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CAROLINA FAGUNDES ASSUMPÇÃO

COMPOSTOS BIOATIVOS EM ÓLEOS E RESÍDUOS DE SEMENTES DE UVAS ORGÂNICAS E CONVENCIONAIS

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CAROLINA FAGUNDES ASSUMPÇÃO

COMPOSTOS BIOATIVOS EM ÓLEOS E RESÍDUOS DE SEMENTES DE UVAS ORGÂNICAS E CONVENCIONAIS

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Orientador: Prof. Dr. Alessandro de Oliveira Rios

Co-orientador: Profª. Drª. Simone Hickmann Flôres

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Carolina Fagundes Assumpção
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DISSERTAÇÃO
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Aprovada em: 11/02/2014
Pela Comissão Examinadora:

Prof. Dr. Alessandro de Oliveira Rios
Orientador – PPGCTA/UFRGS

Profª. Drª. Simone Hickmann Flôres
Co-orientador – PPGCTA/UFRGS

Profª. Drª. Graciela Cristina dos Santos
Banca

Prof. Dr. Eliseu Rodrigues
Banca – UFRGS

Prof. Dr. Plinho Francisco Hertz
Banca - UFRGS

Homologada em: 11/02/2014
Por:

Prof. Dr. Marco Antônio Zachia Ayub
Coordenador do Programa de Pós Graduação em Ciência e Tecnologia de Alimentos

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RESUMO

A indústria de alimentos é responsável pela produção de grandes quantidades de resíduos que podem representar altos custos quando não utilizados. Entre estes resíduos estão as sementes oriundas do processamento de uva, uma das frutas mais consumidas no mundo. Além do aproveitamento de resíduos e consequentemente novas formas de consumo, este material tem sido relatado como fonte natural rica em compostos bioativos. Neste contexto, o consumo de alimentos mais saudáveis também tem impulsionado a busca e a preferência por alimentos orgânicos. Assim, para investigar o potencial de utilização de resíduo da indústria de processamento de suco de uva (*Vitis labrusca*, cv. "Bordô" e "Isabel") foi analisado o resíduo da obtenção do óleo de sementes de uvas orgânicas e convencionais quanto ao teor de carotenoides e atividade antioxidante em comparação à semente integral. Os resíduos orgânicos e convencionais apresentaram os maiores teores de carotenoides totais, pró-vitamina A e atividade antioxidante por DPPH em relação a semente integral. O resíduo convencional destacou-se pela maior atividade antioxidante por TRAP e pelos teores mais significativos de carotenoides presentes no extrato antioxidante. também foram determinados O conteúdo de compostos bioativos e a estabilidade oxidativa dos óleos obtidos da prensagem das sementes orgânicas e convencionais Bordô e Isabel. Não foi observada diferença significativa entre as amostras quanto à atividade antioxidante por TRAP, porém os óleos Bordôs apresentaram-se mais estáveis ao aquecimento, com teores mais significativos de luteína, α e β-caroteno e α-tocoferol. A fim de investigar a qualidade, estabilidade oxidativa e conteúdo de compostos bioativos nestes óleos, foi realizado o refino do óleo Bordô orgânico. Não foi observada presença de β-caroteno e zeaxantina no óleo refinado e o óleo virgem de mesmo cultivar apresentou maior estabilidade ao aquecimento. Neste contexto, os resultados sugerem o consumo de óleos virgens devido a presença e maior quantidade de compostos bioativos. Não foi observada diferença significativa entre os diferentes modos de cultivo, sugerindo que a preferência por alimentos orgânicos pode restringir-se à motivos como ausência de agrotóxicos e apelo socioambiental. Os resíduos obtidos do processamento de uva, inclusive da obtenção do óleo destacaram-se pela alta atividade antioxidante e quantidade significativa de carotenoides, o que sugere sua inserção na alimentação como fonte natural rica em compostos bioativos.

Palavras-chave: atividade antioxidante, carotenoides, tocoferol, estabilidade oxidativa, índice de peróxidos, *Vitis labrusca*.

