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ATIVIDADE BIOLÓGICA DE DIFERENTES SUCOS DE UVA E SEUS PRINCIPAIS CONSTITUINTES

CAROLINE DANI

Caxias do Sul

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Tese apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, visando a obtenção de grau de Doutora em Biotecnologia.

Orientadores: Prof. Dr. João A. P. Henriques

Profª Drª Mirian Salvador

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Prof. Dr. João Antônio Pêgas Henriques

Prof. Dra. Mirian Salvador

Prof. Dr. Guido Lenz

Prof. Dr. Roberto Moura

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APRESENTAÇÃO

Em 1992, Renaud e Lorgeril chamaram a atenção da comunidade científica e médica para o fato de que a incidência de doença cardíaca isquêmica era menor na França do que em outros países industrializados, apesar deste país possuir uma dieta rica em gorduras e uma alta incidência de fumantes na população. Este fato foi atribuído ao consumo regular de vinho pelos franceses, tendo como conseqüência a elevação dos níveis de HDL (high density lipoprotein ou lipoproteína de alta densidade), conhecido como o "bom colesterol". O Paradoxo Francês de Renaud e Lorgeril chamou muita a atenção dos pesquisadores, da imprensa mundial e conseqüentemente, do público.

Entretanto, conhece-se também os comprometimentos que o consumo exagerado de álcool pode trazer ao ser humano. Assim sendo, pensou-se em estudar os benefícios potenciais que o suco de uva poderia gerar à saúde humana. A região nordeste do Rio Grande do Sul é a principal produtora de suco de uva no país.

Durante o meu mestrado entitulado "Atividade antioxidante, mutagênica e antimutagênica de sucos de uva" determinou-se a composição nutricional, conteúdo fenólico e a atividade antioxidante de sucos de uva tintos (variedade Bordô) e brancos (Niágara) orgânicos e convencionais produzidos em escala piloto e adquiridos comercialmente. Os resultados obtidos foram publicados na revista Food Chemical and Toxicology (2007) e mostraram diferenças importantes na composição fenólica entre sucos orgânicos e convencionais. Desta forma, durante o meu doutorado, buscou-se verificar as atividades benéficas do suco de uva tinto, orgânico e convencional, *in vivo,* utilizando-se como modelo biológico ratos Wistar. Avaliou-se ainda a influência de alguns polifenóis isolados na proteção contra danos causados por diferentes agentes estressores em linhagens de levedura *Saccharomyes cerevisiae* proficientes e deficientes em defesas oxidantes.

A tese está dividida em sete partes: 1) introdução, onde se caracterizou o suco de uva, bem como seus principais constituintes e sua atividade benéfica à saúde; 2) objetivos; 3) parte experimental, divididas em 7 capítulos, os quais correspondem aos artigos publicados, submetidos para publicação e em preparação; 4) discussão geral; 5) conclusões; 6) perspectivas; 7) referências; e 8) anexos.

Os anexos estão divididos em 3 partes: a) artigo publicado com os dados do mestrado, que instigou o tema desta tese, b) artigo de revisão publicado na Revista Ciência e Movimento do Centro Universitário Metodista IPA, e c) curriculum vitae da autora.

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BER	Enzima de reparo de base
CAT	enzima catalase
DNA	ácido desoxirribunucléico
$DPPH^{\bullet}$	1,1-difenil, 2-picrilhidrazil
ER	Espécies reativas
ERN	Espécio Reativa de Nitrogênio
HDL-Colesterol	Colesterol *High density level*
O_2	gás oxigênio
$O_2^{\bullet -}$	radical superóxido
$OH^{\bullet}$	radical hidroxil
$^1O_2$	oxigênio singleto
SCGE	*single cell gel eletrophoresis*
SNC	Sistema Nervoso Central
SOD	enzima superóxido dismutase
TBARS	Espécies reativas do ácido tiobarbitúrico

RESUMO

Embora os efeitos benéficos da ingestão moderada do vinho sejam bem conhecidos, a capacidade antioxidante de sucos de uva é ainda pouco estudada. O suco de uva é um alimento composto por uma importante quantidade de polifenóis e pode ser incluído entre os alimentos com potencial antioxidante. Seu estudo é muito relevante pelo amplo consumo desta bebida e a importância econômica que apresenta em nossa região. Atualmente contamos com diferentes sucos de uva no mercado, brancos, tintos e roses, orgânicos ou convencionais. O suco de uva possui vários nutrientes e compostos bioativos com atividades antioxidante, antimutagênica, anticarcinogênica e antiaterogênica, entre outras. Os polifenóis são nutrientes de grande importância no suco de uva, visto que a presença destes pode contribuir para coloração, acidez e outras características importantes do suco. Aos polifenóis, já são atribuídas várias atividades benéficas a saúde do homem. Entretanto, pouco se sabia sobre a atividade do suco, o qual é uma substância complexa. Neste sentido, este estudo buscou avaliar: 1) atividade antioxidante e antimutagênica de diferentes sucos de uva e a influência de metais presentes nestes sucos; 2) atividade antioxidante do suco de uva rose; 3) atividade neuroprotetora e hepatoprotera do suco de uva em ratos Wistar jovens; 4) atividade antioxidante sérica do suco de uva em ratos Wistar jovens; 5) atividade neuroprotetora, hepatoprotetora, antigenotóxica em ratos Wistar envelhecidos; e 6) proteção do resveratrol e da catequina frente a diferentes agentes estressores em diferentes linhagens da levedura *S. cerevisiae.* Alguns metais apresentaram correlações positivas com estas atividades, como o Manganês, e negativas como, por exemplo, o Enxofre. O conteúdo de minerais variou entre sucos brancos e tintos. Entretanto, entre os mais prevalentes, está o potássio. Sendo assim, as correlações apresentadas também diferenciam entre os sucos brancos e tintos. Modelos *in vivo* contaram com grupos de ratos Wistar (machos)

que foram tratados por gavagem duas vezes ao dia durante 30 dias, tendo no total um volume de suco de uva corresponde ao seu peso. Os ratos foram divididos em três grupos: controle (recebeu salina), suco de uva convencional e suco de uva orgânico. No 30º dia os animais recebiam uma dose equivalente ao seu peso de um agente estressor (CCl4) e após cinco horas foram sacrificados. Foi possível observar que os sucos de uva estudados possuem atividade antioxidante e antimutagênica importante no modelo da levedura *S. cerevisiae*. No modelo *in vivo* (ratos Wistar) observou-se uma ação neuroprotetora, hepatoprotetora e antioxidante sérica em ratos jovens. Nos ratos mais velhos observou-se também ação antigenotóxica importante. Nestes trabalhos, observou-se importantes correlações entre as atividades benéficas e o conteúdo polifenólico, principalmente referente ao conteúdo de resveratrol e catequina, não-flavonóide e flavonóide, respectivamente. Observando-se que o suco de uva é uma mistura complexa, foram realizados estudos envolvendo estes polifenóis a fim de analisar a influência dos mesmos isoladamente no modelo da levedura *S. cerevisiae*. Foi possível observar pelos resultados que ambos os polifenóis, quando em quantidades semelhantes, são antioxidantes em potencial, sem diferença estatística. Entretanto, utilizando-se linhagens proficientes e deficientes nos sistemas de defesa antioxidante, foi possível observar que os danos causados pelos agentes estressores são revertidos por ambos polifenóis pela ação principalmente da enzima catalase. Estudos adicionais com modelos específicos para determinadas doenças ou até mesmo com humanos fazem-se necessários, entretanto pode-se concluir que o suco de uva é um alimento rico em polifenóis, vitamina C, minerais e que possui uma importante atividade antioxidante tanto *in vitro* como *in vivo.*

ABSTRACT

Although the beneficial effects of the moderate intake of wine are well known, the antioxidant capacity of grape juices is still little reported. Grape juice is a food which has important amount of polyphenols, thus, it can be included among the foods with high antioxidant potential. Studies with grape juice are very relevant since this beverage is widely consumed and presents economical importance in our region. Nowadays there are several types of grape juices in the market; white, purple and rose, organic or conventional. Grape juice has many nutrients and bioactive compounds with antioxidant, antimutagenic, anticarcinogenic and antiteratogenic activities. Polyphenols are components which play important roles in grape juice, since their presence contributes to the coloring, acidity and other characteristics of the juice. Several beneficial activities to human health are attributed to polyphenols. However, studies related to juice beneficial activity, a complex substance, were scarce. This study aimed to assess: 1) antioxidant and antimutagenic activities of different grape juices and the metals influence in these activities; 2) antioxidant activity of rose grape juice; 3) neuroprotective and hepatoprotective activities of grape juice in young Wistar rats; 4) serum antioxidant activity of grape juice in young Wistar rats; 5) neuroprotective, hepatoprotective and antigenotoxic activities in old Wistar rats; and 6) protective activity of resveratrol and catechin against distinct stressor agents in different strains of *S. cerevisiae* yeast. Some metals like Manganese and Sulfur showed positive and negative correlations with these activities, respectively. Moreover, mineral content varied between white and purple juices. However, Potassium was the most prevalent mineral (in both juices). Thus, the correlations reported could also distinguish white from purple juices. Wistar rats (male) were used as *in vivo* models. They were treated by gavage twice a day, for 30 days, receiving in total a volume of grape juice

corresponding to their weight. Rats were divided into three groups: control (saline), organic and conventional grape juice. On the 30th day, half of each group received a single dose of the stressor agent CCl_4 equivalent to their weight. After six hours, the animals were sacrificed. Experiments with the moldel yeast *S. cerevisiae* demonstrated that the grape juices studied present important antioxidant and antimutagenic activities. The *in vivo* model (Wistar rats) showed neuroprotective, hepatoprotective and seric antioxidant actions of grape juices in young rats. In oldest rats, antigenotoxic activity was found. These studies demonstrated important correlations between beneficial activities and polyphenolic content, mainly related to resveratrol and catechin, non-flavonoid and flavonoid compounds, respectively. Since grape juice is a complex mixture of compounds, involving these polyphenols were carried out in order to analyze their isolated influence on *S. cerevisiae* yeast model. Our results suggest that both polyphenols when applied in similar amountm have the same antioxidant potential. However, when studying strains proficient and deficient in antioxidant defense systems, the damages from stressor agents are reverted by both polyphenols, mainly under the action of catalase enzyme. Further studies with specific models of certain diseases or even with humans are needed. Our findings demonstrated that grape juice is a food rich in polyphenols, vitamin C and minerals which plays important *in vitro* and *in vivo* antioxidant activities.

1. INTRODUÇÃO

1.1 Uva e seus derivados: benefícios à saúde humana

A uva é uma das frutas mais cultivadas no mundo e, juntamente com seus derivados, faz parte da dieta de diversos países, principalmente os localizados na região do Mediterrâneo (Ollala et al., 2004). Entre os derivados da uva, o mais estudado é o vinho, principalmente pelo seu benefício á saúde humana. Estudos epidemiológicos têm revelado uma correlação inversa entre consumo de vinho tinto e a incidência de doenças cardiovasculares, um fenômeno comumente conhecido como "Paradoxo Francês" (Reunad & Logeril, 1992; Vidavalur et al., 2006). Entre os benefícios relacionados à ingesta de vinho ao ser humano, inclui-se a redução da morbidade e mortalidade cardiovasculares, de câncer, doença de Alzheimer e melhoras no déficit cognitivo (Vidavalur et al., 2006; Opie & Leocur, 2007). Entretanto, um dos questionamentos gerados por estes estudos era se as atividades benéficas estavam ligadas à presença do álcool ou dos compostos fenólicos. Desta forma, com a finalidade de responder esta questão, estudos buscaram comparar as atividades benéficas do vinho com outras bebidas alcoólicas, tais como: cerveja, vinho branco e destilados, e com bebidas não-alcóolicas como o suco de uva e o vinho desalcolizado. Nestes estudos, observou-se que em humanos saudáveis, apenas os tomadores de vinho tinto melhoraram o fluxo coronariano, bem como também foram os únicos a aumentar os níveis de HDL-Colesterol e os níveis de antioxidantes plasmáticos, quando comparados a humanos que ingeriram as mesmas quantidades (em g/álcool) de vinho branco ou vodka (Opie & Lecour, 2007). Ainda, quando comparados tomadores de vinho tinto e vodka, apenas os referentes à primeira bebida apresentaram uma inibição no fator NF-Kappa-B durante lipemia pós-prandial, provendo assim um mecanismo antiinflamatório (Cruz et al., 2006).

Em adição, Vinson et al. (2001) compararam a eficácia do vinho tinto, vinho tinto sem álcool e o suco de uva em reduzir os eventos ateroscleróticos em hamster. Água e etanol (6,75%) foram dados ao grupo controle. A concentração de polifenóis das três bebidas foi normalizada a um mesmo valor para o teste com os animais. Os resultados mostraram que o etanol e todas as bebidas geraram uma redução significativa na aterosclerose. Observaram também que o suco de uva foi mais efetivo do que o vinho tinto e o vinho desalcolizado em inibir a aterosclerose, bem como se mostrou mais antioxidante. Comparando o vinho tinto e o vinho tinto sem álcool, verificaram que os efeitos não diferiram estatisticamente, o que demonstra que o etanol não exerce uma função importante nos benefícios agregados à ingesta moderada de vinho. Sendo assim, este fato sugeriu que os compostos fenólicos, presentes em quantidade significativa no vinho são os componentes responsáveis, em parte, pelos benefícios observados (Saiko et al., 2008).

Ainda é importante ressaltar que alguns estudos mostram que o alto consumo de álcool está relacionado ao aparecimento de diversas doenças, entre elas: cirrose, câncer de fígado, câncer do trato superior digestivo (boca, esôfago, laringe e faringe), bem como doenças cardíacas (Da Luz & Coimbra, 2004). Estas evidências vêm contribuindo para que vários grupos de pesquisas venham buscando maiores explicações a respeito do benefício da ingestão do suco de uva, o qual possui a vantagem de ser um produto não alcoólico e que pode ser consumido pela maioria das pessoas, inclusive as portadoras de algumas doenças (hepatite) e as crianças.

1.2. Suco de uva

Segundo a legislação brasileira, Lei N^{o}. 7678 artigo 4º, parágrafo 5^{o}, de 8 de novembro de 1988, suco de uva é a bebida extraída da uva através de processo

tecnológico adequado, não fermentado, não alcoólico, de cor, aroma e sabor característico, submetido a tratamento que assegure a sua conservação e apresentação até o momento do consumo (Rizzon et al., 1998). O suco de uva pode ser elaborado a partir de variedades *Vitis labrusca* ou *Vitis vinifera*, a primeira comumente utilizada na América e a segunda, na Europa. Dentre as variedades empregadas para elaboração de suco na América estão: Bordo, Concord e Isabel (para produção de sucos tintos), Niágara (sucos brancos) e Goethe (suco rose) (Rizzon et al., 1998; ANEXO A).

Além das diferentes variedades de uvas, o mercado brasileiro vem contando com duas classes distintas de sucos de uva. A primeira, denominada convencional, é elaborada a partir de uvas provenientes de vinhedos que receberam tratamento com fitodefensivos. A segunda, denominada orgânica, elaborada a partir de uvas colhidas de vinhedos nos quais o uso de fitodefensivos e/ou engenharia genética são proibidos (Wang et al., 2008). Alguns estudos têm relatado diferenças no conteúdo fenólico e nutricional de frutas (morangos, pêssegos e ameixa) produzidas pelo método tradicional (convencional) e orgânico (Asami et al., 2003; Lombardi-Boccia et al., 2004).

Recentemente, Dani et al. (2007) (ANEXO A) realizaram um estudo com sucos de uva brancos (Niágara) e tintos (Bordo), orgânicos e convencionais, mostrando que os sucos elaborados com uvas provenientes de cultivos orgânicos apresentavam um teor fenólico superior ao suco produzido a partir de uvas de cultivos convencionais. Este fato pode ser explicado pelo fato dos compostos fenólicos serem metabólitos secundários das plantas, produzidos e acumulados durante situações de estresse. Como os pesticidas não são utilizados no cultivo orgânico, as plantas tornam-se mais suscetíveis à ação de patógenos, produzindo uma quantidade maior destes compostos a fim proporcionar a defesa da planta contra estes agressores (Olsson et al., 2007).

Diferentes metodologias podem ser utilizadas na elaboração de sucos de uva. Na produção de sucos tintos, a polpa é aquecida juntamente com a casca e a semente, a fim de incorporar a coloração característica, resultando, também, em um produto com um maior conteúdo de polifenóis, os quais estão localizados principalmente nestes compartimentos na uva (Fuleki & Ricardo-da-Silva, 2003). Neste cenário, recentemente foi demonstrado que os sucos de uva tintos produzidos segundo processo descrito acima apresentam um maior conteúdo de compostos fenólicos, carboidratos e valor calórico, quando comparado com os sucos brancos (ANEXO A). A produção de suco de uva branco é caracterizada por metodologia a frio, na qual, não há aquecimento da casca (ANEXO A).

1.3. Polifenóis

Os constituintes fenólicos são de grande importância na enologia pelas características, direta ou indiretamente, ligadas à qualidade do vinho e suco, especialmente em relação à cor e à adstringência (Riberéau-Gayon et al., 2003). Estes compostos também são de interesse nutricional e farmacológico (entre eles antioxidante, antiinflamatório e antiplaquetário) (Riberéau-Gayon et al., 2003; Ferguson &Philipot, 2008).

Em videiras, a biossíntese de polifenóis é diretamente influenciada pela variedade de uva e de suas características genéticas (Bowers et al., 1993; Boulton et al., 1995). Diversos fatores vitícolas estão intrinsecamente ligados ao processo de biossíntese de polifenóis. Entre eles: porta-enxerto utilizado (influência na susceptibilidade a doenças), temperatura (gradiente térmico ideal entre o dia e a noite próximo de 15° C), umidade (incidência de doenças fúngicas), exposição solar (horas de incidência direta dos raios do sol sobre as uvas em processo de maturação), tipo de solo e adubação (altos teores de matéria orgânica favorecem um rendimento excessivo,

prejudicando a qualidade do fruto) e o manejo do dossel vegetativo (maior controle sobre maturação e rendimento) (Boulton et al., 1995; Cortell et al., 2005).

Os polifenóis possuem, pelo menos, um anel aromático no qual, um dos hidrogênios é substituído por um grupamento hidroxila. Podem ser classificados segundo o tipo de esqueleto principal, dividindo-se em flavonóides e não-flavonóides (Ferguson, 2001; D'Archivio et al., 2007).

1.3.1 Flavonóides

As substâncias relacionadas a esta grande família são divididas em várias subclasses que se distingüem pelo grau de oxidação de seu núcleo pirano (Manach et al., 2004) (Figura 1).

Segundo Riberéau-Gayon et al. (2003), os flavonóides baseiam-se, na forma geral, em estruturas do fenil-2-benzopirona, e estão principalmente representados na uva pelos flavonóis. Entretanto, os flavonóides em seu sentido amplo, compreendem igualmente os antocianos e os 3-flavanóis. Encontram-se na uva, também, outros grupos de menor importância como os di-hidroflavonóis (flavanóis) e, nas folhas, as flavanas (Cheynier et al., 2000).

Os taninos são, por definição, substâncias capazes de combinar-se com as proteínas e com outros polímeros vegetais como os polissacarídeos, sendo os responsáveis pela estrutura e adstringência dos vinhos, e também estão presentes no suco. Sua configuração química é composta por moléculas fenólicas relativamente volumosas, resultantes da polimerização de moléculas elementares de função fenol. Estas estruturas estão subdivididas em taninos hidrolisáveis ou gálicos e os taninos condensados ou catéquicos (Riberéau-Gayon et al., 2003).

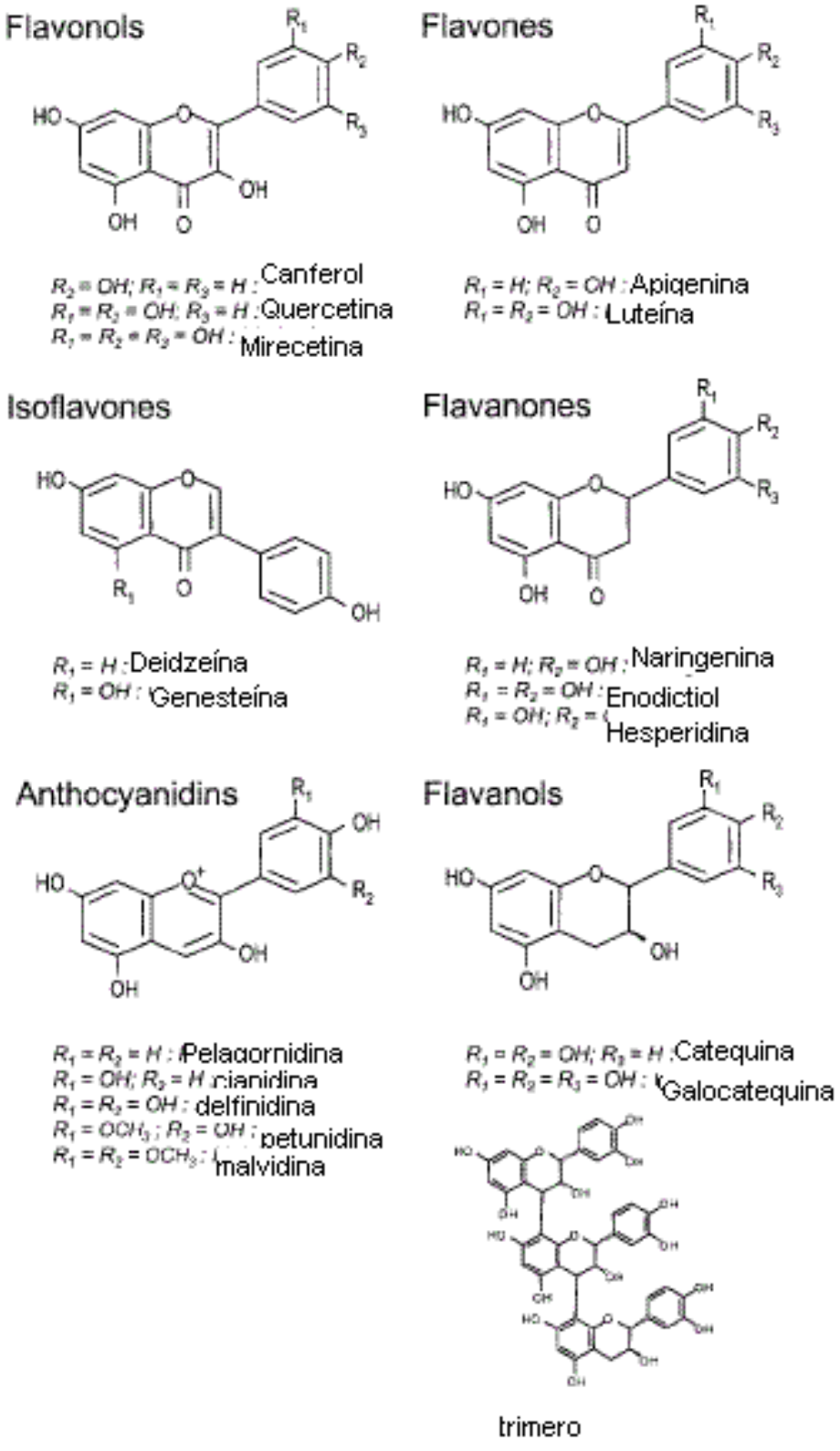

Figura 1. Estruturas químicas dos flavonóides (adaptada de Manach et al., 2004).

Os taninos condensados da uva e do vinho são polímeros complexos de 3-flavanóis ou catequinas, cujas unidades estruturais de base são a catequina e a epicatequina. Os 3-flavanóis estão presentes na uva na forma de monômeros e, em menor forma, representados polimericamente como constituintes dos taninos catéquicos. As principais formas presentes na uva são a (+)-catequina e seu isômero (-)-epicatequina.

As procianidinas dímeras podem ser subdivididas em dois tipo ('A' e 'B') (Figura 2). As procianidinas do tipo B ($C_{30}H_{26}O_{12}$) são dímeros resultantes da condensação das unidades 3-flavanóis unidas entre elas por ligações C4-C8 (B1 a B4) ou C4-C6 (B5 a B8). As procianidinas do tipo A ($C_{30}H_{24}O_{12}$) são dímeros que possuem uniões interflavanos C4-C8 ou C4-C6 e uma ligação éter entre os carbonos C5 ou C7 da unidade terminal com o carbono C2 da unidade superior. As procianidinas trímeras também podem ser classificadas em duas categorias ('C' e 'D') (Figura 2). As procianidinas trímeros do tipo C apresentam uniões interflavanos correspondentes ao tipo B dos dímeros, enquanto que as procianidinas trímeras do tipo D possuem uma ligação interflavano do tipo B e outra do tipo A (Riberéau-Gayon et al., 2003; Mayer et al., 2008).

O suco de uva possui principalmente, (+)-catequina, (-)-epicatequina e quatro procianidinas (B1, B2, B3 e B4). Sendo que as concentrações são influenciadas pelo método de elaboração escolhido, quente ou frio, e ainda a variedade de uva utilizada (Fuleki & Ricardo-da-Silva, 2003)

Fazem parte dos compostos flavonóides, também, um grupo especial de substâncias denominadas antocianos que constituem uma vasta família de polifenóis em plantas e são responsáveis pela coloração de muitas frutas e flores (Wang et al., 2003). De forma geral, estes pigmentos são também encontrados na polpa das cepas tintórias.

No nível subcelular, estão presentes no vacúolo, onde podem estar inclusos em organelas especializadas, definidas como antocianoplastos (Cheynier et al., 2000; Riberéau-Gayon et al., 2003).

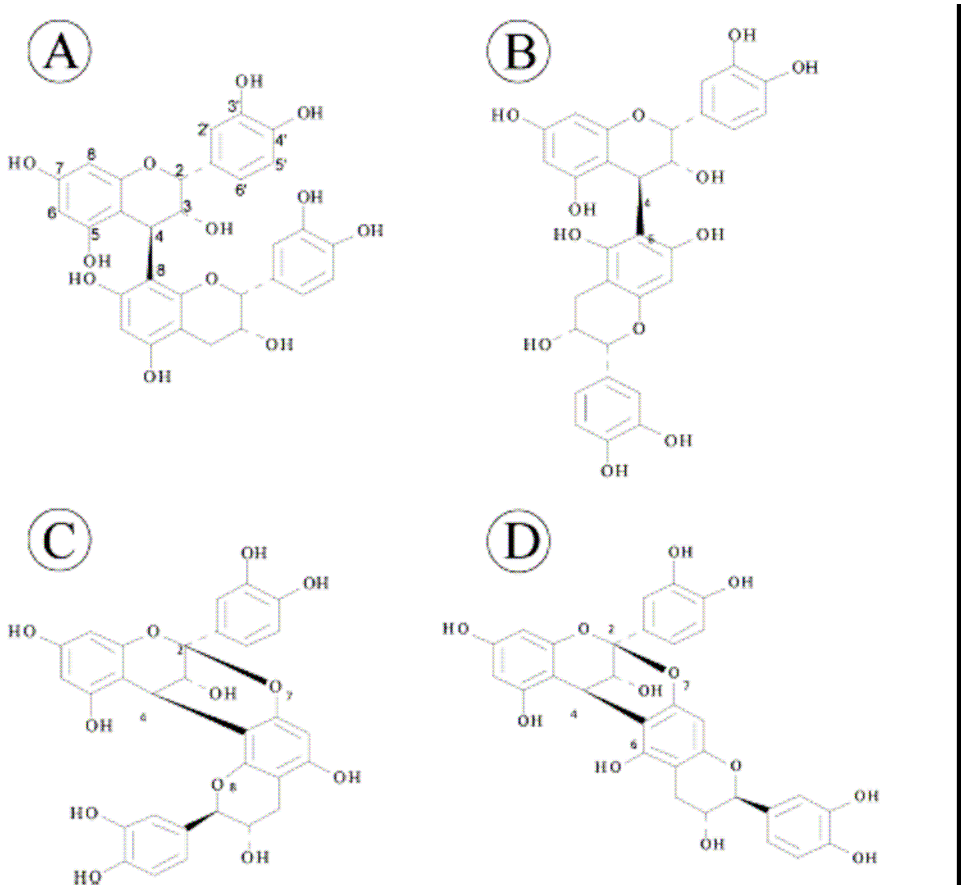

Figura 2. Principais procianidinas presentes na uva (A, B, C e D) de acordo com o explicado no texto acima (adaptado de Mayer et al., 2008)

Embora seja relatada atividade antioxidante para os flavonóides, torna-se interessante salientar que muitos podem exercer efeitos pró-oxidantes, principalmente os que possuem o anel pirogalol (Trueba & Sánchez, 2001). Este efeito está relacionado, no entanto, com outros aspectos, tais como exposição a metais de transição, níveis de estresse celular, pH e concentração do antioxidante no meio (Ferguson, 2001).

1.3.2. Não-flavonóides

Entre os compostos denominados não-flavonóides, os derivados do ácido benzóico e cinâmico e os estilbenos merecem atenção especial. O resveratrol (trans 3,5,4' – trihidroxiestilbeno) (Figura 3) é um importante exemplar dos polifenóis presentes nas uvas e seus derivados (Sun et al., 2001; Kundu & Surh, 2008).

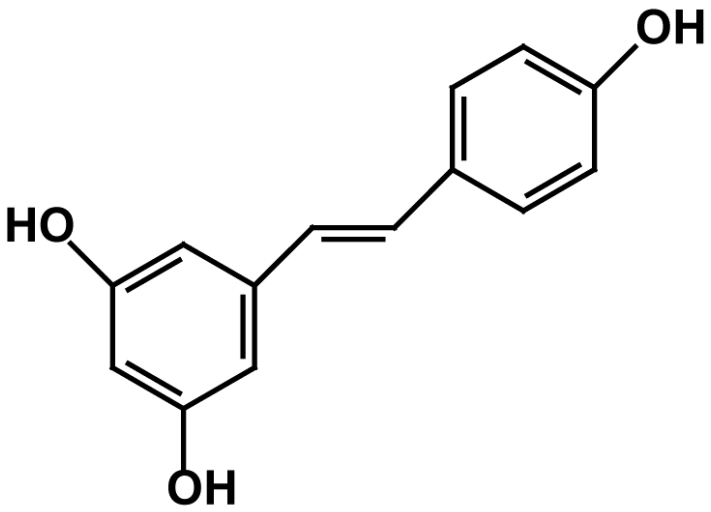

Figura 3. Molécula do trans-resveratrol

Foi isolado primeiramente em 1940 como um ingrediente das raizes do *Veratrum grandiflorum* e tem sido encontrado desde então em uma variedade de aproximadamente 70 espécies da planta, incluindo uvas, *blueberries* e amendoins (para revisão, ver Saiko et al., 2008). O resveratrol é um polifenol e foi classificado como um fitoalexina fúngico (para revisão, ver Saiko et al., 2008). Foi identificado, posteriormente, em 1963, como o componente ativo das raizes secas do *Polygonum cuspidatum*, igualmente chamado Ko-jo-kon no Japão, e usado na medicina asiática tradicional para tratamento de dermatites e hiperlipemias (Burns et al., 2002; Saiko et al., 2008)

O resveratrol foi detectado nas uvas (V*itis vinifera*) em 1976, e então no vinho em 1992 (para revisão, ver Saiko et al., 2008). Nas uvas, especialmente quando contaminado com o fungo *Botritis cinerea*, o resveratrol é sintetizado exclusivamente

na epiderme da folha e nas cascas da uva, mas não na polpa (Craysi et al., 2002; Saiko et al., 2008). Embora o conteúdo de não flavonóides, especialmente o resveratrol, seja bem conhecido em vinhos (Fuleki & Ricardo-da-Silva, 2003; Kasdallah-Grissa et al., 2007; Gambuti et al., 2007) existem poucos estudos que mostram o conteúdo deste polifenol em sucos de uva (Soleas et al., 1995; Yasui et al., 1997, Romero-Pérez et al., 1999; Wang et al., 2002).

1.4. Composição mineral

Além dos compostos fenólicos, os sucos também apresentam outros componentes, entre eles os minerais, que também podem ser eleitos como responsáveis ou capazes de influenciar atividades biológicas em humanos. Ollala et al. (2004) mostraram em trabalho realizado com uvas e sucos de uva produzidos e comercializados na Espanha, a partir de *Vitis vinifera*, que ambos possuem concentrações significativas de cobre e zinco. Foi possível observar, neste trabalho, que a variação na quantidade de minerais pode ser visualizada entre as diferentes amostras, estando fortemente influenciadas pelo tipo de solo, processos agrícolas utilizados e variedade de uva utilizada (Ollala et al., 2004).

Os minerais são introduzidos no ambiente por fonte natural e antropogênica. Esses elementos estão presentes em quase todos os organismos e exibem uma variedade de funções biológicas. Por exemplo, traços de alguns elementos, tais como cromo (Cr), selênio (Se) e níquel (Ni) são requeridos por seus benefícios nutricionais, entre eles: metabolismo de diversas enzimas, o equilíbrio ácido-base, a pressão osmótica, a atividade muscular e nervosa, facilitam a transferência de compostos essenciais através das membranas e, em alguns casos, fazem parte dos elementos constituintes dos tecidos do organismo (para revisão, ver Valko, 2005). O magnésio (Mg), por exemplo, é um co-

fator para DNA polimerase e um protetor efetivo contra carcinogênese induzida *in vivo* pelo Ni (Rojas et al., 1999).

O ferro (Fe), o cobre (Cu), o zinco (Zn) e Se são importantes componentes de moléculas envolvidas na estabilidade genômica, mas também podem apresentar efeitos adversos (Rojas et al., 1999; Beyersmann and Hartwig, 2008). Cr, Cd (Cádmio), Ni e Pb (Chumbo) podem, também, ter efeitos genotóxicos e/ou carcinogênicos em humanos (Beyersmann and Hartwig, 2008).

A mutagenicidade de minerais pode ocorrer por vários mecanismos, tanto diretos quanto indiretos. Os mecanismos diretos incluem: 1) interação com diferentes bases do DNA, alterando o pareamento das bases ou disponibilidade do substrato para replicação de DNA ou transcrição de RNA; 2) interação de íons metais com DNA polimerases, diminuindo a fidelidade da síntese de DNA; e 3) interação de íons metais com as ligações fosfodiésteres do DNA, alterando a estrutura do DNA e a formação de pontes DNA-proteína (Tkeshelashvili et al., 1991). Já o mecanismo indireto, pode ocorrer pela formação de $OH^{\cdot}$ pela reação do tipo Fenton (Fenton-like) *in situ* no DNA (Meneghini, 1997); ou pela formação de ERO e ERN ($^1O_2$, $OH^{\cdot}$, $NO^{\cdot}$ e H_2O_2). A reação de Fenton consiste na decomposição de H_2O_2 mediada por sais de ferro que gera $OH^{\cdot}$ (Figura 3). A adição de um agente redutor, como o ascorbato, leva a um ciclo que aumenta o dano às biomoléculas (McNaught & Wilkinson, 1997). A reação de Fenton pode ser mediada por outros metais como o cobre (Fenton-like).

Figura 4. Reação de Fenton (adaptada de Halliwell, 2008)

A reação de Haber-Weiss consiste de duas etapas (Figura 5), sendo a segunda

reação uma fonte potencial de OH•. Acredita-se que os complexos contendo Fe(III) podem catalizar esta reação, primeiro sendo reduzido a Fe(II) por $O_2^{\bullet-}$ e então reoxidado por H_2O_2. A exemplo das reações Fenton-like, a reação de Haber-Weiss também pode ser catalizada por outros metais (McNaught & Wilkinson, 1997)

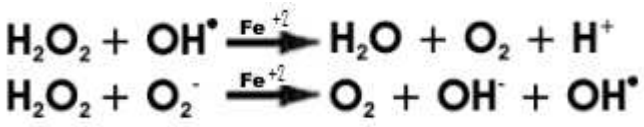

Figura 5. Reação Haber-Weiss (Adaptada de Halliwell, 2008)

Sabe-se que frequentemente a formação dos radicais hidroxil é pelas reações de Fenton e Haber Weiss, sendo que estes radicais são conhecidos por causar dano oxidativo a lipidios, proteínas e ao DNA (Beyersmann & Hartwig, 2008) (Figura 6).

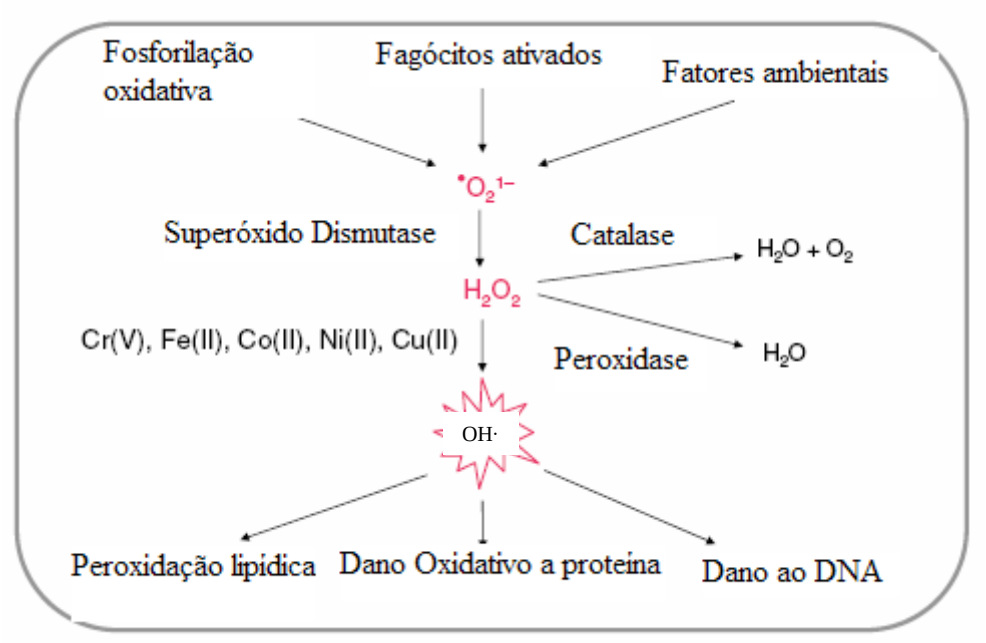

Figura 6. Íons metálicos e estresse oxidativo (modificada de Beyersmann and Hartwig, 2008)

Traços de cobre são essenciais nos organismos, pois esse metal tem papel crucial como co-fator de enzimas, tais como a citocromo c oxidase e a Cu/Zn superóxido

dismutase. Teoricamente, os íons de Cu podem induzir ER e causar danos às biomoléculas, incluindo lipídios e o DNA, provavelmente pela reação de Fenton (Beyersmann & Hartwig, 2008).

1.4. Atividade benéfica do suco de uva

Vários estudos têm mostrado que o consumo de suco de uva pode conferir benefícios à saúde humana (para revisão, ver capítulo VII). O efeito biológico mais estudado dos sucos de uva é o antioxidante (Furhrman et al., 1995; Day et al., 1997; Dávalos et al., 2005; ANEXO A*)*. Dávalos et al. (2005) e Dani et al. (2007) (ANEXO A) mostraram importante atividade antioxidante *in vitro* de sucos de uva *Vitis vinifera* e *Vitis labrusca*, respectivamente. Estudos mostraram também que a ingestão de aproximadamente 125-480 mL/dia de suco de uva tinto convencional, principalmente provindo das variedades *Vitis vinifera*, é capaz de aumentar a atividade antioxidante em homens adultos saudáveis (Day et al., 1997; Frankel et al., 1998; Osman et al., 1998; Freedman et al., 2001; O´Byrne et al., 2002). Ao suco de uva também é atribuído à diminuição de doenças cardiovasculares, principalmente pela sua capacidade em melhorar a função endotelial em homens saudáveis que ingeriram aproximadametne 8 mL/kg/dia de suco de uva (Stein et al., 1999; Chou et al., 2001). A atividade cardioprotetora do suco de uva também está associada à capacidade do mesmo em minimizar processos ateroscleróticos em ratos tratados por duas semanas com suco de uva (Vinson et al., 2001).

Em adição, Keevil et al. (2000) compararam a capacidade de três diferentes sucos: uva, laranja e pomelo (5-7,5 mL/kg/dia, durante 7 a 10 dias), em evitar eventos de agregação plaquetária em humanos sadios e voluntários. Os resultados observados permitiram concluir que apenas o suco de uva apresentou este benefício (Keevil et al., 2000). Suco de uva tinto apresenta uma concentração de polifenóis três vezes superior

ao sucos cítricos, fato que pode, em parte, explicar o efeito potencial dos polifenóis na ação anti-agregação plaquetária. Este efeito está diretamente relacionado com uma redução nos casos de trombose coronariana e risco do infarto do miocárdio (Keevin et al., 2000).

