such as lysine (Table 2) appear to exert influence under the reduction of tissue lipid peroxidation and antioxidant enzyme activity (Martínez-Tomé, García-Carmona & Murcia, 2001). According to with Zhang, Mu & Sun (2012) antioxidative properties as well were attributed to the amino acids such as histidine, methionine, cysteine, tyrosine and phenylalanine, as well as other hydrophobic amino acids. Je, Park & Kim affirm that a strong structure function relationship can be used to explain the antioxidative properties of most amino acids, metionine and cysteine possess a thiol group each; tyrosine and phenylalanine

possess phenolic groups, while histidine has an imidazole ring, which has been shown to be responsible antioxidant properties involving chelating and lipid radical-trapping abilities.

3.3. Carotenoids profile and antioxidant capacity

The Table 3 demonstrate the presence of twenty-three carotenoids (2650.7µg.g^{-1}) in the extract of the *Scenedesmus obliqqus* CPCC05 biomass, the chromatogram is presented in Annex A, were identified or tentatively identified based on the combined information obtained from chromatographic elution on a C30 column, co-chromatography with standards and characteristics of UV–vis and mass spectra (Annex B). The major carotenoids found were all-*trans*-β-carotene (778.2µg.g^{-1} ± 29.9) and all-*trans*-lutein (747.0µg.g^{-1} ± 28.7). In addition, found carotenoids with specific structural characteristics, such as all-*trans*-echinenone (166.8µg.g^{-1} ± 6.4) and 9-*cis*-echinenone (106.51µg.g^{-1} ± 4.0). The antioxidant potential of microalgal carotenoid extract (Table 3), determined according to OU et al. (2013), from the capacity of oxygen radical deactivation for lipophilic samples, the extract presented an antioxidant capacity of 1779.9 µmol TE g^{-1}.

Table 3. Carotenoids profile of the *Scenedesmus obliquus* CPCC05 obtained by HPLC–PDA–MS/MS and antioxidant potential of the carotenoids extract.

Carotenoid	Chemical structure	(µg.g^{-1}dw)[a]
all-*trans*-violaxanthin	$C_{40}H_{56}O_4$	31.7 ± 1.2
9-*cis*-neoxanthin	$C_{40}H_{56}O_4$	60.7 ± 2.3
all-*trans*-luteoxanthin	$C_{40}H_{56}O_4$	54.6 ± 2.0
13-*cis*-antheraxanthin	$C_{40}H_{56}O_3$	nd[b]
15-*cis*-lutein	$C_{40}H_{56}O_2$	nd[b]
all-*trans*-antheraxanthin	$C_{40}H_{56}O_3$	38.2 ± 1.4
all-*trans*-diatoxanthin	$C_{40}H_{54}O_2$	21.1 ± 0.8

all-*trans*-lutein	$C_{40}H_{56}O_2$	747.0 ± 28.7
15-*cis*-zeaxanthin	$C_{40}H_{56}O_2$	38.5 ± 1.4
all-*trans*-zeaxanthin	$C_{40}H_{56}O_3$	262.6 ± 10.1
9-*cis*-lutein	$C_{40}H_{56}O_3$	28.9 ± 1.1
all-*trans*-canthaxanthin	$C_{40}H_{52}O_2$	10.1 ± 0.3
9-*cis*-zeaxanthin	$C_{40}H_{56}O_2$	30.7 ± 1.1
all-*trans*-myxoxanthophyll	$C_{46}H_{66}O_7$	17.5 ± 0.6
5,6-β-carotene-epoxide	$C_{46}H_{56}O$	20.6 ± 0.7
all-*trans*-β-cryptoxanthin	$C_{40}H_{56}O$	23.8 ± 0.9
all-*trans*-crocoxanthin	$C_{40}H_{54}O$	nd[b]
all-*trans*-echinenone	$C_{40}H_{54}O$	166.8 ± 6.4
13-β-carotene	$C_{40}H_{56}$	45.0 ± 1.8
9-*cis*-echinenone	$C_{40}H_{54}O$	106.5 ± 4.0
all-*trans*-α-carotene	$C_{40}H_{56}$	42.0 ± 1.6
all-*trans*-β-carotene	$C_{40}H_{56}$	778.2 ± 29.9
9-*cis*-β-carotene	$C_{40}H_{56}$	124.4 ± 4.7
Total carotenoids		2650.7
ROO lipophilic[c]		1779.9 ± 9.2[d]

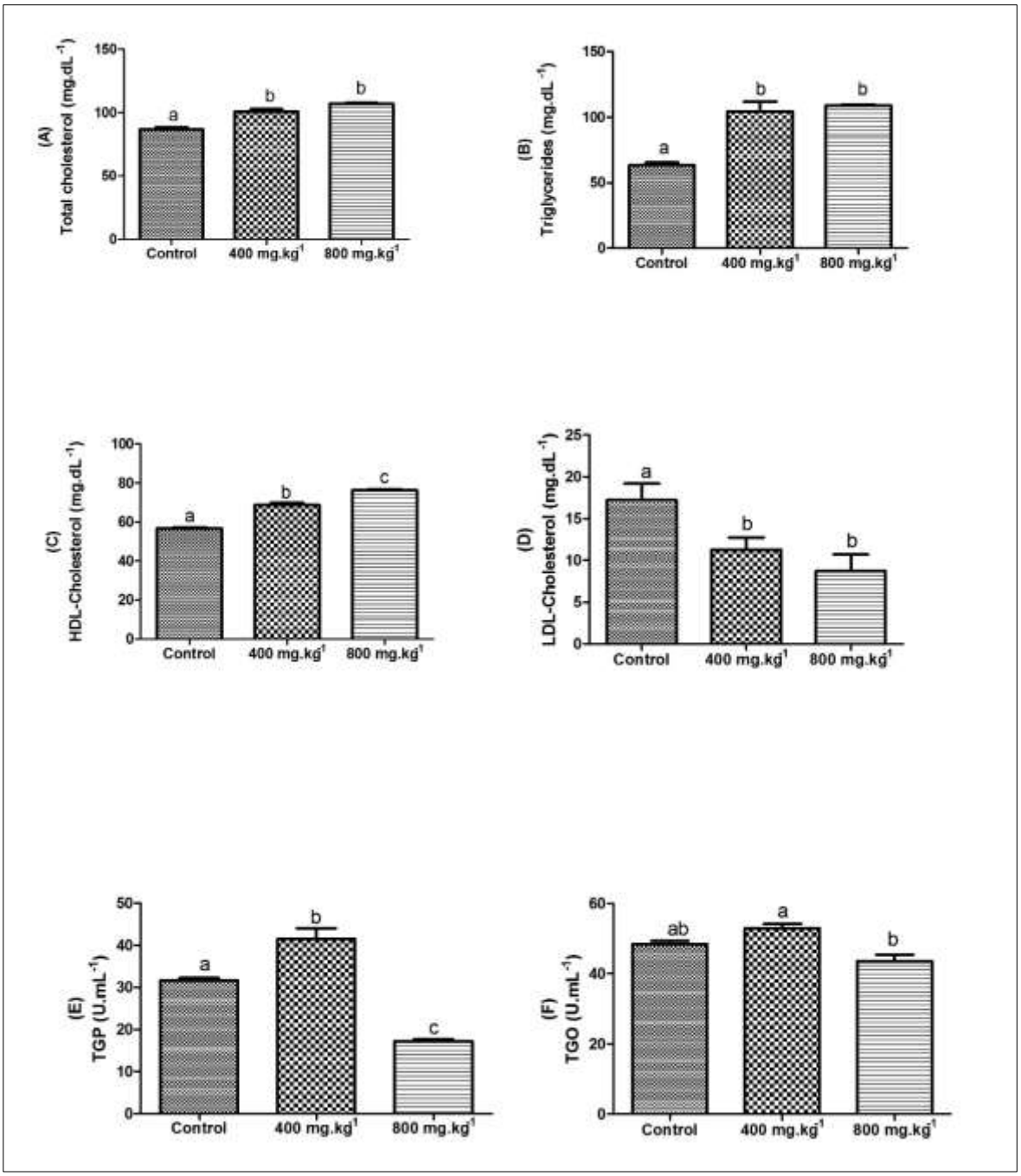
The content of total carotenoids (2650.7µg.g^{-1}) of the biomass represents 3.7 times higher when compared the *Phormidium autumnale* (Rodrigues et al., 2015) this indicates the potential of *Scenedesmus* for the production of pigments. These extract presented an antioxidant capacity of 1779.9 µmolTE.g^{-1}, this result is about three times more efficient when compared to the values reported by Ahmed et al. (2014) for the carotenoid extract of *Dunaliella salina* (577 µmolTE.g^{-1}). Furthermore, this result is substantially higher than those reported in the literature for carotenoids from conventional sources, Marineli et al. (2014) reported 6.48 µmolTE.g^{-1} for chia seeds, Wu et al. (2004) reported 5.57 µmolTE.g^{-1} for Brazilian nuts. It is suggested that this difference occurs due to the presence of specific carotenoids in microalgae, which present a stretching in the system of double conjugated bonds, thus giving them a