ABSTRACT

The food industry is responsible for producing large quantities of waste that may represent high costs when not used. Some of these residues are the seeds derived from the processing of grapes, one of the most consumed fruits worldwide. In addition to the reuse of waste and consequently new forms of consumption, this material has been reported to be rich natural source of bioactive compounds. In this context, the consumption of healthier foods has also driven the search and preference for organic foods. Therefore, to investigate the potential use of grape processing waste (*Vitis labrusca*, variety "Bordô" and "Isabel"), the residues obtained from the extraction of oil from organic and conventional grape seeds were examined to determine their content of carotenoids and antioxidant activity compared with those found in the whole seed. The organic and conventional residues exhibited higher levels of total carotenoids, provitamin A, and antioxidant activity by DPPH compared with the corresponding whole seeds. The conventional residue presented the highest antioxidant activity by TRAP and the highest levels of carotenoids in the antioxidant extract. The content of bioactive compounds and the oxidative stability of the oils obtained from the pressing of organic and conventional seeds "Bordô" and "Isabel" were also determined. No significant difference in the antioxidant activity by TRAP was observed between the samples, but the "Bordô" oils were more stable when heated and exhibited higher levels of lutein, α and β-carotene, and α-tocopherol. To investigate the quality, oxidative stability, and content of bioactive compounds in these oils, the organic "Bordô" oil was refined. The refined oil did not contain β-carotene and zeaxanthin, and the virgin oil of the same variety showed greater stability to heating. Thus, the results recommend the consumption of virgin oils due to their larger content of bioactive compounds. No significant difference was observed between the different modes of cultivation, suggesting that the preference for organic foods can be restricted to reasons such as the absence of pesticides and environmental appeal. The residues obtained from grape processing, including the residues obtained from the extraction of oil, exhibited high antioxidant activity and a high amount of carotenoids, suggesting their inclusion in foods as a natural source rich in bioactive compounds.

Keywords: antioxidant activity, carotenoids, tocopherol, oxidative stability, peroxide index,

Vitis labrusca.

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1. INTRODUÇÃO

A população atual tem demandado uma crescente busca por meios que favoreçam uma vida saudável, impulsionando pesquisas à novas substâncias que satisfaçam tais necessidades. Entre essas substâncias encontram-se os compostos fenólicos, com destaque para investigações voltadas aos polifenois presentes na uva e seus subprodutos (Sautter et al., 2005).

Uvas contêm quantidades significativas de substâncias fitoquímicas que conferem características como cor, aroma e adstringência a vinhos, além das propriedades benéficas à saúde humana. O grupo mais estudado de substâncias fitoquímicas em uvas é o dos polifenois, que são metabólitos secundários produzidos durante o processo de crescimento fisiológico da planta em resposta a vários tipos de estresse (German & Walzem, 2000).

As atividades biológicas destas moléculas têm sido extensivamente estudadas nas últimas décadas, fornecendo fortes evidências do seu potencial benefício à saúde, principalmente devido às propriedades antioxidantes, uma vez que agem seqüestrando radicais livres ou quelando metais e prevenindo a peroxidação lipídica e danos ao DNA (Blokhina et al., 2003). Além disso, como antioxidantes, polifenois protegem constituintes celulares contra danos oxidativos e, portanto, limitam o risco do desenvolvimento de doenças degenerativas, como a doença de Alzheimer (Anastasiadi et al., 2010).

O bagaço de uva é o resíduo sólido da indústria de vinho e sucos e, geralmente, consiste em casca e sementes e engaços, sendo usado como ração animal, majoritariamente, devido ao alto conteúdo de fibras. Entretanto, sementes de uva têm sido comercializadas para a extração de óleo e tem havido um aumento na demanda por esse tipo de produto, devido a sua importância como fonte de antioxidantes (Ahmadi & Siahsar, 2011). O óleo da semente

de uva tem sido estudado como uma possível fonte de ácidos graxos como o linoléico, que está associado à promoção da saúde cardiovascular por regular a produção da lipoproteína de baixa densidade (LDL) e beneficiar sua depuração (Beveridge et al. 2005).

Sementes e cascas de uva são boas fontes de compostos bioativos como ácido gálico, catequina, epicatequina (Yilmaz & Toledo, 2004), carotenoides e tocoferois, apresentando-se como matérias-primas adequadas à produção de ingredientes e suplementos alimentares antioxidantes.