Corroborando com essas observações, Shanmuganayagam et al. (2007) mostraram ainda que o consumo diário de suco de uva está envolvido na redução do desenvolvimento de ateromas, agregação plaquetária e nos níveis de colesterol sérico em coelhos hipercolesterolêmicos tratados com 225 mL de suco de uva tinto por dia, durante 96 dias. Ao suco de uva também pode ser atribuída a capacidade de ser antiinflamatório, estudo este realizado em indivíduos previamente diagnosticados como portadores de doenças coronarianas, que receberam suco de uva durante duas semanas (Albers et al., 2004).

Aos constituintes do suco de uva tinto também foi atribuída a capacidade em inibir processos tumorogênicos na glândula mamária de ratos, induzida por DMBA (7,12-dimetilbenzo[a]antraceno), tratados por oito semanas (Singletary et al., 2003). Neste cenário, Park et al. (2003), utilizando o teste Cometa (*Single cell gel electrophoresis*, SCGE), mostraram que o consumo de suco de uva leva a uma redução significativa nos danos ao DNA de homens saudáveis que ingeriram 480 mL/dia de suco por oito semanas, quando comparado com níveis anteriores à suplementação.

Recentemente, Shukitt-Hale et al. (2006) mostraram que o suco de uva Concord é capaz de melhorar a capacidade cognitiva e motora durante o envelhecimento de ratos, os quais receberam suco de uva em três concentrações (0, 10 e 50% de suco) por seis semanas para análise da atividade motora, e durante 8 semanas para avaliação da cognição.

Além das atividades benéficas ao ser humano descritas, o suco de uva e demais derivados da uva possuem um importante papel econômico para o País, principalmente para o Estado do Rio Grande do Sul e para a região da Serra Gaúcha, justificando assim o seu estudo. Acredita-se que no ano de 2008 tenham sido processados em torno 600 milhões de quilos de uva, sendo que as variedades *Vitis labrusca* correspondem a 88% do total produzido. Neste ano, foram produzidos aproximadamente 12 milhões de litros de suco de uva no país, sendo que em torno de 500 mil litros são sucos orgânicos (IBRAVIN, 2008). Destes 12 milhões, em torno de 95% é suco tinto, principalmente das variedades Bordo, Isabel e Concord (todas *Vitis labrusca)*, uma vez que, no Brasil, não se produz suco com *Vitis vinifera*. A produção de suco de uva branco, principalmente a partir da variedade Niágara (*Vitis labrusca*), corresponde a uma parcela pequena da produção, atingindo em torno de 7% da produção de suco total. A comercialização de sucos de uva no Brasil vem apresentando um acréscimo expressivo. No ano 2002, comercializava-se aproximadamente 7,2 milhões de litros de suco de uva. Em 2007, este valor passou para próximo de 19,7 milhões (IBRAVIN, 2008), sendo que o grande crescimento se deu entre os anos 2005 e 2007, correspondente a 97% de aumento. Segundo o ranking divulgado pela Associação Brasileira das Indústrias de Refrigerantes e das Bebidas Não-Alcoólicas (Abir), o suco de uva pronto para beber é o mais vendido no país, se mantendo a frente do de pêssego e de laranja, correspondendo a 20,8% do mercado em sucos prontos para beber (Abir, 2008).

1.5. Avaliação da atividade antioxidante, antimutagênica e antigenotóxica.

1.5.1 Avaliação da Atividade antioxidante

A atividade antioxidante pode ser avaliada por ensaios *in vitro* e *in vivo*. Diferentes metodologias têm sido desenvolvidas para obter uma avaliação, seja qualitativa ou quantitativa, da capacidade antioxidante de compostos, tanto por meio de testes sem a utilização de células ou utilizando culturas celulares. Com o crescente interesse na função e diversidade de antioxidantes em alimentos, várias metodologias rápidas *in vitro* para medir esta atividade foram desenvolvidas. Dentre os ensaios *in vitro* existentes, um dos mais utilizados é o radical DPPH$^{\bullet}$ (1,1 difenil 2-picrilhidrazil), que quantifica a capacidade de varredura dos antioxidantes (Katsube et al., 2004). Esse método é amplamente utilizado para avaliar a atividade antioxidante de forma rápida e econômica, além de gerar um radical estável e de fácil obtenção (Mensor et al., 2001; Katsube et al., 2004). Halliwell (2008) ressalta a importância de serem realizados testes *in vitro* e *in vivo*, visto que determinadas substâncias foram consideradas antioxidantes em modelos *in vitro*, e posteriormente, quando avaliadas *in vivo*, não apresentaram os mesmos resultados. Fato este associado principalmente à biodisponibilidade e capacidade de metabolização de diversos polifenóis estudados (Soleas et al., 2006; Halliwell, 2008).

Desta forma, ensaios *in vivo* utilizando microrganismos têm se mostrado muito adequados na triagem rotineira de vários produtos, sendo testes rápidos, sensíveis, econômicos e reprodutíveis (Rabello-Gay et al., 1991). A descrição do ciclo de vida da levedura *Saccharomyces cerevisiae* e o conhecimento das características genéticas básicas, além da facilidade de manipulação e, principalmente, do fato deste microrganismo ser eucarioto, proporcionaram a grande difusão deste sistema biológico como metodologia experimental para estudos da atividade antioxidante (Lopes et al., 2004; Raspor et al., 2005; Spada et al., 2008) de inúmeros compostos (Henriques et al., 2001). Os dados obtidos nesse tipo de teste apresentam uma correlação de,

aproximadamente, 70% em relação ao observado no homem (Rabello-Gay et al., 1991; Kim et al., 2005). O estudo da atividade antioxidante baseia-se na avaliação do crescimento celular após tratamentos realizados na presença e ausência do composto a ser testado adicionado de um agente reconhecidamente formador de espécies reativas, como por exemplo, o peróxido de hidrogênio (Spada et al., 2008).

Além dos estudos com microrganismos, ensaios utilizando roedores têm se mostrado bastante úteis na determinação da atividade antioxidante de diferentes compostos tanto a nível sérico como tecidual (Dal-Pizzol et al., 2001; Ferreira et al., 2006). A utilização deste modelo permite também a avaliação do estresse oxidativo em diferentes tecidos (fígado, sistema nervoso central, entre outros) (Kappel et al., 2008; Caregnato et al., 2008). Neste modelo é possível avaliar o nível de peroxidação lipídica (Produção de MDA), peroxidação protéica (ensaio do carbonil), bem como medir a atividade das enzimas antioxidantes superóxido dismutase (SOD) e catalase (CAT). A enzima superóxido dismutase (SOD) é responsável por dismutar ânions superóxido em H_2O_2 e O_2. (Fridovich, 1998) e a enzima catalase (CAT) dismuta o peróxido de hidrogênio em água e oxigênio (Fridovich, 1998). Estas enzimas são responsáveis, juntamente com a glutationa peroxidase (GPx), pelas defesas antioxidantes enzimáticas do organismo, sendo que estas procedem de maneira orquestrada para eliminar os sub-produtos do metabolismo oxidativo (Figura 7).

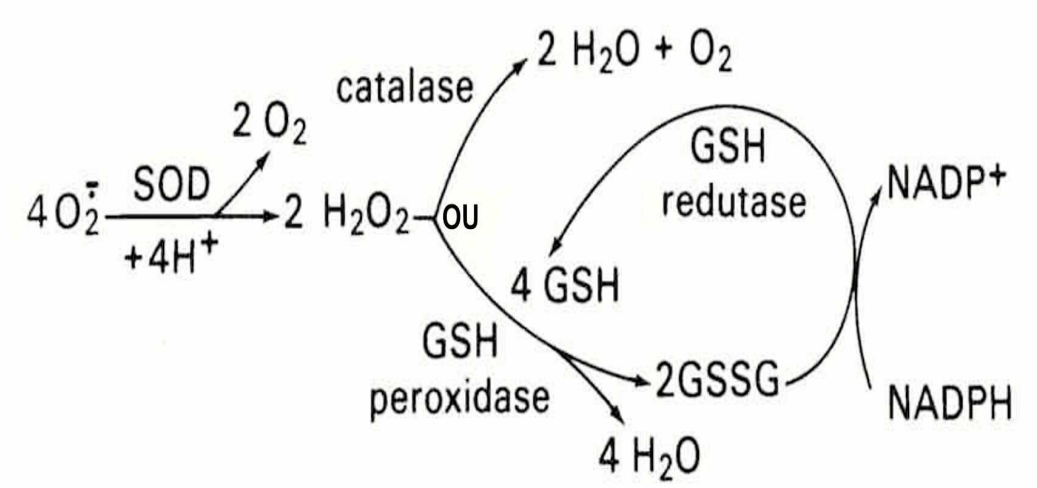

Figura 7. Principais defesas antioxidantes enzimáticas. Adaptado de Proctor & Reynolds, 1984. (GSH peroxidase: glutationa peroxidase, GSH redutase: Glutationa redutase)

1.5.2 Avaliação da atividade mutagênicae antimutagênica

A levedura *S. cerevisiae* tem sido amplamente estudada, tornando-se uma ferramenta importante nas pesquisas sobre mutagênese. Os ensaios com levedura são de grande utilidade na determinação de agentes mutagênicos ambientais ou farmacológicos (Poli et al., 1999; Teerziyska et al., 2000). Esses ensaios são rápidos, sensíveis, econômicos e reprodutíveis, apresentando resultados confiáveis na identificação biológica. Além disso, a levedura possui um sistema endógeno de ativação metabólica constituído de um complexo enzimático (citocromo P-450) de detoxificação, que dispensa a adição de um sistema exógeno, tendo, por essa razão, uma vantagem relativa aos ensaios bacterianos (Paula-Ramos et al., 1991; Moreno et al., 1991; Poli et al., 1999). Experimentos de mutações reversas são os mais comumente utilizados. Baseiam-se na restauração ou compensação de um defeito gênico responsável por um requerimento nutricional (Zimmermann, 1984).

A restauração se deve a uma reversão exata do efeito original, enquanto que a compensação pode ser causada por uma mutação secundária dentro do gene (mutação supressora interna) ou por uma mutação externa, como no caso dos alelos sem sentido (mutação *nonsense*, que resulta na alteração de um códon que codifica um aminoácido para um códon de terminação) (Hawthorne & Leopold, 1974; Atkin et al., 1993). Reversão de auxotrofia para prototrofia pode ser causada por uma substituição, inserção ou deleção de pares de bases ou, ainda, por um a mutação induzida por supressor do gene mutante original (para revisão, ver Henriques et al., 1997; da Costa Medina et al., 2008). Para que seja identificada a mutação reversa, é necessária a utilização de uma linhagem com marcas genéticas adequadas, como, por exemplo, a linhagem haplóide de *S. cerevisiae* XV185-14c, isolada por Von Borstel (Parry & Parry, 1984; Boeira et al., 2002; Rosa et al., 2004). Essa linhagem permite a detecção de dois tipos de mutações lócus específicas: reversões do alelo ocre lys1-1 (alteração para o códon UAA de término de cadeia) ou do alelo missense his1-7 (códon alterado codifica um aminoácido diferente) e reversões por deslocamento de quadro de leitura do DNA (*frameshift*) verificadas no lócus hom3-10. As células revertentes podem ser detectadas pela semeadura em placas contendo meio seletivo, no qual o fator de crescimento inicialmente requerido não está presente, ou está em quantidades muito pequenas, permitindo somente um crescimento inicial (Zimmermann, 1984; Pungartnik et al., 2005).

Os testes usados em avaliações de atividade mutagênicas e carcinogênicos podem ser utilizados para identificar substâncias antimutagênicas e anticarcinogênicas (De Flora et al., 1998; De Flora et al., 2001). Substâncias que apresentam atividade antimutagênica em leveduras também podem ter este efeito antimutagênico em sistemas de cultura com células de mamíferos (Raspor et al., 2005). Para que uma substância

possa ser considerada antimutagênica ela precisa reverter a mutagênese induzida por um agente mutagênico, como por exemplo, o peróxido de hidrogênio (H_2O_2) (Henriques *et al.*, 2001). O H_2O_2 não é tóxico sozinho, mas é altamente reativo *in vivo* pela reação de Fenton e reação de Haber-Weiss, o qual reage parcialmente com íons metálicos tais como o Fe^{+2} ou Cu^{+} para formar radical hidroxil ($OH\cdot$), que pode danificar diretamente o DNA, e assim gerar mutações (Halliwell e Gutteridge, 1999).

1.5.3. Atividade genotóxica e antigenotóxica

A avaliação do potencial genotóxico de um agente pode ser determinada por várias metodologias, entre elas o ensaio Cometa. O ensaio Cometa, conhecido como SGCE (*Single Cell Gel Electrophoresis*), tem sido amplamente utilizado pela comunidade científica como um teste rápido *in vivo* e *in vitro* para detectar genotoxicidade como conseqüência de danos ao DNA. O teste Cometa é utilizado amplamente em genética médica, genética toxicológica e ecotoxicológica, em diagnósticos e tratamentos médicos, medicina ambiental e ocupacional, biomonitoramento ambiental, dentre outras aplicações (Da Silva et al., 2000).

Em condições alcalinas, o ensaio Cometa detecta quebras simples e duplas, além de sítios álcali-lábeis. Dentre as vantagens do teste, destacam-se: sensibilidade para detecção de baixos níveis de danos ao DNA; pequeno número de células; flexibilidade; economia; facilidade de aplicação; habilidade para conduzir estudos usando um pequeno número de amostras; rapidez (curto período de tempo para o experimento); pode ser usado em humanos e em estudos ambientais, e para avaliar reparo de DNA (Tice et al., 2000; Hartmann et al., 2003; Contijo & Tice, 2003; Collins, 2004). As células englobadas em gel sobre uma lâmina são submetidas a uma corrente elétrica, que faz migrar para fora do núcleo os segmentos de DNA livres, resultantes de quebras. Após a eletroforese, as células que apresentam um núcleo redondo são identificadas

como normais, sem dano reconhecível no DNA. Por outro lado, as células lesadas são identificadas visualmente por uma espécie de cauda, como de um cometa, formada pelos fragmentos de DNA. Estes fragmentos podem se apresentar em diferentes tamanhos, e ainda estar associados ao núcleo por uma cadeia simples. Para alguns autores o tamanho da cauda é proporcional ao dano que foi causado, mas somente é de consenso que a visualização do "Cometa", significa dano ao DNA, podendo ser quebra simples, duplas e/ou lesões álcali-lábeis (Tice et al., 2000; Hartmann et al., 2003; Maluf &Erdtmann, 2003; Collins, 2004). Os danos no DNA, avaliados pelo ensaio Cometa, são classificados de acordo com a migração do DNA em 4 classes, podendo ser de zero (nenhum dano) até 4 (dano máximo). Além disso, o ensaio Cometa permite a visualização de células apoptóticas (Figura 8) (Franke et al., 2005a; 2005b; Prá et al., 2005; Franke et al., 2006).

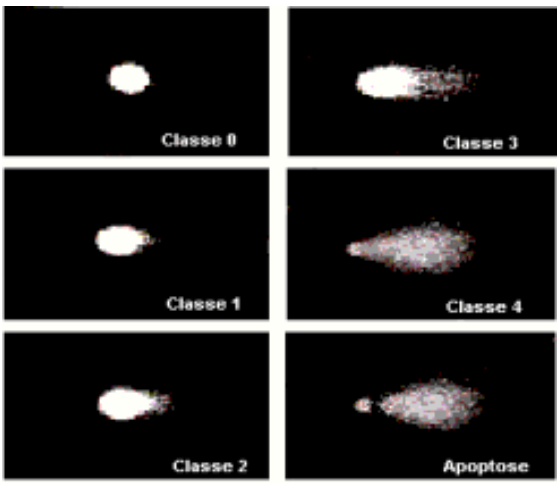

Figura 8 . Classes de dano no DNA das células como visualizado no ensaio Cometa.

O ensaio Cometa também pode ser utilizado para avaliar os efeitos antigenotóxicos de diferentes substâncias (sucos, frutas, polifenóis isolados e extratos de plantas) frente a agentes reconhecidamente genotóxicos (Jang et al., 2008). A proteção de extratos vegetais contra agentes de ação direta sobre o DNA está relacionada, provavelmente, com a variedade de compostos polifenóis que podem

interferir com o citocromo P450-oxidase, mediando o metabolismo de xenobióticos, e interagir diretamente com pró-mutagenos e/ou seus metabólitos ou por suas propriedades antioxidantes (Marnewick et al., 2000). Para avaliar a ação antigenotóxica podem-se utilizar diferentes estratégias, como pré-tratamento, co-tratamento ou pós-tratamento (De Flora, 2001).

2. OBJETIVOS

2.1. Objetivo Geral

O objetivo deste trabalho foi avaliar comparativamente a atividade antioxidante, mutagênica/antimutagênica de sucos de uva elaborados a partir de *Vitis labrusca*, variedades Bordo (tinto), Niágara (Branco) e Rose (Goethe), obtidos a partir de cultivos orgânicos e convencionais.

2.2 Objetivos específicos

- Analisar a composição nutricional, mineral, e o conteúdo de polifenóis e de vitamina C dos sucos de uva;
- Avaliar a atividade antioxidante de sucos de uva tintos, brancos e rose, bem como atividade mutagênica e antimutagênica de sucos tintos e brancos, utilizando como modelo a levedura *Saccharomyces cerevisiae*;
- Avaliar o nível de peroxidação lipídica e de oxidação de proteínas (carbonil) no soro, fígado e tecidos do sistema nervoso (estriado e substância nigra) de ratos Wistar tratados com suco de uva tinto orgânico e convencional;
- Avaliar a atividade das enzimas antioxidantes superóxido dismutase e catalase em soro, sangue total e homogeinizado de tecidos (fígado, estriado e substância nigra) de ratos suplementados com suco de uva tinto orgânico e convencional;
- Avaliar a atividade genotóxica/antigenotóxica (Ensaio Cometa) em ratos suplementados com sucos de uva tinto (Bordo) orgânico e convencional;
- Avaliar a capacidade do resveratrol e da catequina em modular o nível de estresse oxidativo na levedura *Saccharomyce cerevisiae* frente à exposição aguda ao cádmio, peróxido de hidrogênio e tetracloreto de carbono;
- Relacionar os resultados obtidos nos ensaios biológicos com a composição química dos sucos de uva.

3. CAPÍTULOS

3.1. CAPÍTULO I

MINERAL CONTENT OF GRAPE JUICES AND ITS INFLUENCE ON THE ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITIES IN *SACCHAROMYCES CEREVISIAE*

Artigo a ser submetido para a revista Biometals

Neste artigo, demonstrou-se a composição mineral pela metodologia de PIXE (*Particle-Induced X-ray Emission*), a atividade antioxidante e mutagênica/antimutagênica pelo modelo da levedura *S. cerevisiae*, de sucos de uva brancos e tintos. Importantes diferenças entre a composição mineral dos sucos tintos e brancos foram observadas, onde os tintos apresentaram valores superiores de cloro, cálcio e magnésio. Observou-se também que o mineral mais abundante no suco é o potássio. Neste trabalho, mostrou-se também que ambos os sucos, tintos e brancos, apresentaram importante atividade antioxidante *in vivo* e antimutagênica revertendo danos causados pelo peróxido de hidrogênio. Entretanto os sucos brancos possuem atividade antioxidante *in vivo* superior aos tintos. Correlações apresentadas entre a concentração de minerais e as atividades biológicas indicam que estes minerais podem, em parte, influenciar as atividades benéficas atribuídas aos sucos de uva.

MINERAL CONTENT FROM GRAPE JUICES AND ITS INFLUENCE IN THE ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITIES IN *SACCHAROMYCES CEREVISIAE*

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Abstract

Grape juices are an important source of food antioxidants. Unfortunately, there are not enough data about the mineral composition and the influence of frappe juice on antioxidant, mutagenic and antimutagenic activities in eukaryote cells. Thus, the purpose of this work was to evaluate the mineral contents (Mg, Si, P, S, Cl, K, Ca, Mn, Fe, Cu, Zn) of *Vitis labrusca* juices. And also, we evaluate the potential antioxidant, mutagenic and/or antimutagenic activities in the yeast *Saccharomyces cerevisiae.* The results indicated that potassium is presented at higher concentration among all the minerals assayed. All grape juices showed significant antioxidant and animutagenic activities in *S. cerevisiae*. In this sense, these activities showed a positive correlation with copper and zinc contents but a negative correlation with chloride and silicium contents. These results demonstrate that the mineral composition of grape juices can exert a potential antioxidant and antimutagenic protection in *S. cerevisiae* cells.

KEYWORDS: grape juice; minerals; antioxidant; mutagenicity, antimutagenicity

Introduction

Diets have an important role in the prevention of some pathologies related to oxidative stress, such as atherosclerosis and cancer [1]. Grape juices are a source of different antioxidants elements and compounds, mainly polyphenols [2] and many different minerals [3]. In red fortified wines produced with polyvarietal grapes from a 60-70 year old vineyard cadmium (Cd), chromium (Cr), cupper (Cu), iron (Fe), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn) were presented [4]. Interestingly, there is only one report about the presence of Zn and Cu in grape juices from *Vitis vinifera* white and red grapes [3].

While some minerals are essential for human nutrition (e.g., Fe, Cu, Se and Zn), others like Cr, Cd, Ni, As and Pb can induce DNA damage by direct interaction, and some studies have shown these metals also to induce DNA strand breaks and chromosomal aberrations in cultivated mammalian cells [5,6] or through the formation of reactive oxygen species (ROS) [7,8]. Apparently, Fe, Cu, Zn and Se are necessary to keep the genetic stability; however, these minerals could have carcinogenic, mutagenic and oxidative effects in specific physiological conditions [8-10].

This study is the first to show the mineral composition of a variety of grapes (purple and white) and its relationship with the biological effects of grapes juices. It was determined the mineral composition, of grape juices and theirs antioxidant, mutagenic and antimutagenic activities of white and purple *Vitis labrusca* juices using *S. cerevisiae* as a biological model.

Material and methods

Grapes and Grape Juices

Grape juice were produced from *Vitis labrusca*, varieties Bordo and Niagara, and classified in purple and white grape juices, respectively. Purple juices were hot extracted using pulp, seeds,

and skins [2]. For white juices the skin was removed before extraction. Validity periods were observed, and the same juices were used for the entire study.

Mineral composition of grape juices

Mineral contents in grape juices were quantified by means of Particle-induced X-ray emission (PIXE) method [11]. Briefly, each sample was positioned in the proton beam by means of an electric-mechanical system. The characteristic X-rays induced by the proton beam were detected by an HPGe detector from EG&G (GLP series, EG&G Ortec, CA, USA), with an energy resolution of 180 eV at 5.9 keV. The electronics consisted of a Telenec 245 amplifier associated with a PCA3 multichannel analyzer (Oxford Instrument, TN, USA) running in a PC-compatible computer. The GUPIX code [12] was used for data analysishe mineral analysed were Fe, Mn, Ca, K, Cl, S, P, Cu, Zn, Si, and Mg.

Antioxidant Activity

The evaluation of *in vivo* antioxidant activity was carried out using the yeast *S. cerevisiae* strain XV185-14c *(MATα, ade2-2, arg4-17, his1-7, lys1-1, trp5-48, hom3-10)* kindly provided by Dr. R. C. Von Borstel (Genetics Department, Alberta University, Canada). Yeast cells suspensions containing 2×10^7 cells/mL (exponential phase), with or without hydrogen peroxide (H_2O_2; 75 mM) and juices (20% v/v) were incubated for 1 h. After incubation, samples were serially diluted to 2×10^3 cells/mL in saline solution [0.9 % (w/v) NaCl], plated onto YPD [0.5% (w/v) yeast extract, 2% (w/v) bacto-peptone, 2% (w/v) glucose, and 2% (w/v) bacto-agar for solid medium] and incubated at 28 ºC for 48 h. Colonies were then counted and compared to those obtained on control plates, which were assumed to represent 100% of yeast cells survival.

Mutagenic and antimutagenic activities of grape juices

The yeast XV185-14c strain was also used for mutagenic and antimutagenic assays. A cell suspension containing 2×10^8 cells/mL was prepared from a yeast culture in exponential phase of growth and incubated for 3 h in two different grape juice concentrations (10% and 25% v/v). Survival was determined by colony counting in Synthetic Complete Medium –SC (contained 0.67% yeast nitrogen base with no amino acids, 2% glucose, 2% bacto-agar and 0.25% $(NH_4)_2SO_4$ (3-5 days, 28^{o}C), and mutation induction (*LYS*+, *HIS*+ or *HOMO*+ revertants) was performed on corresponding deficient media as described by Zimmermann [13]. Plating was done in triplicate for each dose. As a positive control of mutagenesis it was employed 4-nitroquinolein-1-oxide (4NQO; 5μM).

Antimutagenic assay was also performed with the same yeast strain, which was done by cells' co-treatment with with grape juices (10%) and H_2O_2 (50 mM) for 4 h at 28 ºC with shaking. Samples were plated onto SC, SC-his, SC-lys and SC-hom media. Hydrogen peroxide (50 mM) was used as positive control. Plating was done in triplicate for each dose.

Statistical Analyses

Values were determined as parametrical or non-parametrical by the Kolmogorov-Smirnoff test. Data were submitted to analysis of variance (ANOVA), and means were compared using the test of Tukey. Groups were compared using Student's t-test and Mann-Whitney test (test U). Relationships between variables were assessed using Pearson's product-moment correlation coefficient.

Results and Discussion

To our knowledge, this is the first study of grape juices elaborated from *Vitis labrusca,* Bordo and Niagara cultivars, are important source of minerals, having *in vivo* antioxidant and

antimutagenic activities. Also, there is only one study showing that grapes juices from *Vitis vinifera* contains Cu and Zn [3], indicating that is necessary more studies in this field are needed.

The results obtained about mineral content by means of PIXE analysis demonstrated that each grape juice has an individual mineral profile (Table 1). Among the assayed potassium is the one with highest concentration in both purple and white grape juices (Table 1). Interestingly, purple grape juices contains significant levels of Cl, P and Mg when compared to white grape juices (Table 1). By its turn, white grape juices are richer in Cu content compared to purple ones (Table 1).

Metals are introduced into our environment both from natural and anthropogenic sources. These elements are found practically in all living organisms, and exhibit a wide range of biological functions, such as components of enzymatic and redox systems, and asenzyme activators. Metals are ingested daily by humans as part of their diet. For instance, spinach and cauliflower concentrate cadmium, while lead is found in Brussel sprouts and Chinese beets. In seafood, such as oysters and fish, Pb, Cd, Hg and As are commonly found. Several trace elements, such as Cr, Se and Zn are required for the nutritional well-being of humans and animals. Recent studies suggest that ultra-trace elements such as As and Ni may also have a role both in animal and human nutrition. [8].

The antioxidant, mutagenic, and antimutagenic effects of white and purple grape juices and a possible correlation with mineral content was analyzed by employing the yeast *S. cerevisiae.* Many studies used *S. cerevisiae* as an important model to evaluate antioxidant [14-16], mutagenic and antimutagenic activities of different compounds and elements present in foods.

The highest non-cytotoxic concentration [20% (v/v)] of grape juices was used in the *in vivo* antioxidant assay. All juices showed significant antioxidant activity, decreasing or preventing

oxidative damage induced by H_2O_2 (Figure 1). White juices presented a higher protecting effects when compared to purple juices and the positive control (Fig. 1). Analyzing only the purple grape juices, we observed that the antioxidant activity showed a positive correlation with Cu ($r=0.785$, $p<0.05$) and a negative correlation with Mg content ($r=-0.982$, $p<0.01$; Table 2). No correlation between mineral content and antioxidant protection was observed in white grape juices. Mutagenic assay was performed employing two grape juice concentrations [10 and 25% (v/v)]. None of the analyzed grape juices presented mutagenic activity at any concentration.

In addition, we found an important correlation between mineral content and antimutagenic activities (Table 4). Analyzing only purple grape juices we observed a positive correlation between antimutagenic activity for *lys locus* with Cu and Mn ($r=0.761$, $p<0.01$). For the same locus, Cl, Si and S content showed a negative correlation with antimutagenic activity ($r= -0.761$; -0.828; -0.761, $p<0.05$, respectively). Negative correlations between antimutagenic activity and Cl, Si and S were observed in the *lys* and *hom* loci (Table 5).

If considering only white grape juices a negative correlation between antimutagenic activity (*hom locus*) and Si, S and Mg content was found (Table 5). A positive correlation was observed between antimutagenic activity (*lys locus*) and Zn content ($r=0.741$, $p<0.05$; Table 2), antimutagenic activity (*his locus*) and Mg content ($r=0.781$, $p<0.05$; Table 2) and also antimutagenic activity (*hom locus*) and Cu content ($r=0.781$, $p<0.05$) (Table 2).

It was already observed that Cu content showed a negative correlation with DNA damage in a study made with orange juices [17]. In addition, Zn is a component of more than 70 different enzymes with functions in many aspects of cellular metabolism, involving metabolism of proteins, lipids and carbohydrates [18], being also an important antioxidant and decreasing ROS production [19]. Some studies have reported the ability of Zn to interact with essential elements such as Cu and Fe, decreasing their content in tissues and retarding the oxidative processes [20].

In this sense, the Cl contents showed a negative correlation with the antimutagenic activity of the purple grape juices. In some situations, the Cl^- ion could complex with hydroxyl radicals and form hypochlorous acid (HOCl), which induces important damages to the cells [1]. Thus, the negative correlation observed between antioxidant and antimutagenic activity and Si content can be partially explained because this mineral is able to form free radicals ($Si^{\cdot}$ and $SiO^{\cdot}$) [1] that induce the peroxidation of unsaturated fatty acids, phospholipids, glycolipids, sterols, amino acids and sulfhydryl groups of the transmembrane proteins [21]. Manganese content showed contraditory effects as we obsvered both negative and positive correlations between this mineral and the antimutagenic activity of grape juices. Mn can act as both pro- and antioxidant and that oxidative stress-related effects of Mn are dependent not only on the intracellular concentrations of the metal, but also the exposure duration, secondary oxidative challenges, and the overall oxidant buffering capacity of the cells [22].

Although the influence of minerals in the physiology of the yeast cells should be extensively studied, our results demonstrated that the mineral contents can be responsible for theirs antioxidant and antimutagenic properties, two characteristics that are present in wines [23]. In conclusion, grape juices elaborated from *V. labrusca* cultivars are a good source of micronutrients which present a correlation with the antioxidant and antimutagenic effects, a characteristic of grape juices and wine, contributing to prevent some diseases caused by oxidative stress, such as atherosclerosis, cancer and neurodegenerative diseases like Parkinson.

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Tables

Table 1. Grape juices mineral composition.

Grape juice	Mineral composition (mg/L)[a]										
	Fe	Mn	Ca	K	Cl	S	P	Cu	Zn	Si	Mg
Purple grape juice	19.80±8.10	3.25±0.43	117.2±12.41	321.4±15.3	27.3±0.57	21.5±0.86	31.75±1.29	5.25±1.70	7.00±1.67	9.90±1.27	97.4±0.23
White grape juice	20.30±0.37	2.56±0.20	67.8±2.03*	262.4±15.65*	25.23±1.05	20.6±1.07	31.1±2.18	21.9±12.1*	11.7±2.70	10.5±0.93	94.5±2.26

[a] Mean ±SD; *$p<0.05$ differently between purple and white grape juice.

Table 2. Correlations between antioxidant and antimutagenic activities and mineral composition in purple grape juices.

Correlation	Cl	Cu	Si	S	Mn	Mg
In vivo antioxidant activity	WC	0.785*	WC	WC	0.785*	-0.982**
Antimutagenic activity (*Lys*)	-0.761*	0.761**	-0.828**	-0.761*	0.761*	WC
Antimutagenic activity (*His*)	-WC	-0.859**	0.737*	WC	-0.859*	0.908**
Antimutagenic activity (*Hom*)	-0.781*	0.976**	-0.926**	-0.781**	0.976**	-0.781**

Numbers indicate the r value; *p<0.05, **p<0.01

WC= without correlation.

Table 3. Induction of punctual mutation (*his1-7*), ochre allele (*lys1-1*), and *frameshift* mutation (*hom 3-10*) phase in *S. cerevisiae* strain XV185-14C exponential growth after treatment with the juices (10% v/v) for 3 h.

Samples[#]	Survival (%)	His1/10^7 survivors [a]	Lys1/10^7 survivors [a]	Hom3/10^7 survivors [b]
0	100.0 (234) [c]	9.82 ± 1.54 [d] (69)	7.11 ± 1.61 (50)	3.54 ± 0.22 (25)
Purple grape juice	86.82 (209)	9.72 ± 1.0 (70)	5.65 ± 0.61 (39)	3.96± 0.47 (28)
White grape juice	92.0 (220)	6.62 ± 1.60 (44)	6.71 ± 0.78 (49)	2.92± 0.62 (21)

[a] Locus-specific revertants.

[b] Locus non-specific revertants (forward mutations).

[c] Numbers in parenthesis are the actual numbers of colonies scored in three plates for each dose.

[d] Mean and standard deviation of three independent experiments.

Values of the positive control 4NQO (5µM) locus *hys* (16.01±0.05), locus *lys* (16.25±2.48), and locus *homo* (18.51±4.13).

Table 4. Induction of point mutation (*his 1-7*), ochre allele (*lys1-1*), and *frameshift* mutation (*hom 3-10*) in exponentially growth cells of *Saccharomyces cerevisiae* strain XV185-14C after co-treatment with the juices (10% v/v) and hydrogen peroxide 50 mM for 3 h.

Samples	Survival (%)	His $1/10^7$ survivors [a]	Lys1/10^7 survivors [a]	Hom3/10^7 survivors [b]
0 (Control)	100.0	1.58± 0.83	0.00 ± 0.00	0.00 ± 0,00
H_2O_2 50Mm	45.0	24.70 ± 1.09*	3.18± 1.14*	5.74± 0.66*
Purple grape juice (10%)+ H_2O_2	86.9	5.78± 1.65*(**)	2.02± 0.80*(**)	2.84± 0.84*(**)
White grape juice (10%)+ H_2O_2	88.6	2.94± 1.10**	0.80± 0.23*(**)	2.14± 0.34*(**)

[a]Locus-specific revertants.

[b]Locus non-specific revertants (forward mutations).

*Values statistically different in relation to the control by analysis of variance (ANOVA) and Tukey pos-test, for $p<0.05$

**Values statistically different in relation to H_2O_2 by analysis of variance (ANOVA) and Tukey pos-test, for $p<0.01$

Table 5. Correlations antimutagenic activities and mineral composition in white grape juices.

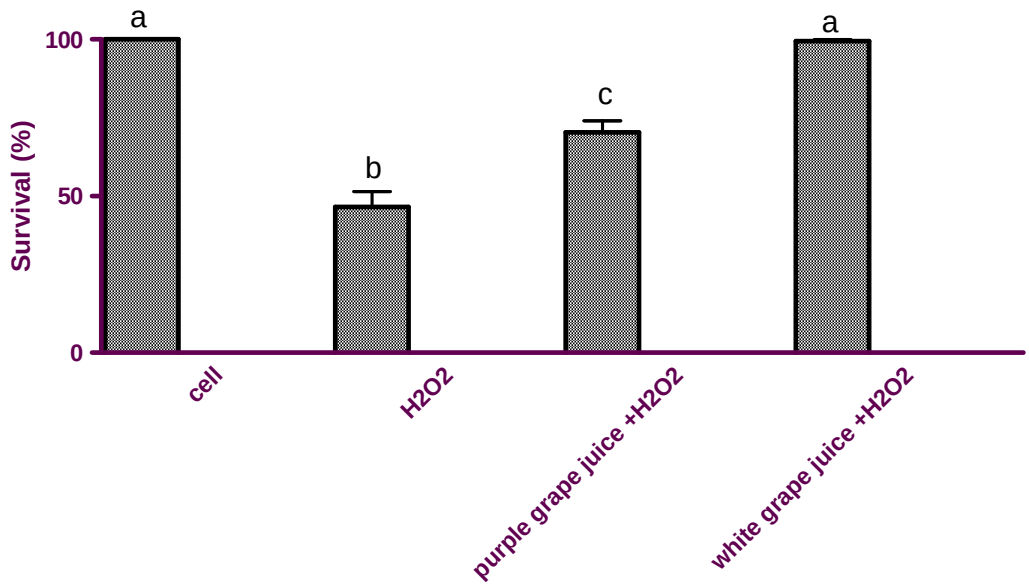
Correlation	Cu	Si	S	Zn	Mg
Antimutagenic activity (*Lys*)	WC	WC	WC	0.741*	WC
Antimutagenic activity (*His*)	WC	WC	WC	WC	0.781*
Antimutagenic activity (*Hom*)	0.781*	-0.781**	-0.781**	WC	-0.781*

r value; *p<0.05, **p<0.01

WC= without correlation

Figure legend

Figure 1. Survival (%) of the yeast *Saccharomyces cerevisiae* treated with hydrogen peroxide at 75mM in the presence or absence of different grape juices (20% v/v).



3.2 CAPÍTULO II

ANTIOXIDANT ACTIVITY, PHENOLIC AND MINERAL CONTENT OF ROSE GRAPE JUICE

Artigo publicado no Journal of Medicinal Food

Este estudo teve por objetivo avaliar a atividade antioxidante *in vitro* e *in vivo*, assim como determinar o conteúdo de polifenóis totais, ácido ascórbico e conteúdo mineral, em um suco de uva rose, produzido a partir de da variedade Goethe (*Vitis labrusca)*. Os resultados mostraram que o suco de uva rose é uma grande fonte de polifenóis, superior aos sucos tinto (Bordo) e branco (Niágara), estudados em outro trabalho do grupo (Anexo A). De todos os metais analisados, o potássio, o cálcio, o magnésio e o ferro mostraram os valores mais elevados. Este suco também apresentou importante atividade antioxidante *in vitro*, avaliado pela metodologia da capacidade de varredura do radical DPPH• e *in vivo*, utilizando o modelo da levedura *S. cerevisae*.

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Short Communication

Antioxidant Activity and Phenolic and Mineral Content of Rose Grape Juice

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ABSTRACT There are many studies related to the antioxidant activity of grape products; however, they concern only purple and white grape varieties. Up to now, there are no reports of studies on the Goethe rose grape variety, either on its antioxidant activity or on its phenolic and mineral quantification. Thus, the purpose of this study was to evaluate *in vitro* and *in vivo* antioxidant activity, as well as to quantify total phenolic compounds, ascorbic acid, and mineral content, in a Goethe rose grape juice. The results obtained showed that the Goethe rose grape juice is a great polyphenol source, which contains catechin, epicatechin, and procyanidins (B_1, B_2, B_3, and B_4). Of all metals analyzed, potassium, calcium, magnesium, and iron showed the highest values. We found that this rose grape juice shows an important antioxidant activity in *in vitro* (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity) and *in vivo* (using the *Saccharomyces cerevisiae* yeast cells) assays. The antioxidant activity could be explained by the significant phenolic content and ascorbic acid levels found in the juice. The results showed that rose grape juice is an excellent antioxidant source, which could contribute to the prevention of many diseases related to oxidative stress, such as atherosclerosis and Parkinson's disease.

KEY WORDS: • *antioxidant* • *ascorbic acid* • *grape juice* • *phenolic compounds*

INTRODUCTION

IT HAS ALREADY BEEN REPORTED that a moderate intake of grape products—wines or juices—have health protection effects. In this sense, some of these activities can be attributed to the phenolic compounds present in grapes.[1] Grape juices can be made from hundreds of different grape varieties, but the *Vitis labrusca* species, which includes the Goethe variety, is the most commonly used. This is a rose grape variety, also known as Roger's, originally from the United States, but which can also be found in several other countries, *e.g.*, Brazil, China, Germany, France, India, and Italy, according to the 2002 European *Vitis* Database.[2]

As already reported in other studies, white and purple grape juices are a source of antioxidants, especially of phenolic compounds such as catechin and procyanidins, which have well-known antioxidant activity.[3,4] Neuroprotective and hepatoprotective effects in Wistar rats were also atributed to grape juices.[5,6] However, no study has yet been conducted to quantify the chemical and phenolic compounds of rose grape juice (RGJ) and their antioxidant activity.

Thus, the purpose of this study was to evaluate the capacity of RGJ to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^•$) (*in vitro* assay) as well as its *in vivo* antioxidant activity, by using *Saccharomyces cerevisiae* yeast cells exposed to hydrogen peroxide (H_2O_2). This study was undertaken to extend the database on (+)-catechin, (−)-epicatechin, procyanidins, and vitamin C amount and mineral content in RGJ.

MATERIALS AND METHODS

Samples

The RGJ, produced in 2005 from *V. labrusca* Goethe variety grapes, was kindly donated by a local winery and was kept in a controlled-temperature room. The main characteristics of the juice are shown in Table 1.