greater protective effect against oxidative stresses, since this effect is directly related to the size of the chromophore.

3.4. Serum analysis

In observing Figure 1, the doses of 400 and 800mg.kg^{-1}, caused significant changes ($p<0.05$) serum lipid profile of the mice. For both doses, there was an increase in total cholesterol (TC) (Figure 1A), triglycerides (TAG) (Figure 1B) and HDL (Figure 1C) relative to the control, an increased dose-dependent were observed for HDL. In contrast for the LDL (Figure 1D), occurred a significant reduction of 41% and 55% for 400 and 800mg.kg^{-1}, respectively. Regarding hepatic enzymes, 800mg.kg^{-1} of biomass reduced the activity of TGP (45%) and TGO (7%) in the serum of the mice, although the latter with no statistical difference ($p <0.05$).

Figure 1. Serum total cholesterol (A), triglycerides (B), high-density lipoprotein (HDL) (C), low-density lipoprotein (LDL) (D), pyruvic (E) and oxaloacetic (F) transaminase enzymes. Data expressed in mean ±SEM. Different letters indicates statistical differences according with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p<0.05$).



The two doses of microalgal biomass caused a significant increase in serum total cholesterol, this can be explained by increased levels of triglycerides and HLD. Parks (2001) documented that high carbohydrate intake might lead to an increase in blood triglycerides. This may explain what occurred in the present study since that the *S. obliquus* carbohydrates content can reach up to 20% (Becker, 2007). According to Ochoa et al. (2016), carbohydrates in levels above that required for the organism normal energetic balance are metabolized to triacylglycerol and stored in adipose tissue as an energy reserve. However, weight gain in the mice at the end of the experiment was not observed (Annex C).

The results found for HDL and LDL were positive for both doses. The increase in HDL related to LDL decrease corroborates with its functions exerted on the bloodstream. For while LDL carries a large fraction of cholesterol, depositing it in the artery wall and causing a possible obstruction, the best known of the potentially antiatherogenic functions of HDLs is its ability to promote the efflux of this cholesterol for reuse or excretion by the liver (Rosenson et al., 2011; Cusi, 2012). Considering the above, it can be inferred that *Scenedesmus obliquus* biomass can play a protective character against the risk factors for cardiovascular diseases.

As regards liver enzymes, the serum content of TGP and TGO decreased for the 800mg.kg^{-1} dose showed no adverse effect at this dose. Elevated levels of these enzymes may evidence hepatic damage induced by toxic effects of exogenous substances that may promote hepatocyte rupture resulting from necrosis or changes in cell membrane permeability (Frank et al., 2001).

3.5. Antioxidant capacity in tissues

The results that determine the antioxidant status of the heart are shown in Figure 2. It was observed that in this tissue the dose of 400mg.kg^{-1} did not significantly alter the activities of SOD (Figure 2A), CAT (Figure 2B) and GPx (Figure 2C) in relation to the control. However, significantly reduced levels of GR (Figure 2D), GSH (Figure 2E) and TBARS (Figure 2F), there was still an increase in the antioxidant potential determined by ORAC (Figure 2G). The dose of 800mg.kg^{-1} presented similar results, except for CAT (Figure 3B), which significantly reduced the control. A linear trend line showed a considerable negative correlation coefficient ($p < 0.01$) between TBARS and ORAC levels the heart ($r = -0.81$).

The biomass of *Scenedesmus obliquus* CPCC05 showed protective character to the heart, independent of dose. The increase in the antioxidant potential in the heart seems to have been the main responsible for the protection since there was a considerable negative correlation between the ORAC and the TBARS. This decrease in lipid peroxidation corroborates the results obtained by Carfagna et al. (2015) after supplementation of the diet of Wistar rats with microalgal biomass *Galdieria sulfuraria* and can be widely associated with the presence of bioactive. Antioxidant enzymes and GSH do not appear to have a large share of the elimination of the reactive species in this tissue since they have remained unchanged or reduced.

Figure 2. Heart enzymatic, non-enzimatic antioxidant status, lipid peroxidation and antioxidant capacity. Superoxide dismutase activity – SOD (A), Catalase activity – CAT (B), Glutathione Peroxidase activity GPx – (C), Glutathione Reductase activity – GR (D), Glutathione reduced activity – GSH (E), Lipid peroxidation by the TBARS assay (F) and antioxidant capacity as evaluated by the ORAC (G). Data expressed in mean ±SEM. Different letters indicates statistical differences according with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p<0.05$).