Há uma tendência entre a população atual de selecionar o consumo de frutas e hortaliças de acordo com o modo de cultivo (Rigon, 2002). Segundo Kokuszka (2005), o método de produção de alimentos está diretamente relacionado ao seu valor nutricional e, neste contexto, a agricultura orgânica surgiu como uma alternativa, promovendo o uso eficiente de fontes naturais, buscando sustentabilidade e maximização do bem estar social (BRASIL, 2003). Segundo Ruth et al. (2011), o cultivo orgânico visa melhorar a biodiversidade, os ciclos biológicos e a atividade biológica do solo, atingindo os sistemas naturais ideais que são socialmente, ecologicamente e economicamente sustentáveis.

De acordo com Cardoso et al. (2011) há uma percepção geral entre a população de que alimentos orgânicos são mais saudáveis e nutritivos que os convencionais. Entretanto, evidências científicas ainda são insuficientes para confirmar ou rejeitar essa afirmação, uma vez que os dados comparativos são inadequados ou inconsistentes devido à heterogeneidade do material e metodologia utilizados.

Devido ao crescente comércio e consumo de alimentos orgânicos e à busca por uma alimentação rica em compostos bioativos, o presente trabalho investigou a influência do método de cultivo na qualidade, estabilidade e teores de compostos bioativos em subprotudos da agroindústria de sucos de uvas orgânicas e convencionais.

1.1. Objetivos

a) Investigar a influência do modo de cultivo no teor de carotenoides e na atividade antioxidante de sementes de uva cultivadas por sistema convencional e orgânico (*Vitis labrusca* Bordô) oriundas da produção de suco integral de uva e nos resíduos provenientes da obtenção do óleo extraído das sementes.

b) Avaliar a qualidade de óleos de sementes de uvas orgânicas e convencionais (*Vitis labrusca* cv. Bordô e Isabel) pela identificação e quantificação de compostos bioativos, determinação de suas características físico-químicas e estabilidade oxidativa.

c) Avaliar a qualidade de óleo de semente de uva orgânico (*Vitis labrusca* cv. Bordô), virgem e refinado, pela identificação e quantificação de compostos bioativos, determinação de suas características físico-químicas e de estabilidade oxidativa.

1.2. Resíduos da agroindústria de uva

O processamento de alimentos gera uma grande quantidade de subprodutos, sendo que o seu descarte representa alto custo à indústria além de impacto ambiental negativo.

O despejo em locais inapropriados de resíduos contendo matéria orgânica biodegradável pode conduzir a problemas ambientais crônicos (Casazza et al., 2012). Com o passar dos anos, um número cada vez maior de produtores tem demonstrado interesse em converter os resíduos agroindustriais em subprodutos rentáveis e com alto valor agregado (Makris et al., 2007).

Esses resíduos incluem itens como sementes, cascas, caules e folhas, que contêm grande variedade de moléculas biológicas ativas, como polifenois antioxidantes, que por sua vez tem aplicação nas indústrias farmacêutica e de alimentos (Bucić-Kojić et al., 2009). Nas últimas décadas, pesquisas têm demonstrado que muitos subprodutos poderiam ser utilizados como fonte potencial de compostos bioativos, sendo que tal exploração ainda é negligenciada devido, por exemplo, à falta de informação das técnicas apropriadas de extração dos compostos (Wijngaard et al., 2012).

A pesquisa que envolve o uso de produtos de resíduos é uma tendência na comunidade científica. Normalmente, esses resíduos são descartados, queimados ou usados como ração animal (Gomez et al., 1996).

Além disso, há um grande interesse na indústria de alimentos em extratos brutos de frutos e plantas ricos em polifenois devido à capacidade que estes compostos possuem de retardar a degradação oxidativa de lipídeos e, desse modo, aumentar a qualidade e o valor nutritivo de um alimento (Moure et al., 2001) sendo uma fonte alternativa de antioxidantes naturais em substituição aos sintéticos.