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TABLE 1. MAIN CHARACTERISTICS OF THE GOETHE RGJ

Parameter analyzed	*Mean ± SD*
Total acidity (g/100 mL)	0.497 ± 0.02
Volatile acidity (g/100 mL)	0.018 ± 0.01
Ethylic alcohol (% vol/vol)	0.10 ± 0.00
Relative density 20/20°C	1.0770 ± 0.03
pH	3.37 ± 0.01
Carbohydrates (%)	12.51 ± 0.05
Proteins (%)	0.24 ± 0.05
Alimentary fiber (%)	0.51 ± 0.03
Humidity (%)	86.48 ± 0.02
Ash (%)	0.25 ± 0.00
Caloric value (kJ/100 mL)	214 ± 0.20

Chemicals

DPPH•, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, gallic acid, and procyanidins B_1, B_2, B_3, and B_4 were all obtained from Sigma-Aldrich (St. Louis, MO). The anthocyanin pigments cyanidin-3-glucoside, delfinidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside were obtained from Extrasynthese (Gennay, France). Methyl parathion was obtained from Bayer, and the acetylcholinesterase kit was bought from UFRJ (Rio de Janeiro, Brazil). All other chemicals were purchased from E. Merck (Darmstadt, Germany).

Chemical analysis and nutritional evaluation of the grape juice

Alcoholic grade, total acidity, volatile acidity, pH, total SO_2, and ascorbic acid were determined using the methods described by Zoecklein *et al.*[7] All analyses were performed in duplicate. The levels of carbohydrates, food fiber, saturated fats, proteins, humidity, and caloric value were determined according to the official methodologies of analysis of the Association of Official Analytical Chemists International.[8]

Phenolic compound content

Total phenol content was quantified using the Singleton and Rossi modification of the Folin-Ciocalteau colorimetric method.[9] High-performance liquid chromatography analysis was used to quantify individual phenolic compounds. Before high-performance liquid chromatography analysis, 5 mL of each sample was filtered through a cellulose membrane with a 0.20-mm diameter. The equipment used in the analysis consisted of a liquid gradient chromatographic system (LC-DAD Series 1100, Hewlett Packard, Palo Alto, CA) with a diode array detector system. A Zorbax 300 SB C_{18} (12 mm × 4.6 mm × 5 μm) precolumn and a C_{18}-ODS (150 mm × 4 mm × 5 μm) column (Agilent Technologies, Palo Alto) were used.

In order to quantify procyanidins B_1, B_2, B_3, and B_4, (+)-catechin, (−)-epicatechin, and gallic acid we used a mobile phase with solvent A (50 m*M* ammonium hydroxide diphosphate, pH 2.6), solvent B (20% solvent A and 80% acetonitrile), and solvent C (0.2 *M* orthophosphoric acid, pH 1.5), in a constant flow of 0.5 mL/minute, in a controlled-temperature room at 40°C. The peak was detected at 204 nm, and the amount of sample injected was 5 μL. The elution conditions were standardized according to the procedure of Lamuela-Raventós and Waterhouse.[10]

Particle-induced X-ray emission (PIXE)

Metals quantified in grapes were analyzed by means of the PIXE method.[11] In this assay, 400 mL of RGJ was dried and transformed in tablets. PIXE analysis was carried out at the 3-MV Tandetron accelerator facility at the Instituto de Física of the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. All measurements were performed using a 2-MeV proton beam with an average current of 5 nA. The acquisition time for each sample was approximately 10–20 minutes. The beam spot at the target position was about 9 mm^2. The filters containing grape juice, blank, and calibration targets were placed in a target holder, which accommodates up to 10 samples. Each sample was positioned in the proton beam by means of an electric-mechanical system. The characteristic X-rays induced by the proton beam were detected by an high-purity germanium detector (GLP series, EG&G ORTEC®, CA), with an energy resolution of 180 eV at 5.9 keV. The detector was positioned at 45°C in relation to the beam axis. The electronics consisted of a Telenec 245 amplifier associated with a PCA3 multichannel analyzer (Oxford Instrument, Memphis, TN), running on a PC-compatible computer. GUPIX code[12] was used for data analysis.

Antioxidant activity

The antioxidant activity of the juice was measured by *in vitro* (DPPH• radical scavenging activity) and *in vivo* (*S. cerevisiae* yeast cells) assays.

Scavenging of DPPH• was measured using a method modified from that of Yamaguchi *et al.*,[13] in which grape juice solutions were added to obtain final concentrations of 0.1%, 1.0%, 10.0%, 50.0%, and 100.0% (vol/vol). The tubes were stored in the dark for 20 minutes, after which absorbance was measured at 517 nm. The results were expressed as the concentration of juice necessary to scavenge 50% of DPPH• radical (IC_{50}). The control used distilled water instead of antioxidant solutions. Catechin was used as the standard.

The determination of the *in vivo* antioxidant activity of the juice was performed using eukaryotic cells of *S. cerevisiae* XV 185-14c yeast (*MATα, ade2-2, arg4-17, his1-7, lys1-1, trp5-48, hom3-10*), kindly provided by Dr. R.C. Von Borstel, Department of Genetics, University of Alberta, Edmonton, AB, Canada, treated with H_2O_2 to induce oxidative stress.[14–16] Suspensions containing 2×10^7 cells/mL (exponential phase), with or without H_2O_2 (75 m*M*) and different concentrations of the juice, were incubated for 1 hour.

TABLE 2. MAIN DIFFERENCES BETWEEN RGJ AND WHITE AND PURPLE COMMERCIAL GRAPE JUICES

	RGJ	*White Niagara*[a]	*Purple Bordo*[a]
Ascorbic acid (mg/L)	48.4 ± 0.10	17.6 ± 0.30	30.8 ± 0.40
Carbohydrate (%)	12.51 ± 0.05	11.19 ± 0.03	9.43 ± 0.01
Alimentary fiber (%)	0.51 ± 0.03	0.271 ± 0.01	0.010 ± 0.00
Total phenolic content (mg of catechin/mL)	156.6 ± 5.15	39.95 ± 1.06	119 ± 3.53

[a]Data from Dani *et al.*[4]

The juice concentrations chosen for this assay were 10%, 25%, and 50% (vol/vol), the last one being the highest noncytotoxic dose. After incubation, samples were diluted in 0.9% (wt/vol) saline solution, plated onto YPD (0.5% yeast extract, 2% bactopeptone, and 2% glucose), and incubated for 48 hours at 28°C. Colonies were then counted and compared to the control plates, which were considered to represent 100% of survival of yeast cells.

Statistical analyses

Except for catechin, epicatechin, and procyanidins, all assays were performed in triplicate. Data were analyzed by analysis of variance, and means were compared using Tukey's test. All tests used the SPSS version 12.0 software package (SPSS, Chicago, IL).

RESULTS

The main findings about RGJ are shown in Table 1. Through these results we can observed that RGJ is an important nutritional source. Total phenolic compounds found in RGJ were 156.6 ± 5.15 mg of catechin/mL of juice. Specific phenolic compounds found and their contents were catechin (2.2 ppm), epicatechin (1.68 ppm), and procyanidins B_1 (4.22 ppm), B_2 (1.95 ppm), B_3 (2.14 ppm), and B_4 (1.71 ppm). In a comparison with White Niagara and Purple Bordo, it was seen that RGJ presented higher levels of ascorbic acid, carbohydrates, and fibers among these three kinds of juices (Table 2).

Fifteen metals were analyzed in RGJ. The main metals found were potassium (290.9 ppm), calcium (97.7 ppm), magnesium (96.5 ppm), and iron (65.8 ppm). Phosphorus, chloride, sulfur, copper, zinc, and manganese were found in lower levels (<10 ppm). The remaining five metals (sodium, aluminum, silicon, titanium, and nickel) were below the level of detection.

In the *in vitro* assay, RGJ showed an IC_{50} value of 14% (catechin standard, 8.03%). In the *in vivo* assay, noncytotoxic concentrations (10%, 25%, and 50% vol/vol) of RGJ were chosen for the test. RGJ was able to inhibit damages caused by the stressing agent (H_2O_2) in all concentrations. The 50% and 25% (vol/vol) concentrations showed the same antioxidant activity, both being better than the 10% (vol/vol) concentration (Fig. 1).

DISCUSSION

It is well known that grapes and grape juices are an important nutritional source. RGJ was shown to be an important nutritional source with significant vitamin C and polyphenol contents. RGJ presented higher values of ascorbic acid, carbohydrates, and alimentary fiber than Niagara white and Bordo purple commercial grape juices analyzed in another study[4] conducted by our group (Table 2). The difference could be attributed to several factors, such as grape cultivar, soil, climate, processing methods, etc.[3]

Although there are other juices richer in ascorbic acid content than RGJ, *e.g.*, orange (1,379 mg/L) and grapefruit (337 mg/L), RGJ juice showed a significant ascorbic acid content (48.4 mg/L) similar to other fruit juices such as peach (66 mg/L) and apple (57 mg/L).[17] Vitamin C plays a protective role against reactive species generated during photosynthesis and the respiration processes in plants[18] and also has antioxidant and antimutagenic effects.[18,19]

RGJ was richer in total phenolic (156.6 ± 5.15 mg of catechin/mL) content than the Bordo (119 ± 3.53) and Niagara (39.95 ± 1.06) commercial grape juices (Table 2) analyzed by our group.[4] Many factors could influence the phenolic content, for example, the grape juice production process and the ripeness level.[3,20] According to the literature, the main compounds found in grape juices made from *Vitis vinifera* and *V. labrusca* are (+)-catechin, (−)-epicatechin, and the procyanidins dimers.[21] RGJ showed higher catechin (2.2 ppm) values when compared with a *V. labrusca* purple grape

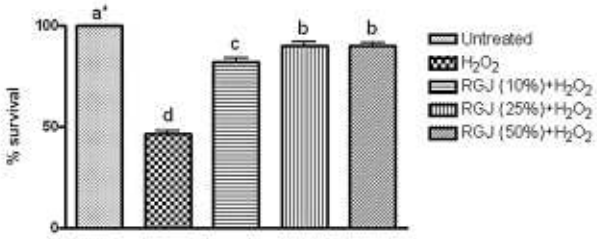

FIG. 1. Survival of *S. cerevisiae* strains untreated and treated with RGJ and/or the stressing agent H_2O_2 (75 m*M*). Different letters correspond to values statistically different by analysis of variance and Tukey's post-test: $^*P < .05$.

juice (1.2 ppm) (Concord variety).[2] This difference occurs because phenolic compounds are secondary metabolites produced and accumulated in plant tissues, and changes in phytopathogenesis, among other factors, may result in different concentrations of these compounds in plant organs.[22]

This is the first study showing the mineral composition of the *V. labrusca* grape juice. Minerals, such as iron, magnesium, sodium, and others, have important physiological functions in the human body.[23] Although more studies are necessary, RGJ is capable of supplying minerals important to human health.

Except for one study showing *in vitro* antioxidant activity of white grape juice,[24] all other studies about antioxidant activity of grape juices are related to juices produced with *V. vinifera* purple grapes.[1,24,25] This is the first study that showed the *in vitro* and *in vivo* antioxidant activity of RGJ from *V. labrusca* species (Goethe variety). *In vivo* antioxidant activity was assessed using *S. cerevisiae* yeasts cells, which are a useful model to screen natural antioxidants.[14–16] In this model, RGJ was able to protect yeast cells from damages induced by H_2O_2.[3] This important biological activity could be attributed, at least, in part, to high levels of phenolic compounds and ascorbic acid found in RGJ. In fact, it has already been shown that phenolic compounds and ascorbic acid contents are related to antioxidant activity.[22,26,27]

Although the results show an important antioxidant activity of RGJ, in both *in vitro* and *in vivo* assays, more studies are needed. The data are very useful since RGJ could become an important component in the diet of adults and children. Our findings show that RGJ could be considered a potentially active compound to be used in conditions where reactive oxygen species are involved, thus protecting against important diseases, such as arteriosclerosis, Parkinson's disease, cancer, and others.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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3.3. CAPÍTULO III

INTAKE OF PURPLE GRAPE JUICE AS A HEPATOPROTECTIVE AGENT IN WISTAR RATS

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Neste estudo, avaliou-se a possível atividade antioxidante *in vivo* (ratos Wistar) dos sucos de uva tintos, orgânico e convencional. Os resultados obtidos permitiram observar que ambos os sucos foram capazes de reduzir níveis do peroxidação lídica e proteíca, bem como modular a ação das enzimas antioxidantes SOD e CAT, reduzindo a razão SOD/CAT, no fígado. No plasma, foi possível observar que somente o suco orgânico reduziu os níveis de peroxidação lipídica, entretanto ambos reduziram os níveis de oxidação protéica. Além desta atividade, o suco de uva orgânico apresentou uma redução significativa na razão SOD/CAT no plasma quando comparado com o grupo que recebeu o suco convencional. Visto que o suco orgânico possui níveis de polifenóis totais e isolados estatisticamente superiores aos convencionais, é possível concluir que estes compostos são muito importantes na atividade benéfica apresentada por este modelo.

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Intake of Purple Grape Juice as a Hepatoprotective Agent in Wistar Rats

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ABSTRACT Grape juice is a source of polyphenols, as catechin, anthocyanidins, resveratrol, and others. Some health benefits have been attributed to these compounds (*e.g.*, antioxidant and antitumorigenic properties). In this study, we investigated the possible antioxidant activity of two different grape juices: organic purple grape juice and conventional purple grape juice. The antioxidant activity of both grape juices was evaluated by an animal model of three groups: control and organic and conventional juices. After 30 days, all animals were sacrificed, and blood and liver were collected to evaluate lipid peroxidation level (thiobarbituric acid-reactive substances [TBARS] assay), protein oxidative level (carbonyl assay), and catalase (CAT) and superoxide dismutase (SOD) activities. The group treated with organic grape juice showed the highest SOD and CAT activities in both plasma and liver when compared with the conventional and control groups ($P < .05$). In plasma, we observed a positive correlation among SOD and CAT activities, resveratrol, and all anthocyanin contents, suggesting that these polyphenols may be, at least in part, responsible for this increased antioxidant defense. The grape juices were capable of reducing carbonyl and lipid peroxidation levels in plasma and liver. However, in plasma, the organic group showed lower carbonyl and TBARS levels when compared to the conventional grape juice group ($P < .05$). Our findings suggest that the intake of purple grape juice, especially of organic juice, induces a better antioxidant capacity when compared to conventional juice and that this may be an important issue for further investigations in the area of biochemical functional foods.

KEY WORDS: • *antioxidant* • *hepatoprotective* • *oxidative stress* • *phenolic content*

INTRODUCTION

THE LIVER REGULATES many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions.[1] Additionally, the liver is the key organ of metabolism and excretion, and it is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. Thus, liver diseases remain one of the more serious health problems. The CCl_4-induced hepatotoxicity model is frequently used for investigating hepatoprotective effects of drugs and plant extracts.[2,3]

CCl_4-induced toxicity is a well-characterized murine model for the study of oxidative damage *in vivo*. The toxicity of CCl_4 results from its reductive dehalogenation by the liver cytochrome P450 enzyme system into the trichloromethyl free radical, which readily interacts with molecular oxygen to form trichloromethyl peroxyl radicals.[4] Both radicals are able to attack proteins and lipids or still abstract hydrogen atoms from an unsaturated lipid, leading to membrane lipid peroxidation, cellular dysfunction, and, finally, to cell necrosis.[5]

In a recent study, grape leaf extracts were able to reduce the damage caused by CCl_4.[6] The possible hepatoprotective activity of purple grape juices, either organic or conventional, has not been reported so far.

Grape juice is a very rich source of polyphenols, such as flavonoids, tannins, and resveratrol.[7] Although there are studies reporting that *Vitis vinifera* grape juices show antioxidant activity,[8–10] there is no reference in literature about this on *Vitis labrusca* cultivars. Presently, there is an increasing interest in a healthier and more environmentally friendly production method for fruits. Organic production is a cultivation method characterized by restrictions against the use of synthetic pesticides and fertilizers, as well as of genetic engineering.[11]

Given these considerations, the aim of the present study was to investigate the beneficial effects of two different purple grape juices—organic and conventional—in reducing the damage to liver and the oxidative stress in plasma and liver, using the well-established murine model.

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MATERIALS AND METHODS

Grape juices

The grape juice samples used in this study were produced from *V. labrusca*, variety Bordo, vintage 2005. The organic juices were produced with organically cultivated grapes (no pesticides) and were obtained from Cooperativa Aecia (Antonio Prado, RS, Brazil), which received the ECOVIDA certificate, a guarantee of organic food production. The conventional juices were obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Both grape juices were kindly donated by these wineries.

Phenolic compounds

Total phenolic content was measured by using the modification of Singleton *et al.*[12] of the Folin-Ciocalteu colorimetric method. High-performance liquid chromatography (HPLC) analysis was used in order to quantify the presence of individual phenolic compounds. Prior to HPLC analysis, 5 mL of each sample was filtered through a cellulose membrane with a 0.20-mm diameter. The equipment used in the analysis consisted of a chromatographic system of liquid gradient, LC-DAD Series 1100 chromatograph (Hewlett-Packard, CA, USA), with a detector system of diode array. A Zorbax 300 SB C18 precolumn (12 mm × 4.6 mm, 5 μm particle size) and C18-ODS column (150 mm × 4 mm, 5 μm particle size) were used in the equipment.

In order to quantify the resveratrol compound, we used a mobile phase of ultrapure water and acetonitrile (75:25 vol/vol) (pH 3.0), at a constant flow of 1.0 mL/minute for 20 minutes, in a controlled-temperature room at 20°C. The peak was detected at 306 nm, and the amount of sample injected was 20 μL.[13]

In order to determine cyanidin-3-glucoside, delphidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside, a mobile phase with solvents A (ultrapure water, formic acid, and acetonitrile [87:10:3 by volume]) and B (ultrapure water, formic acid, and acetonitrile [40:10:50 by volume]), at a constant flow of 0.8 mL/minute, in a controlled-temperature room at 25°C, was applied. The peak was detected at 518 nm, and the amount of sample injected was 50 μL. The elution conditions were 50–60% (30 minutes), 60–100% (30 minutes), and 100–50% (10 minutes).[14]

Animals

Twenty-four male Wistar rats (60 days old, weighing 200 ± 50 g) from our breeding colony were used in the experiments. The animals were handled under standard laboratory conditions of a 12-hour light/dark cycle and fixed temperature (25 ± 2°C). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with the U.S. National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* with the approval of the local ethics committee.

Treatment

The animals were randomly allocated into one of the three experimental groups ($n = 8$): group 1 served as the control and received vehicle saline, and conventional or organic purple grape juices were given to groups 2 and 3, respectively. The doses of purple grape juice were determined by calculating the amount of juice that would be consumed daily in average by a 70-kg male human.[15] Juices were administered to the rats (7 μL of grape juice/g of body weight) twice a day. During the experiment, the amount was adjusted according to the animals' weight. Before sacrifice, the animals' blood was colleted and kept in heparin-coated tubes. On day 30, half of the animals received a single intraperitoneal CCl_4 (3 mL/kg) dose. The animals that received CCl_4 or only vehicle (mineral oil [control]) were killed 6 hours later by decapitation. Liver samples were isolated and stored at −70°C until analysis.

Oxidative stress analyses

We used the thiobarbituric acid-reactive species (TBARS) output during an acid-heating reaction as an index of lipid peroxidation, which is widely adopted as a sensitive method for the measurement of lipid peroxidation, as previously de-

Table 1. Total Phenolic Content and Levels of Resveratrol and Anthocyanins (Cyanidin, Delphinidin, Peonidin, and Malvidin) in Organic and Conventional Grape Juices

	Grape juice	
	Organic	*Conventional*
Total phenolic compounds (mg of catechin/mL)	262.50 ± 0.70*	119.59 ± 3.53
Resveratrol amount (ppm)	0.213 ± 0.005*	0.075 ± 0.010
Cyanidin (ppm)	11.79 ± 0.42*	0.76 ± 0.04
Delphinidin (ppm)	26.30 ± 1.15*	4.10 ± 0.40
Peonidin (ppm)	19.21 ± 1.43*	8.59 ± 0.82
Malvidin (ppm)	232.46 ± 4.25*	95.26 ± 1.95

Data are mean ± SD values.
*Statistically different between the two grape juices ($P < .05$).

scribed.[16] In brief, the samples were mixed with 10% trichloroacetic acid and 0.67% thiobarbituric acid and then heated in a boiling water bath for 15 minutes. TBARS were determined by absorbance at 535 nm.

The oxidative damage to proteins was assessed by determining carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described.[17] In brief, proteins were precipitated by addition of 20% trichloroacetic acid and redissolved in dinitrophenylhydrazine, and the absorbance was read at 370 nm.

Antioxidant enzyme assays were performed in tissue homogenates, as previously described. Catalase (CAT) activity was assayed by measuring the rate of decrease in H_2O_2 absorbance at 240 nm.[18] Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline autooxidation at 480 nm, as previously described.[19]

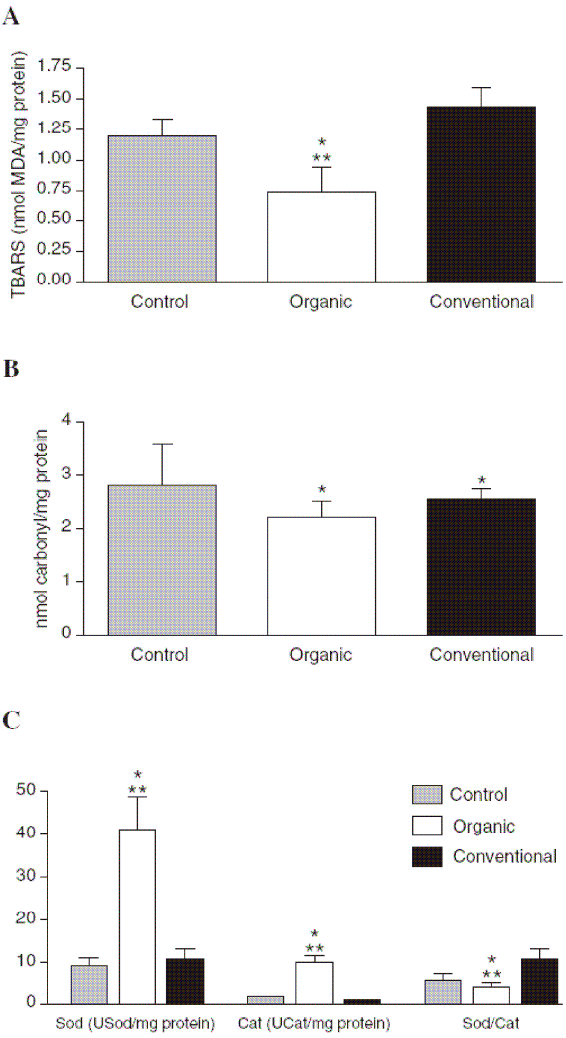

FIG. 1. (**A**) TBARS level, (**B**), carbonyl levels (protein oxidation assay), and (**C**) antioxidant enzyme activities and their ratio in the plasma of rats treated with different grape juices. Control rats received saline. Data are mean ± SD values. $^{*}P < .05$ compared to control; $^{**}P < .05$ compared to conventional grape juice treatment. MDA, malondialdehyde.

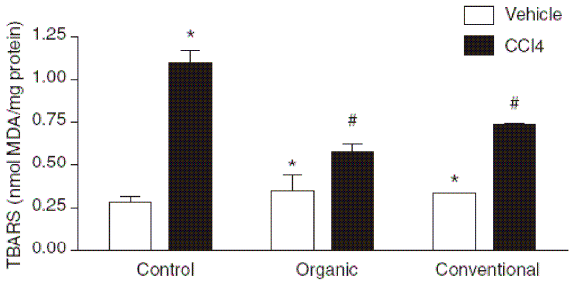

FIG. 2. TBARS content in liver of chronic grape juice-treated Wistar rats. Data are mean ± SD values. $^{*}P < .05$ compared to control + vehicle; $^{\#}P < .05$ compared to control + CCl_4. MDA, malondialdehyde.

Statistical analyses

Biochemical data are expressed as mean ± SEM values, and analysis of variance and Tukey's test were performed using the SPSS (Chicago, IL) version 12.0 package. All tests were performed in duplicate. Pearson's correlation coefficient was used to test the correlation between polyphenol content and the assays.

RESULTS

Table 1 shows the content of phenolic compounds in the two types of purple grape juice used in this study. The two types present a statistical difference in the content of total phenolic compounds ($P < .05$), especially in resveratrol amount; the organic purple grape juice had higher amounts in both parameters. Important differences could be observed between both grape juices with regard to content of anthocyanins (malvidin, cyanidin, delphinidin, and peonidin); we also observed that the organic juice is richer in the amount of all phenolics than the conventional juice (Table 1).

In this study, we have demonstrated that grape juice, especially the organic one, was capable of altering oxidative parameters in plasma. It was observed that the animals that received organic grape juice showed lower plasma lipid peroxidation levels when compared to conventional grape juice and control groups ($P < .05$) (Fig. 1A). We found a negative correlation between lipid peroxidation (TBARS) and total phenolic content ($r = -0.511$) and resveratrol ($r = -0.546$), cyanidin ($r = -0.604$), peonidin ($r = -0.512$), delphinidin ($r = -0.593$), and malvidin ($r = -0.526$) con-

tents ($P < .01$ for all comparisons). Indeed, the protein oxidative damage decreased after treatment with both grape juices, showing a significant difference in relation to the control ($P < .05$) (Fig. 1B). In this assay, the polyphenol content showed an important correlation; we also observed a negative correlation between carbonyl content and polyphenol total amount ($r = -0.799$) and resveratrol ($r = -0.679$), cyanidin ($r = -0.604$), peonidin ($r = -698$), delphinidin ($r = -0.629$), and malvidin ($r = -0.692$) contents ($P < .05$ for all comparisons). When analyzing the activities of antioxidant enzymes, we observed that the group treated with organic grape juice had higher SOD and CAT activities as compared to the conventional grape juice and control groups. We observed a positive correlation between the SOD and CAT activities ($r = 0.707$; $P < .01$). We observed a positive correlation between SOD and CAT activities and total phenolic content ($r = 0.773$ and 0.578, respectively) and also between resveratrol ($r = 0.775$ and 0.602), cyanidin ($r = -0.775$ and 0.632), peonidin ($r = 0.687$ and 0.578), delphinidin ($r = 0.766$ and 0.629), and malvidin ($r = 0.721$ and 0.588) contents ($P < .01$ for all comparisons). This suggests that these polyphenols may be responsible for this increased antioxidant defense. The SOD/CAT ratio of the organic group presented the lowest level when compared to conventional juice and control groups ($P < .05$) (Fig. 1C). This ratio showed a negative correlation with the content of phenolic compounds; we observed this correlation with resveratrol ($r = -0.621$), cyanidin ($r = -0.608$), peonidin ($r = -0.609$), delphinidin ($r = -0.619$), and malvidin ($r = -0.615$) ($P < .05$ for all comparison).

CCl_4 damage was quantified through the lipid peroxidation detection assay, and the level of lipid peroxides was significantly increased in the liver of rats after the CCl_4 injection ($P < .05$) (Fig. 2). However, after treatment with organic grape juice these levels decreased significantly ($P < .05$) when compared to conventional grape juice and control treatments (Fig. 2). This could be explained by the phenolic content; we observed a negative correlation between liver lipid peroxidation and total phenolic content ($r = -0.511$) and resveratrol ($r = -0.546$), cyanidin ($r = -0.604$), peonidin ($r = -0.512$), delphinidin ($r = -0.593$), and malvidin ($r = -0.526$) contents ($P < .05$ for all comparisons).

Figure 3 shows the capacity of CCl_4 to induce protein oxidative damage in liver when compared to control ($P < .05$). A significant attenuation of the oxidative damage induced by the CCl_4 injection can be observed in the groups that were given grape juice ($P < .05$), but the group that received conventional grape juice showed lower values (higher protection against damage) when compared to the group that

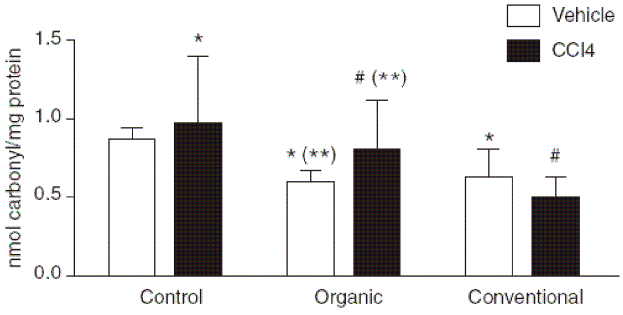

FIG. 3. Liver protein peroxidation (carbonyl) levels in liver. Data are mean ± SD values. $^{*}P < .05$ as compared to control + vehicle; $^{\#}P < .05$ compared to control + CCl_4; $^{**}P < .05$ compared to conventional grape juice treatments.

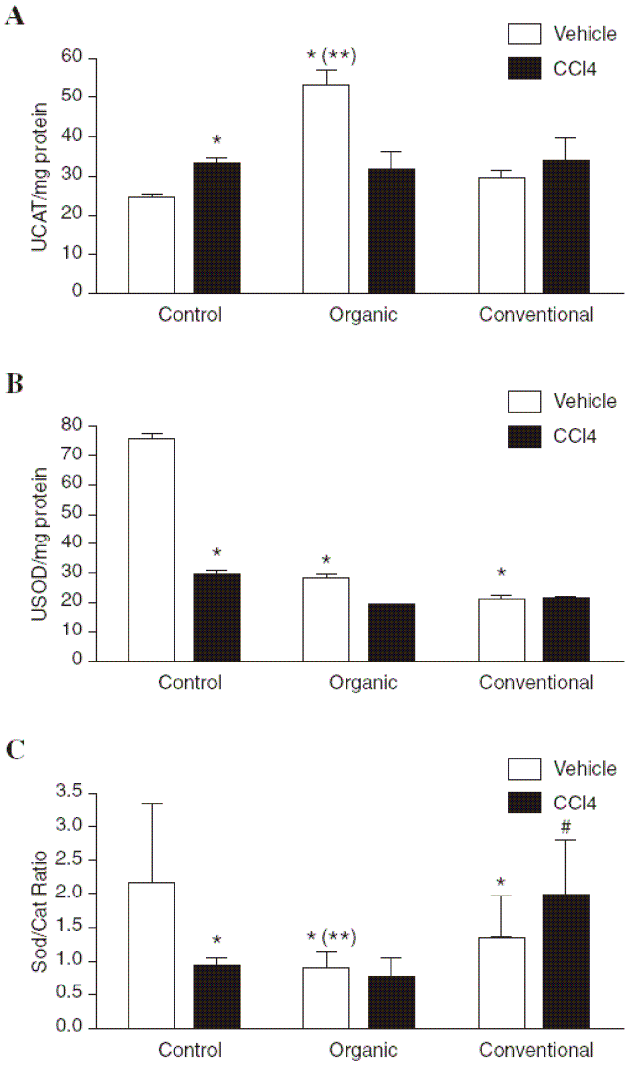

FIG. 4. Levels of enzyme activity—(A) CAT, (B) SOD, and (C) SOD/CAT ratio—in liver in rats treated with different grape juices and that received CCl_4 or vehicle injection. $^{*}P < .05$ compared to vehicle; $^{\#}P < .05$ compared to CCl_4; $^{**}P < .05$ compared to organic and conventional grape juice treatments.

received organic grape juice ($P < .05$) (Fig. 3). However, in the groups that received vehicle in addition to grape juice, the organic grape juice group showed a more significant decrease when compared to the conventional grape juice group, but both grape juices provided protection when compared to the vehicle-only group ($P < .05$) (Fig. 3).

Figure 4 shows the effects of grape juice treatment on CAT and SOD and on the ratio of both enzymes' activities in liver. Modifications in CAT activity in the liver were observed between the control and CCl_4-treated groups, with the CCl_4 group showing higher values ($P < .05$) (Fig. 4A). However, a significant increase in CAT activity was also observed in the organic grape juice group when compared to the conventional grape juice and control groups that had both received vehicle in addition ($P < .05$). We observed a correlation between CAT activity and total phenolic content ($r = 0.372$) and resveratrol ($r = 0.380$), cyanidin ($r = 0.376$), delphinidin ($r = 0.381$), peonidin ($r = 0.373$), and malvidin ($r = 0.376$) contents ($P < .01$ for all comparisons). SOD activity was reduced in CCl_4-treated rat liver ($P < .05$) (Fig. 4B). We observed that both juices reduced SOD activity in liver when compared to the control group that received vehicle only ($P < .05$) (Fig. 4B). The liver SOD/CAT ratio decreased significantly when compared to the control and conventional groups (Fig. 4C).

DISCUSSION

In our study we observed that chronic treatment with grape juice was able to reduce the lipid peroxidation level in liver and plasma after CCl_4 injection, whereas the organic juice induced a more significant reduction than the conventional juice. These protection activities could indicate a hepatoprotective action of grape juice since the CCl_4 damage was smaller after grape juice intake.

Orhan *et al.*[6] showed that the ethanol extract of *V. vinifera*—despite not having measured its active compounds in their study—was capable of inducing a possible hepatoprotective action. They believed that it could be due to (1) inhibiting the cytochrome P450-dependent oxygenase activity, (2) preventing lipid peroxidation, and (3) stabilizing the hepatocyte membrane, induced by polyphenolic compounds.

We also observed that CCl_4 injection increased lipid peroxidation level 6 hours after a single intraperitoneal injection, suggesting that this agent makes a good topic for investigating the antioxidant effect of chronic treatment with grape juice. The phenolic content of the juices could be a possible explanation for this effect. Organic grape juice showed higher contents of total phenolic compounds and other phenolics, such as resveratrol and anthocyanins, associated with antioxidant activities.[9,20–23]

Phenolic compounds are secondary metabolites produced and accumulated in plant tissues. Depending on the presence of biotic and abiotic factors (*e.g.*, phytopathogenesis, water availability), different amounts of these compounds in plant organs would result. Actually, organic farming is a small-scale practice, in which there is no use of chemical protective substances like pesticides or artificial fertilizers to promote plant growth. Since pesticides are not used, the plants are more susceptible to the action of phytopathogenic organisms, resulting in the production of larger amounts of phenolic compounds.[11] This study shows that the choice of agricultural practice used for grapes (organic vs. conventional) results in different amounts of total phenolic compounds, resveratrol, and anthocyanins. The correlations observed between amount of resveratrol and anthocyanins with antioxidant enzyme activity could help explain why some important studies attributed health benefits[9,20–23] to grape juice intake, as we have shown our study.

Our study further showed that especially after treatment with organic grape juice the liver SOD/CAT ratio was lower than that in the control group. This parameter is very important because as a result of an imbalance between these two enzymes oxidative stress may be induced, and it participates in some diseases.[24,25] SOD activity leads to the production of hydrogen peroxide, which can react with iron via the Fenton reaction to generate hydroxyl radicals, which are thought to be the most toxic oxygen molecules *in vivo*.[26] CAT could scavenge an excess of hydrogen peroxide, avoiding its potential role as an oxidative stress-facilitating molecule. Our results showed that grape juice treatment induced CAT activity in a different way than SOD, reducing the SOD/CAT ratio and suggesting a better antioxidant protective status once there would be no extra hydrogen peroxide to overcome Fenton chemistry.

In conclusion, based on earlier reports[27–29] providing evidence of antiplatelet and antioxidant benefits from grape consumption and on our results showing reduced oxidative stress in liver and plasma, it seems reasonable to recommend that moderate quantities of purple grape juice be regularly included in daily servings of fruits and vegetables in order to help maintain a healthy life by attenuating oxidative damage and providing hepatoprotective action, at least in some in the organs studied.

ACKNOWLEDGMENTS

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3.4. CAPÍTULO IV

PROTECTIVE EFFECTS OF PURPLE GRAPE JUICE ON CARBON TETRACHLORIDE-INDUCED OXIDATIVE STRESS IN BRAINS OF ADULT WISTAR RATS

Artigo publicado no Journal of Medicinal Food, 11(1) 2008, 55-61

Neste estudo, objetivou-se avaliar a proteção de sucos de uva tintos, orgânico e convencional, aos danos oxidativos presentes em tecidos do Sistema Nervoso Central (estriado e substância nigra) de ratos Wistar. Os ratos foram divididos em grupos de acordo com o tratamento: controle (salina), convencional e orgânico, e tratado durante 30 dias. Os resultados mostram que os dois sucos estudados foram capazes de reduzir os níveis de peroxidação lipídica em ambos os tecidos. Quando avaliada a capacidade de reduzir os níveis de oxidação protéica, foi possível observar que, na substância nigra, ambos os sucos possuíram esta atividade, entretanto no estriado esta proteção foi conferida somente pelo suco orgânico. A atividade neuroprotetora também pode ser atribuída aos sucos pela capacidade dos mesmos em reduzir os níveis da razão SOD/CAT em ambos tecidos. Estes resultados permitem que novamente se mostre a influência do conteúdo fenólico a atividade benéfica do suco de uva.

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Protective Effects of Purple Grape Juice on Carbon Tetrachloride-Induced Oxidative Stress in Brains of Adult Wistar Rats

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ABSTRACT The antioxidant properties of purple grape juice, organic and conventional, in brain tissues are not well known. In this study our objective was to evaluate the antioxidant activity in substantia nigra and striatum of rats chronically treated with organic or conventional purple grape juice and to correlate the results obtained with the polyphenol content (total polyphenolic content, resveratrol, and anthocyanins [malvidin, delphinidin, peonidin, and cyanidin]). We observed that CCl_4 damage decreased significantly in the grape juice-treated groups when compared with the control group. In the grape juice-treated groups we further observed a decrease of lipid (thiobarbituric acid-reactive substances assay) and protein (carbonyl) peroxidation, as well as a significant antioxidant protection through the increase of enzyme activity. Antioxidant activities were significantly correlated with polyphenol content. These findings demonstrated that both grape juices have potent antioxidant properties and these activities could be at least attributed to the high phenolic content present in these juices.

KEY WORDS: • *antioxidant* • *grape juice* • *neuroprotection* • *oxidative stress* • *phenolic content*

INTRODUCTION

NOWADAYS SEVERAL STUDIES have shown that a high consumption of vegetables and fruits is consistently associated with a low risk of oxidative stress-induced diseases. Polyphenols are the main compounds believed to be responsible for this protection. These classes of compounds are found in many fruits, like grapes and its products.[1] Grape juice is a very rich source of polyphenols, such as flavonoids and anthocyanides, and nonflavonoids, such as resveratrol.[2]

CCl_4-induced toxicity is a well-characterized murine model for oxidative damage *in vivo*. The toxicity of CCl_4 results from its reductive dehalogenation by the cytochrome P450 enzyme system into a trichloromethyl free radical, which readily interacts with molecular oxygen to form the trichloromethyl peroxyl radical.[3]

Several studies have demonstrated that the liver is not the only target organ of CCl_4. CCl_4 has been reported to cause lipid peroxidation in other organs such as kidney, heart, and brain.[3,4] In the present study, levels of thiobarbituric acid-reactive substances (TBARS) and carbonyl proteins and activities of antioxidant enzymes were quantified in brain structures to examine the antioxidant activity with purple grape juice pretreatment.

The substantia nigra and the striatum, components of the dopaminergic pathway, play an important role in the regulation of movements and of some cognitive functions. The high metabolic rate and the oxidative degradation of dopamine by the mitochondrial monoamine oxidase in these structures contribute to the oxidative damage in biomolecules. Indeed, oxidative stress is involved in some pathologies that affect the nigrostriatal axis.[5]

There is an increasing interest in healthier and more environmentally friendly production methods for different fruits. Nowadays organic production is not one cultivation method, but many, characterized by restrictions against the use of synthetic pesticides and synthetic fertilizers, although the detailed regulations of what can be called organically cultivated vary.[6,7] Nevertheless, consumers today buy products that are labeled "organically cultivated" in stores, and many expect the quality to be superior to that of conventionally cultivated products in terms of contents that make the products healthier.[8] There are some studies that showed some differences in antioxidant activities and in the phenolic content in fruit juices obtained from organic and conventional procedures, but until now these studies are inconclusive.[6,8]

Although there are many brain diseases caused by free radicals, like Parkinson's disease, we did not find consistent data in literature suggesting a relationship between grape

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juice intake and antioxidant protection to the central nervous system. One study showed that different Concord grape juice concentrations (10% and 50%) were beneficial in reversing the course of neuronal and behavioral aging.[9] Given these considerations, the aim of the present study was to investigate the beneficial effects of organic and conventional purple grape juices in reducing the oxidative stress in the striatum and the substantia nigra isolated from 60-day-old Wistar rat brains.

MATERIALS AND METHODS

Grapes and grape juices

The grape juice samples used in this work were made with grapes of the *Vitis labrusca* Bordo variety. The organic juices were obtained from the Cooperativa Aecia (Antonio Prado, RS, Brazil) and were certified by ECOVIDA, while the conventional juices were obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Throughout the tests, we observed the expiry dates of the juices and always used the same trademarks.

Phenolic compounds

Total phenolic content was measured using the modification of Singleton *et al.*[10] of the Folin-Ciocalteau colorimetric method. High-performance liquid chromatography (HPLC) analysis was used in order to quantify the presence of individual phenolic compounds. Before the HPLC analysis, 5 mL of each sample was filtered through a cellulose membrane (diameter, 0.20 mm). The equipment used in the analysis consisted of an LC-DAD Series 1100 liquid chromatographic system (Hewlett-Packard, Palo Alto, CA) with a diode array detector system.