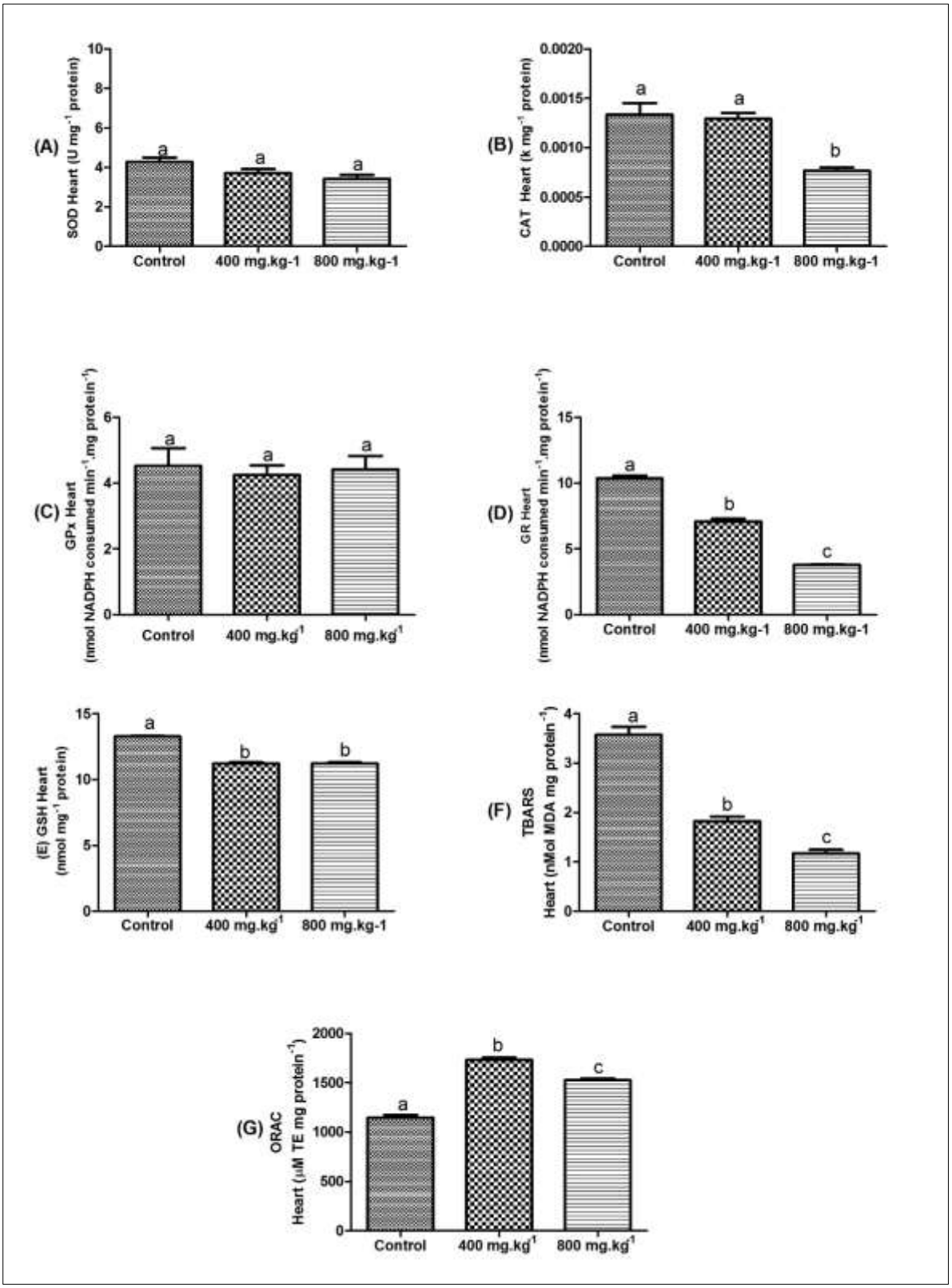
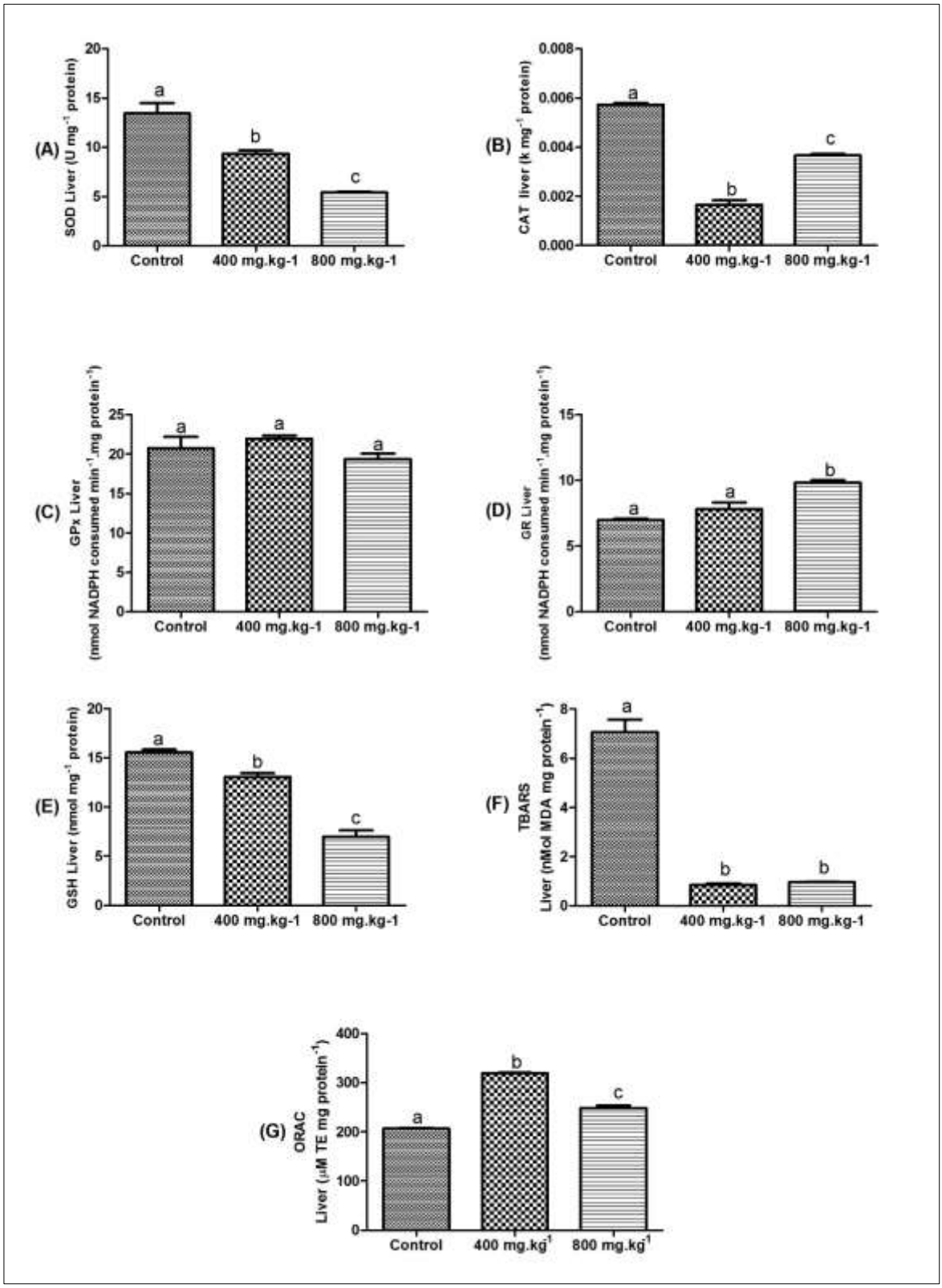