Uma das frutas mais consumidas mundialmente é a uva (*Vitis vinifera*), sendo que 80 % do total produzido é destinado à produção de vinho (Lutterodt et al., 2011), sendo a cultura da uva é uma das mais difundidas (Soto et al., 2012). Em algumas regiões do Brasil a indústria de processamento de uva desempenha um papel socioeconômico fundamental, portanto reutilizar os resíduos desse tipo de indústria é de grande valia e pode representar uma diminuição dos custos de produção e criação de novas formas de consumo.

Subprodutos dos processamentos de vinho e suco de uva representam uma fonte abundante de compostos fenólicos. Após a prensagem das uvas para extração do suco, o resíduo obtido é conhecido como bagaço, sendo constituído de sementes, casca e caules (Chamorro et al., 2012). As sementes representam de 20 a 26 % do resíduo, sendo fonte de

proteínas e óleo rico em vitamina E, composto que desenvolve papel importante na saúde humana (Ahmadi & Siahsar, 2011). Além disso, em muitos países, como o Brasil, sementes de uva são consideradas material de descarte pela maioria das agroindústrias.

Uvas e o extrato de sementes de uva são as principais fontes de compostos fenólicos como antocianinas, flavanois, catequinas e proantocianidinas e, conforme relatado por inúmeros pesquisadores, compostos fenólicos são capazes de promover saúde, protegendo contra doenças devido a sua alta atividade antioxidante (Hogan et al., 2010). Alimentos ricos em antioxidantes têm sido associados à redução de riscos de doenças crônicas, incluindo câncer e doenças coronárias (Choi et al., 2010).

O grupo de fitoquímicos mais estudado da uva é o dos polifenois (ou compostos fenólicos), metabólitos secundários com diversas estruturas químicas funcionais e que são produzidos durante o processo de crescimento fisiológico da planta e/ou como resposta a alguma forma de estresse ambiental (Naczk & Shahidi, 2004).

A composição das sementes de uva é aproximadamente de 40 % de fibras; 16 % de lipídeos; 11 % de proteínas e 7 % de compostos fenólicos complexos como taninos, açúcares, minerais e outras substâncias (Campos et al. 2008). A casca de uva, por exemplo, é fonte de antocianidinas e antocianinas, pigmentos naturais com propriedades antioxidantes que agem por meio da inibição da lipoperoxidação, além de apresentar atividades antimutagênicas (Pedreschi & Cisneros-Zevallos, 2006).

As sementes constituem uma porção considerável do bagaço, somando cerca de 38 a 52 % em base seca, permitindo extração do óleo que é rico em ácidos graxos insaturados, principalmente ácido linoléico (Maier et al., 2009).

O óleo de semente de uva é produzido a partir das sementes obtidas do bagaço restante da produção de sucos e vinhos, representando um potencial produto de agregação de valor à agroindústria (Lutterodt et al. 2011). O óleo obtido por prensagem a frio, ou seja, que não é

exposto a tratamentos térmicos ou químicos, tende a reter maior quantidade de compostos bioativos como antioxidantes naturais (Yu et al., 2005). Óleos obtidos por tal técnica podem representar fonte de compostos benéficos, como compostos fenólicos antioxidantes (Bail et al., 2008), além de outros fitoquímicos (Crews et al., 2006).

O óleo das sementes de uva tem apresentado aumento da produção industrial pela crescente demanda nos setores farmacêutico e de alimentos e segundo Russ & Meyer-Pittroff (2004), dependendo da colheita, das condições gerais das sementes e da extração utilizada para obtenção de óleo o rendimento pode atingir entre 13,5 e 14,5 % do volume total de sementes secas.

O óleo de semente de uva oferece vários benefícios à saúde humana devido ao seu alto conteúdo de ácidos graxos insaturados e compostos antioxidantes, como monômeros de flavan-3-ol, ácidos fenólicos e proantocianidinas oligoméricas (Prasain et al., 2009), além de apresentar atividade antimicrobiana (Palma et al., 1999).

Após a extração do óleo da semente, outro resíduo pode ser obtido, representando o potencial desse tipo de agroindústria. Estudos de Luther et al. (2007) relatam que este resíduo é fonte de proantocianidinas, as quais podem induzir a apoptose e inibir metástases em células cancerígenas de mama e cólon.