Resveratrol analysis. In order to quantify the *trans*-resveratrol compound, we used a mobile phase of ultrapure water and acetonitrile (75:25 vol/vol) (pH 3.0) in a constant flow of 1.0 mL/minute for 20 minutes in a controlled-temperature room at 20°C. The peak was detected at 306 nm, and the amount of sample injected was 20 μL.[11]

Anthocyanin analysis. In order to determine cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside, we used a mobile phase with solvents A (ultrapure water, formic acid, and acetonitrile) and B (ultrapure water, formic acid, and acetonitrile) in a constant flow of 0.8 mL/minute in a controlled-temperature room at 25°C. The peak was detected at 518 nm, and the amount of sample injected was 50 μL.[12]

Animals

Twenty-four male Wistar rats (60 days old, weighing 200 ± 50 g) from our breeding colony were used in the experiments. The animals were handled under standard laboratory conditions of a 12-hour light/dark cycle and fixed temperature (25 ± 2°C). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with the U.S. National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* with the approval of the local ethics committee.

Chronic intake

The animals were randomly allocated to one of the three experimental groups ($n = 8$): group 1 served as the control and received saline, and groups 2 and 3 were given purple grape juice (conventional and organic, respectively). The doses of purple grape juice were determined by calculating the daily amount of juice consumed on average by a 70-kg human male. As a reference, we used a study with humans who received 480 mL/day.[13] The amount of juice was administered to the rats according to their body weight. We gave 7 μL of grape juice/g of body weight, twice a day; however, during the experiment the amount varied. On day 30, half of the animals received a single intraperitoneal dose of CCl_4 (3 mL/kg). The animals that received CCl_4 (positive control) or animals that received only oil (vehicle) (negative control) were killed 6 hours later by decapitation. Brain structures were isolated and stored at −70°C until analysis.

Oxidative stress parameters

As an index of lipid peroxidation we used the formation of TBARS during an acid-heating reaction, which is widely adopted as a sensitive method for measurement of lipid peroxidation, as previously described.[14]

The oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine, as previously described.[15]

Antioxidant enzyme assays were performed in tissue homogenates, as previously described.[3] Catalase (CAT) activity was assayed by measuring the rate of decrease in H_2O_2 absorbance at 240 nm.[16] Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline autooxidation, as previously described.[17]

Statistical analyses

Statistics were calculated by means of analysis of variance and Tukey's test using the SPSS (Chicago, IL) version 12.0 package. All tests were performed in duplicate. Pearson's correlation coefficient was used to test correlation between polyphenol content and the assays.

RESULTS

Table 1 presents additional information about the content of phenolic compounds in the two types of purple grape juice used in this study. There is a statistical difference in the content of total phenolic compounds ($P < .05$) between both juices, especially in the resveratrol amount; the organic purple grape juice had higher amounts in both parameters. It is possible to observe an important difference between these

TABLE 1. TOTAL PHENOLIC CONTENT AND LEVELS OF RESVERATROL AND ANTHOCYANINS (CYANIDIN, DELPHINIDIN, PEONIDIN, AND MALVIDIN) IN ORGANIC AND CONVENTIONAL GRAPE JUICES

	Grape juice	
	Organic	*Conventional*
Total phenolic compounds (mg of catechin/mL)	262.50 ± 0.70*	119.59 ± 3.53
Resveratrol amount (ppm)	0.213 ± 0.005*	0.075 ± 0.010
Cyanidin (ppm)	11.79 ± 0.42*	0.76 ± 0.04
Delphinidin (ppm)	26.30 ± 1.15*	4.10 ± 0.40
Malvidin (ppm)	232.46 ± 4.25*	95.26 ± 1.95

Data are mean ± SD values.
*Statistically difference between the two grape juices ($P < .05$).

two grape juices in content of anthocyanins (malvidin, cianidin, delphinidin, and peonidin): the organic juice was richer than the conventional one (Table 1).

CCl_4 induced an increase in lipid peroxidation in the striatum and substantia nigra when compared to the control group ($P < .05$). The chronic treatment with both grape juices did not induce significant differences in the striatum when compared to the control group, but we observed a significant reduction in TBARS in the substantia nigra of isolated organic juice-treated rats ($P < .05$). Both grape juices had a protective effect when comparing the CCl_4-induced groups (Fig. 1).

Concerning protein peroxidation damage (carbonyl assay), there is a significant difference between the CCl_4 and vehicle groups, in which CCl_4 increased the protein oxidative level as expected (Fig. 2). However, after the intake of organic grape juice, carbonyl levels showed an important and significant attenuation in the striatum when compared with the negative (vehicle) and positive (CCl_4) controls (Fig. 2A). This decrease showed a positive correlation with all phenolic compounds: resveratrol ($r = 0.786$), malvidin ($r = 0.796$), delphinidin ($r = 0.796$), peonidin ($r = 0.786$), cianidin ($r = 0.786$), and total phenolic content ($r = 0.693$) (all $P < .05$).. In the substantia nigra both organic and conventional grape juices were capable of reducing the carbonyl levels when compared to CCl_4-induced groups, but the conventional juice showed a better performance than the organic juice (Fig. 2B). In these groups the decrease in carbonyl levels showed also an important correlation with the grape juice phenolic content: resveratrol ($r = 0.493$), malvidin ($r = 0.534$), delphinidin ($r = 0.493$), peonidin ($r = 0.453$), cianidin ($r = 0.493$), and total phenolic content ($r = 0.530$) (all $P < .05$).

Both grape juice-treated groups showed higher CAT activity ($P < .05$) in the striatum as compared to the negative and positive controls. In this same structure we observed a significant increase in the positive control (CCl_4) when compared with the negative control (Fig. 3A). CAT activity was increased in both grape juice groups when compared with the positive control group in striatum. In this tissue, there was a positive correlation between CAT activity and total polyphenol content ($r = 0.539$; $P < .01$), resveratrol ($r = 0.552$; $P < .01$), cyanidin ($r = 0.450$; $P < .01$), delphinidin ($r = 0.476$; $P < .05$), peonidin ($r = 0.538$; $P < .01$), and malvidin ($r = 0.533$; $P < .01$). Because of this positive correlation, the increase of CAT activity could be explained, in part, by the presence of these compounds. Furthermore, the positive control group showed a significant decrease ($P <$

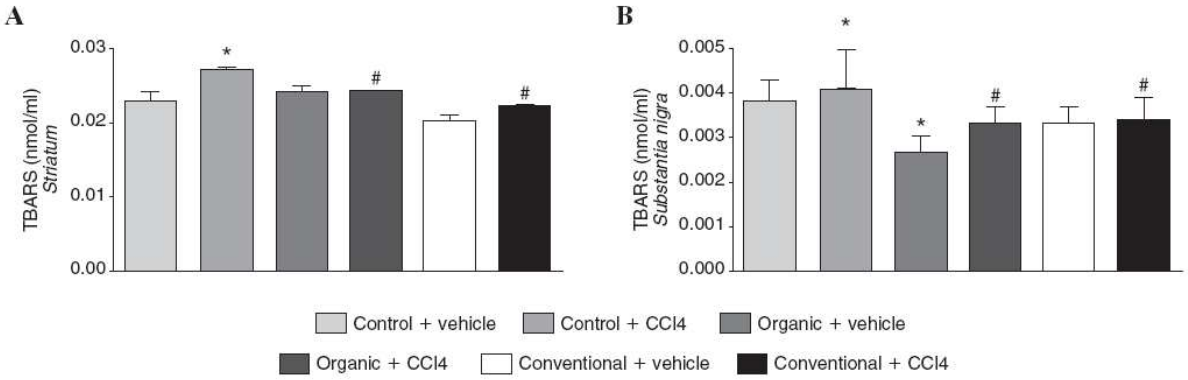

FIG. 1. TBARS level in (**A**) striatum and (**B**) substantia nigra with different grape juice treatments. *$P < .05$ compared to control + vehicle; #$P < .05$ comparing the grape juice treatments to control + CCl_4.

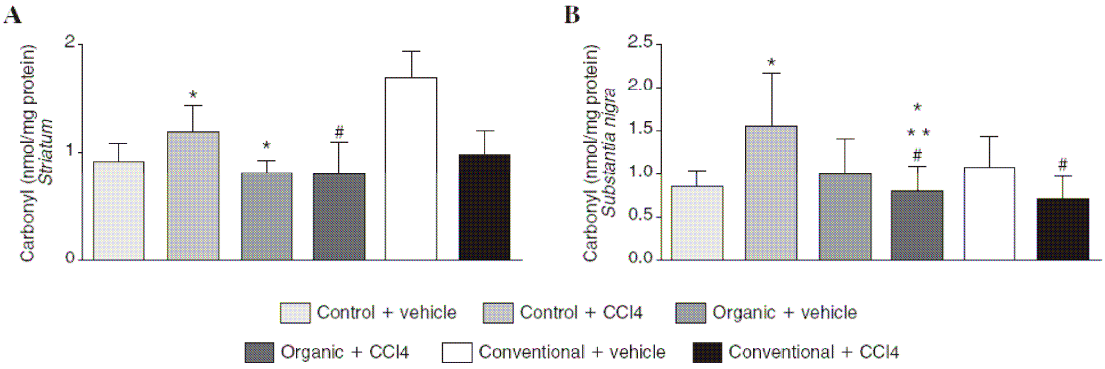

FIG. 2. Protein peroxidation level (carbonyl assay) in (**A**) striatum and (**B**) substantia nigra with different grape juice treatments. $^{*}P < .05$ compared to control + vehicle; $^{**}P < .05$ comparing the organic and conventional grape juice treatments; $^{\#}P < .05$ comparing the grape juice treatments to control + CCl_4.

.005) in the substantia nigra when compared with the negative control (Fig. 3B). A positive correlation was observed when for CAT activity with total phenolic and peonidin content ($r = 0.437$ and 0.434, respectively; $P < .05$).

When analyzing the SOD activity it was observed that the organic grape juice combined with CCl_4 increased the activity of this enzyme in the striatum when compared with the positive control, showing a positive correlation with resveratrol content ($r = 0.633$; $P < .05$) (Fig. 4A). In the substantia nigra the organic grape juice group presented the highest values of SOD activity when compared with both controls, negative and positive (Fig. 4B). A positive correlation was observed between SOD activity and contents of the phenolic compounds resveratrol ($r = 0.693$; $P < .01$), cyanidin ($r = 0.689$; $P < .05$), peonidin ($r = 0.682$; $P < .01$), delphinidin ($r = 0.695$; $P < .01$), and malvidin ($r = 0.687$; $P < .01$) and total phenolic content ($r = 0.681$; $P < .05$), showing an important action of these compounds in increasing this enzyme activity after CCl_4 exposure.

We observed a significant decrease of the SOD/CAT ratio in the striatum and the substantia nigra in both chronic grape juice intake groups when compared with the positive control; however, the organic juice was able to reduce this ratio in the striatum more than the conventional juice (Fig. 5). Indeed, in both grape juice-treated groups the SOD/CAT ratio showed lowest levels in the striatum and substantia nigra when compared with the negative control (vehicle), but the organic grape juice reduced this level more significantly in the striatum ($P < .005$) (Fig. 5). This high reduction property of organic grape juice could be explained, at least in part, as being due to the rich phenolic content analyzed in this juice, which in striatum and substantia nigra showed a negative correlation with the SOD/CAT ratio. In striatum this correlation was observed with resveratrol ($r = -0.577$; $P < .01$), cyanidin ($r = -0.587$; $P < .01$), peonidin ($r = -0.591$; $P < .01$), delphinidin ($r = -0.501$; $P < .05$), and malvidin ($r = -0.587$; $P < .01$).

DISCUSSION

Organisms can suffer oxidative damage, yet the animal brain is often said to be especially sensitive; one reason is its high oxygen consumption and high lipid content. The

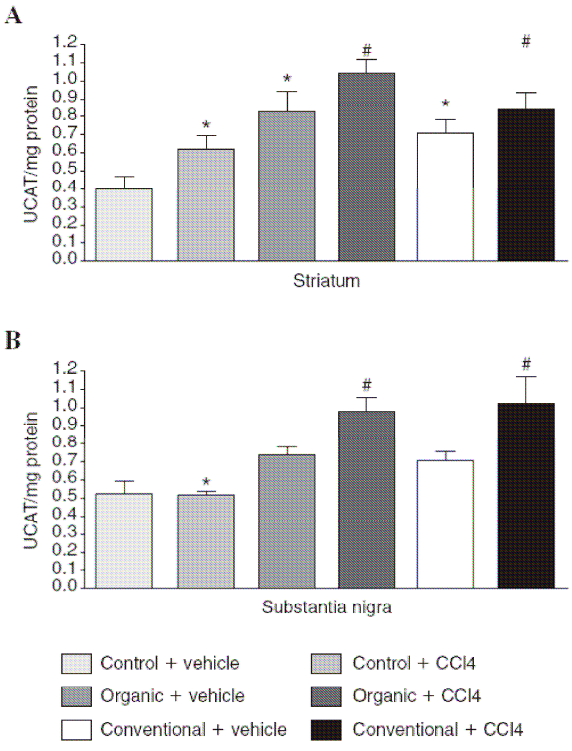

FIG. 3. CAT activity level (in units [U] of enzyme activity) in (**A**) striatum and (**B**) substantia nigra with different grape juice treatments. $^{*}P < .05$ compared to control + vehicle; $^{\#}P < .05$ comparing the grape juice treatments to control + CCl_4.

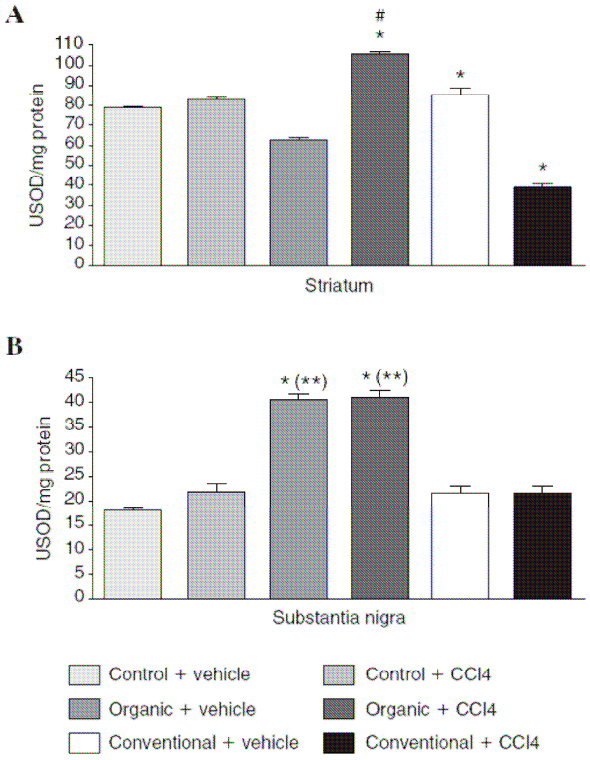

FIG. 4. SOD activity level (in units [U] of enzyme activity) in (**A**) striatum and (**B**) substantia nigra with different grape juice treatments. $^{*}P < .05$ compared to control + vehicle; $^{**}P < .05$ comparing the organic and conventional grape juice treatments; $^{\#}P < .05$ comparing the grape juice treatments to control + CCl_4.

present study is the first one to show that purple grape juice can reduce oxidative stress in structures of the nervous system, such as the substantia nigra and the striatum. In another study, the intake of juice obtained from the Concord grape, a variety of purple grape, was able to enhance motor performance and dopamine release.[9]

In our study we observed that chronic grape juice intake reduced lipid peroxidation and oxidative protein damage after treatment with CCl_4, known as an oxidative stressor.[3] The reduction of the lipid peroxidation level in brain structures was observed in other studies with rats that received grape seed extract.[18] In the present study, the reduction observed could be explained, at least in part, by the phenolic content—of which grape products show an important amount.

This fact could be observed in our study: positive correlations were observed between the phenolic content and the reduction of the lipid peroxidation and protein oxidation (carbonyl) inducted by CCl_4. CCl_4-induced toxicity is a well-characterized murine model for oxidative damage *in vivo*, because the radicals produced by this agent are able to attack proteins and lipids or still abstract hydrogen atoms from an unsaturated lipid, leading to membrane lipid peroxidation, cellular dysfunction, and finally cell necrosis.[19] Against these damages the organisms have important antioxidant defenses, such as SOD and CAT enzymes; however, other nonenzymatic compounds can reduce this damage, such as the polyphenol compounds. Many biological activities are attributed to phenolic compounds, *e.g.*, antioxidant, anti-inflammatory, and anti-oncogenic.[20] Data from a recent study suggest that *Vitis vinifera* can be used as a chemopreventive agent against oxidative stress and carcinogenesis, mainly because of their phenolic compounds.[21]

Park *et al.*[13] showed in their study that purple grape juice was capable of reducing DNA damage, as evidenced by the Comet assay. It has already been reported that the compounds present in grape juice can inhibit (1) platelet activity,[22] (2) low-density lipoprotein oxidation and oxidative damage to DNA,[13] and (3) coronary disease and atherosclerosis.[1]

Phenolic compounds are secondary metabolites produced and accumulated in plant tissues. Nowadays organic farming is a widely utilized small-scale practice, in which no chemical substances like pesticides or artificial fertilizers to

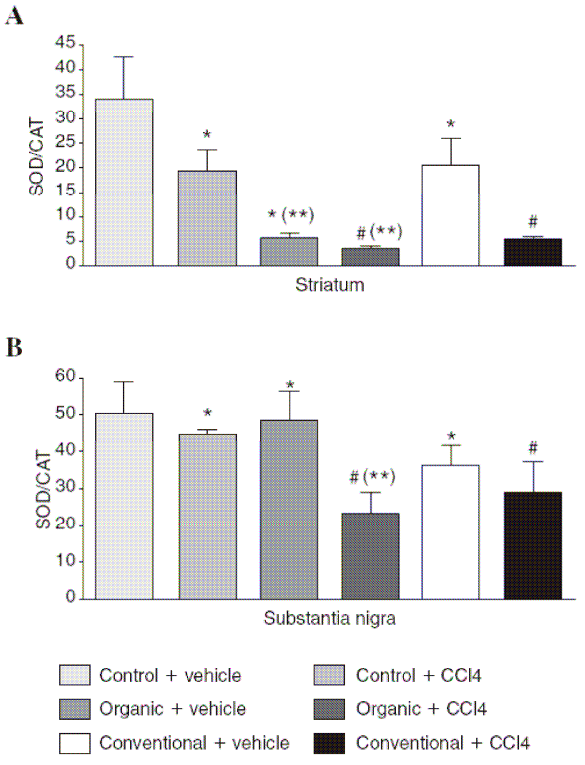

FIG. 5. SOD/CAT ratio in (**A**) striatum and (**B**) substantia nigra with different grape juice treatments. $^{*}P < .05$ compared to control + vehicle; $^{**}P < .05$ comparing the organic and conventional grape juice treatments; $^{\#}P < .05$ comparing the grape juice treatments to control + CCl_4.

promote plant growth are used. Since pesticides are not used, plants are more susceptible to the action of phytopathogenic organisms, resulting in the production of larger amounts of phenolic compounds.[20] We observed that depending on which agricultural practice is chosen (organic vs. conventional), it will result in different amounts of resveratrol, anthocyanins, and tannins, with the organic practice being richest in these compounds (Table 1).

The brain has antioxidant defenses that could act against free radicals, but with aging these defenses decrease. As defenses one can cite enzymes such as SOD and CAT. SOD plays a key role in detoxifying superoxide anions, which otherwise damage the cell membranes and macromolecules.[23] Clinical studies demonstrated a reduction of SOD activity in parkinsonism associated with other neurodegenerative disorders, showing the key role played by this enzyme in fighting free radicals produced in the brain.[24,25]

In the present study, CAT activity was increased after chronic intake of purple grape juice, especially in the organic juice group, suggesting that a high content in phenolic compounds is capable of increasing the activity of the enzymatic antioxidant defenses. SOD activity was significantly increased in the purple grape juice chronic intake groups, once more suggesting that the juice is capable of increasing the activity of the enzymatic antioxidant defenses. Indeed, the organic grape juice group showed the highest increase, a fact that could be explained by the richer content of total phenolics and higher levels of resveratrol and anthocyanins found in the organic juice. Recently, a study reported that resveratrol was capable of reducing the lipid peroxidation and increasing activities of the antioxidant enzymes SOD and CAT.[26] Furthermore, this increased enzymatic activity was also observed in other studies that reported higher SOD and CAT activities in brain regions of rats treated with grape seed extracts.[18] Balu *et al.*[18] attributed this increase to the free radical quenching action of the dihydroxyl (catechol) structure in the B-ring of proanthocyanin present in grape seeds.

We could also observe that after chronic intake of grape juice the SOD/CAT ratio was lower in this group than in the control. In the striatum, the organic grape juice group showed the lowest ratio level when compared with the negative and positive control groups. The SOD/CAT ratio is a very important parameter because an imbalance between these two enzymes of the antioxidant defenses may induce an increase in oxidative stress level and thus to the development of many diseases associated with it.[27] SOD activity leads to the production of hydrogen peroxide, which reacts with iron to generate hydroxyl radicals via the Fenton reaction, which in turn are thought to be the most toxic oxygen molecules *in vivo*.[28] CAT could clean up an excess of hydrogen peroxide, thus diminishing its oxidative effects. Our results showed that treatment with grape juice can induce an increase in CAT and SOD activity and reduce the SOD/CAT ratio, suggesting an important antioxidant activity of grape juice, and with no reactive species being produced in excess, proving a grape juice health benefit shown in another study.[29]

In conclusion, the results of the present study suggest that grape juice intake enhances antioxidant status in rats by reducing the levels of lipid peroxidation end products, whose accumulation would otherwise play a key role in brain aging, and reduces the incidence of brain diseases, such as Parkinson's, Alzheimer's, and other diseases. Further studies are needed to obtain a better understanding of the molecular mechanism by which chronic grape juice intake may modulate CAT and SOD activities.

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3.5. CAPÍTULO V

ANTIOXIDANT AND ANTIGENOTOXIC ACTIVITIES OF PURPLE GRAPE JUICE- ORGANIC AND CONVENTIONAL – IN ADULT RATS

Artigo submetido à Journal of Medicinal Food

(Recentemente enviamos as correções sugeridas pelos Revisores da Revista)

Neste trabalho, avaliou-se a capacidade dos sucos de uva tintos, orgânico e convencional, em reduzir os danos oxidativos, em diferentes tecidos, em ratos Wistar adultos. Os resultados obtidos permitem observar que ambos os sucos de uva foram capazes reduzir os níveis de peroxidação lipídica no córtex, substância nigra e no hipocampo, entretanto na substância nigra o suco orgânico apresentou uma redução estatisticamente maior que o suco convencional. A redução nos níveis de peroxidação lipídica no plasma foi observada somente no grupo que recebeu o suco convencional. No córtex, se verificou uma redução significativa na razão SOD/CAT nos grupos que receberam os sucos orgânicos e convencionais. Entretanto, a redução foi estatisticamente menor no grupo que recebeu suco convencional. No hipocampo, também se observou que ambos os sucos reduziram este parâmetro, mas diferentemente neste tecido, o suco orgânico apresentou valores inferiores ao convencional. Neste estudo, também foi possível observar que ambos os sucos apresentaram importante atividade antigenotóxica, onde ambos os sucos mostraram-se capazes de reduzir o dano ao DNA. Sendo assim, os dados sugerem que os sucos de uva tintos são importantes “escudos” contra estes danos acumulados durante este processo, visto que são antioxidantes, ou seja, capazes de minimizar os danos causados pelo estresse oxidativo.

Antioxidant and antigenotoxic activities of purple grape juice- organic and conventional – in adult rats

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Running title: Purple grape protection in old rats.

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Abstract

Oxidative damage to biomolecules occurs by the accumulation of free-radicals molecular damage and/or a diminution of antioxidant protection. The aim of this study was to evaluate the protection of organic and conventional purple grape juices in brain, liver and plasma from adult Wistar rats (7 months old) against the oxidative damage provoked by carbon tetrachloride (CCl_4). Adult rats were divided into three groups (control, conventional and organic purple grape juice). Half of the rats received CCl_4 and the other half received the vehicle (vegetable oil). The chemical analytical determination showed that with organic purple grape juices showed the highest levels of total phenolic, resveratrol and catechins contents.. Considering the treatment groups, it was observed that in all tissues (brain structures and liver) and plasma CCl4 treatment increased the lipid peroxidation (LP) levels. Both grape juices were capable to reduce LP levels in cerebral cortex and hippocampus, however in striatum and substantia nigra only the organic grape juice reduced LP level. The CCl_4 provoked an increase in catalase (CAT) activity in cerebral cortex, hippocampus and substantia nigra, and also in superoxide dismutase (SOD) activity in substantia nigra. This increase was reduced by the both juices in substantia nigra and hippocampus structures ($p<0.05$). In the alkaline version of the comet assay performed on whole blood it was observed that CCl_4 was capable to induce mainly 4 and 3 DNA damage class frequencies, which was reduced significantly in groups that received both purple grape juices.This implies that both grape juices have an important antigenotoxic activity.

Key Words: grape juice, oxidative stress, DNA damage

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Introduction

Epidemiological studies have suggested an inverse relation between the consumption of polyphenol-rich foods and beverages and the risk of degenerative diseases, particularly cancers and cardiovascular diseases, mainly by their antioxidant capacity [1,2]. Some researches have shown, also, that diets rich in fruits and vegetables are beneficial in both forestalling and reversing the deleterious effects of aging on neuronal communication and behavior [3,4,5]. In these studies, the observed protection may be the result of the antioxidant and anti-inflammatory properties of the polyphenolic compounds found in these fruits and vegetables [6]. Grape juice is a very rich source of polyphenols, such as flavonoids, tannins, and resveratrol [7], showing important antioxidant activity *in vitro* [7].

However, the question remains if properties demonstrated in *in vitro* studies are relevant to protect against oxidative damage *in vivo*. Indeed, in previous study [8,9] we observed that chronic intake of grape juices – organic and conventional – was able to reduce oxidative stress damage in brain tissues (striatum and substantia nigra), liver and plasma in young rats (2 months old), using the CCl_4 as the damage oxidative inducer.

CCl_4-induced toxicity is a well-characterized murine model for oxidative damage *in vivo.* The toxicity of CCl_4 results from its reductive dehalogenation by the cytochrome P450 enzyme system into a trichloromethyl free radical, which readily interacts with molecular oxygen to form the trichloromethyl peroxyl radical. Several studies have demonstrated that the liver is not the only target organ of CCl_4. CCl_4 has been reported to cause lipid peroxidation in other organs such as kidney, heart, and brain [10,11].

The aging is a process that accumulates oxidative damage leading to DNA, lipids, and protein damages. Such free radical-mediated damages are prevalent during aging and are associated with diseases like Alzheimer and Parkinson [12,13]. In this study we choose rats

older than the first studies [8,9] to verify if the grape juice protection is also present in older individuals. Consequently, the aim of the present study was to investigate the beneficial effects of two different purple grape juices—organic and conventional—in reducing oxidative stress generated by CCl_4 in 7 months-old rats.

2 Material and Methods

2.1 Grapes and Grape Juices

The grape juices used in this work were elaborated with grapes of the *Vitis labrusca* Bordo variety. Organic grape juice was produced with grapes cultivated with no pesticides [7], certified by ECOVIDA, and was obtained from Cooperativa Aecia (Antonio Prado, Rio Grande do Sul, RS, Brazil). Conventional juice was produced with grapes cultivated using traditional methods [7], and was obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Throughout the tests, we observed the expiry dates of the juices and always used the same trademarks.

2.2 Phenolic Compounds

Total phenolic content was quantified using a Singleton and Rossi's modification of the Folin-Ciocalteau colorimetric method [14]. High performance liquid chromatography (HPLC) analysis was used in order to measure the presence of phenolic compounds. Before the HPLC analysis, 5 mL of each sample were filtered through a cellulose membrane of 0.20 mm diameter. The equipment used in the analysis consisted of liquid chromatographic system, LC-DAD Series 1100 (Palo Alto, CA), with a diode array detector system (DAD).

2.2.1 Resveratrol analysis

In order to quantify resveratrol, we used a mobile phase of ultra-pure water and acetonitrile (75:25 v/v) pH 3.0, in a constant flow of 1.0 mL.min-1 for 20 min, in a controlled-

temperature room at 20 °C. The peak was detected at 306 nm, and the amount of sample injected was 20 μL [15].

2.2.2. Catechins analysis. In order to determine the catechins ((+)-catechin and (-)-epicatechin) it was used a mobile phase with solvent A (ammonium hydroxide diphosphate 50 mMol.L-1, pH 2.6), solvent B (20 % of solvent A and 80 % of acetonitril) and solvent C (ortophosphoric acid 0.2 Mol.L-1, pH 1.5), in a constant flow of 0.5 mL.min-1, in a controlled-temperature room at 40 °C. The peak was detected in 204 nm, and the amount of sample injected was 5 μL. The elution conditions were: 100 % of solvent A for 5 min; 96 % of solvent A and 4 % of solvent B for 10 min; 92 % of solvent A and 8 % of solvent B for 10 min; 8 % of solvent B and 92 % of solvent C for 20 min; 30 % of solvent B and 70 % of solvent C for 5 min; 40 % of solvent B and 60 % of solvent C for 5 min; 80 % of solvent B and 20 % of solvent C for 5 min and, 100 % of solvent A for 5 min[16].

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2.3 Animals

Twenty-four male Wistar rats (7 months old) from our breeding colony were used in the experiments. The animals were handled under standard laboratory conditions of a 12-h light/dark cycle and fixed temperature (25±2 ºC). Food and water were available *ad libitum*. All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals with the approval of the local ethics committee.

2.4 Chronic intake

The animals were randomly allocated into one of the three experimental groups (n=8). Group 1 served as control and received saline, groups 2 and 3 were given purple grape juice (conventional and organic, respectively). The doses of purple grape juice were determined by calculating the daily amount of juice consumed on average by a 70-kg human male. As a

reference, we used a study with humans who received 480 mL/day[17]. The amount of juice was administered to the rats according to their body weight. The rats received by oral gavage 7 µL of grape juice/g of body weight, twice a day. On day 30, half of the animals received a single intraperitoneal dose of CCl_4 (3 mL/kg). The animals that received CCl_4 (positive control) or animals that received only vegetable oil (vehicle) (negative control) were killed 6 hours later by decapitation. The dose of CCl_4 was previously chosen on the basis of pilot experiments, which indicated that this dose demonstrated significant increase in protein and lipid oxidative damage and animals survival of 100% [18].

The whole blood was colleted with heparin, one part was for Comet assay, the other part was centrifuged and the plasma isolated and stored until analysis (TBARS, CAT e SOD activities). Brain structures (cerebral cortex, hippocampus, striatum and substantia nigra) and liver were isolated and stored at -70 ºC until analysis (TBARS, CAT e SOD activities).

2.5 Oxidative stress parameters

As an index of lipid peroxidation, we used the formation of thiobarbituric acid reactive species (TBARS) during an acid-heating reaction, which is widely adopted as a sensitive method for measuring lipid peroxidation, as previously described [19]. Antioxidant enzyme assays were performed in plasma and tissue homogenates, as previously described [20]. Catalase (CAT) activity was assayed by measuring the rate of decrease in H_2O_2 absorbance at 240 nm, and the results are expressed in UCAT/g protein [21]. Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline autoxidation, as previously described, and the results are expressed in USOD/mg protein [22].

2.6 Comet assays (CA)

The standard protocol for preparation and analysis of the CA followed Tice et al [23]. The slides were prepared by mixing 5 μL whole blood, collected in the group that received only vehicle (saline, conventional or organic grape juices) with 90 μL low melting point agarose (0.75%). The mixture (cells/agarose) was added to a fully frosted microscope slide coated with a layer of 300 μL of normal melting agarose (1%). After solidification, the cover slip was gently removed and the slides were placed in lyses solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5, with freshly added 1 % Triton X-100 and 10 % dimethyl sulfoxide [DMSO]) for a minimum of 1 h and a maximum of 7 days. Subsequently, the slides were incubated in freshly prepared alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 12.6) for 20 min. The DNA was electrophoresed for 20 min at 25 V (0.90 V/cm) and 300 mA; the buffer was neutralized with 0.4 M Tris (pH 7.5). Finally, the DNA was stained with silver nitrate [24]. The slides were coded for blind analysis. In order to ensure adequate eletrophoresis conditions and efficiency, negative and positive controls (human blood) were used for each experiment. Images of 100 randomly selected cells (50 cells from two replicated slides) were analyzed from each animal. Cells were also scored visually according to tail size into five classes, from no tails (0), to maximally long tails (4), resulting in a single DNA damage score for each subject, and consequently for each study group. The results were expressed in damage class frequency (%) [25].

2.6 Statistical Analysis

The statistical analysis was done by means of analysis of variance (ANOVA) and Tukey's test using the SPSS 12.0 package. All tests were performed in duplicate. Pearson's correlation coefficient was used to test correlation between polyphenol content and the results of the assays.

3. Results

It can be seen in the Table 1 that the organic grape juice present a higher levels of total phenolic compounds , resveratrol and catechin than those observed in conventional purple grape juice ($p<0.05$). It was observed that CCl_4 induced an increase in lipid peroxidation in all tissues (Table 2) when compared to the control group ($p<0.05$). The chronic treatment with both grape juices reduces peroxidation levels in the cerebral cortex and hippocampus (Table 2). In the substantia nigra and striatum, only the organic grape juice was capable of reducing the CCl_4-induced peroxidation levels. In plasma (Table 2) only the conventional grape juice group showed this decrease. If considering all the groups that received the vegetable oil (vehicle), both grape juices were capable of reducing TBARS levels in cerebral cortex when compared to the control (saline). In substantia nigra only the organic was able to reduce the TBARS levels, and in plasma, only the conventional (Table 2).

CCl_4 induces a reduction in SOD activity in cerebral cortex, hippocampus, plasma and liver (Table 3). However, in substantia nigra, this activity increased when compared to the control ($p<0.05$). The SOD activity depletion was restored by both grape juices in hippocampus, however only the conventional grape juice restored this activity in plasma. In the striatum, CCl_4 did not alter the values when compared with the control; however, if comparing the CCl_4 group with the grape juice groups it was observed that both grape juices reduced SOD activity. Considering only the vehicle groups both grapes juices increased SOD activity in substantia nigra and liver. However, in hippocampus and cerebral cortex only the conventional group increased this enzyme activity, while in striatum and plasma the organic group showed the highest level ($p<0.05$) (Table 3).

The group that received CCl_4 showed a significant reduction in CAT activity in the striatum and plasma (Table 4). However, in the plasma only the organic grape juice was

capable to increase CAT activity. In cerebral cortex, substantia nigra and hippocampus the CCl_4 provoked an increase in CAT activity. In substantia nigra, both grape juice were able to reduce this increase, but the organic juice was better (reduced more). In hippocampus, only the organic juice was able to reduce this raise.

If considering the vehicle groups, it was observed that both grape juice increased CAT activity in cerebral cortex and substantia nigra when compared to the control ($p<0.05$); in the plasma the organic grape juice was capable to increase this enzyme activity. However, in the striatum both grape juices reduced CAT activity (Table 4).

In the striatum, we observed a negative correlation between CAT activity and total phenolic content and resveratrol ($r=-0.492$ and $r=-0.328$, $p<0.05$), respectively. We also observed a significant increase of SOD/CAT ratio in the liver of rats that received CCl_4. However, in this tissue, only the organic grape juice decreased these levels (Table5). In cortex, both grape juices were capable to reduce this parameter if comparing with the vehicle or CCl_4 group.

In Cometa assay the internal controls (human blood) showed low damage in the negative control [Damage 1 (12%); Damage 0 (88%)] and, high damage in positive control-MMS [Damage 4 (15%), 3 (22%), 2 (32%), 1(19%) and 0 (19%). In Figure 1, it was observed that CCl_4 was capable to induce DNA damage identified by the increase of damage 4 and 3 frequencies if comparing with the control group (received only the oil). And in the groups that received the grape juice, it was observed a significative reduction of the damage 4, 3 and 2 frequencies. However this reduction was more expressive in the organic group.

4. Discussion

Polyphenol-rich food with health benefits has become a more common element in food marketing[26,27]. In a recent study, purple grape juice was the beverage that showed the highest antioxidant properties *in vitro*, higher than e.g. orange, açai and apple juices[28]. The antioxidant activity was also observed in healthy individuals who received 100 mL/day of purple grape juice during 14 days. These individuals showed an increase in plasmatic antioxidant activity[28]. Grape juice benefits were also demonstrated in a previous study, with younger rats (2 moths). We observed that chronic treatment with grape juice was able to reduce lipid peroxidation and protein oxidation levels in liver, plasma and brain structures after CCl_4 injection[8,9]. Indeed, it was observed that the organic juice was capable of reducing lipid peroxidation in the plasma and substantia nigra more significantly than the conventional juice. This was also observed in protein oxidation, where the organic juice showed better results in the liver and striatum. These protection activities–observed in young rats–could indicate a neuro- and hepatoprotective action of the purple grape juice since the CCl_4 damage was reduced after grape juice intake[8,9].

Considering that ageing is a process where there is an accumulation of oxidative damage and that oxidative stress is considered a risk factor and a contributor to age-related increase of oxidized lipids and proteins[29], this study evaluated the *in vivo* antioxidant and antigenotoxic effects of grape juice on the rat model of CCl_4-induced toxicity, using 7-months old rats, older than the previous study with 2-months old rats[8,9].

The findings of the present study conducted with older rats suggest a reduction of lipid peroxidation in the brain structures and plasma in groups that received the purple grape juices. However, we observed that the organic grape juice was more efficient than the conventional one. The explanation could be the higher total phenolic, resveratrol and catechin contents (Table 1) of this juice. These compounds have been reported in many

studies for their beneficial properties[30]. Although the lipid peroxidation decrease was observed in some structures, it is important to highlight the reduction observed in the hippocampus, because this structure is one of the brain regions with an ability to generate neurons (neurogenesis), a feature that decreases with ageing[31,32].

When comparing the results obtained in this study with those from assays performed with younger rats[31,32], we observe that the LP levels in older (7 months) rats were higher than those observed in younger rats (2008a). Moreover, it can also be seen that, in young rats, both grape juices were capable of reducing lipid peroxidation induced by CCl_4. These differences might be explained by an inefficient endogenous antioxidant system as found in ageing rats[33] and by the difference in the brain tissues, since the brain is vulnerable to oxidative damage due, amongst others[34], to the high utilization of oxygen and the large amount of easily oxidizable polyunsaturated fatty acid[34]. Although we did not find any correlation between TBARS level and polyphenol content, we may attribute the decrease in the peroxidation level to these compounds, which are present in both juices, but especially in the organic one.

According to Park et al.[17], healthy men who received grape juice (480 mL) daily for 8 weeks showed depletion of free radical levels. Recently, a similar study was conducted, in which either grape juice (10 ml/kg/day) or α-tocopherol (400 IU/day) was given for 2 weeks to healthy subjects who were otherwise on a flavonoid-restricted diet. In both treatments, serum oxygen radical antioxidant capacity (ORAC) and LDL oxidation resistance were increased[35], implying that the grape juice had a possible free radical scavenging effect. More specifically, grape juice supplementation reduced protein oxidation more efficiently than α-tocopherol supplementation. The authors suggested that

grape juice supplementation provided a strong antioxidant activity[35]. Moreover, in another study, purple grape juice was capable of reducing lipid peroxidation induced by cadmium in older (13 months) rats[36].

The free radical damages could be prevented or repaired by the antioxidant defense. In the human body, there are two major enzymes, SOD and CAT. SOD plays a key role in detoxifying superoxide anions, which otherwise damage the cell membranes and macromolecules[37]. CAT has the ability to detoxify H_2O_2 radicals. Release of H_2O_2 promotes the formation of numerous other oxidant species that greatly contribute to oxidative stress leading to pathogenesis[38]. Clinical studies demonstrated a reduction of SOD activity in Parkinsonism associated with other neurodegenerative disorders, showing the key role played by this enzyme in fighting free radicals produced in the brain[39,40]. In our results, we observed that in some tissues (cerebral cortex, hippocampus, liver, and plasma) CCl_4 decreases SOD activity. According to Chang et al [41], the decrease of SOD activity by CCl_4 could result from the consumption of SOD to compensate excessive peroxyl radicals derived from CCl4's metabolite. In a further study, the authors showed that the decreased SOD activity in aged tissue was brought back to near normal level upon grape seed extract administration, perhaps because this extract acts as a potent scavenger of superoxide radicals and metal chelator[42]. The reduction caused by CCl_4 was increased by both grape juices in the hippocampus.