Figure 3 shows the antioxidant status of the liver. In this tissue, the activity of SOD (Figure 3A) significantly reduced with increasing microalgal biomass dosage. There was a reduction in CAT activity (Figure 3B) for both dosages. No difference was observed for GPx (Figure 3C). The activity of GR (Figure 3D) increased about 12% and 40% according to the dose increased, although only the group 800mg.kg^{-1} presented a significant difference in relation to the control. On the other hand, GSH reduced 16% and 56% as doses were increased. TBARS levels (Figure 3F) also decreased in the biomass groups (7.06, 0.86 and 0.96 nmol MDA.mg^{-1} protein). The ORAC test (Figure 3G) showed an increased antioxidant potential for two doses in relation control. A linear trend line showed a considerable negative correlation coefficient ($p < 0.01$) between TBARS and ORAC levels the liver ($r = -0.79$).

The same protective effect of the heart was verified for the liver, the administration of different doses of microalgal biomass resulted in significant decreases in TBARS levels (87% and 86%), corroborating results found in the literature (Carfagna et al. , 2015). Besides, the biomass still resulted in a significant increase in the content of GR for the dose of 800mg.kg^{-1}, according to Silva et al. 2014, this may have occurred because this enzyme is involved in GSH recovery.

Figure 3. Liver enzymatic, non-enzimatic antioxidant status, lipid peroxidation and antioxidant capacity. Superoxide dismutase activity – SOD (A), Catalase activity – CAT (B), Glutathione Peroxidase activity GPx – (C), Glutathione Reductase activity – GR (D), Glutathione reduced activity – GSH (E), Lipid peroxidation by the TBARS assay (F) and antioxidant capacity as evaluated by the ORAC (G). Data expressed in mean ±SEM. Different letters indicates statistical differences according with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p<0.05$).



The Figure 4 shows the antioxidant status of the kidney. The SOD (Figure 4A) not presented significant alteration, as well as the GR (Figure 4D). CAT levels (Figure 4B) were significantly reduced in the two doses studied. For the 400mg.kg^{-1} group there was a 59% increase in the activity of the GPx enzyme (Figure 4C) in relation to the control. For GSH values (Figure 4D), the increase occurred about 94% for the dose of 800mg.kg^{-1}. The TBARS values decreased from control to both doses. The antioxidant potential in this tissue expressed an atypical behavior, since the larger dose of microalgae administered resulted in a reduction of 27% in this marker when compared to the control. Unlike the other tissues, no negative correlation ($p < 0.01$) was observed between the levels of TBARS and ORAC in the kidney ($r = -0.11$).

Although the levels of TBARS in the kidneys decreased significantly in all the groups that ingested a biomass, there was a reduction in the antioxidant potential of this tissue for a dose of 800 mg.kg^{-1}. Thus, the protective effect observed in the kidneys can be conducted by an increase of GSH activity. This could be supported by the presence of phenolic compounds in the microalgae biomass (Madaame et al., 2015). According to Vanzo et al. (2008), the kidneys are responsible for the catabolism of phenolic compounds, widely distributed in this tissue and related to the increase in the expression of the endogenous antioxidant system (Yeh & Yen, 2006).

Figure 4. Kidney enzymatic, non-enzimatic antioxidant status, lipid peroxidation and antioxidant capacity. Superoxide dismutase activity – SOD (A), Catalase activity – CAT (B), Glutathione Peroxidase activity GPx – (C), Glutathione Reductase activity – GR (D), Glutathione reduced activity – GSH (E), Lipid peroxidation by the TBARS assay (F) and antioxidant capacity as evaluated by the ORAC (G). Data expressed in mean ±SEM. Different letters indicates statistical differences according with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p<0.05$).