O resíduo da obtenção do óleo não tem recebido devida atenção, mas pode representar uma fonte rica em antioxidantes naturais e outros compostos bioativos, segundo Luther et al. (2007). Os autores relataram que o extrato etanólico de resíduo de óleo de semente de uva Chardonnay não apensas suprimiu a peroxidação lipídica em óleo de peixe, mas também protegeu o ácido eicosapentanoico (EPA) e o ácido docosahexanoico (DHA), os ácidos graxos ω-3 mais bioativos, contra a degradação oxidativa. Maier et al. (2009) também relataram resultados favoráveis aos resíduos da obtenção do óleo, observando que este tipo de subprotudo é rico em polifenois com propriedades antioxidantes.

A caracterização de compostos bioativos deste resíduo e a demonstração do potencial das suas propriedades benéficas podem levar à agregação de valor e maior à utilização desse tipo de alimento.

1.3. Compostos bioativos em resíduos da agroindústria de uva

Substâncias bioativas ocorrem em pequenas quantidades em alimentos, podendo ser obtidas de uma dieta equilibrada, levando ao fortalecimento do sistema endógeno e redução do estresse oxidativo, além da diminuição do risco de doenças, uma vez que podem neutralizar os radicais livres que resultam de processos oxidativos intracelulares (Volp et al., 2009). Nos últimos anos, pesquisas têm demonstrado um considerável interesse na composição química de frutas e sementes, sendo que os resultados obtidos demonstram que algumas plantas são ricas em compostos bioativos e, dessa forma, fontes alternativas de matéria prima com quantidades viáveis destas substâncias para processos industriais. Além disso, segundo Holser et al. (2004), óleos de sementes podem ser fontes de nutrientes e bioativos, agregando valor a alimentos processados.

Segundo Nakagawa et al., 1999, estudos *in vivo* baseados em uma dieta antioxidante, incluindo compostos como α-tocoferol, β-caroteno e ácido ascórbico, protegem contra danos oxidativos. Diversos estudos *in vitro* têm demonstrado que compostos fenólicos reduzem a oxidação da lipoproteína de baixa densidade (LDL), particularmente aqueles com múltiplas hidroxilas, os quais são mais eficientes na prevenção desse tipo de oxidação e, consequentemente, da aterogênese.

Há mais de uma década, pesquisas têm sido desenvolvidas a fim de desvendar integralmente a ação de moléculas bioativas relatadas como protetoras das células contra danos por radicais livres, com efeitos antibacterianos, anticancerígenos e antimutagênicos.

Seu uso como quimiopreventivo também já foi sugerido (Miyake et al., 1999), uma vez que pode inibir a geração de radicais livres, os quais são responsáveis por danos ao DNA.

Todos os organismos estão expostos à espécies reativas de oxigênio (ROS, *Reactive Oxigen Species*) ou metabólitos reativos de oxigênio, como peróxido de hidrogênio (H_2O_2), ânions superóxidos ($-O_2^-$) ou radicais hidroxila (-OH), como subprodutos do metabolismo oxidativo ou por meio de compostos geradores de radicais livres (Yu, 1994). Radicais livres e ROS estão intimamente relacionados a várias doenças degenerativas, incluindo aterosclerose, envelhecimento precoce e doenças cardiovasculares (Choi et al., 2010).

Os benefícios das uvas estão associados a dois fatores: a presença de ácidos graxos poliinsaturados (PUFA), geralmente nas sementes; e a presença de compostos fenólicos pelo seu alto potencial antioxidante, e logo pela sua capacidade de prevenir a oxidação de substratos biológicos (Jacob et al., 2008).

Dentre os compostos fenólicos, as sementes de uva possuem quantidade significativa de ácido gálico, catequina e epicatequina, além de inúmeros tipos de procianidinas (Terra et al., 2007). Investigações recentes indicam inúmeras atividades biológicas de extratos de sementes de uva, como propriedades antioxidantes, efeitos radioprotetores, prevenção de catarata, efeitos anti-hiperglicêmicos, além da modulação de expressão de sistemas enzimáticos antioxidantes (Lutterodt et al., 2011).