Our current results show that after CCl_4 injection CAT activity was increased in the cerebral cortex, substantia nigra, and hippocampus. This increase was diminished in the substantia nigra in both purple grape juice groups. However, in the hippocampus only the organic juice was capable of reducing the activity of this enzyme. This could be explained by the higher content in phenolic compounds of this juice. When comparing the results

from the young rats' study[8,9], we observe that SOD and CAT activities are higher in the older rats than the younger ones in control groups (saline). This might be explained by the fact that the older rats have a greater oxidative damage, requiring a higher antioxidant activity than the younger ones. This was also observed in the prior study comparing only the vehicle group –that received oil–, which showed that CAT activity was increased in the striatum and liver after chronic intake of purple grape juice, especially in the group that received organic juice. This suggests that a high content in phenolic compounds is capable of increasing the activity of the enzymatic antioxidant defenses. When comparing only the groups that received vehicle, the results of our study showed that both purple grape juices increase CAT activity in the cerebral cortex and substantia nigra. However, in liver, only the organic juice was capable of increasing the activity of this enzyme. This was also observed in young rats[9]. This increase might be explained by the phenolic content and the ability to remove toxic reactive species present in a normal physiological function[43].

When analyzing the results of SOD and CAT activities, we observed that CCl4 brought about a reduction in SOD activity and an increase in CAT activity. This imbalance might explain the increase of oxidative damage, proven by the high LP observed in older rats. SOD/CAT ratio is a very important parameter because an imbalance between these two enzymes of the antioxidant defenses may induce an increase in oxidative stress level and thus lead to the development of many diseases associated with it[44]. SOD activity leads to the production of hydrogen peroxide, which reacts with iron to generate hydroxyl radicals via the Fenton reaction, which in turn are thought to be the most toxic oxygen molecules *in vivo*[33]. CAT could clean up an excess of hydrogen peroxide, thus reducing its oxidative effects. The differences found among all structures could be explained, at least in part, by the fact that, with age, some structures show a greater accumulation of oxidative

damage than others, particularly in the striatum, since this area is especially vulnerable to oxidative stress during ageing. The reason behind it may be that accelerated oxidation of dopamine by monoamine oxidase in the nerve terminals of the striatal neurons increases H_2O_2 production in these neurons[45].

In a recent article, it was observed that the chronic intake of both purple grape juices, but especially the organic juice, reduced the SOD/CAT ratio–increased by the CCl_4 injection–in the striatum and substantia nigra [9]. Indeed, our present study, this ratio decrease was observed in the cerebral cortex, substantia nigra, and liver. However, in liver, only the organic juice was capable of reducing the ratio, and in the substantia nigra, the organic juice also performed best. In agreement with other studies[3,46,47], we observed that the combinations of antioxidant polyphenolics found in fruits and vegetables may show efficacy in ageing. These secondary compounds serve a variety of functions that enhance the plants' survivability. Moreover, they may be responsible for the putative multitude of beneficial effects of fruits and vegetables on health-related issues[48].

Purple grape juice– organic and conventional –were capable of reducing DNA damage caused by CCl_4 in older (7 months) rats. Indeed, our findings suggest that the purple grape juices were capable of reducing the high damage frequency when compared with the control that received only vegetable oil. In this sense, Park et al.[17] showed by Comet assay that purple *Vitis vinifera* grape juice was capable of reducing DNA damage in healthy individuals. This protection – that the compounds present in grape juice can inhibit the oxidative damage to DNA – has already been reported by Ferguson[6]. Comet assay conducted in other studies with fruits and their juices, e.g. orange juice, furnished evidence of important antigenotoxic activity in rats' blood[49, 50,51].

Although further research is necessary, our study shows that a supplementation with grape juice could at least help preventing or decreasing the damages caused by oxidative stress, especially those associated with ageing, as Parkinson's and Alzheimer's diseases, and others.

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Legend to figure

Figure 1. Damage class frequency in whole blood rats that received both grape juices, organic and conventional, comparing with control.

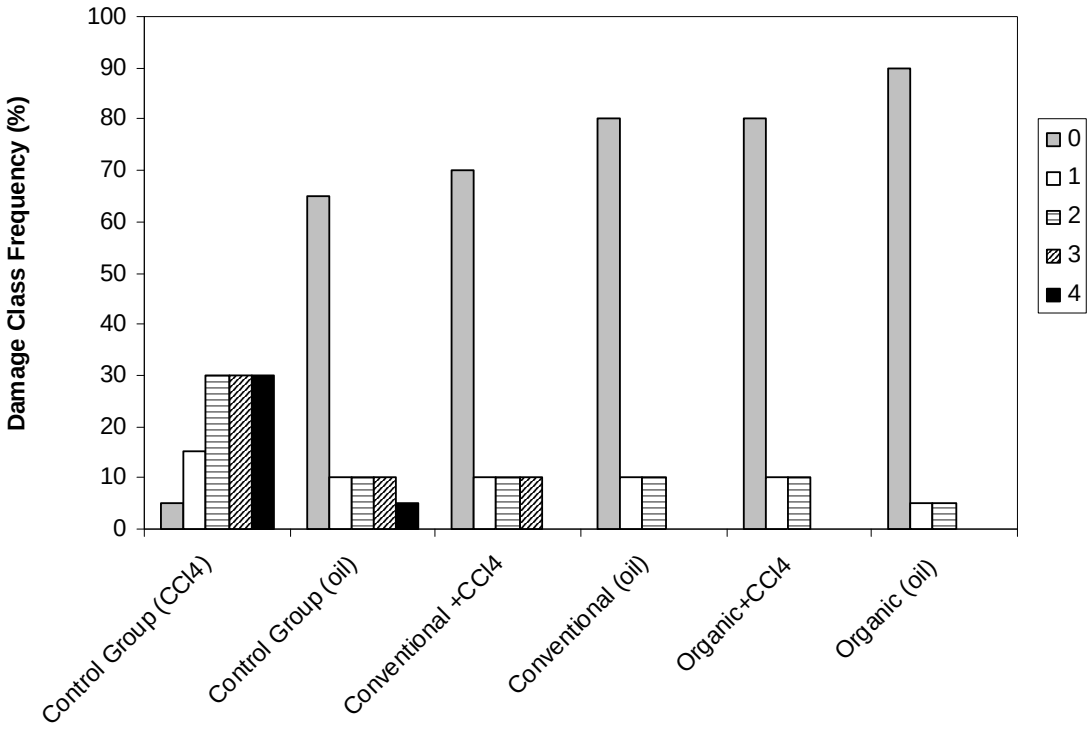

Table 1. Total phenolic content (mg catechin/mL), resveratrol and catechins amount (ppm) in grape juices (organic and conventional)

Grape juices	Total phenolic compounds (mg catechin/ mL)	Resveratrol (ppm)	Catechins (ppm)
Organic	271.5±12.02	0.214±0.0023	36.58±0.020
Conventional	120.67±1.87*	0.073±0.0021*	24.13±0.001*

*p<0.05 between the juices.

Table 2. TBARS level (nmol/ml) in the cerebral cortex, substantia nigra, hippocampus, striatum, liver and plasma of rats treated with conventional and organic grape juices.

Treatments	Cerebral Cortex	Substantia nigra	Hippocampus	Striatum	Liver	Plasma
Control	4.55±1.58	2.06±0.04	1.91±0.10	1.43±0.08	2.55±0.19	69.06±5.07
CCl4	7.46±2.56*	2.53±0.73*	14.00±0.10 *	2.82±0.77*	5.27±1.19 *	80.55±6.69 *
Conventional	1.41±0.62*(**)	3.02±1.09*	2.90±0.41 (**)	2.90±0.74*	6.27±1.09 *	51.03±2.06 * (**)
Conventional + CCl4	0.98±0.04*(**)	2.81±0.44*	7.85±1.56 *(**)	2.12±0.50*	4.10±2.38 *	62.06±7.68 (**)
Organic	2.62±0.74*(**)	2.10±0.48*(**)#	2.45±0.36 *(**)	1.62±0.18**	5.70±1.23 *	71.82±6.00 *#
Organic + CCl4	3.81±0.64* (**)#	2.17±0.45*(**)#	3.85±0.54*(**)#	1.30±0.27**	6.33±1.01*	82.75±0.65*#

*p<0.05, different from control, ** p<0.05, different from CCl4, # p<0.05 different between grape juices treatments Control rats received saline. Data are mean + SD values.

Table 3. Superoxide Dismutase (USOD/mg protein) activity in the cerebral cortex, substantia nigra, hippocampus, striatum, liver and plasma of rats treated with conventional and organic grape juices

Treatments	Cerebral Cortex	Substantia nigra	Hippocampus	Striatum	Liver	Plasma
Control	62.11±12.35	4.33±0.24	15.03±1.21	207.2±84.84	33.93±0.90	23.84±3.82
CCl4	35.92±4.09 *	21.64±5.84 *	6.93±0.14 *	187.5±59.41	30.93±1.94 *	5.85±0.69 *
Conventional	130.05±35.00 *(**)	119.05±24.45 *(**)	22.08±1.01 *(**)	36.85±5.62 *(**)	42.70±2.42 *(**)	11.27±0.32 *(**)#
Conventional + CCl4	22.01±6.00*	32.32±6.08 *(**)	95.23±10.95 * (**)	14.96±4.18 *(**)	18.55±5.30 *(**)	7.72±0.85 * (**)#
Organic	45.71±1.63 (*)#	31.22±1.54 *(**)#	6.39±0.72 *#	354.19±103.3#	43.93±2.35 *(**)	45.15±1.78 *(**)
Organic + CCl4	11.00±1.20 * (**)	27.30±1.56 *(**)	26.78±9.18 **(#)	91.16±23.84 *(**)#	12.04±1.19 *(**)#	4.95±1.10 *

*p<0.05, different from control, ** p<0.05, different from CCl4, # p<0.05 different between grape juices treatments Control rats received saline. Data are mean + SD values.

Table 4. Catalase activity (UCAT/mg protein) in the cerebral cortex, substantia nigra, hippocampus, striatum, liver and plasma of rats treated with conventional and organic grape juices

Treatments	Cerebral Cortex	Substantia nigra	Hippocampus	Striatum	Liver	Plasma
Control	1.62±0.31	3.19±1.52	1.78±0.55	161.08±48.2	2.95±0.65	3.08±0.50
CCl4	3.72±0.29	9.72±3.97	33.61 ±13.50*	26.03±6.60*	3.04 ±0.70	0.85 ±0.23*
Conventional	62.67±23.26*(**)	8.18±2.68	1.08±0.31 (**)	13.62±3.66*	2.05±1.26	5.30±1.35 (**)
Conventional + CCl4	11.99±4.40*(**)	3.39±0.95(**)	29.02±2.23 *	1.95±0.67*(**)	2.10±1.26	1.42±0.28 *
Organic	3.11±0.25*(#)	7.74±1.64*(**)	2.94±1.11**	29.66±5.07*	5.09±1.06 *(**)#	1.20±0.40*#
Organic + CCl4	3.31±1.13*(#)	0.84±0.13*(**)#	5.03±1.35 (**)#	21.58±5.07*#	8.03±0.84 *(**)#	2.10±0.07 *(**)

*p<0.05, different from control, ** p<0.05, different from CCl4, # p<0.05 different between grape juices treatments Control rats received saline. Data are mean + SD values.

Table 5. SOD/CAT ratio in the cerebral cortex, substantia nigra, hippocampus, striatum, liver and plasma of rats treated with different grape juices

Treatments	Cerebral Cortex	Substantia nigra	Hippocampus	Striatum	Liver	Plasma
Control	38.20±0.46	1.63±1.09	23.22±3.33	6.29±2.59	8.64±1.89	7.75±0.35
CCl4	9.30±0.62*	2.73±0.67	0.60±0.19	10.22±2.80	17.40±2.22*	7.54±1.19
Conventional	0.15±0.02*(**)	13.63±1.92	20.1±0.62**	4.70±2.21	10.06±2.31*	2.24±0.72
Conventional + CCl4	7.33±1.35*(**)	10.05±1.10	27.0±0.12**	8.55±2.94	11.50±6.7	5.12±0.17
Organic	14.39±1.35*(**)#	4.26±0.80	0.85±0.15*(#)	10.31±2.38	13.25±3.88	42.18±18.11*(**)#
Organic + CCl4	3.80±1.69*(**)#	33.02±9.41*(**)#	2.80±0.48*(#)	7.73±3.17	3.26±1.22*(**)#	16.35±1.62*(**)#

*p<0.05, different from control, ** p<0.05, different from CCl4, # p<0.05 different between grape juices treatments. Control rats received saline. Data are mean + SD values.

3.6 CAPÍTULO VI

ANTIOXIDANT PROTECTION OF RESVERATROL AND CATHECHIN IN *S. CEREVISIAE*

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Neste trabalho, avaliou-se a capacidade do resveratrol e da catequina em proteger a levedura *Saccharomyces cerevisiae* contra conhecidos agentes estressores: peróxido de hidrogênio, tetracloreto de carbono e cádmio. Linhagens da levedura *S. cerevisae* proficiente e deficientes nas defesas antioxidantes (superóxido dismutases, catalase ou glutatione redutase) foram utilizadas. Frente a todos os agentes estressores e em todas as linhagens, ambos os polifenóis aumentaram o percentual de sobrevivência da levedura, embora a proteção tenha sido menor frente ao peróxido de hidrogênio. Entretanto, utilizando linhagens deficientes nas defesas antioxidantes, mostrou-se possíveis mecanismos antioxidantes destes polifenóis. Os resultados propuseram que, provavelmente, a enzima catalase seja a mais envolvida nestes mecanismos.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antioxidant Protection of Resveratrol and Catechin in *Saccharomyces cerevisiae*

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Moderate consumption of red wine reduces the risk of heart disease and extends lifespan, but the relative contribution of wine polyphenols to these effects is unclear. In this work, the capacity of resveratrol and catechin to protect the eukaryotic microorganism *Saccharomyces cerevisiae* against oxidative stress caused by different agents, hydrogen peroxide, carbon tetrachloride, and cadmium, was evaluated. Under all stress conditions, both polyphenols increased tolerance, although their protection was more evident under peroxide exposure. By using mutant strains deficient in specific antioxidant defense systems (superoxide dismutases, catalase, or glutathione), it was observed that increased H_2O_2 tolerance produced by both polyphenols was associated with catalase, as well as the rise in survival rates caused by resveratrol under CCl_4. The acquisition of tolerance was correlated with a reduction in lipid peroxidation, indicating that the antioxidant property of resveratrol and catechin involves protection against membrane oxidation.

KEYWORDS: Catechin; resveratrol; oxidative stress; lipid peroxidation; *Saccharomyces cerevisiae*

INTRODUCTION

Oxidative stress has been correlated with aging and diseases. On the other hand, phytochemicals present in fruits and vegetables may have antioxidant effects that protect from the oxidative damage arising from metabolic and exogenous sources (*1*). Although the protective effects have been primarily attributed to the well-known antioxidants, such as vitamins C and E and β-carotene, plant phenolics also seem to play a significant role. Grapes are rich in phenolic compounds, such as flavonoids (catechin, epicatechin, quercetin, anthocyanins, and procyanidins), and resveratrol (*2*, *3*). Increasing evidence indicates the importance of wine consumption in the daily diet since it is supposed to be one of the explanations for the "French paradox"—the low incidence of heart disease and cancer in France in spite of high fat consumption (*4*).

Resveratrol (3,5,4′-trihydroxystilbene) is one of most important polyphenols found in red wine. It is associated with a surprising number of health benefits, most notably the mitigation of age-related diseases, including neurodegeneration, carcinogenesis, and atherosclerosis (*5*). Catechin is a flavan-3-ol, namely, 2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran) present mainly in white wine (*3*). It has been shown that catechin is very effective in blocking the growth of human cell lines originating from prostate (*6*) and breast (*7*) cancers and is also a potential antioxidant and antimutagenic agent.

Despite the studies that showed the antioxidant properties of resveratrol and catechin, the molecular mechanisms of how they function in vivo remain unclear. These polyphenols show different bioavailabilities (*8*), which make it difficult to determine the antioxidant potential of each one. In addition, their protective effects have been reported to be more pronounced in vitro, using high, nonphysiological concentrations (*9*).

The bioavailability appears to differ greatly between the various polyphenols, and the most abundant polyphenols in our diet are not necessarily those that have the best bioavailability profile (*8*). Recently, we found that Wistar rats that consumed purple grape juice daily showed protection against oxidative stress, but the results obtained did not allow us to state which polyphenol was absorbed most or gave the best protection (*2*).

The aim of this work was to evaluate the mechanism by which resveratrol and catechin protect *Saccharomyces cerevisiae*, a useful model to screen in vivo for natural antioxidants: Its entire genome sequence has been elucidated, and it is a genetically tractable organism, amenable to modifications such as gene disruption or mutation, which facilitates the identification of gene targets of chemicals or drugs or stress, such as oxidative stress, response pathways (*10*). *S. cerevisiae* has similar antioxidant responses to mammals, and 30% of known genes involved in human disease have yeast orthologues, that is, functional homologues (*11*). Furthermore, by using this microorganism, the differences in bioavailability of polyphenols would be discarded. We tested different oxidative stresses, generated by carbon tetrachloride, hydrogen peroxide, or cadmium. The toxicity of CCl_4 results from its reductive dehalogenation by cytochrome P450 into trichloromethyl free radical, which readily

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interacts with molecular oxygen to form trichloromethyl peroxyl radicals (*12*). Both radicals are able to attack proteins and lipids or to remove hydrogen atoms from unsaturated lipids leading to membrane lipid peroxidation, cellular dysfunction, and finally cell necrosis (*13*). Cadmium is an environmental carcinogenic pollutant that inactivates several proteins involved in DNA repair systems and creates an oxidative stress that can result in additional DNA lesions (*14*). H_2O_2 can generate hydroxyl radical, the most reactive and toxic reactive oxygen species (ROS) (*15*).

MATERIALS AND METHODS

Chemical Reagents. H_2O_2 was purchased from Merck; dimethyl sulfoxide (DMSO), resveratrol, and catechin were acquired from Sigma-Aldrich. Media components were obtained from Difco.

***S. cerevisiae* Strains and Growth Conditions.** Wild-type (WT) strain BY4741 (*MATa*, *his3*, *leu2*, *met15*, and *ura3*) and its isogenic mutants *sod1*Δ, *sod2*Δ, *ctt1*Δ, and *gsh1*Δ, harboring the genes *SOD1*, *SOD2*, *CTT1*, or *GSH1*, respectively, interrupted by gene *KanMX4*, were acquired from Euroscarf (Frankfurt, Germany). Stocks of yeast strains were maintained on solid 2% YPD (1% yeast extract, 2% glucose, 2% peptone, and 2% agar); in the case of the mutant strains, the medium also contained 0.02% geneticine. For all experiments, cells were grown in liquid YPD medium using an orbital shaker at 28 °C and 160 rpm with the ratio of flask volume/medium of 5/1.

In Vivo Antioxidant Analysis. Yeast cells at the midlong phase (10^6 cells/mL) were reinoculated in fresh medium (the initial cell concentration was 10^5 cells/mL), containing or not the antioxidant agent (10 μg/mL catechin or resveratrol), and incubated, for 1 h, at 28 °C/160 rpm. To choose the doses of the polyphenols used in the adaptive treatments, cells were exposed to increased concentrations of resveratrol or catechin and then spotted adjacently on YPD agar plates incorporating CCl_4, peroxide, or cadmium. The concentration chosen was the lowest that could improve cell growth as compared to cohorts exposed to stress without being treated with polyphenol. Both cultures, treated or not with polyphenol, were subjected to oxidative stress (2.5 mM H_2O_2, 10 mM CCl_4, or 2.5 mM $CdSO_4$) at 28 °C/160 rpm for 1 h. Cell viability was analyzed by plating, in triplicate, on solid YPD medium, after proper dilution. Plates were incubated at 28 °C for 72 h, and the colonies were counted. The number of colonies in each plate was between 150 and 200. Tolerance was expressed as a percentage of survival (*16*).

Detection of Lipid Peroxidation. Cells (50 mg) were centrifuged at 2000*g* for 2 min and washed twice with distilled Millipore purified water. The pellets were resuspended in 0.5 mL of 10% trichloroacetic (w/v), and 1.5 g of glass beads was added. The samples were lysed by six cycles of 20 s agitation on a vortex followed by 20 s on ice. Extracts were centrifuged at 2000*g* for 3 min, and the supernatant was mixed with 0.1 mL of 0.1 M EDTA and 0.6 mL of 1% (w/v) thiobarbituric acid in 0.05 M NaOH. The reaction mixture was incubated in a boiling water bath for 15 min, and after the mixture was cooled, the absorbance was measured at 532 nm (*17*).

Intracellular Oxidation. The oxidant-sensitive probe 2′7′-dichlorofluorescein diacetate was used to measure intracellular oxidation (*18*). Fluorescence was measured using a Photo Technology International (PTI) spectrofluorimeter set at an excitation wavelength of 504 nm and an emission wavelength of 524 nm. A fresh 5 mM stock solution of 2′,7′-dichlorofluorescein diacetate dissolved in ethanol was added to the culture (the final concentration was 10 μM) and incubation at 28 °C continued for 15 min to allow uptake of the probe. The culture was divided according to treatment (with or without polyphenol). After 1 h, the oxidative agent (CCl_4, Cd^{2+}, or H_2O_2) was added. Thereafter, 50 mg of cells was harvested by centrifugation and washed twice with water. The pellets were resuspended in 500 μL of water, and 1.5 g of glass beads was added. The samples were lysed by three cycles of 1 min agitation on a vortex mixer followed by 1 min on ice. The supernatant solutions were obtained after centrifugation at 25000*g* for 5 min and diluted 6-fold with water, and then, the fluorescence was measured. As a control, the fluorescence was analyzed in cells that had not been exposed to oxidative stress.

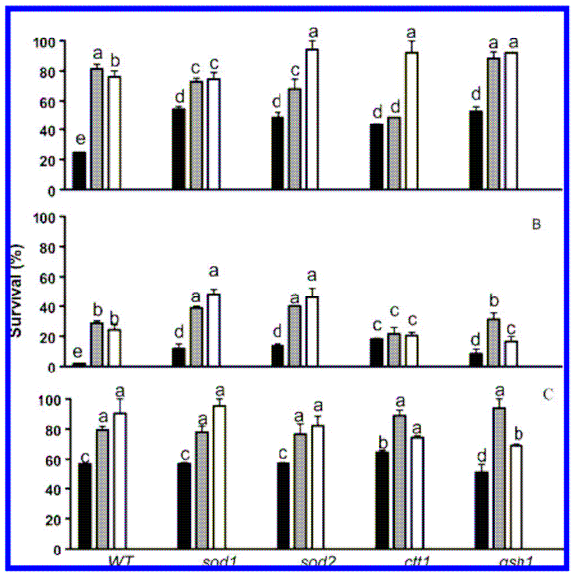

Figure 1. Effect of resveratrol and catechin on survival rates of cells stressed with 10 mM CCl_4 (**A**), 2.5 mM H_2O_2 (**B**), or 2.5 mM $CdSO_4$ (**C**). Black bars mean that cells were directly stressed, gray bars mean that cells were adapted with resveratrol and stressed, and white bars mean that cells were adapted with catechin and stressed. Data represent the means ± SDs of at least three independent experiments. Each stress was analyzed separately to determine statistical differences (different letters mean statistically different results in each oxidative stress group; $p < 0.05$).

Statistical Analyses. The statistics were done by means of analysis of variance and Tukey's test using the SPSS 12.0 package. The latter denotes homogeneity between experimental groups at $p < 0.05$. In all figures and tables, different letters mean statistically different results.

RESULTS AND DISCUSSION

Resveratrol and Catechin Increase Oxidative Stress Tolerance in *S. cerevisiae*. According to **Figure 1**, 2.5 mM H_2O_2 was the most toxic stress for all strains tested, followed by CCl_4 and Cd^{2+}. This result was expected since H_2O_2 generates the most toxic and highly reactive hydroxyl radical, against which the organisms have no defense (*15*). Cells of the WT strain acquired tolerance to all stresses when preadapted with resveratrol or catechin. The increased tolerance caused by each polyphenol was similar, and the best protection was observed under peroxide.

Molecular studies have revealed that phenolics can modulate the cell response by interacting with a wide spectrum of molecular targets, such as protein kinases, transcription factors NF-κβ and c-JUN, and antioxidant detoxifying enzymes (*5*). To investigate the involvement of some antioxidant defense system in the mechanism of acquisition of tolerance, mutant strains deficient in superoxide dismutase, catalase, or glutathione synthesis were used. Free radical scavenging enzymes such as catalase and superoxide dismutase are the first line of cell defense against oxidative injury. The equilibrium between these enzymes is a major process for the effective removal of ROS (*19*). *S. cerevisiae* possesses two isoforms of superoxide dismutase (the cytosolic Sod1 and the mitochondrial Sod2) and two isoforms of catalase, although only the cytosolic Ctt1 seems

Table 1. Effect of Resveratrol and Catechin on Lipid Peroxidation

stress	treatment	WT	*ctt1*
CCl_4	without polyphenol	1.5 ± 0.2[a] a[b]	1.6 ± 0.1 a
	resveratrol	0.9 ± 0.1 b	0.5 ± 0.0 c
	catechin	0.9 ± 0.1 b	1.4 ± 0.0 d
H_2O_2	without polyphenol	2.6 ± 0.1 a	2.7 ± 0.1 a
	resveratrol	1.1 ± 0.1 b	1.5 ± 0.0 c
	catechin	1.2 ± 0.1 b	1.0 ± 0.2 b
Cd	without polyphenol	1.2 ± 0.1 a	1.4 ± 0.1 c
	resveratrol	0.8 ± 0.1 b	0.4 ± 0.0 d
	catechin	0.6 ± 0.0 b	0.7 ± 0.2 b

[a] The results were expressed as a ratio between lipid peroxidation levels of stressed, adapted or not with polyphenol, and nonstressed cells. Data represent the means ± SDs of at least three independent experiments. [b] Each stress was analyzed separately to determine statistical differences (different letters mean statistically different results at $p < 0.05$).

to be directly involved in oxidative stress (*20*). The second line of defense consists of nonenzymatic scavengers. The most important one is glutathione (GSH), present from bacteria to higher eukaryotes and whose synthesis in *S. cerevisiae* requires the enzymes Gsh1 and Gsh2 (*21*).

Under our experimental conditions, cells fermented glucose, and thus, some of the intracellular antioxidants were absent or present at very low concentrations. Catalase and superoxide dismutase activities and glutathione levels increase significantly only when cells are breathing (*22*). Therefore, although we did not test it in this study, we can assume that if a mutant strain deficient in a specific antioxidant system is not able to acquire tolerance after the adaptive treatment, this might mean that the protection mechanism caused by polyphenol involves the induction of this antioxidant.

Catechin was capable of increasing the tolerance of all mutant strains to CCl_4, indicating that both Sods, Ctt1, and GSH are not necessary for this adaptive treatment (**Figure 1A**). On the other hand, the protection caused by resveratrol against CCl_4 seems to need the cytosolic catalase, since the *ctt1* strain was not able to acquire tolerance when adapted with resveratrol. Ctt1 seems also to be involved in the protection mechanism achieved by both polyphenols against H_2O_2 (**Figure 1B**). Against cadmium stress, both polyphenols were capable of increasing the survival rates of all mutants, although in the *gsh1* strain the protection caused by catechin was slightly less effective than in WT (**Figure 1C**).

Levels of Lipid Peroxidation and Intracellular Oxidation. Next, intracellular oxidation and lipid peroxidation were analyzed to understand how catechin and resveratrol protect cells against the oxidative damage caused by CCl_4, H_2O_2, and Cd^{2+}. One of the targets of free radical attack is the membrane, leading to lipid peroxidation, cell leakage, and death. **Table 1** shows that all stresses increased the levels of lipid peroxidation. Both polyphenols showed similar capacities of reducing the oxidative damage to membrane. Peroxide stress was most aggressive to the membrane, which is in accordance with the low survival rates (**Figure 1**). However, the treatment with polyphenols practically inhibited the increase in lipid peroxidation caused by peroxide in WT (the increase fell from 160% to around 10%). CCl_4 and Cd^{2+} produced a more modest increase in lipid peroxidation (50 and 20%, respectively), which was suppressed by resveratrol and catechin. The protection conferred by resveratrol and catechin against membrane oxidation appears to be directly correlated to the acquisition of tolerance, since the greatest increase in survival rates was reached during peroxide exposure. While the phenols increased almost 20-fold the tolerance to peroxide, reducing to around 10% the rise in

Table 2. Enhancement of Intracellular Oxidation Produced in Response to Peroxide or Carbon Tetrachloride Stress

stress	rreatment	WT	*ctt1*
CCl_4	without polyphenol	4.9 ± 0.1[a] a[b]	2.0 ± 0.1 b
	resveratrol	3.3 ± 0.4 c	2.6 ± 0.0 d
	catechin	3.4 ± 0.3 c	0.8 ± 0.1 c
H_2O_2	without polyphenol	12.2 ± 0.3 d	1.6 ± 0.1 b
	resveratrol	4.6 + 0.3 c	1.9 + 0.4 b
	catechin	10.2 ± 0.5 a	2.1 ± 0.2 a

[a] The results were expressed as a ratio between fluorescence of stressed, adapted or not with polyphenol, and nonstressed cells. Data represent the means ± SDs of at least three independent experiments. [b] Each stress was analyzed separately to determine statistical differences (different letters mean statistically different results at $p < 0.05$).

the lipid peroxidation levels, under carbon tetrachloride and cadmium stresses, the increase in the survival rates did not exceed 3-fold, and the reduction in the levels of peroxidation was smaller.

Because cytosolic catalase might be involved in the protection mechanism achieved by polyphenols (**Figures 1A,B**), peroxidation was also investigated in the *ctt1* mutant strain. However, the behavior of the mutant was similar to that of the WT strain. In the mutant, both polyphenols were able to reduce the levels of lipid peroxidation caused by all stresses, although the *ctt1* strain had not acquired tolerance after polyphenol treatment and peroxide stress or after catechin adaptation and carbon tetrachloride stress. These results indicate that Ctt1 is not involved in the protection conferred by catechin and resveratrol against lipid oxidation.

Polyphenols exhibit a wide range of biological effects (*23*); many of them have been attributed to their free radical scavenging activity. To determine whether resveratrol and catechin are responsible for the increase in tolerance by decreasing reactive oxidative species concentration, the level of intracellular oxidation was measured by using the fluorescent probe 2′,7′-dichlorofluorescein diacetate. This probe is widely used to evaluate the enhancement of reactive oxidative species after oxidative stress since, once inside the cell, it becomes susceptible to attack by reactive oxidative species, producing a more fluorescent compound (*24*). According to **Table 2**, after direct exposure of the WT strain to peroxide and CCl_4, there was an increase in intracellular oxidation. There was a greater increase after H_2O_2, which can be correlated with the higher sensitivity shown by cells under this stress condition (**Figure 1**). Cell exposure to 2.5 mM Cd_2SO_4 for 60 min did not increase the levels of intracellular oxidation (data not shown).

Similarly to what occurred with lipid peroxidation, both catechin and resveratrol decreased the levels of reactive oxidative species produced by peroxide or carbon tetrachloride (**Table 2**). After resveratrol treatment, the levels of reactive oxidative species produced in response to peroxide were almost 3-fold lower, suggesting that resveratrol has a high capacity to eliminate hydroxyl radicals formed by a Fenton reaction. In the absence of cytoplasmic catalase, the levels of reactive oxidative species produced by carbon tetrachloride were only reduced when the mutant was adapted with catechin, the same treatment that led to acquisition of tolerance of *ctt1* strain (**Figure 1A**). In the mutant, neither catechin nor resveratrol was able to reduce the increase in the levels of intracellular oxidation caused by peroxide; coincidentally, neither treatment increased the tolerance of this strain (**Figure 1B**). Considering these results, Ctt1 seems to contribute to the elimination of reactive oxidative species achieved by resveratrol under CCl_4 and H_2O_2 stresses as well as by catechin under H_2O_2 exposure.

The mutant deficient in Ctt1 showed an increase in intracellular oxidation caused by CCl_4 and H_2O_2 lower than the WT strain. This could be associated with super expression of other antioxidant systems as a form of compensation. Several other studies have shown that a deficiency in one antioxidant system is overcome by an increase in the remaining defense system (*25, 26*).

The medicinal actions of resveratrol and catechin are mostly attributed to their antioxidant capacity and free radical scavenging potential, since oxidative stress is involved in aging as well as in the onset and evolution of more than 100 diseases (*27*). However, the true antioxidant effect of these polyphenols and the mechanisms by which they protect the organisms against oxidative stress have not yet been elucidated. The antioxidant potential of catechin and resveratrol has been investigated mainly through in vitro analyses, although several studies have shown that phenolics are extensively metabolized in vivo, resulting in significant alteration in their redox potentials (*28*). Therefore, it is essential to screen the efficacy of these compounds in vivo. The physiological significance of dietary antioxidants depends on their absorption and biotransformation mechanism. In animal models, it is more difficult to interpret the results, which warrants further investigation on the bioavailability of the polyphenols.

As previously observed (*25, 29, 30*), peroxide, carbon tetrachloride, and cadmium stresses caused damage in *S. cerevisiae* cells. Peroxide and carbon tetrachloride produced free radicals, verified by the increase in the levels of intracellular oxidation, and all stresses tested were able to attack the membrane, leading to lipid peroxidation. Both resveratrol and catechin increased the tolerance to all oxidative conditions, and their protective effects were similar (**Figure 1**).

Resveratrol and catechin reduced intracellular oxidation and lipid peroxidation, which could explain why cells acquired tolerance when adapted with these polyphenols. By reducing the ROS level, biomolecules become less prone to oxidation. The prevention of low-density protein (LDL) oxidation seems to protect against heart diseases, while the prevention of DNA oxidation diminishes genomic instability and the chances of developing cancer (*31*). Heart disease and cancer are the two leading causes of death worldwide.

The antioxidant properties of polyphenols seem to be associated with their capacity to donate hydrogen to free radicals, leading to the formation of stable molecules. Resveratrol and catechin reduced the levels of ROS produced in response to H_2O_2 or CCl_4 (**Table 2**). These stresses generate different free radicals, which contribute to the increase in the levels of intracellular oxidation. Cadmium stress, achieved by submitting cells to 2.5 mM $CdSO_4$ for 1 h, did not increase the level of intracellular oxidation but did induce lipid peroxidation (**Table 1**). The toxicity of this metal is associated with its attack against a membrane. Polyphenols are preferentially incorporated into membrane lipid bilayers and act as hydrogen donors, trapping free radicals and inhibiting the formation of lipid radicals (*5*). According to our results, both resveratrol and catechin reduced the level of lipid peroxidation caused by peroxide, carbon tetrachloride, and cadmium (**Table 1**).

While there has been a major focus on the antioxidant properties, there is an emerging view that polyphenols, and their in vivo metabolites, may affect signaling pathways that modulate cell response (*5*). According to the literature, the addition of polyphenols to commonly used cell culture media leads to the generation of substantial amounts of hydrogen peroxide (*32*). Such H_2O_2 generation could explain the increased tolerance to oxidative stress after adaptive treatments with polyphenols. Several studies show that treatment of yeast (and even human cells) with low concentrations of H_2O_2 induces adaptive responses, which protect cells from the lethal effects of a subsequent challenge with higher concentrations of oxidants (*25*). In silico data mining with Yeast Microarray Global Viewer (*33*) revealed that peroxide treatment preferentially activates genes involved with H_2O_2 degradation, such as *CTT1*.

According to our results, in the *ctt1* mutant strain, pretreatment with resveratrol or catechin did not increase tolerance to peroxide, nor did resveratrol induce tolerance to CCl_4. Catalase activity is very low in cells that are fermenting but increases linearly over a wide range of H_2O_2 concentrations, thereby maintaining a controlled intracellular peroxide concentration and avoiding oxidative damage to membranes, one of the main causes of several diseases and aging (*15*). Taken together, these results suggest that high levels of ROS could be reduced after resveratrol and catechin treatment, presumably by the activation of cellular defenses, like Ctt1. Therefore, we can conclude that if the same concentration of polyphenol is used and ignoring the differences in metabolism and permeability, both resveratrol and catechin achieved excellent protection against oxidative stress, which has been implicated in the etiology and progression of several acute and chronic disorders.

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3.7 CAPÍTULO VII

GRAPE JUICE: ITS COMPOUNDS AND HEALTH BENEFITS

Capítulo enviado para uma série de livros: Researches in Bioactive Natural Products

Vários estudos na literatura têm mostrado que o suco de uva é um composto com importante atividade benéfica a saúde e com um considerável conteúdo de polifenóis. Sendo assim, neste capítulo procurou-se fazer uma revisão da literatura científica internacional, relatando os principais compostos fenólicos presentes nos sucos de uva produzidos a partir de diferentes espécies (*Vitis labrusca* e *Vitis vinifera*), bem como de diferentes cultivares (brancas e tintas).

GRAPE JUICE: ITS COMPOUNDS AND HEALTH BENEFITS

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ABSTRACT

Experimental data have increasingly suggested that cellular oxidative damage has a relevant pathophysiological role in several types of human diseases, such as atherosclerosis and cancer (Ames *et al.*, 1993). In order to minimize oxidative stress our cells have developed a complex biochemical redox mechanism, consisting of both enzymatic and non-enzymatic components (Park *et al*., 2003). Moreover, the diet, especially the consumption of fruits and vegetables, also has an important role in the maintenance of physiological redox equilibrium. These foods supply several antioxidants, including several polyphenolic compounds to the body. Grapes are rich in phenolic compounds, such as flavonoids (catechin, epicatechin, quercetin, anthocyanins, procyanidins), and resveratrol (3,5,4`-trihydroxy-stilbene), which are mainly found in red grape products (Wang *et al*., 2002; Soleas *et al*., 1997; Fuleki and Ricardo da- Silva, 2003). In this chapter we review the main constituents of purple and white grape juices and their health benefices. All findings suggest that grape juices induce significant antioxidant, antiplaquetary, antitumoral and antimutagenic activities, and this may be an important issue for further investigations in the area of biochemical functional foods.

Grape juice and its constituents

Grapes are the most widely grown fruit in the world, second to oranges, and represent an essential component in the Mediterranean diet and culture (Olalla et al., 2004). In North America, purple commercial grape juice is primarily made from *Vitis labrusca* cv. Concord grapes. Niagara grapes, another *labrusca*-type cultivar, is responsible for the typical flavor of commercial white grape juice. Both of these cultivars are extensively grown for juice production in the Niagara region of the province of Ontario, Canada (Sun et al., 2001). Wine, grape and grape products contribute to $162 Billion to US economy, according to study by MKF Research LLC of Napa Valley unveiled on Capitol Hill by the Congressional Wine Caucus on January 17 (2007). Research documenting many positive health benefits associated with the consumption of grapes and grape products are increasing the market for these products.

Actually there are several types of grape juices in worldwide markets. At first, grape juice can be manufactured with any variety of grapes, where they reach an appropriate maturation. Grape juices produced in traditional wine countries are elaborated with *Vitis vinifera* grapes, from white or purple cultivars. On the other hand, the Brazilian grape juices are manufactured with *Vitis labrusca* grapes, known as american or hybrid, mainly Bordo and Concord (purple types), Niagara (white ones) and Rose (Goethe) (Rizzon et al., 1998; Dani et al., 2007).

The chemical composition of grape juice slightly differs from the fruit, except for the higher amounts of raw fiber and oil, found in seed. The technology of preparation, mainly related to temperature and extraction time, regulates the solubility and diffusion

intensity of the compounds, from the skin into the must. This is an outstanding influence on the chemical composition and on the type of the final product (Rizzon et al., 1998). In general, white, purple and rose grape juices with different nutritional characteristics and phenolic content can be obtained, although there is little study about it.

Besides the different varieties of grapes, the actual market counts on the conventional and the organic juice classes. This last juice belongs to the organic farming, which is currently practiced worldwide, and does not use chemical substances, such as pesticides and synthetic fertilizers. Some studies have reported differences in phenolic and nutritional contents of fruits (strawberry, peach and plum) growth according to the traditional and organic methods (Asami et al., 2003; Lombardi-Boccia et al., 2004). However, there is no agreement on which method is better and neither on how the agricultural practice could possibly influence the product's final chemical content.

A recent study with eight different types of grape juices, white (Niagara) or purple (Bordo), manufactured with organically- or conventionally-produced grapes assessed polyphenol content of different kinds of juices (Dani et al., 2007). Depending on the agricultural method, organic juices presented higher polyphenol content when compared to juices manufactured with conventionally-grown grapes (Dani et al., 2007). This fact could be explained because phenolic compounds are secondary metabolites produced and accumulated in plant tissues, during stress situation. As pesticides are not used in organic farming, plants are more often attacked by phytopathogensa situation which causes the plant to produce higher amounts of phenolic compounds as a means to defend itself (Soleas et al., 1997).