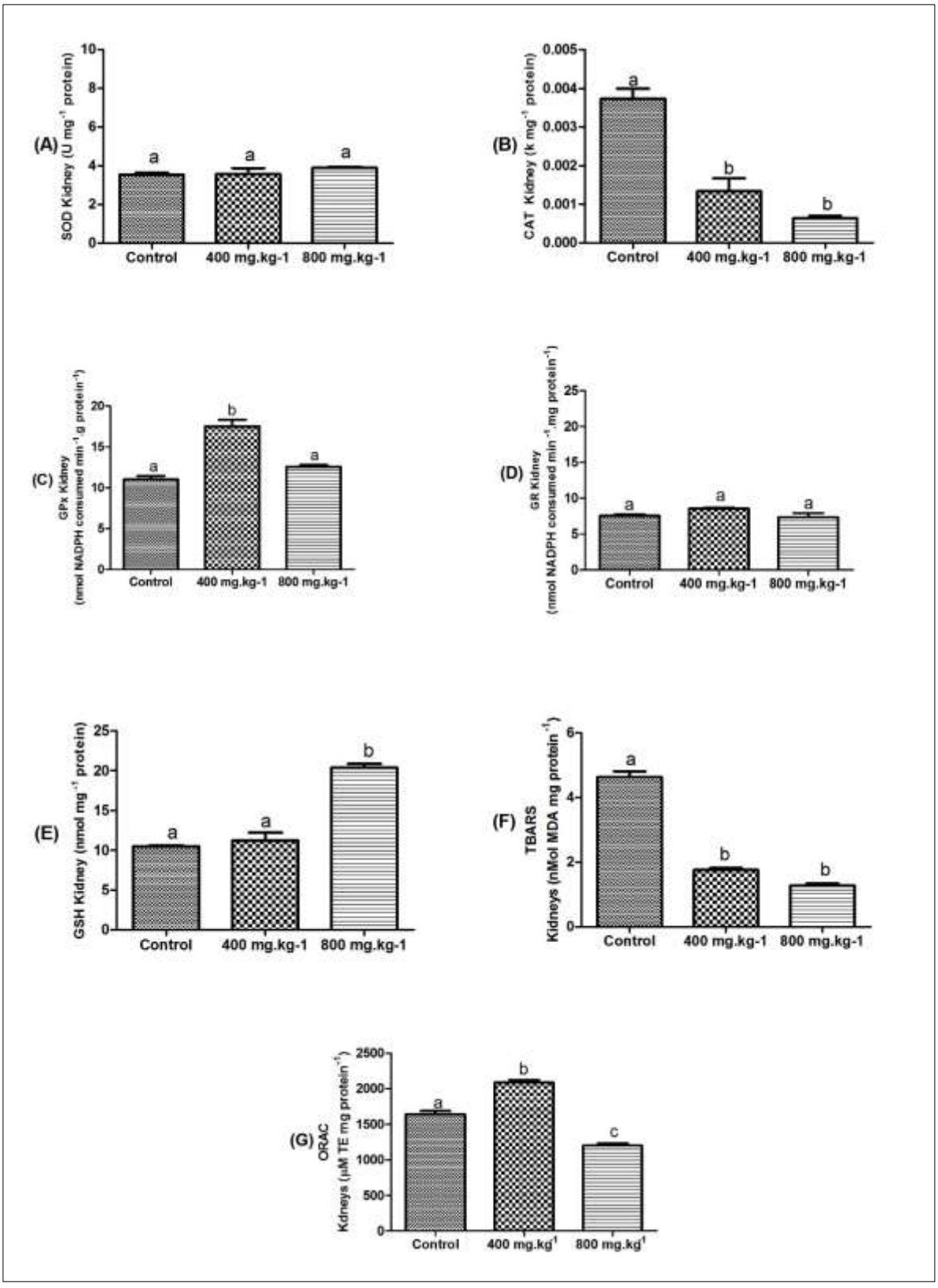
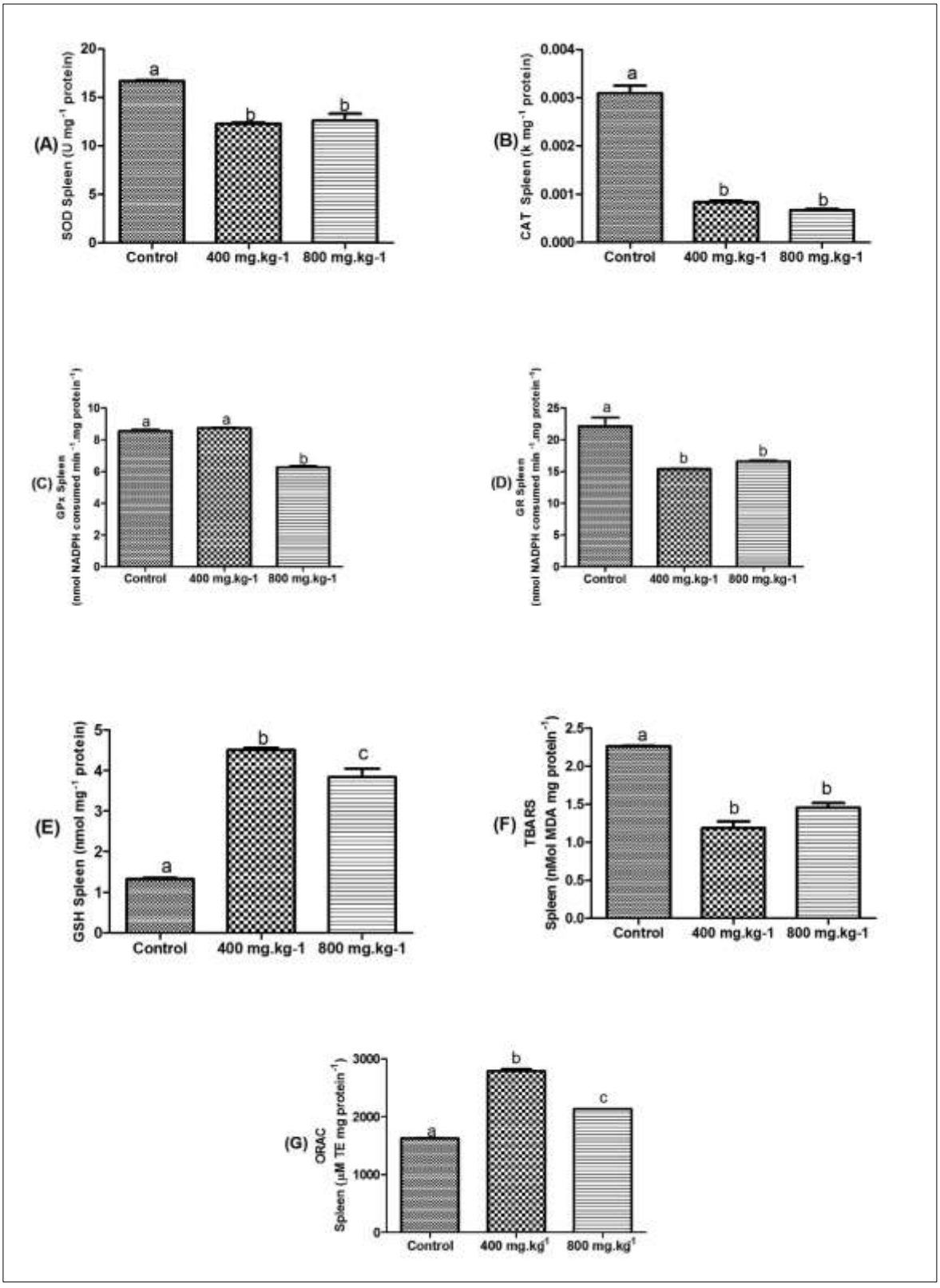


Figure 5 shows the antioxidant status of the spleen. SOD levels (Figure 5A) of the ingested groups of 400 and 800mg.kg^{-1} significantly decreased control, 26% and 24%, respectively. Likewise, CAT levels (Figure 5B) were reduced, 74% and 77%, respectively. For GPx values (Figure 5C), only 800mg.kg^{-1} biomass of *S. obliquus* CPCC05 presented a significant difference. There was a decrease in GR levels (Figure 5D) for both groups in relation to the control, this result corroborates with the increase of GSH levels (Figure 5E), since the GR enzyme acts on GSH recovery. TBARS levels (Figure 5F) were significantly reduced (47 and 36%). As regards the antioxidant potential (Figure 5G), both doses caused an increase. A linear trend line showed a high negative correlation coefficient ($p < 0.01$) between TBARS and ORAC levels the spleen ($r= -0.94$).

Finally, the antioxidant status to the spleen, as well as the heart and liver demonstrated the protective effect against lipid peroxidation, mainly supported by the high negative linear correlation between TBARS and ORAC. In addition, an increase in GSH levels also improved the status.

The influence of microalgal biomass doses on the activity of antioxidant enzymes in the tissues was not conclusive for this, studies that evaluate the expression of these enzymes are necessary.

Figure 5. Spleen enzymatic, non-enzimatic antioxidant status, lipid peroxidation and antioxidant capacity. Superoxide dismutase activity – SOD (A), Catalase activity – CAT (B), Glutathione Peroxidase activity GPx – (C), Glutathione Reductase activity – GR (D), Glutathione reduced activity – GSH (E), Lipid peroxidation by the TBARS assay (F) and antioxidant capacity as evaluated by the ORAC (G). Data expressed in mean ±SEM. Different letters indicates statistical differences according with one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($p<0.05$).



4. Conclusions

According to the results, *Scenedesmus obliquus* CPCC05 presented a valuable content of biocompounds, such as PUFAs (α-linolenic acid, linoleic acid and oleic acid), amino acids that have been associated with bioactive properties and carotenoids with high antioxidant potential.