Different methodologies are applied in grape juice manufacturing. When purple juices are produced, the pulp is heated along with the skin and seed, resulting in a higher incorporation of phenolic compounds into the juice (Fuleki & Ricardo-da-Silva, 2003). Purple grape juices produced with skin heating showed a higher phenolic compound content when compared to white juices (Table 1), and also high carbohydrate and caloric levels (Dani et al., 2007).

Phenolic constituents are very important to enology because they are directly or indirectly related to wine and juice's quality, especially with regard to their color and astringency, and have also nutritional and pharmacological activities (Riberéau-Gayon et al. 2003). The polyphenol structure has at least one aromatic ring, in which (at least), one hydrogen is replaced with a hydroxyl group. They can be classified as flavonoid and non-flavonoid compounds (Riberéau-Gayon et al., 2003; Ferguson, 2001).

Flavonoids include the anthocyans, quercetin, catechin, epicatechin and procyanidins (Ferguson, 2001). Grape juice presents mainly (+)-catechin, (-)-epicatechin and four procyanidins (B1, B2, B3 e B4) (Table 1). The concentration of these compounds can change according to the pressing method (hot or cold maceration), to the cultivar and, to a lesser degree, pasteurization and vintage (Fuleki & Ricardo-da-Silva, 2003). Anthocyanins are responsible for many of the fruit and floral colors observed in nature. In Concord grape juice the major anthocyanins are delphinidin, cyanidin, petunidin, malvidin, and peonidin, in this order of quantity (Wang et al., 2003).

Among the compounds named non-flavonoid, stilbenes, benzoic and cinnamic acid derivatives deserve special attention. Resveratrol, a stilbene, is the major component of the polyphenols from grapes and their products (Sun et al., 2001). It is a phytoalexin present in

grapevines (Flanzy, 2003), which was originally identified as the active ingredient of an Oriental herb (Kojo-kan), used for treatment of a wide variety of diseases including dermatitis, gonorrhea, fever, hyperlipidemia, arteriosclerosis, and inflammation (Sun et al., 2001). Altought non-flavonoids contents, especially resveratrol, are well-known in wines (Fuleki e Ricardo-da-Silva, 2003) there are very few studies about the content of these compounds in grape juices (Table 1), opening an interesting possibility of new studies about this issue.

Phenolic content of different varieties of grape juices, most of them originated from *Vitis labrusca* grapes, are shown in Table 1. It is possible to notice that polyphenol content can be modify according to the variety and the elaboration process of the juices.

1. **Table 1**. Phenolic content of different varieties of grape juices

Specie of cultivar	Variety	Main Characteristic	Catechin (ppm)	Epicatechin (ppm)	Procyanidin B1 (ppm)	Procyanidin B2 (ppm)	Procyanidin B3 (ppm)	Procyanidin B4 (ppm)	Resveratrol (ppm)	Total phenolic content	Authors
Vitis labrusca	Bordo	Conventional purple grape juice	2.06	22.13	1.33	1.83	7.95	4.66	0.075	119.59*	Dani et al., 2007
Vitis labrusca	Niagara	Conventional White grape juice	7.39	5.95	7.53	1.32	13.06	2.45	ND	48.05*	Dani et al., 2007
Vitis labrusca	Bordo	Organic Purple grape juice	33.89	2.72	7.53	2.32	10.03	0.64	0.213	262.50 *	Dani et al., 2007
Vitis labrusca	Niagara	Organic White grape juice	0.90	1.81	3.45	1.58	18.5	3.59	ND	60.20*	Dani et al., 2007
Vitis labrusca	Concord	Conventional Purple grape juice	5.53	6.89	18.03	11.61	1.53	1.01	ND	145.81*	Fuleki and Ricardo-da-Silva, 2003
Vitis vinifera	Vincent	Conventional White grape juice	18.41	33.11	32.14	17.99	6.55	11.12	ND	ND	Fuleki and Ricardo-da-Silva, 2003
Vitis labrusca	Niagara	Conventional white grape juice l	0.98	1.07	ND	0.35	0.04	0.16	ND	32.81 **	Fuleki and Ricardo-da-Silva, 2003
Vitis labrusca	Concord	conventional purple grape juice	ND	ND	ND	ND	ND	ND	ND	2.06***	Seeram et al., 2008
Vitis vinifera	NI	Conventional White grape juice	ND	ND	ND	ND	ND	ND	ND	327**	Frankel et al., 1998
Vitis labrusca	Concord	Conventional Purple grape juice	ND	ND	ND	ND	ND	ND	ND	1742**	Frankel et al., 1998
Vitis labrusca	Concord	conventional Purple grape juice concentrate	ND	ND	ND	ND	ND	ND	ND	977 ***	Singletary et al., 2003

ND = not determined; * in mg catechin/mL; ** mg of galic acid/ L; *** mg of gal

Grape juice and its health benefits

It has been already reported that grape juice can prevent: (i) platelet aggregation, (ii) LDL oxidation and oxidative damage to DNA, (iii) coronary disease and atherosclerosis (Table 2). The most studied biological effect of the grapes juices is their antioxidant activity, which can be observed in in vitro, ex vivo and in vivo assays. In in vitro and ex vivo assays, purple grape juices, mainly the organic ones, showed a better antioxidant activity, which is positively correlated to resveratrol, catechin, and total phenolic contents (Dani et al., 2007; Ferguson, 2001). On the other hand, in *in vivo assays (*using the *Saccharomyces cerevisiae* yeast model), white juices present a better protection activity against damages generated by hydrogen peroxide. Among the purples grape juices, the organic ones showed a better antioxidant activity, which is positivelly correlated to resveratrol content (unpublished data from our group).

The disparities related to results obtained through *in vitro* and *in vivo* assays could be attributed, at least, in part, to phenols metabolism. I*n vivo* antioxidant effects depend on polyphenols bioavailabity and metabolism (Vinson et al., 2004), which can be influenced by their structure, absorption and interaction with other compounds (Manach et al. 2004).

Oxidative stress is considered as a major risk factor that contributes to age-related increase in lipid peroxidation and declined antioxidants in the central nervous system during aging (Balu et al., 2005). Several reports have shown that long term polyphenols supplementation improves cognitive performance in old Wistar rats, mainly because the capacity to these polyphenol in prevent the oxidative stress damage (Joseph et al., 1999; Bastianetto & Quirion, 2002).

The results from a study of Barbara Shukitt-Hale et al., (2006), which evaluates the effects of Concord grape juice on cognitive and motor deficits in aging, suggest that it may

take a higher concentration of grape juice to enhance motor performance, whereas lower concentration may be sufficient to alter cognitive performance. A study with striatum and substantia nigra isolated from adult Wistar rats was the pioneer to show that purple grape juices can reduce oxidative stress in brain structures (Dani et al., 2008b). This result is corroborated by Balu et al., (2005), whom found normal levels of lipid peroxidation and antioxidant defenses in grape seed extract-supplemented aged rats.

Additionally, some studies showed that the intake of approximately 125-480mL/day of conventional purple grape juice elaborated from *Vitis vinifera* grapes is able to increase antioxidant levels in men (Day et al., 1997; Osman et al., 1998; Freedman et al., 2001; O'Byrne et al., 2002). This demonstrates that the diet is one defense strategy to prevent, intercept, or repair age-induced oxidative stress. In fact, all kind of fruit intake is associated with a lowered risk of degenerative disease, whereas the lack of adequate consumption of fruits and vegetables is linked to cancer incidence (Ames et al., 1993).

It is also attributed to the grape juice the decrease of cardiovascular diseases (Vinson et al., 2001; Singletary et al., 2003; Sanchez-Moreno et al., 1999). Platelet aggregation can be reduced by the intake (5-7.5 mL/kg/ day) of grape juice for one week, which is not observed for orange or grapefruit juices intake (Keevil et al. 1999). Purple grape juice presents a concentration of total polyphenol three times higher than citric juices, which indicates the potential effect of polyphneols on platelet aggregation. This effect could consequently reduce thrombosis coronary and myocardium infarct risks (Keevin et al., 2000). Intake of purple grape juice improves, also, endothelium function in patients with atherosclerotic vascular disease (Chou et al., 2001).

Inhibition of chemically induced rat mammary tumorigenesis was observed for Concord grape juice constituents, suggesting a potential breast cancer prevention (Singletary et al., 2003). Park et al. (2003) showed that grape juice consumption result in a pronounced reduction in the levels of DNA damages, when compared to the pre-supplementation level. Additionally, both purple and white grape juices showed antimutagenic activity in *Saccharomyces cerevisiae* yeast, which is positive correlated with the phenolic content of the juices. In fact, polyphenol antimutagenic activity (flavonoids or non-flavonoids) has been already reported in literature (Ferguson, 2001).

Briefly, this review shows that grape juices, both purple and white, are rich in several bioactive compounds, which are able to decrease oxidative stress damages, assisting in many important diseases such as cancer, coronary heart disease, neurological diseases, and others. Besides, grape juice is a non-alcoholic beverage, which can be include in diets of children and eldery peoples, where an the alcoholic beverage is not indicated.

Table 2. Beneficial effects related for different grape juices

Specie of cultivar	Variety	Main Caracheristic	**Biological activity**	**References**
Vitis labrusca	Bordo	Convetional Purple grape juice	Antioxidant activity	Dani et al., 2007; Dani et al., 2008a ; Dani et al., 2008b
Vitis labrusca	Niagara	Conventional White grape juice	Antioxidant activity	Dani et al., 2007
Vitis labrusca	Bordo	Organic Purple Grape juice	Antioxidant activity	Dani et al., 2007; Dani et al., 2008a ; 2008b
Vitis labrusca	Niagara	Organic White Grape juice	Antioxidant activity	Dani et al., 2007
Vitis vinífera	ND	Conventional purple grape juice	Antioxidant activity	Sanchez-Moreno et al., 1999
Vitis vinifera	*ND*	Conventional purple grape juice	Antioxidant activity	Day et al., 1997
Vitis labrusca	Concord	Conventional purple grape juice	Antioxidant activity	Osman et al., 1998 Freedman et al., 2001 O'Byrne et al., 2002 Dávalos et al., 2005 Wang et al., 1996
Vitis labrusca	Bordo and Niagara	Conventional Purple and white, respectively grape juice	Antioxidant activity	Dani et al., 2007 Fuhrman et al., 1995

Vitis labrusca	Concord	Conventional Purple grape juice	Inhibition of platelet aggregation	Carbonaro et al., 2002 Keevil et al., 2000. Osman et al., 1998 Demrow et al., 1995
Vitis labrusca	Concord	Conventional purple grape juice	Antioxidant activity	Abu-Amsha et al., 1996; Durak et al., 1999 Frankel et al., 1998 Stein et al., 1999
Vitis vinifera	*ND*	Conventional purple grape juice	Antioxidant activity	Day et al., 1997
Vitis labrusca	Concord	Conventional purple grape juice	Inhibiton of atherosclerosis	Vinson et al., 2001
Vitis labrusca	Concord	Conventional purple grape juice	Antithrombotic and vasodilatory activities	Folts et al., 2002
Vitis labrusca	Concord	Conventional purple grape juice	Antithrombotic activity	Demrow et al., 1995
Vitis labrusca	Concord	Conventional purple grape juice	Antiinflammatory activity	Albers et al., 2004
Vitis vinifera	*ND*	Conventional purple grape	Vasodilatory activity	Takahara et al., 2005

		juice		
Vitis labrusca	Concord	Conventional purple grape juice	Vasodilatory activity	Chou et al., 2001 Stein et al., 1999
Vitis labrusca	Concord	Conventional purple grape juice	Benefits on cognitive and motor deficits in aging	Barbara Shukitt-Hale et al., 2006 Park et al., 2003
Vitis labrusca	Concord	Conventional purple grape juice	Antitumoral activity	Park et al., 2003
Vitis labrusca	Concord	Conventional purple grape juice	Antitumoral activity	Singletary et al., 2003

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4. DISCUSSÃO

Atualmente existem várias evidências epidemiológicas correlacionando dietas ricas em frutas e vegetais frescos e a redução da incidência de doenças associadas ao envelhecimento e a perturbação da estabilidade genômica, como cânceres, doenças cardiovasculares e neurodegenerativas (para revisão, ver Ames, 2003; Fenech et al., 2005). Esta proteção está diretamente associada à presença de polifenóis em várias frutas, verduras e seus derivados (Fenech & Ferguson, 2001; Ames et al., 2005; Fenech, 2005; Fenech et al., 2005).

Anteriormente os alimentos eram avaliados em função da presença de certos nutrientes, como proteínas, carboidratos e lipídeos. Entretanto, nos últimos anos, passaram a despertar também interesse em relação a compostos que protegem o organismo, entre eles os polifenóis e as vitaminas (Ames, 2001; para revisão, ver Fenech, 2008). O suco de uva possui uma quantidade considerável de polifenóis (Anexo A), com relação aos quais já foram relatados diversos benefícios à saúde humana, tais como a capacidade de influenciar no status oxidativo e na estabilidade genômica de forma direta ou indireta (Figura 9; Ferguson, 2001).

Desta forma, vários estudos têm demonstrado que o suco de uva pode ser incluído entre os alimentos com benefícios potenciais à saúde humana (Figura 10, Capítulo VII). Estes benefícios são encontrados principalmente nos sucos produzidos com variedades *Vitis vinifera* (Keevil et al., 2000; Singletary et al., 2003; Park et al., 2003). Entretanto, pouco se conhece à respeito destes benefícios dos sucos produzidos com variedades *Vitis labrusca*, utilizadas no Brasil e em toda a América. Estes estudos assumem particular interesse visto a importância econômica que o setor da uva e seus derivados têm na Região Nordeste do Estado do Rio Grande do Sul, bem como em todo o País.

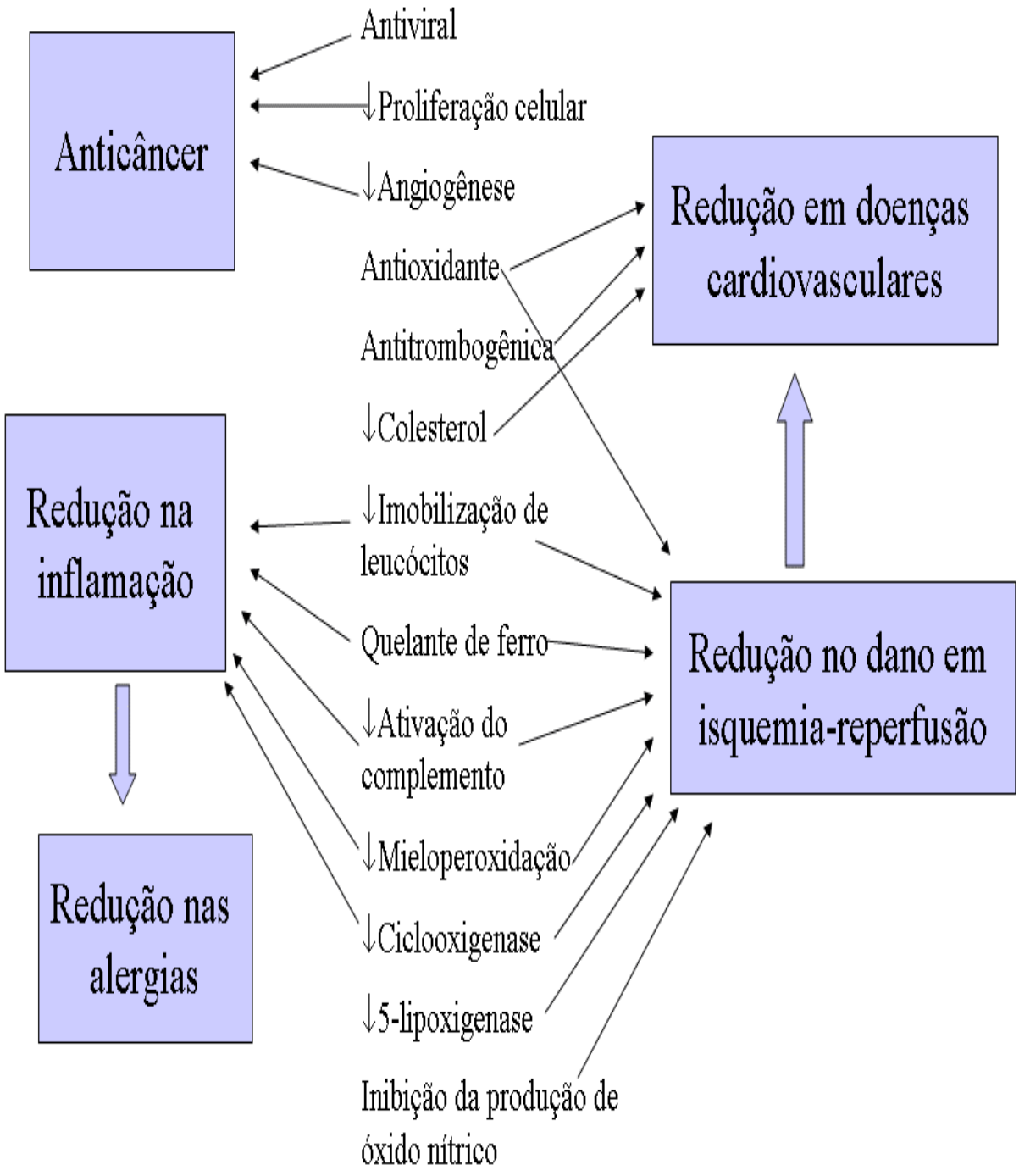

Figura 9. Hipóteses de possíveis ações dos polifenóis e a sua interação com diferentes doenças (Adaptado de Nijveldt et al., 2001 e Ferguson, 2001)

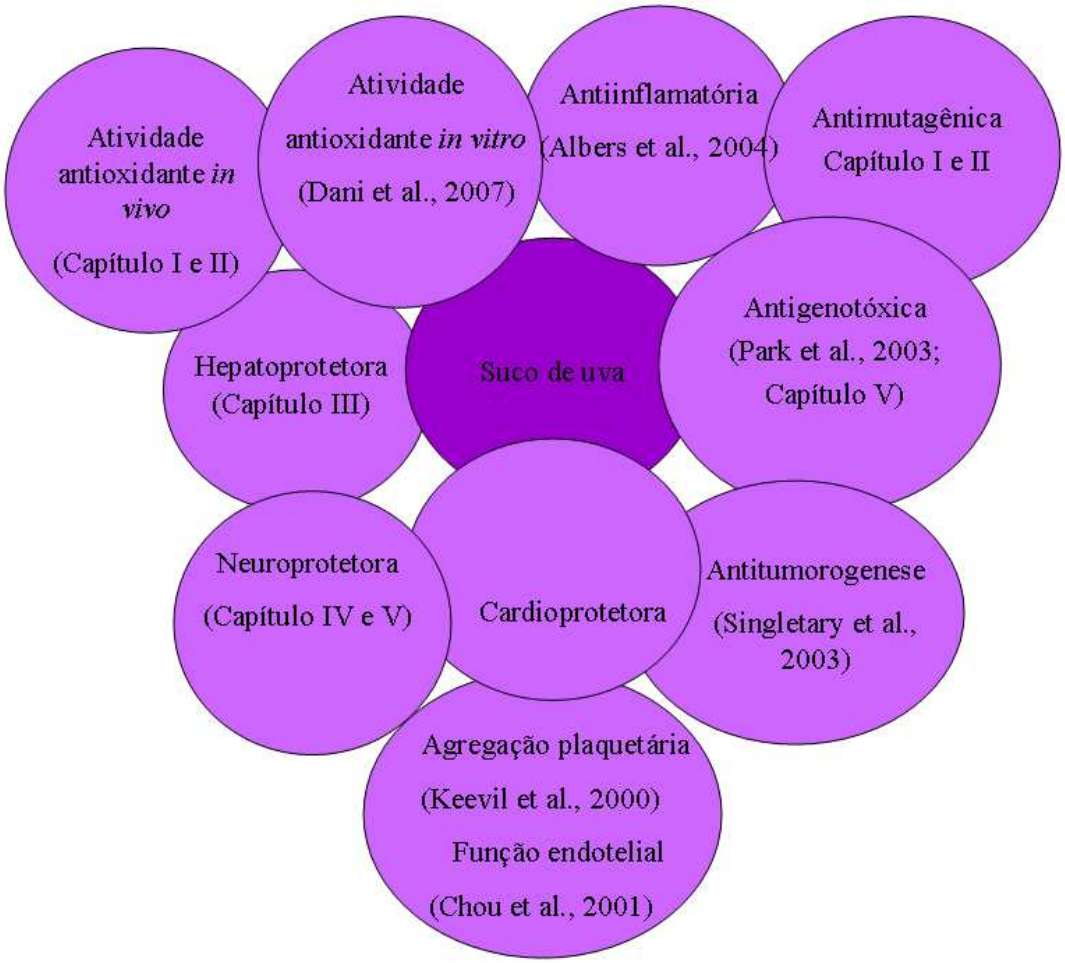

Figura 10. Principais benefícios relatados ao suco de uva na literatura e nesta tese.

De modo geral, podem-se obter sucos de uva brancos, tintos e rosados com características nutricionais e composições fenólicas distintas (Capítulo I, Capítulo II e Anexo A). Além das diferentes variedades de uvas que podem ser utilizadas, o mercado atual conta com outras classes de sucos, os convencionais e os orgânicos, sendo os últimos produzidos sem a presença tanto de agrotóxicos ou engenharia genética (IFOAM, 2008). Sendo assim, no presente trabalho foram avaliadas nove amostras de suco de uva: quatro brancos (Niagara), quatro tintos (Bordo) e um rose (Goethe).

Recentemente, Dani et al. (2007) (Anexo A) avaliaram a atividade antioxidante e a composição nutricional de oito amostras de suco de uva: brancos (Niagara) e tintos (Bordo), produzidos com uvas orgânicas e convencionais, bem como elaborados em

escala piloto e comercial. Neste trabalho, observou-se que os sucos orgânicos (brancos e tintos) apresentaram, em geral, maior composição fenólica do que os convencionais. Convêm ressaltar que diferenças significativas também foram encontradas entre sucos brancos e tintos, onde os últimos apresentaram os maiores valores. Os sucos orgânicos mostraram também valores superiores no conteúdo de resveratrol e antocianidinas (Anexo A). Estas diferenças podem ser explicadas pela escolha na metodologia de produção dos sucos (brancos e tintos), bem como pelo manejo orgânico, que se caracteriza por produzir mais metabólitos secundários em virtude de estresse pelo não uso de agrotóxicos (Soleas, 1997).

No capítulo II, compararam-se os resultados obtidos com o suco rose produzido com a variedade Goethe, *Vitis labrusca* com aqueles obtidos com sucos brancos e tintos convencionais comerciais (Anexo A). Observou-se que o suco rose possui valores de compostos fenólicos e ácido ascórbico superiores (Capítulo II, Tabela 2) aos demais sucos tintos e brancos (Anexo A).

Entre os polifenóis, o que vem chamando mais atenção da comunidade científica nos últimos dez anos é o resveratrol (um estilbeno, da família dos não flavonóides) (Figura 11).

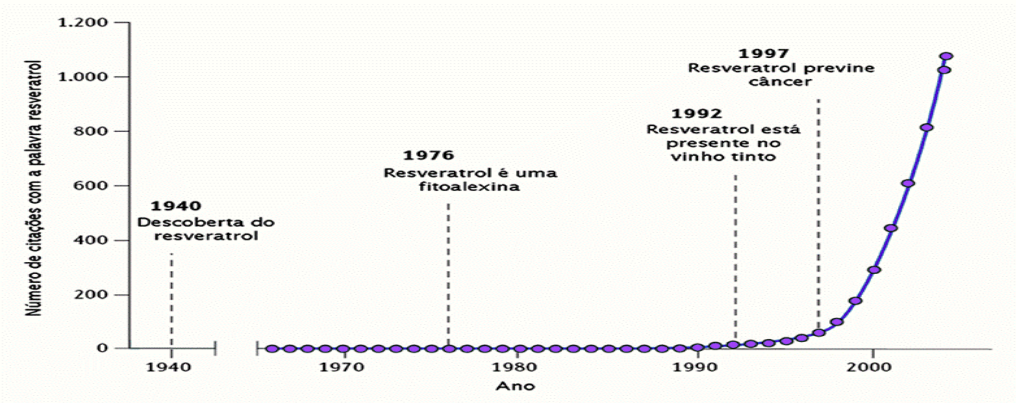

Figura 11. Número de citações científicas no PUBMED com a palavra resveratrol

Este crescente interesse tem levado a numerosos estudos que objetivaram a determinação deste composto em todos os vinhos comerciais (Roldan et al., 2003), estabelecendo que os níveis de resveratrol são maiores nos vinhos tintos do que nos brancos, devido fundamentalmente a técnica de vinificação empregada (Roldan et al., 2003). Por outro lado, a quantidade de resveratrol está diretamente correlacionada com a influência de alguns fatores no cultivo da uva, como a variedade de uva, clima, solo e estado sanitário da videira. De acordo com Jeandet et al. (1995), o conteúdo de resveratrol nos vinhos produzidos a partir de variedades tintas é três vezes superior aos produzidos com variedades brancas quando cultivados com nas mesmas condições. Muitas das variações observadas entre os diferentes produtos podem ser explicadas parcialmente pelas propriedades intrínsecas dos cultivares, como a idade da videira, o grau de maturação da uva, e o nível de infecção pelo *Botrytis cinerea*. Neste mesmo cenário, radiação solar, temperatura, umidade e precipitação, podem influenciar no conteúdo de resveratrol em todos os derivados da uva, visto que a síntese deste composto está relacionada à ativação de mecanismos de defesa da planta antes do dano ou por ataque de algum patógeno (Roldan et al., 2003).

Diferenças observadas no conteúdo de resveratrol entre sucos e vinhos (Tabela 2) se devem, provavelmente, ao fato dos vinhos passarem por processo de fermentação em contato com as cascas (parte da uva que concentra o resveratrol), diferente dos sucos, que não passam por este processo. Conseqüentemente, na literatura existem poucos estudos que relatam a quantidade deste componente em suco de uva. Na tabela 1 é possível observar que a variedade de uva utilizada, bem como as características específicas do suco e do sistema de condução (orgânico e convencional) influenciam na quantidade deste composto.

Tabela 1. Quantidade de resveratrol em sucos e vinhos.

Suco/Vinho	Espécie da uva utilizada	Resveratrol	Referência
Suco orgânico comercial	*Vitis labrusca*	0,22±0,03 mg/L	ANEXO A
Suco orgânico escala piloto	*Vitis labrusca*	0,18±0,02 mg/L	ANEXO A
Suco convencional comercial	*Vitis labrusca*	0,08±0,02 mg/L	ANEXO A
Suco convencional escala piloto	*Vitis labrusca*	0,07±0,03 mg/L	ANEXO A
Concord Grape Juice	*Vitis labrusca*	0,228 mg/L	Wang et al., 2002
Suco de uva Japonês	Não especificado	0,04 a 0,44 mg/L	Yasui et al., 1997
Suco de uva Varietal	Não especificado	0,003 a 0,015 mg/L	Soleas et al., 1995
Suco de uva tinto	*Vitis vinifera*	0,5 (0,09 a 1,09) mg/L	Romero-Pérez et al., 1999
Suco de uva branco	*Vitis vinifera*	0,05 (nd a 0,19) mg/L	Romero-Pérez et al., 1999
Vinho Brasileiro Cabernet Sauvignon	*Vitis vinifera*	2,01 mg/L	Souto et al., 2001
Vinho Brasileiro Merlot	*Vitis vinifera*	5,10 mg/L	Souto et al., 2001

Além da composição nutricional e fenólica de diferentes sucos de uva, na tabela 1 do Capítulo I está apresentada a composição mineral dos sucos brancos e tintos produzidos com variedades *Vitis labrusca* determinada pela técnica de PIXE. Pode-se observar importantes diferenças na composição mineral entre estes sucos, tais como o maior conteúdo de cobre nos sucos brancos do que os tintos. Este resultado é similar ao encontrado por Ollala et al.(2004) em sucos produzidos a partir de *Vitis vinifera*. De

acordo com Angelova et al. (1999), a variação observada pode estar fortemente relacionada com o tipo de solo e os processos agrícolas envolvidos.

Em adição, Ollala et al. (2004) observaram que os sucos tintos têm valores maiores de zinco do que os sucos brancos (*Vitis vinifera).* Esta diferença, entretanto, não foi observada nos resultados apresentados na Tabela 1 do Capítulo I. Os valores de Cu e Zn em sucos de uva (*Vitis vinifera),* apresentados por Ollala et al. (2004) e Onianwa et al. (1999) (Tabela 2), são inferiores aos do Capítulo I, com suco de uva (*Vitis labrusca*). Este fato pode, em parte, ser explicado pelas distintas variedades de uva utilizadas.

Tabela 2. Valores médios de Cu e Zn encontrados em sucos de uva.

Suco	Cu (mg/L)	Zn(mg/L)	Referência
Tinto (*Vitis vinifera)*	0,05	0,55	Ollala et al.(2004)
Tinto (*Vitis labrusca*)	5,25	7,00	Capítulo I
Branco (*Vitis vinifera*)	0,06	0,41	Ollala et al.(2004)
Branco (*Vitis labrusca*)	21,9	11,7	Capítulo I
Suco de uva (Nigéria)- variedade não informada	0,001 a 0,27	0,23 a 1,96	Onianwa et al., 1999.
Recomendações diárias em adolescentes	1,5-3 mg	11-40 mg	National Research Council, 1989.

Os resultados apresentados na tabela mostram que os sucos são importantes fontes nutricionais de cobre e zinco, e pode-se afirmar que a quantidade requerida pelo National Research Council (1989) seria mais facilmente atingida com os sucos produzidos com *Vitis labrusca*, mostrando assim a importância deste produto. Além dos teores/da quantidades de cobre e o zinco apresentados(as) no Capítulo I (Tabela 1), foi determinada a presença de outros minerais, como ferro, manganês, cálcio, potássio,

cloro, enxofre, fósforo, cobre, zinco, silício e magnésio. Dentre estes minerais o presente em maior quantidade para ambos os sucos (brancos e tintos) foi o potássio, seguido de magnésio e cálcio (Capítulo I, Tabela 1).

Atualmente, a determinação de minerais em uvas e sucos tem sido considerada relevante não apenas por questões nutricionais, mas também do ponto de vista tecnológico. Estes minerais podem ter uma influência considerável no processo de produção, podendo, em alguns casos, exercer efeitos negativos como reações de oxi-redução, precipitação e/ou alterações nas características organolépticas dos derivados da uva (Ollala et al., 2004). Do ponto de vista nutricional, pode-se afirmar que o suco de uva é uma fonte de minerais, os quais possuem importantes funções fisiológicas no organismo humano, tais como atuar como co-fator de enzimas, auxiliar na estabilização da membrana plasmática, participar do mecanismo de homeostase e no o equilíbrio ácido-base, influenciar a atividade muscular e nervosa, atuar/estar envolvido na sinalização e na progressão celular (Halliwell & Gutteridge, 1999). A tabela 3 mostra as recomendações para ingestão diária de minerais para homens adultos saudáveis. Baseada nestas recomendações e a partir do conteúdo mineral conhecido destes sucos (Tabela 1, Capítulo I), é possível estimar que 500 mL de suco *Vitis labrusca* por dia (em torno de 2 copos) é capaz de suprir 100% a necessidade diária de ferro, manganês, cobre e zinco. Desta forma, este conjunto de observações nos leva a propor a importância à saúde humana que a ingesta diária deste produto pode gerar nos seres humanos, visto a influência de vários minerais em diversas funções fisiológicas do corpo humano.

Tabela 3. Recomendações diárias de ingesta de minerais sugeridas a adultos.

Minerais	Referências para ingestão diária			
	Homens adultos		Mulheres adultas	
Cálcio	1200 mg	2500 mg	1200 mg	2500 mg
Cromo	30 µg	ND	25 µg	ND
Cobre	900 µg	10 mg	900 µg	10 mg
Ferro	8 mg	45 mg	18 mg	45 mg
Magnésio	420 mg	350 mg	320 mg	350 mg
Manganês	2,3 mg	11 mg	1,8 mg	11 mg
Fósforo	700 mg	4000 mg	700 mg	4000 mg
Selênio	55 µg	400 µg	55 µg	400 µg
Silício	ND	ND	ND	ND
Zinco	11 mg	40 mg	8 mg	40 mg

Dados adaptados do NATIONAL RESEARCH COUNCIL, Recommended Dietary Allowances (1989).

Além da composição fenólica e mineral, ao longo trabalho verificou-se que os sucos, quando avaliados em testes *in vitro* (Anexo A; Capítulo II) e *in vivo* (Capítulo I; Capitulo II), podem ser considerados excelentes fontes antioxidantes. Nos testes *in vitro*, os sucos tintos, principalmente os orgânicos, apresentaram atividades antioxidantes elevadas quando comparadas aos sucos brancos. Correlações positivas, tanto para branco como para tintos, entre o conteúdo de polifenóis ($r=0,616$; $p<0,05$), catequina ($r=0,741$; $p<0,05$) e procianidinas (0,525; $p<0,01$) e atividade antioxidante (Anexo A). Os sucos tintos, orgânicos e convencionais, apresentaram correlações positivas também entre o conteúdo de antocianinas e atividade antioxidante *in vitro* (0,781; $p<0,05$). Estas correlações encontram respaldo em diversos trabalhos nos quais se atribui aos polifenóis uma atividade antioxidante *in vitro* significativa (para revisão, ver Ferguson, 2001; Ferguson & Philpot, 2008).

No capítulo I e II, utilizando a levedura *S. cerevisiae* como modelo biológico, observou-se que todos os sucos (brancos, tintos e rose) apresentavam atividade antioxidante *in vivo*. Entretanto, os sucos brancos mostraram maior proteção aos danos gerados pelo peróxido de hidrogênio do que os sucos tintos e o rose, apesar dos brancos apresentarem os menores valores de polifenóis do que os sucos tintos. Este resultado pode ser explicado pelo fato do suco de uva ser uma mistura complexa, formada por outros compostos bioativos como vitaminas e minerais (selênio), os quais, em sinergismo, podem estar relacionados com a atividade protetora aos danos gerados pelas ER (Halliwell, 2008). Em adição, Dávalos et al. (2005) afirma que a atividade antioxidante de derivados da uva é influenciada não apenas pelo conteúdo de polifenóis, mas também por outros componentes como vitaminas. Conteúdo fenólico não é o único fator que influencia na atividade antioxidante de derivados da uva. Franke et al. (2004) atribuem a atividade antioxidante encontrada em sucos de laranja à vitamina E, carotenóides e aos minerais (selênio), podendo estes agir isoladamente ou em sinergismo.

Além da atividade antioxidante, os sucos de uva, tintos e brancos, apresentaram atividade antimutagênica importante, sendo capazes de reduzir a freqüência de mutações induzidas pelo agente reconhecidamente mutagênico peróxido de hidrogênio (Tabela 4 do Capítulo I). Existem consideráveis estudos que relatam atividade antimutagênica de polifenóis (para revisão, ver Ferguson, 2001). Entretanto, o conteúdo de minerais parece, também, estar correlacionado com esta atividade, uma vez que correlações positivas foram observadas entre o conteúdo de Zn (0,741, $p<0,05$) e Mg (0,781, $p<0,05$) em sucos brancos. É importante ressaltar que zinco e magnésio têm grande importância na estabilidade genômica, sendo co-fatores de enzimas envolvidas nos processos de reparo por excisão de bases (BER), por excisão de nucleotídeos

(NER) e nos múltiplos estágios de formação de tumores (Hartiwig, 2001). Em adição, tem sido mostrado que o Mg é um co-fator para a DNA-polimerase e um protetor efetivo contra carcinogênese (Rojas et al., 1999).

Nos capítulos III e IV, foram avaliadas a capacidade antioxidante, neuroprotetora, hepatoprotetora e antigenotóxica de dois sucos de uva tintos comerciais (orgânicos e convencionais), utilizando-se ratos machos Wistar (jovens e envelhecidos) tratados com tetracloreto de carbono. Observou-se que ambos os sucos foram capazes de reduzir os níveis de peroxidação lipídica (TBARS) nos tecidos do SNC, estriado e substância nigra, como também no fígado de ratos jovens (Figura 1A e 1B do Capítulo IV e Figura 2 do Capítulo III, respectivamente). Entretanto, quando avaliados em ratos envelhecidos, observou-se que a redução nos níveis de TBARS não ocorreu em todos as estruturas (Capítulo V, Tabelas 1 a 6). Esta redução foi observada apenas no córtex e no hipocampo nos ratos que ingeriram ambos os sucos de uva. Entretanto, somente o suco orgânico foi capaz de reduzir os níveis de TBARS na substância nigra. Neste cenário, recentemente, Balu et al. (2006) visualizaram diferenças quanto aos níveis de estresse oxidativo entre grupos de ratos jovens e velhos, tratados com extrato de semente de uva (*Vitis vinifera*). Os ratos envelhecidos apresentaram um aumento na produção de anions superóxido e nos danos ao DNA em diferentes tecidos do SNC. Apesar da diminuição nos níveis de TBARS não ser observada em todos os tecidos, a neuroproteção observada, principalmente no hipocampo, tem suma importância, visto este tecido corresponde a uma das regiões de neurogênese, atividade esta diminuída com o envelhecimento (Kempermann et al., 1998; Kuhn et al., 1996). Em adição, o hipocampo e o córtex são conhecidos por estarem envolvidos em um papel crucial na memória (Hale et al., 1996). É importante ressaltar, ainda, que o hipocampo é uma das estruturas

do SNC mais suscetível à danos oxidativos, principalmente por eventos de peroxidação lipídica (Cechetti et al., 2008).

No Capítulo V, figura 2, mostrou-se que sucos tintos (orgânico e convencional) possuem atividade antigenotóxica, quando avaliada pelo teste Cometa em linfócitos de sangue periféricos em ratos envelhecidos tratados com tetracloreto de carbono. Nesse mesmo sentido, Franke et al.(2005a; 2005b;2006) mostraram que o suco de laranja também possui atividade antigenotóxica, a qual foi atribuída ao conteúdo de vitamina C e polifenóis. Neste sentido, Park et al. (2003) afirmam que um dos possíveis mecanismos pelos quais o suco de uva reduz o dano ao DNA seria pelo conteúdo fenólico, visto que estes podem agir como seqüestradores de radicais livres e assim reduzir a liberação destes no plasma. Esses autores também demonstraram que o consumo de suco de uva em homens saudáveis por 8 semanas resultou em uma pronunciada redução nos níveis de dano ao DNA comparada ao controle (homens que não fizeram esta ingesta). Segundo Park et al. (2003), o dano ao DNA é conhecido por ser o marcador biológico sensível na avaliação do estresse oxidativo, representando o não balanço entre geração de espécies reativas e deficiências no sistema antioxidante.

O conjunto de resultados descritos nos Capítulos I a V permite identificar correlações importantes entre a atividade antioxidante *in vitro*, *ex vivo* e *in vivo* e o conteúdo de polifenóis, levando-nos, em várias situações, a propor que esta atividade benéfica do suco de uva fosse atribuída aos polifenóis presentes, principalmente o resveratrol, entre os sucos tintos, e a catequina, entre os sucos brancos.

A partir destas observações, no Capítulo VI avaliou-se a atividade isolada do resveratrol e da catequina, utilizando linhagens da levedura *S. cerevisiae* proficientes e/ou deficientes em mecanismos de defesa antioxidante. Os resultados obtidos neste estudo mostraram que ambos resveratrol e catequina são antioxidantes potenciais,

quando presentes em concentrações equimolares, mostrando-se capazes de reduzir os níveis de lipoperoxidação induzida pelos agentes estressores: peróxido de hidrogênio, tetracloreto de carbono e cádmio (Tabela 1 do Capítulo VI). De acordo com os resultados apresentados no Capítulo VI, os pré-tratamentos com resveratrol ou catequina não foram capazes de aumentar a tolerância ao peróxido de hidrogênio em cultura de *S. cerevisiae* deficientes na enzima catalase citosólica (*ctt1*). Entretanto, frente ao CCl_4, apenas a catequina foi capaz de aumentar a tolerância. O conjunto dessas observações sugere que altos níveis de ER poderiam ser reduzidas após tratamento com resveratrol e catequina, presumidamente pela ativação de defesas celulares, principalmente envolvendo a atividade da enzima catalase. Entretanto, vale salientar que a ação dos polifenóis pode ser atribuída ao metabolismo dos mesmos, envolvendo ingestão e biodisponibilidade, influenciada pela estrutura dos compostos, absorção, interação com outros compostos e metabolismo dos mesmos (Manach et al., 2004).

Em sumo, os resultados obtidos neste trabalho nos levam a sugerir que o suco de uva, branco ou tinto, orgânico ou convencional, produzidos a partir de variedades *Vitis labrusca*, é uma bebida rica em vários compostos, os quais, são capazes de diminuir os danos causados pelo estresse oxidativo, possivelmente pela indução das defesas celulares existentes na célula (Halliwell, 2008). Entretanto, é possível observar, também, que estes sucos possuem diferenças quanto à quantidade de polifenóis e nas atividades biológicas testadas. Desta forma, a fim de facilitar esta observação e de propor uma possível escolha do melhor suco, compilou-se na tabela 4 as principais atividades biológicas, bem como o conteúdo de polifenóis dos diferentes sucos estudados, a qual possibilita concluir que o suco que mais se destaca é o suco bordo orgânico comercial, por ser o mais rico em polifenóis e com uma atividade biológica mais expressiva.

Tabela 4. Principais atividades biológicas e conteúdo de polifenóis dos diferentes sucos estudados neste estudo.

Tipo de Suco	Polifenóis Totais	Atividade Biológica				
		Antioxidante *in vivo* (*S. cerevisiae*)	Antimutagênica (*S. cerevisiae*)	Hepatoprotetora	Neuroprotetora	Antigenotóxica
Bordo Orgânico Comercial	2	2	2	1	1	1
Bordo Orgânico Escala Piloto	1	2	2	ND	ND	ND
Bordo Convencional Comercial	5	3	2	1	2	1
Bordo Convencional Escala Piloto	3	2	2	ND	ND	ND
Niagára Orgânico Comercial	6	1	1	ND	ND	ND
Niágara Orgânico Escala Piloto	8	1	1	ND	ND	ND
Niágara Convencional Comercial	7	1	1	ND	ND	ND
Niágara Convencional Escala Piloto	9	1	1	ND	ND	ND
Rose comercial (Goethe)	4	1	Não determinada	ND	ND	ND
Referências	Anexo e Capítulo II	Capítulo I e II.	Capítulo I	Capítulo III	Capítulo IV	Capítulo V

* Os números referem-se ao ranking , onde 1 corresponde ao maior valor e 9 o menor valor.

Em conclusão, estes resultados são importantes visto que a região Nordeste do RS é responsável pela produção de um produto muito gostoso e interessante em termos de manutenção de saúde, e este é um produto disponível a todos, de fácil acesso e comercialização. Em concordância, é possível afirmar que o suco de uva é um alimento com importantes atividades benéficas a saúde, havendo diferenças entre brancos e tintos, e orgânicos e convencionais, entretanto todos apresentaram importante atividade antioxidante. Este produto ainda tem a vantagem de poder ser consumido desde a infância até a velhice, visto que, por não ser alcoólico, não acarreta nenhum dano.

CONCLUSÕES

As avaliações das atividades antioxidante, mutagênica/antimutagênica de sucos de uva, *Vitis labrusca*, tintos (Bordo), brancos (Niágara) e rosado (Goethe), em diferentes sistemas biológicos, permitem concluir que:

a) os sucos de uva tintos apresentaram o maior teor de polifenóis totais, resveratrol, catequina, epicatequina, procianidinas e antocianidinas em relação aos sucos brancos e rose;
b) os sucos orgânicos, tanto brancos quanto tintos, mostraram maior conteúdo de polifenóis totais do que os produzidos pelo do manejo convencional;
c) o suco bordo orgânico produzido em escala piloto possui a maior atividade antioxidante *in vitro*, a qual apresentou correlação positiva com o conteúdo de polifenóis total, procianidinas B1 e B3 e com catequina;
d) os sucos de uva apresentam importante conteúdo mineral, suprindo as necessidades diárias de alguns destes minerais que possuem função fisiológica importante, como por exemplo, o ferro;
e) correlações importantes entre minerais e atividades biológicas foram observadas nos sucos estudos;
f) os sucos de uva brancos mostraram maior atividade antioxidante *in vivo* do que os tintos e o rosado. Entre os sucos tintos, os orgânicos mostraram-se mais efetivos do que os produzidos a partir de manejo convencional;
g) correlações positiva entre o conteúdo de resveratrol e a atividade antioxidante *in vivo* dos sucos tintos foram observadas;
h) todos os sucos possuem importante atividade antimutagênica em células da levedura *S.cerevisiae* tratadas com peróxido de hidrogênio;

i) os sucos tintos convencionais e orgânicos apresentaram atividade neuroprotetora e hepatoprotetora em ratos Wistar jovens, expostos ao tetracloreto de carbono;

j) os grupos de ratos Wistar que receberam suco de uva, orgânico e convencional, apresentaram valores de peroxidação lipídica, protéica e relação SOD/CAT diminuídos em relação ao controle;

k) sucos de uva tinto, orgânico e convencional, apresentaram atividade antigenotóxica quando avaliada em um grupo de ratos mais velhos (7 meses), e também de uma atividade neuroprotetora e hepatoprotetora neste grupo.

l) ambos os polifenóis mostraram-se capazes de aumentar a tolerância frente a diferentes agentes estressores quando avaliados em diferentes linhagens da levedura *S.cerevisiae* proficientes e deficientes nas defesas antioxidantes (sod, cat e gsh); e,

m) neste modelo, observou-se que o aumento na tolerância parece estar associado à atividade da enzima catalase.

PRESPECTIVAS

- Realizar a dosagem de marcadores neurológicos tais como GFAP, S100β, GDNF, NOS neuronal em cérebro e soro de ratos tratados com sucos tintos orgânicos e convencionais.

- Investigar o efeito de diferentes sucos de uva sobre parâmetros comportamentais como memória, atividade locomotora e exploratória.

- Avaliar a atividade antigenotóxica, *in vivo* ou *in vitro*, pelo teste de micronúcleos, de diferentes sucos de uva.

- Determinar a influência da ingestão de sucos de uva em diferentes modelos de ratos que explorem diferentes doenças, tais como Parkinson, doenças cardiovasculares, câncer e Alzheimer.

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ANEXOS

ANEXO A

Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food Chem Toxicol. 2007 Dec;45(12):2574-80. Epub 2007 Jun 28.

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Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes

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Abstract

Although the beneficial effects of moderate wine intake are well-known, data on antioxidant capacity of grape juices are scarce and controversial. The purpose of this study was to quantify total polyphenols, anthocyanins, resveratrol, catechin, epicatechin, procyanidins, and ascorbic acid contents in grape juices, and to assess their possible antioxidant activity. Eight *Vitis labrusca* juices – white or purple, from organically- or conventionally-grown grapes, and obtained in pilot or commercial scale – were used. Organic grape juices showed statistically different ($p < 0.05$) higher values of total polyphenols and resveratrol as compared conventional grape juices. Purple juices presented higher total polyphenol content and *in vitro* antioxidant activity as compared to white juices, and this activity was positively correlated ($r = 0.680$; $p < 0.01$) with total polyphenol content. These results indicate that white and purple grape juices can be used as antioxidants and nutritional sources.

keywords: Grape juice; Antioxidant; Nutritional; Phenolic compounds

1. Introduction

Experimental data have increasingly suggested that cellular oxidative damage induced by reactive species (RS) has a relevant pathophysiological role in several types of human diseases, such as atherosclerosis and cancer (Ames et al., 1993). In order to neutralize these RS, our cells have developed a complex biochemical redox mechanism, consisting of both enzymatic and non-enzymatic components (Park et al., 2003). Moreover, foods, particularly fruits and vegetables, also have an important role in maintaining physiological redox equilibrium. These foods supply several antioxidants, such as vitamin C and several polyphenolic compounds, to the body. Grapes are rich in phenolic compounds, such as flavonoids (catechin, epicatechin, quercetin, anthocyanins and procyanidins), and resveratrol (3,5,4′-trihydroxy-stilbene), which are mainly found in red grape products (Wang et al., 2002; Soleas et al., 1997; Fuleki and Ricardo-da-Silva, 2003). It has been already reported that grape juice compounds can prevent: (i) platelet aggregation, (ii) LDL oxidation and oxidative damage to DNA, (iii) coronary diseases and atherosclerosis (Frankel et al., 1998; Osman et al., 1998; Day et al., 1997; Singletary et al., 2003).

As grape juices are a relevant source of polyphenolic compounds, many people are becoming aware of the importance of their consumption in their daily diet. There is an increasing public concern as to developing healthy habits and eating quality food. Some consumers are also taking into account agricultural methods (conventional or organic) when purchasing their food. Organic agriculture, among other practices, does not use pesticides during the cultivation (IFOAM, 2005). Organic strawberries and tomatoes present higher content of secondary metabolites

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(e.g. polyphenolic compounds), as they suffer more fungal infections, thereby producing a higher level of these metabolites for their defense (Asami et al., 2003; Lombardi-Boccia et al., 2004). To our knowledge, there are no studies in literature on how organic cultivation of grapes may change the chemical characteristics of grapes and their products (wine and juices). In addition, we did not find any studies comparing the biological activities of organic and conventional, white and purple grape juices.

This study aimed at assessing the antioxidant capacity of different types of grape juices (white or purple juices from organically- or conventionally-grown grapes) using standard *in vitro and ex vivo* assays. In addition, (+)-catechin, (−)-epicatechin, *trans*-resveratrol, anthocyanidin, and individual procyanidin contents of juices produced from different *Vitis labrusca* varieties were analyzed by HPLC.

2. Materials and methods

2.1. Chemicals

DPPH•, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, gallic acid, and procyanidins B1, B2, B3 and B4 were obtained from Sigma–Aldrich (St. Louis, MO). The anthocyanin pigments cyanidin-3-glucoside, delphinidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside were obtained from Extrasynthese (Gennay, France). Methyl-parathion was obtained from Bayer, and the acetylcolinesterase kit was purchased from UFRJ (Rio de Janeiro, Brazil). All other chemicals were purchased from E. Merck (Damstadt, Germany).

2.2. Grapes and grape juices

Grape juice samples produced from *V. labrusca,* varieties Bordo and Niagara, were analyzed according to eight different groups (Table 1). Organic grapes cultivated with no pesticides, and commercial organic juices were obtained from Cooperativa Aecia (Antonio Prado, Rio Grande do Sul, RS, Brazil). Conventional grapes, cultivated using traditional methods, and commercial conventional juices were obtained from Vinhos Monte Reale (Flores da Cunha, RS, Brazil). Validity periods were observed, and the same brands were used for the entire study.

Grapes were cultivated in 2005, and all juices were manufactured during the same year. Purple juices were heat-extracted using pulp, seeds, and skin, whereas the skin was removed before extraction in the white juices. Commercial conventional juices were manufactured by heat extraction (approximately 50 °C), with a subsequent pressing in order to separate the pulp, and then submitted to pasteurization (at 85°). All other juices, purple and white, were manufactured by heat extraction, immediately followed by bottling, both at 80 °C.

2.3. Grape juice chemical analysis and nutritional evaluation

Alcoholic grade, total acidity, volatile acidity, pH, total SO_2, and ascorbic acid were determined using the methods described by Zoecklein et al. (2000). All analyses were performed in duplicate. Carbohydrates, food fiber, saturated fats, proteins, and humidity levels, as well as calorie values were determined according to AOAC International official methodologies (AOAC, 1998).

2.4. Pesticide determination

Organophosphorus and carbamate pesticides were determined in juice samples as methyl parathion-equivalent activity, which causes inhibition of the enzyme acetylcholinesterase (AChE), as previously described by Bastos et al. (1991) and Lima et al. (1996). Methyl-parathion (Folidol 600® – Bayer, Brazil) calibration curve was used to express AChE activity in ppm of methyl-parathion.

2.5. Phenolic compound content

Total phenol content was measured using Singleton and Rossi's modification of Folin–Ciocalteau's colorimetric method (Singleton et al., 1999). High performance liquid chromatography (HPLC) analysis was used to quantify the presence of individual phenolic compounds. Before HPLC analysis, 5 mL of each sample were filtered through a 0.20-mm cellulose membrane. The equipment consisted of a liquid-gradient chromatographic system, LC-DAD Series 1100 (Palo Alto, CA), with diode array (DAD) detector system. Zorbax 300 SB C18 pre-column (12 mm × 4.6 mm × 5 μm) and C18-ODS column (150 mm × 4 mm × 5 μm) (Agilent Technologies, USA) were used.

2.5.1. trans-Resveratrol analysis

In order to quantify *trans*-resveratrol, ultra-pure water and acetonitrile mobile phase (75:25 v/v), pH 3.0, in constant flow of 1.0 mL min^{-1} for 20 min in a controlled-temperature room at 20 °C, was used. The peak was detected at 306 nm, after injection of 20-μL samples (Jeandet et al., 1995).

2.5.2. Anthocyanins analysis

In order to determine cyanidin-3-glucoside, delphidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside levels, mobile phase with solvents A: ultra-pure water, formic acid, acetonitrile (87:10:3 v/v/v), and B: ultra-pure water, formic acid, acetonitrile (40:10:50 v/v/v), in constant flow of 0.8 mL min^{-1}, in a temperature-controlled room at 25 °C, was used. The peak was detected at 518 nm, after injection of 50-μL samples (Office International de la Vigne et du Vin, 2003).

2.5.3. Procyanidins analysis

In order to determine procyanidins B_1, B_2, B_3, and B_4, (+)-catechin, (−)-epicatechin, and gallic acid, mobile phase with solvent A (ammonium hydroxide diphosphate 50 mmol L^{-1}, pH 2.6), solvent B (20% of solvent A and 80% of acetonitrile), and solvent C (orthophosphoric acid

Table 1
Analyzed grape juices ($n = 8$)

Name	Cultivar	Agriculture practice	Production scale	Heat treatment
BCC	Bordo	Conventional	Commercial	Pasteurized at 85 °C[a]
BCP	Bordo	Conventional	Pilot	Hot bottled at 80 °C[b]
BOC	Bordo	Organic	Commercial	Hot bottled at 80 °C
BOP	Bordo	Organic	Pilot	Hot bottled at 80 °C
NCC	Niagara	Conventional	Commercial	Pasteurized at 85 °C
NCP	Niagara	Conventional	Pilot	Hot bottled at 80 °C
NOC	Niagara	Organic	Commercial	Hot bottled at 80 °C
NOP	Niagara	Organic	Pilot	Hot bottled at 80 °C

[a] Juice pasteurized at 85 °C, for 3 min in pasteurization unity for fresh juices EFC-250 l/h, ETAL®, before being bottled.
[b] Juice hotted at 80 °C and immediately bottled.

0.2 mol L^{-1}, pH 1.5), in a constant flow of 0.5 mL min^{-1}, in a temperature-controlled room at 40 °C, was used. The peak was detected at 204 nm, after injection of 5-μL samples. Elution conditions were standardized according to Lamuela-Raventós and Waterhouse (1994).

2.6. Antioxidant activity

Juice antioxidant activity was measured by *in vitro* (DPPH· radical-scavenging activity, and sod and cat-like activities) and *ex vivo* (inhibition of serum lipid peroxidation).

2.6.1. Chemical measurement of DPPH·-radical scavenging activity

DPPH· radical scavenging was measured using a modified Yamaguchi et al. (1998) method, in which white and purple grape juice solutions were added to obtain final concentrations of 0.1, 1.0, 10.0, 50.0, and 100.0% v/v. Tubes were stored in the dark for 20 min, after which absorbance was measured at 517 nm. Results were expressed as the amount of juice necessary to scavenge 50% of DPPH· radical (IC_{50}). Distilled water was used instead of antioxidant solutions as control. Catechin was used as standard.

2.6.2. Superoxide dismutase- and catalase-like activities

Superoxide dismutase-like activity was spectrophotometrically determined in grape juice samples by measuring the inhibition of self-catalytic adrenochrome formation rate at 480 nm, in a reaction medium containing 1 mmol L^{-1} adrenaline (pH 2.0), and 50 mmol L^{-1} glycine (pH 10.2). This reaction was performed at 30 °C for 3 min (Bannister and Calabrese, 1987). Results were expressed as the amount of grape juice needed to reduce 50% of adenochrome. Catalase-like activity assay was performed according to the method described by Aebi (1984), by determining hydrogen peroxide decomposition rate at 240 nm. Results were expressed in micromoles of H_2O_2 decomposed per minute.

2.6.3. Inhibition of serum lipid peroxidation assay

Inhibition of serum lipid peroxidation was determined using a modification of the method described by Durak et al. (1999). Pooled fresh human serum of 1 mL, 150 μL of grape juices sample, and 15 μL of $CuSO_4$ (5 mM; positive control) were incubated at 37 °C for 1 h. Oxidative stress levels were then spectrophotometrically measured as thiobarbituric acid reactive substances concentration (TBARS), as described by Wills (1996). TBARS results were expressed as nmol/mL.

2.7. Statistical analyses

Values were determined as being parametrical or non-parametrical by the Kolmogorov–Smirnoff test. All assays were performed in triplicate. Data were submitted to analysis of variance (ANOVA), and means were compared using the test of Tukey. Groups were compared using Student's *t*-test and Mann–Whitney *U*-test. Relationships between variables were assessed using Pearson's product–moment correlation coefficient. SPSS 12.0 software package was used in all statistical analysis.

3. Results

3.1. Grape juice chemical analysis and nutritional evaluation

Grape juice alcohol levels were between 0.03% and 0.3% (v/v), with total acidity between 0.40 and 0.96 g/100 mL tartaric acid. No volatile acidity was detected in any juice. pH values varied from 3.21 to 3.60, and sulfur dioxide from 0.027 to 0.029 g/L. Table 2 shows nutritional analyses and ascorbic acid content. Juices produced at pilot scale presented the highest calorie value and carbohydrate content. Purple grape juices had high vitamin C (ascorbic acid) levels. Except for the purple grape juice produced at pilot scale (BOP), organic juices presented higher ascorbic acid values as compared to conventional juices.

3.2. Pesticide determination

No organophosphorus or carbamate pesticides were detected in the analyzed grape juice samples.

3.3. Total phenolic content

Purple juices presented higher total phenolic content as compared to white juices (Fig. 1). Within agricultural method, organic juices presented higher polyphenol content as compared to juices manufactured with conventionally-grown grapes (Fig. 1).

3.4. Resveratrol and anthocyanins contents

Resveratrol and anthocyanins contents were only measured in purple grape juices, which were heated with the skin, allowing phenolic compound to be transferred to the juice (Fuleki and Ricardo-da-Silva, 2003). Organic juices presented higher resveratrol (Fig. 2) and anthocyanins contents (Table 3) as compared to conventional juices.

Table 2
Nutritional analyses and acid ascorbic level in different grape juices ($n = 8$)

Sample[A]	Calorie values (kJ)	Carbohydrates (%)	Protein (%)	Fiber (%)	Moisture (%)	Ashes (%)	Ascorbic acid (mg %)
BCC	$39.04 \pm 0.05^{d,B}$	9.43 ± 0.01^{d}	0.317 ± 0.05^{d}	0.010 ± 0.00^{e}	90.02 ± 0.02^{b}	0.199 ± 0.00^{b}	30.8 ± 0.40^{c}
BCP	53.68 ± 0.10^{a}	12.93 ± 0.02^{a}	0.487 ± 0.05^{a}	0.250 ± 0.01^{a}	86.12 ± 0.02^{d}	0.197 ± 0.00^{b}	44.0 ± 0.13^{b}
BOC	32.47 ± 0.02^{e}	7.82 ± 0.07^{e}	0.240 ± 0.05^{f}	0.105 ± 0.05^{d}	91.65 ± 0.08^{a}	0.132 ± 0.00^{c}	57.2 ± 0.70^{a}
BOP	48.36 ± 0.08^{b}	11.76 ± 0.02^{b}	0.332 ± 0.05^{b}	0.120 ± 0.00^{c}	87.56 ± 0.01^{c}	0.216 ± 0.00^{a}	30.8 ± 0.90^{c}
NCC	46.03 ± 0.01^{c}	11.19 ± 0.03^{c}	0.310 ± 0.05^{e}	0.271 ± 0.01^{a}	88.10 ± 0.03^{c}	0.110 ± 0.00^{d}	17.6 ± 0.30^{e}
NCP	52.12 ± 1.72^{a}	12.68 ± 0.46^{b}	0.316 ± 0.05^{d}	0.093 ± 0.01^{d}	86.79 ± 0.46^{d}	0.106 ± 0.00^{d}	4.4 ± 0.10^{g}
NOC	35.07 ± 0.09^{e}	8.43 ± 0.02^{e}	0.327 ± 0.01^{c}	0.170 ± 0.05^{b}	90.84 ± 0.02^{ab}	0.224 ± 0.00^{a}	22.0 ± 0.80^{d}
NOP	50.92 ± 0.08^{a}	12.48 ± 0.02^{b}	0.327 ± 0.05^{c}	0.120 ± 0.00^{c}	86.88 ± 0.55^{d}	0.226 ± 0.00^{a}	8.8 ± 0.21^{f}

[A] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: bordo conventional organic; BOP: Bordo organic pilot scale; NCC: niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot Scale.

[B] Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e, f, g at same column are statistically different.

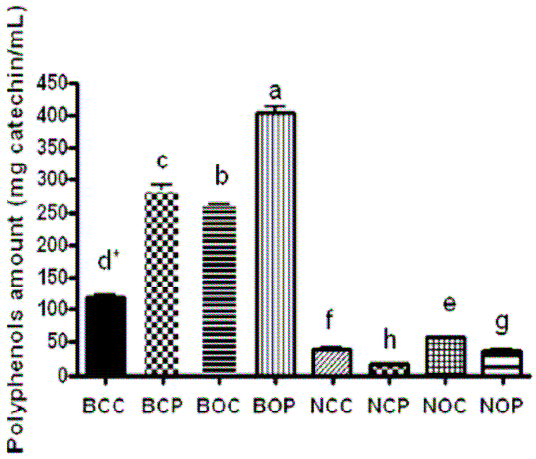

Fig. 1. Polyphenols amount in different grape juices. Values are the mean ±SD of three replicates. BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale. Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e, f, g, h at same figure are statistically significantly different.

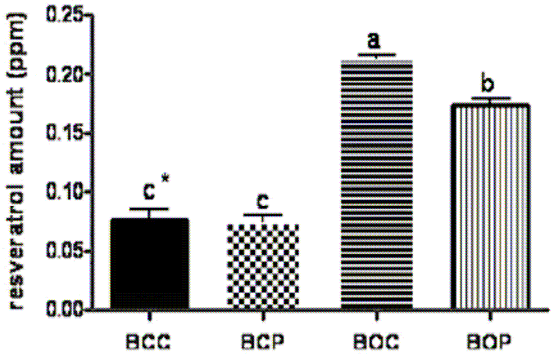

Fig. 2. Resveratrol amount in different purple grape juices. BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale. Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$ that means a, b, c at same figure are statistically significantly different.

3.5. Catechins and procyanidins content

Many differences were observed in the contents of catechins and procyanidins between purple and white juices (Table 4). Except for NCC, purple juices presented higher catechin and epicatechin contents as compared to white juices. Among all evaluated procyanidins, the B_3 fraction presented the highest level in all analyzed juices (Table 4).

3.6. Scavenging of DPPH· radical

Except for NCP and NOP, all juices showed higher or similar antioxidant activity as compared to the standard solution, which contained catechin (Fig. 3). Among all assayed samples, BCP, BOC, BOP, and NOC presented higher antioxidant activity. This activity was positively correlated with total phenolic ($r = 0.616$; $p \leqslant 0.05$), procyanidins B_1 ($r = 0.689$; $p \leqslant 0.05$), B_3 ($r = 0.521$; $p \leqslant 0.05$), and catechin ($r = 0.545$; $p \leqslant 0.05$) contents. Purple juice antioxidant activity was positively correlated with malvidin, cyanidin, peonidin, and delphinidin ($r = 0.781$; $p < 0.01$), catechin ($r = 0.741$; $p < 0.01$), and procyanidin B1 ($r = 0.781$; $p < 0.01$) contents.

3.7. Serum lipid peroxidation inhibition assay

$CuSO_4$-induced lipid peroxidation inhibition was tested for the eight grape juices samples (Table 5). Except for NCP, all grape juices were able to suppress serum lipid peroxidation induced by $CuSO_4$. Among all tested samples, BCP showed the highest lipoperoxidation protection activity (2.94 ± 0.03 nmol/mL) as compared to the $CuSO_4$ control (4.88 ± 0.03 nmol/mL).

3.8. Superoxide dismutase and catalase-like activities

In the present study, superoxide dismutase and catalase-like activities of different grape juices were tested. Among all tested samples (Table 6), BOP showed the highest superoxide-like activity, with the lowest IC50 value (3.52 ± 0.00 ml). There was a positive correlation between sod-like activity and total phenolic content ($r = 0.838$, $p < 0.01$), epicatechin content ($r = 0,824$, $p < 0.01$), and ascorbic acid level ($r = 0.625$, $p < 0.01$). NCP presented the highest

Table 3
Anthocyanins content in different purple grape juices ($n = 4$)

Sample[A]	Cyanidin (ppm)	Delphynidin (ppm)	Peonidin (ppm)	Malvidin (ppm)
BCC	$0.76 \pm 0.04^{B,c}$	4.10 ± 0.40^{c}	8.59 ± 0.82^{a}	95.26 ± 1.95^{d}
BCP	12.98 ± 0.51^{b}	30.22 ± 1.35^{b}	22.82 ± 1.18^{b}	308.76 ± 4.20^{b}
BOC	11.79 ± 0.42^{b}	26.30 ± 1.15^{b}	19.21 ± 1.43^{b}	232.46 ± 4.25^{c}
BOP	20.91 ± 0.83^{a}	49.51 ± 1.80^{a}	32.60 ± 1.78^{c}	425.96 ± 6.36^{a}

Values are the average of three replicates ±SD.
[A] BCC: Bordo Conventional Commercial; BCP: Bordo Conventional Pilot Scale; BOC: Bordo Conventional Organic; BOP: Bordo Organic Pilot Scale.
[B] Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, at same column are statistically different.

Table 4
Catechins and procyanidins content in different grape juices ($n = 8$)

Sample[a]	Catechins (ppm)		Procyanidins (ppm)			
	Catechin	Epicatechin	B1	B2	B3	B4
BCC	$2.06 \pm 0.15^{d,*}$	22.13 ± 1.92^{a}	1.33 ± 0.18^{d}	1.83 ± 0.16^{a}	7.95 ± 0.58^{d}	4.66 ± 0.36^{a}
BCP	86.43 ± 2.31^{a}	2.11 ± 0.20^{c}	7.98 ± 0.67^{b}	1.88 ± 0.15^{a}	27.04 ± 1.32^{a}	2.27 ± 0.27^{b}
BOC	33.89 ± 1.82^{c}	2.72 ± 0.22^{c}	7.53 ± 0.52^{b}	2.32 ± 0.15^{a}	10.03 ± 0.51^{c}	0.64 ± 0.13^{e}
BOP	76.69 ± 2.70^{b}	4.91 ± 0.21^{b}	14.0 ± 0.82^{a}	1.13 ± 0.34^{a}	25.68 ± 1.17^{a}	2.93 ± 0.38^{a}
NCC	7.39 ± 0.52^{d}	5.95 ± 0.32^{b}	7.53 ± 0.31^{b}	1.32 ± 0.42^{a}	13.06 ± 0.62^{c}	2.45 ± 0.26^{b}
NCP	0.79 ± 0.05^{d}	0.97 ± 0.14^{d}	0.93 ± 0.06^{d}	0.94 ± 0.23^{a}	14.78 ± 0.34^{b}	1.68 ± 0.27^{c}
NOC	0.90 ± 0.06^{d}	1.81 ± 0.10^{c}	3.45 ± 0.43^{c}	1.58 ± 0.38^{a}	18.5 ± 0.7^{b}	3.59 ± 0.50^{a}
NOP	0.38 ± 0.02^{d}	0.92 ± 0.16^{d}	0.76 ± 0.11^{d}	0.61 ± 0.10^{b}	7.47 ± 0.28^{d}	1.23 ± 0.24^{d}

Values are the average of three replicates ±SD.

* Different letters correspond to mean values statistically different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e at same column are statistically different.

[a] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale.

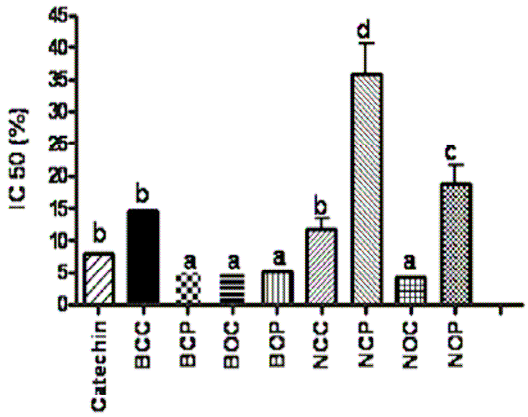

Fig. 3. IC 50 (amount of samples needed to reduce 50% of DPPH.) of different grape juices. Values are the mean ± SD of three replicates. BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale. Different letters correspond to mean values statistically significantly different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c at same figure are statistically.

Table 5
Peroxidation levels of serum treated with or without $CuSO_4$ or grape juice ($n = 8$)

Sample[a]	TBARS (nmol/mL)
Control	$3.29 \pm 0.03^{*}$
$CuSO_4$	$4.88 \pm 0.03^{**}$
$CuSO_4$ + BCC	$4.41 \pm 0.00^{*}$ (**)
$CuSO_4$ + BCP	$2.94 \pm 0.03^{*}$ (**)
$CuSO_4$ + BOC	$4.58 \pm 0.00^{*}$ (**)
$CuSO_4$ + BOP	$3.51 \pm 0.03^{*}$
$CuSO_4$ + NCC	$3.77 \pm 0.03^{*}$
$CuSO_4$ + NCP	$4.90 \pm 0.00^{**}$
$CuSO_4$ + NOC	$4.33 \pm 0.06^{*}$ (**)
$CuSO_4$ + NOP	$4.01 \pm 0.00^{*}$ (**)

Values are the average of three replicates ±SD.

* $p < 0.05$ compared to $CuSO_4$.

** $p < 0.05$ compared to control.

[a] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale.

catalase-like activity (34.37 ± 0.62 μmol of H_2O_2 decomposed/min).

4. Discussion

4.1. Grape juice phenolic compounds, vitamin C, and nutritional analyses

Phenolic compounds are secondary metabolites produced and accumulated in plant tissues. Changes in phytopathogenesis, among others factors, may result in different concentrations of these compounds in plant organs. Organic farming is currently practiced world wide, and does not use chemical substances, such as pesticides and synthetic fertilizers, for growing crops. As pesticides are not used, plants are more susceptible to the action of phytopathogens, and this causes the plant to produce higher amounts of phenolic compounds as a means to defend itself (Soleas et al., 1997). In the present study, it was observed that the choice of agricultural method (organic versus conventional) resulted in different amounts of resveratrol, anthocyanins, and tannins in grape juices (Fig. 2; Tables 3 and 4). It should be noted that the present study is the first to report resveratrol content differences between organic and conventional grape juices. This difference is due to the fact that no pesticides are used in organic vineyards (Carbonaro et al., 2002).

Moreover, different methodologies are applied in grape juice manufacturing. When purple juices are produced, the pulp is heated along with the skin, resulting in the incorporation of phenolic compounds into the juice (Fuleki and Ricardo-da-Silva, 2003). In the present study, we also observed that purple juices presented higher phenolic compound content as compared to white juices, in which manufacturing process the grape skin is not heated (Fig. 1).

Table 6
Sod-like and catalase-like activity in different grape juices ($n = 8$)

Sample[A]	Sod-like activity (IC 50)[B]	Cat-like activity (μmol of H_2O_2 decomposed/min)
BCC	$5.40 \pm 0.05^{a,C}$	3.77 ± 0.02^{a}
BCP	11.64 ± 0.01^{d}	20.27 ± 0.25^{f}
BOC	6.47 ± 0.01^{c}	7.60 ± 0.10^{b}
BOP	3.52 ± 0.02^{b}	9.37 ± 1.87^{c}
NCC	20.53 ± 0.17^{e}	12.12 ± 0.87^{d}
NCP	22.98 ± 0.01^{f}	34.37 ± 0.62^{g}
NOC	87.00 ± 0.25^{h}	15.00 ± 0.10^{e}
NOP	30.42 ± 0.04^{g}	18.87 ± 0.125^{e}

Values are the average of three replicates ±SD.
[A] BCC: Bordo conventional commercial; BCP: Bordo conventional pilot scale; BOC: Bordo conventional organic; BOP: Bordo organic pilot scale; NCC: Niagara conventional commercial; NCP: Niagara conventional pilot scale; NOC: Niagara conventional organic; NOP: Niagara organic pilot scale.
[B] Amount of samples (ml) necessary to reduce 50% the adrenochrome formation.
[C] Different letters correspond to mean values statistically significantly different by analysis of variance (ANOVA) and Tukey post hoc test, for $p < 0.01$, that means a, b, c, d, e, f, g at same column are statistically significantly different.

In addition to phenolic compounds, vitamin C is also present in grape juices. In plants, vitamin C provides protection against RS generated during photosynthesis and respiration processes. Vitamin C is also involved in cell growth, and it is a co-factor of several enzymes participating in the synthesis of anthocyanidins and several secondary metabolites (Barata Soares et al., 2004). Vitamin C levels were positively correlated to total polyphenol ($r = 0.878$; $p < 0.01$), procyanidins B1 ($r = 0.676$; $p < 0.01$) and B2 (0.852; $p < 0.01$), and catechin (0.799; $p < 0.01$) contents. Except for BCP juice, organic juices presented higher vitamin C content as compared to conventional juices (Table 2). Similar results were found by Carbonaro et al. (2002) with organic and conventionally-grown peaches. Significant variation in vitamin C levels was observed between purple and white juices, and this is probably due to grape variety, ripening grade, and hours of sun exposure (Wang et al., 1996).

Several factors may influence grape juice nutritional analyses, such as grape variety, soil, climate, processing methods, etc. (Fuleki and Ricardo-da-Silva, 2003). In the present study, we observed that grape juices produced at pilot scale presented high carbohydrate levels. Although further studies are needed to confirm this hypothesis, this fact may be attributed to manufacturing process (Table 2).

4.2. Grape juice antioxidant properties

Except for one study that showed *in vitro* antioxidant activity of a white grape juice (Dávalos et al., 2005), all other studies on antioxidant activity of grape juices used purple grape juices elaborated from *Vitis vinifera* (Castilla et al., 2006; Rho and Kim, 2006; Day et al., 1997). This is the first study that showed the antioxidant activity of grapes juices produced with *V. labrusca* varieties (Bordo and Niagara), which are frequently used to produce grape juice in South America.

All studied grape juices showed significant *in vitro* antioxidant activity (Fig. 3). In the *ex vivo* assay, only one grape juice (NCP) did not prevent $CuSO_4$-induced peroxidation in serum (Table 5). Indeed, this grape juice presented the lowest total phenolic and ascorbic acid contents. This result suggests that these compounds provide protection by inhibiting lipid peroxidation, as shown by Carbonaro et al. (2002) in peaches. Although the measurement of other possible chemicals found in juices would be important, grape juices are a very complex mixture of compounds. The biologically active compounds are mainly phenolic and ascorbic acid. To our knowledge, the presence of alkaloids and aflatoxins in grapes juices is rare. No references on this issue were found in a recent literature database revision.

It was observed that white juices presented higher catalase-like activity antioxidant response as compared to purple juices (Table 6). In fact, some polyphenols are able to decompose H_2O_2 (Ferguson, 2001), thereby reducing the damage induced by this oxidative agent (Halliwell and Gutteridge, 1999).

This study showed that grape juices elaborated from *V. labrusca* are good antioxidant sources. This biological activity can be influenced by agricultural methods and polyphenols and ascorbic acid levels present in the juices.

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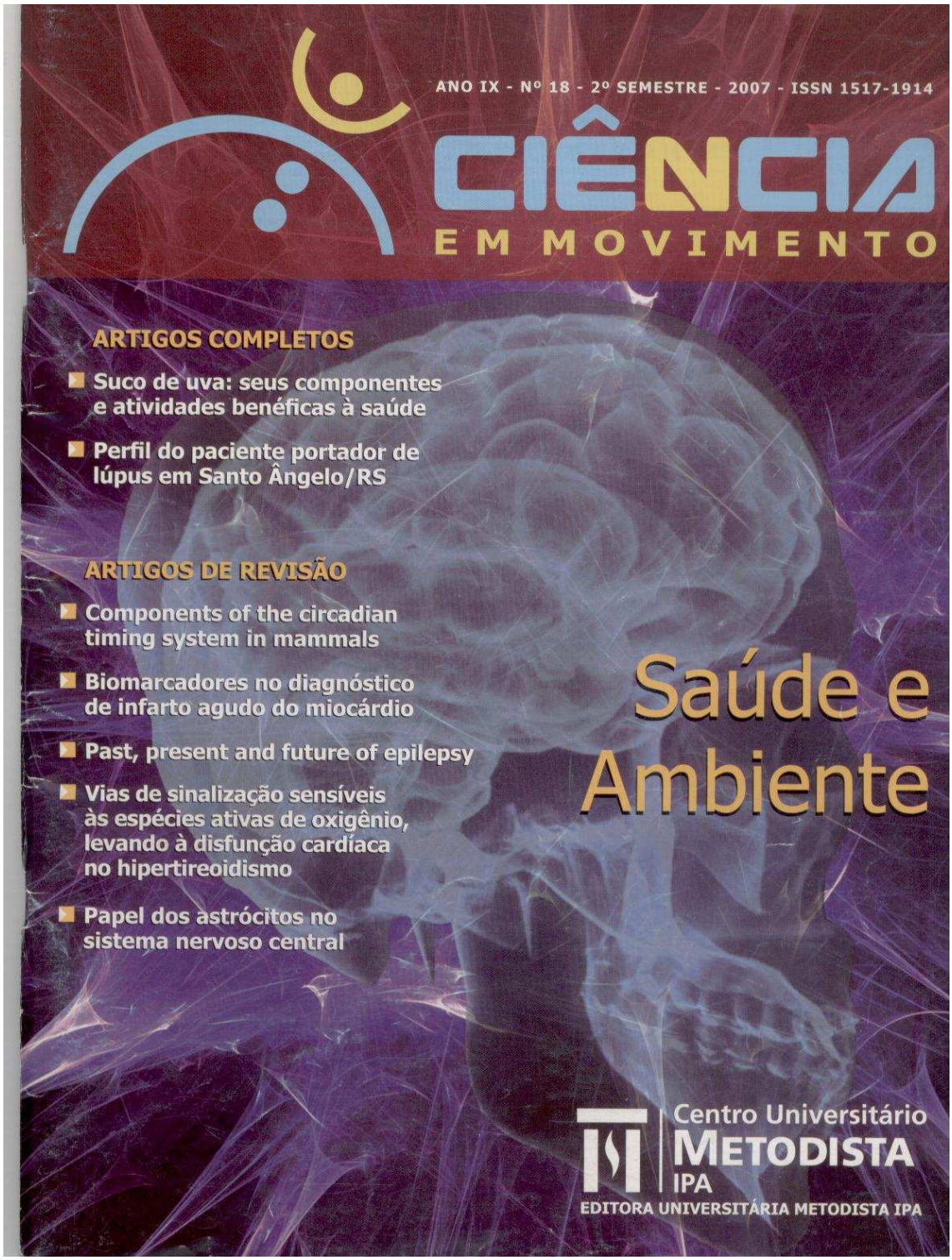
ANEXO B

Suco de uva: seus componentes e atividades benéficas à saúde. Artigo publicado na Revista Ciência e Movimento, ANO IX, 18, 2º semestre, 2007. ISSN 1517-1914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Suco de uva: seus componentes e atividades benéficas à saúde

Grape juice: its components and beneficial activities to the health

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João A.P. Henriques****

RESUMO

Embora os efeitos benéficos da ingestão entrada moderada do vinho sejam bem conhecidos, dados sobre a capacidade antioxidante de sucos de uva é escassa e controversa. A finalidade deste estudo foi mostrar a quantidade importante de polifenóis, por exemplo, as antocianinas, o resveratrol, catequina, epicatequina, procianidinas, bem como o índice ácido ascórbico em sucos de uva, e assim avaliar sua possível atividade antioxidante. Em nosso estudo nós avaliamos oito sucos do labrusca de *Vitis labrusca*, branco ou tinto, a partir de vinhedos orgânicos e convencionais, obtidos em escala piloto ou comercial. Através dos resultados nós pudemos observar que todos os sucos de uva apresentaram um índice total importante de polifenóis, bem como atividade antioxidante in vitro, além disso observou-se que os sucos foram capazes de reduzir os danos causados pelos radicais livres no cérebro e no fígado. Estes resultados indicam que os sucos de uva brancos e tintos podem ser usados como fontes antioxidantes e nutritivas.

Palavras-chave
uva; polifenóis; antioxidante.

ABSTRACT

Although the beneficial effects of moderate wine intake are well-known, data on antioxidant capacity of grape juices are scarce and controversial. The purpose of this study was to show the important total polyphenols, anthocyanins, resveratrol, catechin, epicatechin, procyanidins, and ascorbic acid contents in grape juices, and to assess their possible antioxidant activity. In our study we evaluated eight *Vitis labrusca* juices – white or purple, from organically- or conventionally-grown grapes, and obtained in pilot or commercial scale. Through the results we could observe that all grape juices presented important total polyphenol content and *in vitro* antioxidant activity. Besides, juices were capable to reduce free radical action in brain and liver. These results indicate that white and purple grape juices can be used as antioxidants and nutritional sources.