In relation to the biological assay, biomass presented an important role with regard to lipoproteins, since it increased HDL and reduced LDL, thus reducing the protective effect against atherosclerotic pathologies. Regarding the antioxidant status, the results obtained for the endogenous system did not allow a concise conclusion, and there is a need for the verification of genetic transcription factors. It was possible to observe a protective effect of biomass against cellular peroxidation in all tissues analyzed, due to the reduction in TBARS levels. A correlation test has shown that this protective effect on the heart, liver and spleen is correlated with the increased antioxidant potential in these tissues.

Based on the above, it is suggested that the biomass of Scenedesmus obliquus CPCC05 can be explored as an alternative natural route in the reduction of risk factors, prevention or treatment of pathologies associated with serum lipid profile and cellular integrity.

5. References

ADARME-VEGA, C. T. et al. Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. **Microbial Cell Factories**, v.96, n.11, p. 1–10, 2012.

AEB, I. H. Catalase in vitro. **Methods in Enzymology**. v. 105, p. 121-126, 1984.

AHMED, F. et al. Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. **Food Chemistry**, v.165, n.15, p.300–306, 2014.

BECKER, E.W. micro-algae as a source of protein. **Biotechnology Advances**, v.25, n 2, p. 207–210, 2007.

BRADFORD, M.M. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein–dye binding. **Analytical Biochemistry**, v. 72, n. 1, p. 248–254, 1976.

BRITTON, G. UV/visible spectroscopy. In G., Britton, S., Liaaen-Jensen, H., Pfander (Eds). **Carotenoids: Spectroscopy**. (p.13-62). Badel: Birkhäuser Verlag, 1995.

CARFAGNA, S. et al. Dietary Supplementation with the Microalga *Galdieria sulphuraria* (Rhodophyta) Reduces Prolonged Exercise-Induced Oxidative Stress in Rat Tissues. **Oxidative Medicine and Cellular Longevity**, v.1, p.1–11, 2015.

CARLBERG, I.; MANNERVIK, B., 1985I. Carlberg, B. Mannervik. Glutathione-reductase. **Methods in Enzymology**, v. 113, p. 484–490, 1985.

CHACON-LEE, T. L.; GONZALEZ-MARINO, G. E. Microalgae for “healthy” foods: possibilities and challenges. **Comprehensive Reviews in Food Science and Food Safety** v.9, n. 6, p. 655–675, 2010.

CUSI, K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. **Gastroenterology**, v.142, p.711–725, 2012.

CUSI, K. Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. **Gastroenterology**, v.142, p.711–725, 2012.

DE ROSSO, V. V.; MERCADANTE, A. Z. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. Journal of Agricultural and **Food Chemistry**, v. 55, n. 13, p. 5062–5072, 2007.

DE ROSSO, V.V.; MERCADANTE, A.Z. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits. **Journal of Agricultural and Food Chemistry**, v.55, p.5062– 5072, 2007.

Ellman, G. L. **Tissue sulfhydryl groups**. Archives of Biochemistry and Biophysics, v. 82, n.1, p. 70-77, 1959.

FAURE, P.; LAFOND, J.L**. Measurement of plasma sulfhydryl and carbonyl groups as a possible indicator of protein oxidation**. In: Analysis of free Radicals in biological systems. Ed. Verla Bostonp. 1995. p. 237–248.

FLOHE, L.; GUNZLER, W. A. Assays of glutathione-peroxidase. **Methods in Enzymology**, v. 105, p. 114–121, 1984.

FRANK, H. A. et al. An assessment of the clinical utility of serum ALT and AST in chronic hepatitis C. **Hepatology Research**, v.18, n.1, p.63–71, 2001.

FRIEDEWALD, W. T.; LEVY R. I., FREDRICKSON, D. S. Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. **The American Association of Clinical Chemists**, v. 18, n.6, 499 – 502, 1972.

HAGEN, S.R.; FROST B.; AUGUSTIN, J. Precolumn phenylisothiocyanate derivatization and liquid chromatography of amino-acids in food. Journal of the **Association of Official Analytical Chemists**, v.72, n.6, p.912–916, 1989.

HARTMANN, L; LAGO, B. C. A rapid preparation of fatty methyl esters from lipids. Laboratory Practice, Londres, v. 22, p. 475 – 477, 1973.

HODSON, L.; SKEAFF, C.M.; CHISHOLM, W-A.H. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. **European Journal of Clinical Nutrition**, v.55, p.908–915, 2001.

JACOB-LOPES, E. et al. Bioprocess for the conversion of carbon dioxide from industrial emissions, bioproducts, uses thereof and hybrid photobioreactor. BR Pat. 041028. 15 sep. 33p. 2014.

JACOB-LOPES, E.; QUEIROZ, M. I.; ZEPKA, L. Q.; NETTO, F. M. Protein characterisation of the *Aphanothece Microscopica Nägeli* cyanobacterium cultivated in parboiled rice effluent. **Food Science and Technology**, v. 26, p. 482–488, 2006.

Je, J.Y.; Park P.J.; Kim, S.K. Antioxidant activity of a peptide isolated from Alaska pollack (*Theragra chalcogramma*) frame protein hydrolysate. **Food Research International**, v.38, p.45–50, 2005.
KATAYAMA, S.; MINE, Y. Antioxidative activity of amino acids on tissue oxidative stress in human intestinal epithelial cell model. **Journal of Agricultural and Food Chemistry**, v.55, p. 8458–8464, 2007.

MAADANE, A et al. Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. **Journal of Biotechnology**, v. 215, n.10 p.13–19, 2015.

MANDELLI, F. et al. Identification of carotenoids with high antioxidant capacity produced by extremophile microorganisms. **World Journal of Microbiology and Biotechnology**, v.28, p.1781–1790, 2012.

MARINELI, R. S. et al. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica L*.). **Food Science and Technology**, v.59, n.2, p.1304–1310, 2014.

MARTÍNEZ-TOMÉ, M.; GARCÍA-CARMONA, M.F.; MURCIA, M.A. Comparison of the antioxidant and pro-oxidant activities of broccoli amino acids with those of common food additives. **Journal of the Science of Food and Agriculture**, v.81, p.1019–1026, 2001.

MENSINK, R. P. et al. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. **The American Journal of Clinical Nutrition**, v.77, n.5, p.1146–55, 2003.

OCHOA, M. et al. Obesogenic diets have deleterious effects on fat deposits irrespective of the nature of dietary carbohydrates in a Yucatan minipig model Original. **Nutrition Research**, v.36, n.9, p.947–954, 2016.

OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal-tissues by thiobarbituric acid reaction. **Analytical Biochemistry**, v.95, n. 2, p. 351–358, Jun. 1979.

OU, B. et al., Determination of Total Antioxidant Capacity by Oxygen Radical Absorbance Capacity (ORAC) Using Fluorescein as the Fluorescence Probe: First Action 2012.23. **Journal of AOAC International**, v. 96, n. 6, p.1372-1376, Nov-Dec. 2013.