Key words
grape, polyphenols, antioxidant.

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SUCO DE UVA

A história da elaboração do suco de uva começou nos Estados Unidos, na cidade de Vineland, New Jersey, em 1868. Aplicando as idéias de Louis Pasteur na elaboração de uvas Concord, um grupo de religiosos pôde fazer um "vinho não fermentado sacramental" para uso em sua igreja. Tamanho foi o sucesso que, em 1893, o suco de uva era a bebida favorita nos Estados Unidos. Do momento de sua criação até os dias de hoje, a busca do equilíbrio entre o teor de açúcar e acidez é premissa básica para se produzir um suco de uva de boa qualidade (CAINELLI, 2006).

Segundo a legislação brasileira, Lei Nº. 7678 artigo 4°, parágrafo 5°, de 8 de novembro de 1988, suco de uva é uma bebida extraída da uva através de processo tecnológico adequado, não fermentado, não alcoólico, de cor, aroma e sabor característico, submetido a tratamento que assegure a sua conservação e apresentação até o momento do consumo (RIZZON et al., 1998). O Brasil no cenário internacional é o 21° país em área cultivada, 14° em produção de uvas, 24° em exportação de uva e o 10° país em exportação de suco de uva. (Cadastro Vinícola – IBRAVIN/2006). O suco de uva, bem como todos os produtos provenientes da uva, possui um importante papel econômico na região da Serra Gaúcha, e em todo o Estado do Rio Grande do Sul.

Devido à facilidade de elaboração, aliada às características organolépticas e ao seu valor nutricional, o suco pode contribuir na dieta alimentar. A princípio, o suco pode ser elaborado com uva de qualquer variedade, desde que este alcance uma maturação adequada e apresente bom estado sanitário. O suco produzido em muitos países de tradição vitícola é elaborado com uvas *Vitis vinifera* tanto de cultivares brancas quanto tintas. Já o suco de uva brasileiro é elaborado principalmente com uvas dos grupos das *Vitis labrusca*, ou conhecidas como americanas e híbridas tintas, principalmente Bordo e Concord (RIZZON et al., 1998). No entanto, recentemente, tem sido utilizadas também uvas das variedades Niagara branca (sucos brancos).

Na viticultura convencional, utiliza-se tradicionalmente, herbicidas, inseticidas e fungicidas, principalmente dos grupos químicos ditiocarbamatos e organofosforados (RIZZON et al., 1998). Entretanto os sucos orgânicos são elaborados segundo as diretrizes de padrão de qualidade (1ª ed. de 31 de outubro de 1989) que tiveram por base as normas da IFOAM (International Federation of Organic Moviments), sendo produzidos a partir de uvas colhidas de vinhedos sem uso de fitodefensivos e/ou engenharia genética, entre outras normas pré-estabelecidas pelas diretrizes citadas acima.

A produção de alimentos orgânicos é caracterizada pela ausência ou limitação no uso de produtos químicos sintéticos. Alguns destes podem aumentar ou diminuir a produção de compostos fenólicos em plantas. Isto ocorre, pois, entre outras funções, os compostos fenólicos fazem parte do sistema de defesa da planta, ou seja, o conteúdo nas plantas é influenciado pelas condições de cultivo e colheita, assim como condições de crescimento, grau de amadurecimento, tamanho dos frutos, e a variedade da planta (HERRMANN, 1976).

Geralmente as plantas provindas da agricultura orgânica têm um período de amadurecimento maior em comparação com as convencionais, principalmente devido a uma liberação mais lenta de nutrientes. Como os flavonóides são formados durante o período de amadurecimento, acredita-se que o conteúdo destes seja maior nas planta com manejo orgânico (GRINDER-PEDERSEN et al., 2003). No entanto, um limitado número de estudos tem investigado o efeito da técnica de cultivo no conteúdo fenólico, e os resultados são contraditórios (ASAMI et al., 2003; LOMBARDI-BOCCIA et al., 2004). ASAMI e colaboradores (2003) observaram um aumento significativo na quantidade de polifenóis em morangos orgânicos quando comparados às culturas convencionais. Até o momento não existem estudos comparando o conteúdo polifenólico de sucos de uva orgânicos e convencionais.

O suco de uva, principalmente os elaborados a partir das variedades tintas, assim como o vinho, é uma fonte de flavonóides como, por exemplo, catequina, epicatequina e antocianidinas (FULEKI & RICARDO-DA-SILVA, 2003). Recentemente demonstrou-se que sucos tintos e brancos produzidos a partir de *Vitis vinifera*, apresentam importante atividade antioxidante *in vitro* (DÁVALOS et al., 2005). Entretanto apenas um estudo vem mostrando atividade antioxi-

dante de sucos de uva brancos e tintos (variedades Bordo e Niagara) elaborados a partir de *Vitis labrusca*, variedades estas usadas em larga escala na vitivinicultura brasileira (DANI et al., 2007a). O mesmo estudo ainda compara a atividade antioxidante dos sucos com relação às suas variedades, à prática agrícola (orgânico x convencional) e ao método de elaboração (escala piloto e comercial) (DANI et al., 2007a).

A ingestão de aproximadamente 125-480 mL/dia de suco de uva tinto convencional, principalmente provindos das variedades *Vitis vinifera*, foi capaz de aumentar a atividade antioxidante (DAY et al., 1997; OSMAN et al., 1998; FREEDMAN et al., 2001; O'BYRNE et al., 2002). Ao suco de uva também é atribuído à diminuição de doenças cardiovasculares, e a inibição de processos tumorais, através da redução de danos oxidativos ao DNA (VINSON et al., 2001; SINGLETARY et al., 2003; PARK et al., 2003; SANCHEZ-MORENO et al., 1999), além de ser um inibidor da oxidação da LDL (ABU-AMSHA et al., 1996; WANG et al., 1996; DURAK et al., 1999).

POLIFENÓIS

Os constituintes fenólicos são de grande importância na enologia pelas características direta ou indiretamente ligadas à qualidade do vinho e suco, especialmente em relação à cor e à adstringência. Estes compostos também são de interesse nutricional e farmacológico (RIBERÉAU-GAYON et al. 2003). A uva contém compostos não flavonóides na polpa e flavonóides nas cascas e sementes. Isto permite adaptar as condições de extração dos polifenóis a partir das diferentes partes dos grãos de maneira essencial à composição fenólica dos vinhos e sucos (RIBERÉAU-GAYON et al. 2003)

Os polifenóis possuem, pelo menos, um anel aromático no qual, ao menos um hidrogênio é substituído por um grupamento hidroxila. Podem ser classificados segundo o tipo de esqueleto principal, dividindo-se em flavonóides e os não-flavonóides (FERGUSON, 2001).

Aos polifenóis em geral, atribui-se a capacidade de quelar metais, inibir a atuação do radical livre superóxido e do oxigênio singlet. Além disso, os polifenóis apresentam também, atividades antitrombótica, antinflamatória, antiviral, antialérgica e de proteção aos hepatócitos, como também atividade anticancerígena (por inibição das enzimas topoisomerases I e II) (FERGUSON, 2001; TRUEBA & SÁNCHEZ, 2001; LANDRAULT et al., 2003).

Em videiras, a biossíntese de polifenóis é diretamente influenciada pela variedade de uva, de suas características genéticas, da área de produção bem como do método de elaboração (BOWERS et al., 1993; BOULTON et al., 1995; PÉREZ-MAGARIÑO e GONZÁLEZ-SANJOSÉ, 2004). Diversos fatores vitícolas estão intrinsecamente ligados ao processo de biossíntese, tais como, porta-enxerto utilizado (susceptibilidade a doenças, vigor, etc.), temperatura (gradiente térmico ideal entre o dia e a noite próximo de 15° C), umidade (incidência de doenças fúngicas), exposição solar (horas de incidência direta dos raios do sol sobre as uvas em processo de maturação), tipo de solo e adubação (altos teores de matéria orgânica favorecem um rendimento excessivo, prejudicando a qualidade do fruto), manejo do dossel vegetativo (maior controle sobre maturação e rendimento) (BOULTON et al., 1995; CORTELL et al., 2005), entre outros. A maturação da uva também apresenta um impacto já estabelecido no teor de polifenóis, em virtude da grande variedade de processos físicos e bioquímicos que ocorrem durante o amadurecimento das uvas (GONZÁLEZ-SANJOSÉ, 2006).

A concentração de polifenóis em sucos varia de acordo com diversos fatores, como: método de elaboração escolhido, rendimento da prensagem, tempo e temperatura de maceração, estabilização e filtração (FULEKI & RICARDO-DA-SILVA, 2003), entre outros.

NOSSOS RESULTADOS

Em nosso estudo, oito amostras de suco de uva, brancos (Niagara) e tintos (Bordo), produzidos com uvas orgânicas e convencionais, bem como elaborados em escala piloto e comercial foram utilizadas para avaliar diversos parâmetros nutricionais e antioxidantes (DANI et al., 2007a). Nossos resultados foram obtidos a partir de diferentes metodologias e os mesmos estão expressos na tabela 1. A quantificação de compostos fenólicos totais foi determinada espectrofotometricamente, UV (280nm) utilizando a curva de catequina como padrão (SINGLETON et al., 1996). Os compostos majoritários (catequina,epicatequina, pro-

cianidinas, resveratrol e antocianinas) foram determinados por Cromatografia Líquida de Alta Eficiência (CLAE).

Os sucos tintos apresentaram, em geral, maior conteúdo fenólico (principalmente taninos) do que os brancos e o rosado. Quanto à diferença entre sucos orgânicos e convencionais, os sucos orgânicos incorporaram maior nível de compostos fenólicos, resveratrol, catequinas e antocianidinas (DANI et al., 2007a). Este fato pode ser explicado, pois os compostos fenólicos são metabólitos secundários da planta, produzidos em situação de estresse, que no caso da videira orgânica é gerado, pois ela não recebe proteção dos fitodefensivos, ou outros agentes (SOLEAS, 1997).

O método de elaboração dos sucos também apresenta diferenciações, principalmente na produção de sucos brancos e tintos, onde geralmente os primeiros são produzidos no método a frio e os outros na metodologia a quente, ou seja, se aquece o suco em contato com a casca e semente, fato este que pode ser responsável pela maior incorporação de compostos fenólicos e demais compostos (FULEKI & RICARDO-DA-SILVA, 2003). Em nosso trabalho os sucos elaborados em escala piloto, ou seja, aquecimento para tintos e a frio para os brancos, apresentaram diferenças em alguns parâmetros nutricionais, como carboidratos e valor calórico. Aqueles produzidos em escala piloto, apresentaram maiores níveis de carboidratos e conseqüentemente, uma maior valor calórico (DANI et al., 2007a). Esta afirmação pode vir a contribuir para a escolha do melhor processo de produção de sucos, para a maior incorporação de compostos nutricionais importantes.

Dentre os ensaios *in vitro* existentes, um dos mais utilizados é o radical DPPH• (1,1 difenil 2-picrilhidrazil) que vem sendo utilizado para medir a capacidade de varredura dos antioxidantes (RICE-EVANS *et al.*, 1995). O método de varredura do radical DPPH• (1,1-difenil-2-picrilhidrazil) é um método amplamente utilizado para avaliar a atividade antioxidante em um tempo relativamente curto comparado com outros métodos (BRAND-WILLIANS *et al.*, 1995; MENSOR et al., 2001). Os antioxidantes reagem com o DPPH•, que é um radical livre estável, e provocam a diminuição da coloração indicando o potencial de varredura da solução antioxidante. A atividade da amostra a ser testada é atribuída a sua capacidade de doar elétrons (MURTHY *et al.*, 2002).

Além dos ensaios *in vitro*, testes *ex-vivo*, como a inibição de hemólise pelo radical AAPH e a inibição da peroxidação lipídica em soro, tem sido extensamente utilizados (EDENHARDER & GRÜNHAGE, 2003; BUB *et al.*, 2003). Dados obtidos através de testes *in vitro*, *ex-vivo* e *in vivo* podem apresentar diferentes resultados quanto à avaliação da capacidade antioxidante. Em células, a expressão da atividade antioxidante é mais complexa, pois envolve questões de permeabilidade celular e possível metabolização dos compostos ensaiados, além de um complexo sistema regulatório enzimático de defesas antioxidantes endógenas celulares (RASPOR *et al.*, 2003).

A atividade antioxidante *in vitro* foi comprovada por diversos testes, entre eles a capacidade de varredura do radical DPPH•. Neste teste, os sucos tintos apresentaram uma melhor atividade antioxidante, destacando-se o tinto orgânico comercial (DANI et al., 2007a) SANCHEZ-MORENO et al. (1999) mostraram por meio deste mesmo teste, DPPH•, que vinhos e sucos tintos apresentam atividade antioxidante superior a dos produtos brancos.

Ainda na avaliação da atividade antioxidante *in vitro*, as habilidades de seqüestrar superóxido (Sod-like) e degradar peróxido de hidrogênio (Cat-like) também foram dosadas. Os sucos tintos apresentaram uma melhor atividade Sod-like do que os brancos. Este fato em parte pode ser explicado, pois estes também são os mais ricos em polifenóis e ácido ascórbico (DANI et al., 2007a). Em um estudo realizado observou-se que extratos ricos em polifenóis preservam atividade de enzimas antioxidantes devido a sua atividade seqüestradora de radicais livres e/ou indução de defesa de expressão das enzimas (CHIDAMBARA MURTHY et al., 2002). Com relação à atividade Cat-like, observou-se que os sucos brancos apresentaram uma melhor atividade quando comparados aos tintos (DANI et al., 2007a), valores estes que apresentam uma correlação positiva com a atividade antioxidante *in vivo*, determinada em células da levedura eucariótica *Saccharomyces cerevisiae*.

A atividade antioxidante *ex vivo* foi determinada pelo teste inibição de hemólise, que utiliza como indutor de hemólise o agente estressor o AAPH• e pelo

teste de inibição da peroxidação lipídica do soro, utilizando como agente estressor, o sulfato de cobre. Na inibição da hemólise, todos os sucos apresentaram uma excelente atividade antioxidante, sendo que a maioria conseguiu inibir em 100% a atividade hemolítica dos eritrócitos induzida pelo AAPH•. Este método já foi validado por outros trabalhos nos quais foram considerados excelentes antioxidante compostos que possuíam esta atividade inibitória, como por exemplo, o extrato aquoso de Bidens pilosa e alguns flavonóides isolados (ABAJO et al., 2004; EDENHARDER & GRÜNHAGE, 2003).

Somente o suco niágara convencional escala piloto não apresentou capacidade de inibir a peroxidação lipídica induzida pelo reconhecido agente estressor, sulfato de cobre sendo que este foi o que apresentou o menor conteúdo fenólico e o menor teor de ácido ascórbico. Os demais sucos apresentaram excelente inibição da peroxidação lipídica. Se considerados somente os sucos tintos esta atividade antioxidante pode ser atribuída aos compostos fenólicos e às procianidinas (DANI et al., 2007a). Ainda, atribuí-se aos compostos fenólicos a atividade protetora na inibição da peroxidação lipídica, conforme descrito por CARBONARO et al. (2002) em seus estudos com pêssegos.

Estudos mostraram que o consumo de sucos de frutas e vinho é capaz de reduzir peroxidação lipídica através do teste das espécies reativas do ácido tiobarbitúrico (TBARS), mostrando assim dados que corroboram com a atividade apresentada neste trabalho (FUHRMAN et al., 1995).

Os dados acerca da capacidade antioxidante e/ou mutagênica de suco de uva são poucos e contraditórios (PATRINELLI et al., 1996a; 1996b). Em nosso trabalho os sucos se mostraram excelentes fontes antioxidante, sejam através de testes *in vitro* ou *in vivo*. Nos testes *in vitro* os sucos tintos, principalmente os orgânicos, apresentaram melhores valores, mostrando correlações positivas com o conteúdo fenólico total, resveratrol, catequinas (FERGUSON, 2001). Conforme afirmado por FRANKE et al., 2004, no presente trabalho observou-se uma correlação negativa da atividade antioxidante com a quantidade de açúcar, carboidratos (DANI et al., 2007a).

Os ensaios microbianos têm se mostrado muito adequados na triagem rotineira de vários produtos, sendo testes rápidos, sensíveis, econômicos, reprodutíveis quando comparados a ensaios realizados com animais, e apresentando resultados confiáveis na identificação da atividade biológica (HENRIQUES et al., 1987). Várias razões, tem tornado a levedura *Saccharomyces cerevisiae* um excelente modelo para se estudar oxidação intracelular e estresse oxidativo, sendo que os resultados obtidos nas células de levedura podem ser extrapolados a células humanas (RASPOR et al., 2005). Trata-se de um organismo provido de núcleo e de organelas com metabolismo semelhante à de eucariotos superiores.

Os ensaios com levedura têm sido de grande utilidade na determinação de agentes mutagênicos ambientais ou farmacológicos e servem para complementar os ensaios de mutagenicidade realizados em bactérias (HENRIQUES *et al.*, 1987). A levedura *S. cerevisiae* tem sido amplamente estudada, tornando-se uma ferramenta importante nas pesquisas sobre mutagênese. Experimentos de mutações reversas são os mais comumente utilizados. Estes testes baseiam-se na restauração ou compensação de um defeito gênico responsável por um requerimento nutricional (ZIMMERMANN et al., 1984).

Os testes usados em avaliações de atividade mutagênicas e carcinogênicas também são cada vez mais utilizados para identificar potencial antimutagênico e anticarcinogênico devido a sua flexibilidade para variações metodológicas (DE FLORA et al., 2001). Substâncias que apresentam atividade antimutagênica em leveduras também podem ter este efeito antimutagênico em sistemas de cultura com células de mamíferos (RASPOR et al.,2003). Para que uma substância possa ser considerada antimutagênica ela precisa reverter a mutagênese induzida por um agente mutagênico, como por exemplo, o peróxido de hidrogênio (H_2O_2) (HENRIQUES et al., 2001).

Nos testes *in vivo*, os sucos brancos apresentaram uma melhor atividade protetora aos danos gerados pelo peróxido de hidrogênio. Entre os sucos tintos, os orgânicos mostraram valores melhores quanto à proteção, atividade esta que apresentou correlação positiva com o conteúdo de antioxidante não-flavonóide, o resveratrol. Outro estudo já relatou a capacidade do resveratrol de ser um potente antioxidante e de inibir peroxidação lipídica (VINSON, 1995).

Apesar dos sucos brancos apresentarem os menores valores polifenóis eles apresentaram uma atividade protetora significativamente superior aos sucos tintos quanto aos danos gerados pelo peróxido de hidrogênio e do terc-butil. Esta afirmação pode em parte ser explicada, pois segundo SOLEAS et al. (1997) o conteúdo fenólico não é o único fator que influencia na atividade antioxidante de derivados da uva, existem outros compostos bioativos como vitaminas e minerais (selênio), bem como o sinergismo entre eles pode também estar relacionado com a atividade protetora aos danos gerados pelas espécies reativas de oxigênio (ERO).

As diferenças apresentadas nos efeitos antioxidantes observados em modelos *in vitro, ex vivo* e *in vivo*, poderia ser atribuída ao metabolismo dos polifenóis. Diferenças estas que já foram apresentadas em estudos que mostram que os efeitos antioxidantes *in vivo* dos polifenóis dependem de sua ingestão e da sua biodisponibilidade (VINSON et al., 2004). Segundo MANACH et al. (2004), a biodisponibilidade dos polifenóis pode ser influenciada pela estrutura dos compostos, absorção, interação com outros compostos e metabolismo.

Não foram detectados organofosforados ou pesticidas nas amostras analisadas de suco de uva, em nosso estudo (DANI et al., 2007a). No entanto, as frutas, assim como a uva, também podem causar mutagenicidade e toxicidade, uma vez que a presença de pesticidas e/ou alguns compostos químicos podem apresentar efeitos adversos, podendo atuar na proliferação de tumores (AMES et al., 1997). Além disso, antioxidantes presentes nas frutas podem vir a sofrer auto-oxidação e gerar substâncias reativas pró-oxidantes dependendo das condições celulares (HALLIWELL & GUTTERIDGE, 2000).

Os sucos neste trabalho, em concentrações maiores (50 e 25 %), apresentaram uma fraca atividade antimutagênica, a qual pode ser atribuída em parte ao conteúdo de polifenóis totais, vitaminas, ou até mesmo a combinação destes fatores. Entretanto, na concentração de 10% a maioria dos sucos apresentou atividade antimutagênica importante, sendo capazes de reverter mutações geradas pelo agente reconhecidamente mutante, o peróxido de hidrogênio.

A atividade antimutagênica apresentou correlação positiva com o conteúdo fenólico na maioria dos sucos, sendo que nos tintos esta atividade foi principalmente correlacionada ao conteúdo do resveratrol. A atividade antimutagênica de polifenóis, sejam flavonóides ou não-flavonóides, já foi relatada pela literatura, o que leva a população a busca de dietas ricas nos mesmos (FERGUSON, 2001).

A partir dos resultados discutidos acima, estudos adicionais investigando os efeitos dos sucos de uva em animais são necessários, para auxiliar no melhor entendimento de como os sucos de uva protegem biomoléculas contra dano oxidativo.

Estudos recentes do nosso grupo de pesquisa, utilizando como modelo *in vivo* ratos machos Wistar, avaliaram parâmetros antioxidantes em dois sucos de uva tintos, orgânicos e convencionais, em diversos tecidos (substância nigra, estriado e fígado), bem como no plasma (DANI et al., 2007b; 2007c).

Em um dos estudos, observou-se que os sucos de uva, principalmente os orgânicos, foram capazes de altera os parâmetros oxidativos no plasma e fígado dos animais. O grupo de animais que recebeu diariamente suco de uva orgânico apresentou uma maior redução nos níveis de peroxidação de lipídios (TBARS) quando comparado ao grupo que recebeu o suco convencional e ao grupo controle, que só recebeu salina. No entanto, o dano oxidativo as proteínas diminuiu após o tratamento com ambos os grupos que receberam os sucos, mostrando-se melhor do que o grupo controle. Quando avaliado as enzimas antioxidantes, os grupos tratados com sucos orgânicos apresentaram uma atividade Sod e Cat maiores quando comparados aos grupos tratados com suco de uva convencional e salina (DANI et al., 2007b).

Essas atividades de proteção poderiam indicar uma ação hepatoprotetora do suco de uva. ORHAN e cols (2007) mostraram que o extrato de etanol de *Vitis vinifera* foi capaz de induzir uma possível ação hepatoprotetora, apesar de não terem sido medidos os compostos ativos. Eles acreditam que possa ser devido à (i) inibição da atividade da citocromo P450 oxigenase-dependente; (ii) prevenção da peroxidação lipídica e (iii) estabilização da membrana do hepatócito, induzida pelos compostos fenólicos. Além do mais, outros estudos relatam que, as plantas que não são tratadas com pesticidas e agrotóxicos são mais sus-

cetíveis à ação de organismos fitopatogênicos, o que resulta numa maior produção de compostos fenólicos (BOURN et al., 2002). Isso foi possível observar nesse estudo uma vez que o suco de uva orgânico apresentou um maior conteúdo de compostos fenólicos e conseqüentemente uma melhor atividade antioxidante, dados estes que corroboram com outros estudos (FERGUSON, 2001; MOKNI et al., 2007).

No outro estudo de nosso grupo utilizou-se um grupo de ratos mais velhos e verificou-se a atividade antioxidante dos sucos de uva em amostras de tecido do cérebro (substância nigra e estriado). Esse é primeiro estudo a mostrar que sucos de uva tintos podem reduzir o estresse oxidativo em estruturas do sistema nervoso (DANI et al., 2007c). Nesse estudo observou-se que a ingestão crônica de suco de uva reduziu a peroxidação lipídica e o dano oxidativo à proteína após tratamento com tetracloreto de carbono (CCl_4), reconhecido agente estressor. A redução do nível de peroxidação lipídica em estruturas do cérebro foi relatada em outros estudos com ratos os quais receberam extrato de semente de uva (BALU et al., 2005). Essa redução pode ser em parte explicada pelo conteúdo fenólico, do qual as uvas apresentam uma quantidade importante. O papel de seqüestrar os radicais livres e a capacidade de quelar metais ativos redox tem sido atribuído às uvas e seus derivados (BALU et al., 2005).

Pode-se também observar que a ingestão crônica de suco de uva diminui a proporção Sod/Cat nos grupos tratados com sucos em comparação ao grupo controle. No estriado, o suco de uva orgânico mostrou a menor proporção quando comparado aos grupos controles positivos e negativos. A proporção Sod/Cat é um parâmetro bastante importante porque um desequilíbrio entre essas duas enzimas de defesa antioxidante pode elevar o estresse oxidativo e induzir o desenvolvimento de diversas doenças (DAL-PIZZOL et al., 2001). A atividade da Sod resulta na produção de peróxido de hidrogênio que na reação com ferro gera radical hidroxil via a reação de Fenton. A Cat degrada o peróxido de hidrogênio, diminuindo dessa forma, os efeitos oxidativos (HALLIWELL AND GUTTERIDGE, 1999). Nossos resultados mostram que o tratamento com suco de uva pode induzir um aumento da atividade Cat e Sod e reduzir a proporção Sod/Cat, o que sugere uma importante atividade antioxidante dos sucos de uva (DANI et al., 2007c).

Além do mais, foi possível observar que a ingestão de sucos de uva tintos, orgânicos e convencionais, também reduz danos ao DNA, através do teste Cometa (dados não publicados). PARK et al. (2003) também mostrou em seu estudo que o suco de suco de uva foi capaz de reduzir o dano ao DNA, evidenciado pelo teste Cometa.

Apesar de mais estudos serem necessários, é possível afirmar que o suco é uma excelente fonte nutricional, capaz de fornecer componentes nutricionais e/ou polifenólicos.

O suco de uva, por não ser uma bebida alcoólica, pode ser ingerido por crianças e idosos, que fazem uso da polifarmácia, por exemplo. Estudos relatam que o consumo excessivo e crônico do etanol, que também está presente nos vinhos, resulta em danos a diversos órgãos, incluindo o cérebro e fígado. Isso possivelmente acontece em parte devido à habilidade do etanol em aumentar o e estresse oxidativo em diferentes sistemas celulares, levando ao aumento da produção de ERO e produtos da lipoperoxidação (SUN et al., 2001b; MONTELIU et al., 1995).

Dessa forma, muitos são os projetos desenvolvidos em nossa região para a incorporação do suco de uva na merenda escolar de escolas estudais e municipais. Através deste trabalho pode se afirmar que o suco de uva é um alimento rico em diversos compostos e que se ingerido desde a infância terá grande importância na melhoria da qualidade de vida. Esta melhoria é conquistada pelo fato do suco ter se mostrado um excelente antioxidante, capaz de reduzir os danos gerados pelo estresse oxidativo, reduzindo assim doenças importantes como o câncer, doenças neurológicas, entre outras.

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Tabela 1. Parâmetros Nutricionais e Antioxidantes avaliados em diferentes sucos de uvas (n=8)

Ensaios		BCC A	BCE	BOC	BOE	NCC	NCE	NOC	NOE
Valores de polifenóis (mg ácido gálico/L)		119,50± 3,53 ^{d*}	285,00 ± 7,07^{c}	262,5 ± 0,7^{b}	404,5± 9,19^{a}	39,95 ± 1,5^{f}	16,85 ± 0,91^{h}	57,30 ± 0,7^{e}	38,10 ± 0,7 ^{g}
Catequinas (ppm)	(+)-catequina	2,06	86,43	33,89	76,69	7,39	0,79	0,90	0,38
	(-)-epicatequina	22,13	2,11	2,72	4,91	5,95	0,97	1,81	0,92
Procianidinas (ppm)	B1	1,33	7,98	7,53	14,0	7,53	0,93	3,45	0,76
	B2	1,83	1,88	2,32	1,13	1,32	0,94	1,58	0,61
	B3	7,95	27,04	10,03	25,68	13,06	14,78	18,5	7,47
	B4	4,66	2,27	0,64	2,93	2,45	1,68	3,59	1,23
Antocianinas (ppm)	Cianidina	0,76± 0,04^{b}	12,98 ± 0,51^{b}	11,79 ± 0,42^{b}	20,91 ± 0,83^{a}	ND	ND	ND	ND
	Delfinidina	4,10 ± 0,40^{c}	30,22 ± 1,35^{b}	26,30 ± 1,15^{b}	49,51 ± 1,80^{a}	ND	ND	ND	ND
	Peonidina	8,59 ± 0,82^{a}	22,82 ± 1,18^{b}	19,21 ± 1,43^{b}	32,60 ± 1,78^{c}	ND	ND	ND	ND
	Malvidina	95,26 ± 1,95^{d}	308,76 ± 4,20^{b}	232,46 ± 4,25^{c}	425,96 ± 6,36^{a}	ND	ND	ND	ND
Resveratrol (ppm)		0,075 ± 0,01^{c}	0,074 ± 0,01^{c}	0,213 ± 0,02^{a}	0,173 ± 0,01^{b}	ND	ND	ND	ND
Carboidratos (%)		9,43 ± 0,01^{d}	12,93 ± 0,02^{a}	7,82 ± 0,07^{a}	11,76 ± 0,02^{b}	11,19 ± 0,03^{c}	12,68 ± 0,46^{a}	8,43 ± 0,02^{e}	12,48 ± 0,02^{a}
Valor calórico (Kcal/100mL)		39,04 ± 0,05^{d*}	53,68 ± 0,10^{a}	32,47 ± 0,02^{e}	48,36 ± 0,08^{b}	46,03 ± 0,01^{c}	52,12 ± 1,72^{a}	35,07 ± 0,09^{e}	50,92 ± 0,08^{a}
DPPH* (IC50)		14,50 ± 0,00^{b}	5,48 ± 0,00^{a}	5,36 ± 0,00^{a}	5,18 ± 0,00^{a}	11,74 ± 1,73^{b}	35,75 ± 4,82^{d}	4,34 ± 0,00^{a}	18,82 ± 2,83^{c}
Atividade Sod-like (IC50)		5,40 ± 0,05^{e*}	11,64 ± 0,01^{d}	6,47 ± 0,01^{c}	3,52 ± 0,02^{b}	20,53 ± 0,17^{e}	22,98 ± 0,01^{f}	87,00 ± 0,25^{h}	30,42 ± 0,04^{g}
Atividade Cat-like (µmol de H2O2 decomposto/min)		3,77 ± 0,02^{a}	20,27 ± 0,25^{f}	7,60 ± 0,10^{b}	9,37 ± 1,87^{c}	12,12 ± 0,87^{d}	34,37 ± 0,62^{g}	15,00 ± 0,10^{e}	18,87 ± 0,125^{e}
Sobrevivência (%) H_2O_2 75mM = 46,50±4,94^{c}		51,65 ± 2,10^{c}	81,30 ± 2,10^{b*}	70,25 ± 1,95^{b}	74,40 ± 0,00^{b}	98,95 ± 1,05^{a}	100,00 ± 0,00^{a}	100,00 ± 0,00^{a}	100,00 ± 0,00^{a}
% de Inibição de hemólise		100^{a}	84,55^{b}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
TBARS (nmol/mL) Controle negativo = 3,29±0,03^{a} CuSO4—controle positivo = 4,88±0,03^{b}		4,41 ± 0,00^{c}	2,94 ± 0,03^{d}	4,58 ± 0,00^{e}	3,51 ± 0,03^{a}	3,77 ± 0,03^{a}	4,90 ± 0,00^{b}	4,33 ± 0,06^{c}	4,01 ± 0,00^{f}

^{A}BCC: Bordo Convencional Comercial; BCE: Bordo Convencional Escala Piloto; BOC: Bordo Orgânico Comercial; BOE: Bordo Orgânico Escala Piloto; NCC: Niágara Convencional Comercial; NCE: Niágara Convencional Escala Piloto; NOC: Niágara Orgânico Comercial; NOE: Niágara Orgânico Escala Piloto

ND = NÃO DETERMINADO

*Letras distintas correspondem a valores médios estatisticamente diferentes pela análise de variância (ANOVA) e pós-teste de Tukey p<0,01, para cada ensaio realizado

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Fev a Julho de 2004

Estágio Curricular

6. **Laboratório de Análises Clínicas Vitaclin** Dezembro a Fevereiro 2002/Julho de 2003/ Dezembro a Fevereiro de 2003-

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Participação em projetos

1. Avaliação nutricional, antioxidante e mutagenica/antimutagenica em sucos de uva orgânicos convencionais produzidos por métodos diferenciados.
2. Avaliação Atividade moduladora de suco de uva tinto, orgânicos e convencionais, na genotoxicidade, estresse oxidativo e sistema imune de ratos wistar. avaliação dos mecanismos de proteção do resveratrol em linhagens da levedura *S. cerevisiae* .
3. Avaliação nutricional, antioxidante e genotóxica de espumantes brasileiros.
4. Avaliação da atividade antioxidante e genotóxica de resíduos de vinificação.
5. Avaliação do nível de estresse oxidativo em manipuladores de quimioterápicos.

6. Avaliação do nível de estresse oxidativo em manipuladores de tintas.

PROJETOS

2006-atual: Avaliação Atividade moduladora de suco de uva tinto, orgânicos e convencionais, na genotoxicidade, estresse oxidativo e sistema imune de ratos wistar. avaliação dos mecanismos de proteção do resveratrol em linhagens da levedura *S. cerevisiae* .

Situação: Em Andamento; Natureza: Pesquisa

Integrantes: Caroline Dani (Responsável); Lívia Soldatelli Oliboni, José Cláudio Fonseca Moreira, Felipe Dal Pizzol, Elis Eleutherio, João Antonio Pegas Henriques; Miriam Salvador

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Artigos publicados

1. DANI, Caroline, Bonatto D, Pereira, MD, SALVADOR, Mirian, HENRIQUES, João Antonio Pegas, Eleutherio, E Antioxidant Protection of Resveratrol and Catechin. Journal of Agricultural and Food Chemistry. , v.11, p.4268 - 4272, 2008.

2. DANI, Caroline, OLIBONI, Lívia Soldatelli, PASQUALI, M. A. B., OLIVEIRA, M. R., UMEZU, Fernanda de Medeiros, SALVADOR, Mirian, MOREIRA, J. C. F., HENRIQUES, João Antonio Pegas Intake of Purple Grape Juice as a Hepatoprotective Agent in Wistar Rats. Journal of Medicinal Food. , v.11, p.127-32, 2008.

3. DANI, Caroline, PASQUALI, M. A. B., OLIVEIRA, M. R., UMEZU, Fernanda de Medeiros, SALVADOR, Mirian, HENRIQUES, João Antonio Pegas, MOREIRA, J. C. F.Protective Effects of Purple Grape Juice on Carbon Tetrachloride-Induced Oxidative Stress in Brains of Adult Wistar Rats. Journal of Medicinal Food. , v.11, p.55-61, 2008.

4. DANI, Caroline, OLIBONI, Lívia Soldatelli, VANDERLINDE, Regina, Bonatto D, SALVADOR, Mirian, HENRIQUES, João Antonio Pegas Phenolic content and antioxidant activities of white and purple juices manufactured with organically- or conventionally-produced grapes. Food and Chemical Toxicology. , v.45, p.2574 - 2580, 2007.

5. DANI, Caroline, OLIBONI, Lívia Soldatelli, SALVADOR, Mirian, HENRIQUES, João Antonio Pegas. Suco de uva: seus componentes e atividades benéficas à saúde. Ciência em Movimento. , v.18, p.7 - 18, 2007

6. DANI, Caroline, CASTRO, Simone, WAGNER, Sandrine Comparsi Prevalência da anemia e deficiências nutricionais. Revista Brasileira de Análises Clínicas.40(3) , 2008.

Artigos aceitos para publicação

1. DANI, Caroline, OLIBONI, Lívia Soldatelli, VANDERLINDE, Regina, Pra, D, Dias FJ, YONEAMA, M. L., Bonatto D, SALVADOR, Mirian, HENRIQUES, João Antonio Pegas ANTIOXIDANT ACTIVITY, PHENOLIC AND MINERAL CONTENT OF ROSE GRAPE JUICE. Journal of Medicinal Food. , 2008.

Trabalhos publicados em anais de eventos (resumo)

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Trabalhos de conclusão de curso de graduação

1. Sandra Galliazzi Silva. AVALIAÇÃO DA PREVALÊNCIA DE ENTEROPARASITAS E SUA ASSOCIAÇÃO A PRESENÇA DE ANEMIA E/OU CARÊNCIA NUTRICIONAL ESCOLARES DO MUNICÍPIO DE FLORES DA CUNHA. 2007. Curso (Biomedicina) - Centro Universitário Metodista IPA

2. Juliane dos Santos. Sucos e vinhos: comparação entre suas atividades antioxidantes e composição fenólica. 2007. Curso (Biomedicina) - Centro Universitário Metodista IPA

Orientação de outra natureza

1. Ericksen Borba. Avaliação de enteroparasitoses e anemias em escolares do município de Flores da Cunha. 2008. Orientação de outra natureza (Biomedicina) - Centro Universitário Metodista IPA

Eventos

Participação em eventos

1. Apresentação de Poster / Painel no(a) I Congresso Internacional de Bioanálises, IV Congresso Sul Brasileiro de Biomedicina e VIII Semana Gaúcha de Biomedicina, 2008. (Congresso) Avaliação de prevalência da anemia em escolares do município de Flores da Cunha.

2. Laboratório de Bioética, 2008. (Simpósio).

3. Seminário Docente Extensionista- Extensão nas universidades: espaço de aprimoramento intelectual e humano, 2008. (Seminário).

4. Apresentação Oral no(a) XIX Salão de Iniciação Científica- XVI Feira de Iniciação Científica- II Salão UFRGS Jovem, 2007. (Outra)

Atividade Antioxidante de Sucos de uva brancos e tintos, orgânicos e convencionais.

5. Conferencista no(a) IV Jornada Acadêmica da Biomedicina, 2007. (Seminário)

Atuação do biomédico no RS.

6. Apresentação de Poster / Painel no(a) V MEETING OF SFRBM - SOUTH AMERICAN GROUP / V INTERNATIONAL CONFERENCE ON PEROXYNITRITE AND REACTIVE NITROGEN SPECIES, 2007. (Congresso)

Protection of purple grape juice, organic and conventional, against free radical damages in old Wistar rats. ; ANTIOXIDANT POTENTIAL OF RESVERATROL AND CATECHIN: THE IN VIVO EVIDENCE.

7. Apresentação de Poster / Painel no(a) III Congresso Sul Brasileiro de Biomedicina, 2007. (Congresso)

SUCOS DE UVA BRANCOS E TINTOS, ORGÂNICOS E CONVENCIONAIS: AVALIAÇÃO DA ATIVIDADE ANTIOXIDANTE.

8. Neuroproteção e Produtos Naturais, 2007. (Simpósio)

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9. Apresentação de Poster / Painel no(a) XXXV Reunião Anual da Sociedade Brasileira de Bioquímica e Biologia Molecular, 2006. (Congresso)

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10. Apresentação de Poster / Painel no(a) I Simpósio vinho e saúde, 2005. (Seminário)

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Regular coffe intake is related to increased sperm motility and antioxidant levels in infertile men.

12. Sunrise Free Radical School, 2005. (Simpósio)

.13. V Semana Gaúcha de Biomedicina, 2005. (Seminário)

. 14. Curso Biologia Molecular: Fundamentos e Aplicações, 2004. (Seminário)

. 15. II Jornada de Integração Farmacêutica-Biomédica, 2004. (Simpósio)

. 16. Workshop Internacional Estresse Oxidativo em Gastroenterolgia- Dos modelos experimentais a clínica, 2004. (Congresso)

. 17. Discussão sobre pesquisa utilizando células tronco embrionárias humanas, 2003. (Encontro)

. 18. I Ciclo Biomédico AGAB, 2003. (Congresso)

. 19. Curso Teórico sobre Perfusão Extra-Corpórea, 2003. (Seminário)

. 20. 3ª Semana Gaúcha de Biomedicina, 2003. (Congresso)

. 21. I Ciclo Interno de Palestras do ICSA, 2003. (Seminário)

. 22. Curso de Extensão Habilidades Clínicas Básicas, 2002. (Seminário)

. 23. Reuniâo Científica, 2002. (Encontro)

. 24. Curso de Extensão : Hemoglobinopatias, 2002. (Encontro)

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25. XI Congresso Brasileiro de Biologia Celular, 2002. (Congresso)

26. Curso de Dor, 2002. (Seminário)

27. Curso de Extensão Universitária, 2002. (Outra)

28. Palestra Projeto Genoma, 2001. (Encontro)

29. Curso Básico de Cancerologia, 2001. (Congresso)

30. Hemograma e Citologia Hematológica, 2001. (Seminário)

31. Curso intesivo para auxiliar de farmácia, 2001. (Simpósio)

Bancas

Participação em banca de comissões julgadoras

Outra

1. II SALÃO DE INICIAÇÃO CIENTÍFICA E EXTENSÃO- Centro Universitário Metodista IPA, 2007 Centro Universitário Metodista IPA