PARKS, E. J. Effect of Dietary Carbohydrate on Triglyceride Metabolism in Humans. **The Journal of Nutrition**, v.131, n.10, p.2772–2774, 2001.

PIRES, C. V. et al. Qualidade nutricional e escore químico de aminoácidos de diferentes fontes proteicas. **Food Science and Technology**, vol.26, n.1, .179–187, 2006.

PLAZA, M. Cifuentes, A. Ibanez, E. In the search of new functional food ingredients from algae. **Trends in Food Science & Technology**, v. 19, p.31–39, 2008.

POWLS, R.; BRITTON, G. A. Carotenoprotein, containing violaxanthin, isolated from *Scenedesmus obliquus*. **Biochemical and Biophysical Research**, v.453, n.1, p. 270–276, 1976.

PRASANNA, R. et al. Rediscovering cyanobacteria as valuable sources of bioactive compounds (review). **Applied Biochemistry and Microbiology**, v. 46, n.2, p. 119–134, 2010.

RAPOSO, M. F. D. J. et al. Health applications of bioactive compounds from marine microalgae. **Life Sciences**, v.93, n.15, p.479–486, 2013.

RIPPKA, R. et al. Generic assignments strain histories and properties of pure cultures of cyanobacteria. **Journal General Microbiololy**, v. 111, n. 1, p.1–61, 1979.

RODRIGUES, D. B. et al. Bioactive pigments from microalgae *Phormidium autumnale*. **Food Research International**, v.77, n.2, p.273–279, 2015.

RODRIGUES, D. B. et al. Production of carotenoids from microalgae cultivated using agroindustrial wastes. **Food Research International**, v.65, p.144–148, 2014.

RODRIGUEZ-AMAYA, D. B., KIMURA, M., GODOY, H. T., & AMAYA-FARFAN, J. Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. **Journal of Food Composition and Analysis**, 21, 445–463, 2008.

RODRIGUEZ-AMAYA, D.B. Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of food carotenoids — a review. **Journal Food Composition Analysis**, v.23, p.726–740, 2010.

ROSENSON, R.S. et al. HDL Measures, Particle Heterogeneity, Proposed Nomenclature, and Relation to Atherosclerotic Cardiovascular Events. **Clinical Chemistry**, v.57, p.392–410, 2011.

SAHUA, A. et al. Fatty acids as biomarkers of microalgae. **Phytochemistry**, v.89, p.53–58, 2013.

SON, D. O.; SATSU, H.; SHIMIZU, M. Histidine inhibits oxidative stress- and TNF-alpha-induced interleukin-8 secretion in intestinal epithelial cells. **FEBS Letters**, v.579, p.4671–467, 2005.

SPIES, J. R. Determination of tryptophan in proteins. **Analytical Chemistry**, v.39, n.12, p.1412-1415, 1967.

STAHL, W.; SIES, H. Antioxidant activity of carotenoids. **Molecular Aspects of Medicine**. v.24, n.6, p. 345-51, Dec. 2003.

TABELI, A.F. et al. Fatty acids profiling: A selective criterion for screening microalgae strains for biodiesel production. **Algal Research**, v.2, n.3, p.258–267, 2013.

VAZ, B. S. Moreira, J.B. Morais M.G. Costa, J.A.V. Microalgae as a new source of bioactive compounds in food supplements. **Current Opinion in Food Science**, v.7 p. 73–77, 2016.

WANG, J. From microalgae oil to produce novel structured triacylglycerols enriched with unsaturated fatty acids. **Bioresource Technology**, v. 184, n. 1, p. 405–414, May. 2015.

WHITE, J.A.; HART, R.J.; FRY, J.C. An Evaluation of the Waters Pico-Tag System for the Amino-Acid-Analysis of Food Materials. **The Journal of Automatic Chemistry**, v.8, n.4, p.170–177, 1986.

WINTERBOURN, C. C. et al. The estimation of red cell superoxide dismutase activity. **Journal of Laboratory and Clinical Medicine**, v. 85, n. 2, p. 337–341, 1975.

WU, X. et al. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. **Journal of Agricultural and Food Chemistry**, v.52, p.4026–4037, 2004.

YEH C.T.; YEN, G.C. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance-associated protein 3 mRNA expression. **Journal of Nutrition**, v.136, n.1, p.11–5, 2006.

ZEPKA, L.Q., MERCADANTE, A.Z. Degradation compounds of carotenoids formed during heating of a simulated cashew apple juice. **Food Chemistry**, v.117, p.28–34, 2009.

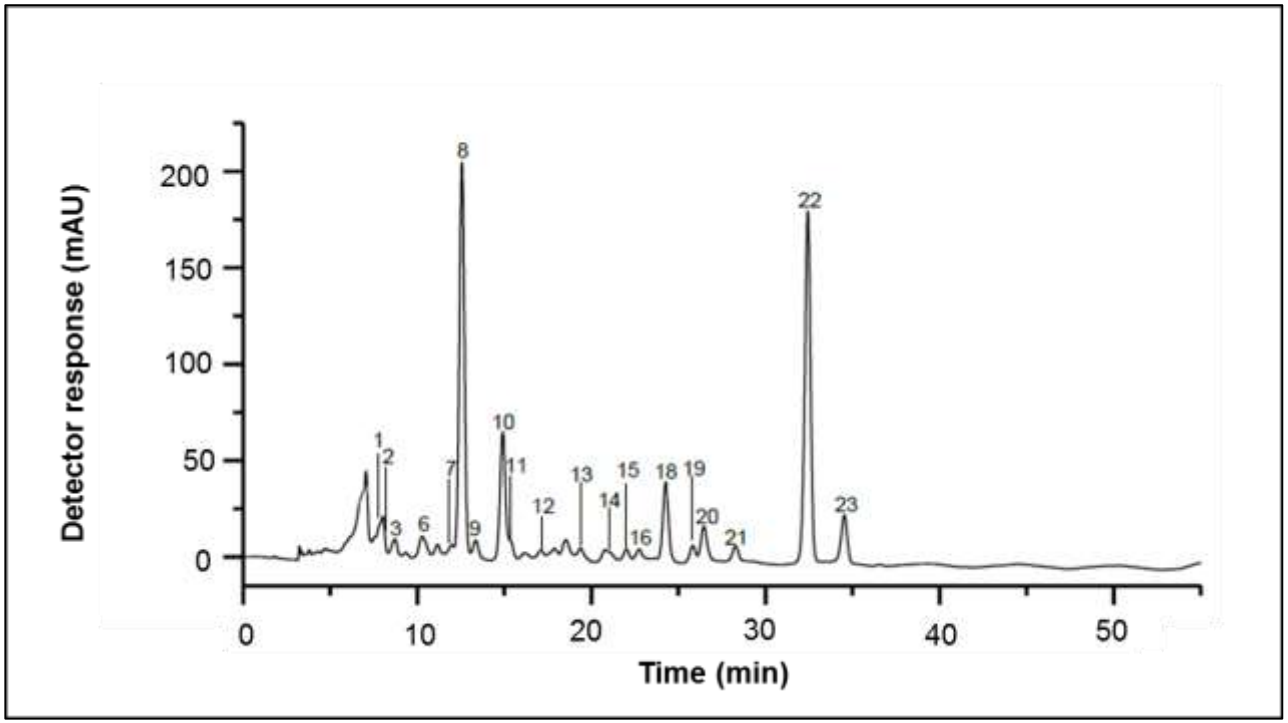
Zhang, M.; Mu, T. H.; Sun, M. J. Sweet potato protein hydrolysates: antioxidant activity and protective effects on oxidative DNA damage. **International Journal of Food Science & Technology**, v.47, p.2304–2310, 2012.

CONCLUSÃO GERAL

Com base nos resultados obtidos no presente estudo, pode-se inferir que a biomassa de *Scenedesmus obliquus* (CPCC05) é fonte potencial de numerosos compostos de interesse em termos de saúde humana. De maneira geral, esses biocompostos foram capazes de modular de forma positiva o conteúdo sérico de liporoteina de altae baixa densidade, bem como exercer um efeito protetor contra peroxidação lipídica celular em todos os tecidos**.** Diante disso, sugere-se que a biomassa de *Scenedesmus obliquus* CPCC05 pode ser explorada como uma via alternativa e natural na diminuição de fatores de risco, prevenção ou tratamento de patologias dependentes do perfil lipídico sérico e integridade celular.

ANEXOS

ANEXO A. CHROMATOGRAM, OBTAINED BY HPLC-PDA, OF THE CAROTENOID EXTRACT FROM *SCENEDESMUS OBLIQUUS* CPCC05 BIOMASS. PEAK IDENTIFICATION AND CHARACTERIZATION IS GIVEN IN ANNEX B



ANEXO B. CHROMATOGRAPHIC, UV–VIS SPECTRUM AND MASS CHARACTERISTICS OF *SCENEDESMUS OBLIQUUS* CPCC05 CAROTENOIDS OBTAINED BY HPLC–PDA–MS/MS

Peak[a]	Carotenoid	t_R (min)[b]	UV-Vis characteristics	Fragment ions (positive mode) *(m/z)*			
			$\lambda_{máx}$ (nm)[c]	III/II (%)[d]	AB/II (%)[e]	$M+H]^+$	MS/MS
1	all-*trans*-violaxanthin	7.6	414, 437, 466	56	0	601	583 $[M+H-18]^+$, 565 $[M+H-18-18]^+$, 509 [M+H–92] $^+$, 221
2	9-*cis*-neoxanthin	7.9-8	328, 412, 435, 464	75	22	601	583 $[M+H-18]^+$, 565 [M+H-18-18] $^+$, 547 [M+H-18-18-18] $^+$, 509 [M+H–92] $^+$
3	all-*trans*-luteoxanthin	8.6-8.7	406, 421, 447	62	0	601	583 $[M+H-18]^+$, 565 [M+H-18-18] $^+$, 509 [M+H–92] $^+$, 491 [M+H–92-18] $^+$, 221
4	13-*cis*-antheraxanthin	9.3	441/443	0	nd[g]	585	567 $[M+H-18]^+$, 549 [M+H-18-18] $^+$, 531 [M+H-18-18-18] $^+$, 220
5	15-*cis*-lutein	10.2	330, 416, 440, 465	14	26	569	551 [M+H-18] $^+$, 533 [M+H-18-18] $^+$
6	all-*trans*-antheraxanthin	10.4	419, 445, 471	50	0	585	567 [M+H-18] $^+$, 549 [M+H-18-18] $^+$, 531 [M+H-18-18-18] $^+$, 493 [M+H-92] $^+$, 221
7	all-*trans*-diatoxanthin	12.0	425, 449, 472	9	nd[g]	567	549 [M+H-18] $^+$, 535, 531 [M+H-18-18] $^+$, 475 [M+H-92] $^+$, 393
8	all-*trans*-lutein	12.5-12.6	420, 444, 472	59	0	569	551 [M+H-18] $^+$ (in source), 533 [M+H-18-18] $^+$, 495 [M+H-18-56] $^+$
9	15-*cis*-zeaxanthin	13.3	420, 449, 474	16	nd[g]	569	551 [M+H-18] $^+$, 533 [M+H-18-18] $^+$, 477 [M+H-92] $^+$
10	all-*trans*-zeaxanthin	14.8-14.9	425, 450, 476	30	0	569	551 [M+H-18] $^+$, 533 [M+H-18-18] $^+$, 477 [M+H-92] $^+$
11	9-*cis*-lutein	15.2	331, 415, 441, 467	50	11	nd[f]	551 [M+H-18] $^+$ (in source), 533 [M+H-18-18] $^+$, 495 [M+H-18-56] $^+$
12	all-*trans*-canthaxanthin	17.0	470/472	0	0	565	547 [M+H-18] $^+$, 509 [M+H-56] $^+$, 459 [M+H-106] $^+$, 363, 203

13	9-*cis*-zeaxanthin	19.3	419, 446, 470	33	nd[g]	569	551 $[M+H-18]^+$, 533 $[M+H-18-18]^+$, 477 $[M+H-92]^+$
14	all-*trans*-myxoxanthophyll	20.9-21.1	449, 473, 505	48	0	nd[f]	nd[f]
15	5,6-β-carotene-epoxide	21.9	419, 445, 473	64	0	553	535 $[M+H-18]^+$, 461 $[M+H-92]^+$, 205
16	all-*trans*-β-cryptoxanthin	22.6-22.7	425, 450, 476	18	0	553	535 $[M+H-18]^+$, 461 $[M+H-92]^+$
17	all-*trans*-crocoxanthin	23.6	419, 444, 466	66	0	551	533 $[M+H-18]^+$, 459 $[M+H-92]^+$, 393
18	all-*trans*-echinenone	24.1-24.2	459/462	0	0	551	533 $[M+H-18]^+$, 427, 203
19	13-β-carotene	25.8-25.9	338, 420, 445, 470	14	48	537	444 $[M+H-92]^+$, 347
20	9-*cis*-echinenone	26.3-26.4	457/454	0	nd[g]	551	533 $[M+H-18]^+$, 471 $[M+H-80]^+$, 427
21	all-*trans*-α-carotene	28.1	419, 445, 473	62	0	537	413, 321
22	all-*trans*-β-carotene	32.2-32.3	425, 451, 478	33	0	537	444 $[M+H-92]^+$, 399, 355
23	9-*cis*-β-carotene	34.3-34.4	421, 446, 472	30	nd[g]	537	444 $[M+H-92]^+$

a: Numbered according to the chromatogram shown in Annex A; b: Linear gradient in methanol and methyl tert-butyl ether; c: Sectral fine structure; d: Ratio of the height of the longest wavelength absorption peak (III) and that of the middle absorption peak (II); e: Ratio of the *cis* peak (AB) and the middle absorption peak (II); f: Carotenoid content in dry weight; g: Not detected.

ANNEX C. BODY WEIGHTS (G) OF THE ANIMALS PER WEEK