Os compostos fenólicos são metabólitos secundários responsáveis por importantes propriedades em alimentos e bebidas, como cor, aroma e adstringência (Brossaud et al., 2001), possuindo muitos efeitos positivos sobre a saúde humana e podendo atuar como sequestradores de radicais livres, doadores de elétrons ou hidrogênio e fortes quelantes de metal, prevenindo a peroxidação lipídica e danos ao DNA, (Blokhina et al., 2003).

A atividade antioxidante de polifenois pode ser manifestada de diferentes maneiras, seja por doação de átomos de hidrogênio, interrupção de reações de oxidação, quelação de

metais pesados ou aceptores de radicais livres. O potencial antioxidante é dependente do número e do arranjo dos grupos hidroxila e da extensão da conjugação estrutural (Mildner-Szkudlarz et al., 2010).

Os compostos intermediários formados pela ação de antioxidantes fenólicos são relativamente estáveis devido à distribuição de carga em todo o sistema de anel aromático. A capacidade antioxidante destes compostos é atribuída ao poder redutor do grupo hidroxila aromático, que neutraliza a reatividade dos radicais livres (Saito et al., 2008).

Polifenois representam um grupo biologicamente relevante de compostos naturais, que tem gerado interesse entre os consumidores e indústrias. Este potencial antioxidante tem levado à incorporação de compostos fenólicos em ingredientes de alimentos e suplementos nutracêuticos, segundo González et al. (2010). De acordo com Chamorro et al. (2012), apesar da possibilidade dos polifenois serem empregados como antioxidantes bioativos, a modificação na composição de seus diferentes polímeros, bem como sua estabilidade e capacidade antioxidante permanecem em estudo.

A síntese de compostos fenólicos em sementes durante o desenvolvimento de plantas depende de fatores climáticos, de cultura e genéticos (Downey et al., 2003). Santos et al. (2011) investigaram compostos fenólicos e ácidos graxos em diferentes cultivares de uva e relataram que as sementes de uva possuem alto conteúdo de óleo; o qual caracteriza-se pela quantidade significativa de ácidos graxos poliinsaturados e ácido oléico. As sementes de uva apresentaram maiores valores detectados para atividade antioxidante e compostos fenólicos. Já as cascas de uva demonstraram possuir composição intermediária entre as da semente e da polpa. Este estudo sugere que uvas são uma potencial fonte de nutrientes, ácidos graxos essenciais e compostos fenólicos.

A atividade antibacteriana de compostos fenólicos da uva já foi estudada *in vitro* por Baydar et al. (2004). Além disso, esses compostos são fontes naturais antioxidantes em

comparação aos sintéticos, largamente utilizados, e sem efeitos indesejáveis em processos enzimáticos ou orgânicos (Rhodes et al., 2006).

Parry et al. (2006) estudando as características químicas e funcionais de farinhas de sementes de diferentes frutas, sugerem que esse tipo de resíduo deve ser incluído na dieta como fonte natural de antioxidantes, além de conter compostos antiproliferativos. Os autores relatam que pesquisas ainda são necessárias no sentido de investigar os efeitos da formulação de um provável alimento, seu processamento, bem como os mecanismos químicos e bioquímicos envolvidos nas propriedades antioxidantes e antiproliferativas, a fim de promover sua utilização como produtos de suplementos alimentares para promoção da saúde e prevenção de doenças.

Catequinas e seus isômeros são os polímeros majoritários na semente de uva. Esses fenólicos têm sido relacionados a polissacarídeos de parede celular, os quais contêm grupos hidrogênio bem como oxigênio e glicosídeos aromáticos que tem a capacidade de formar pontes de hidrogênio e interações hidrofóbicas com polifenois (Downey et al., 2003).

Lutterodt et al. (2011) estudando óleos de sementes de uvas Chardonnay, Concord, Muscadine e Ruby Red, observaram conteúdos de α-tocoferol de (42,5 a 488 $\mu g \cdot g^{-1}$) de resíduo de semente de uva obtida após extração do óleo por prensagem a frio. Já o óleo obtido não apresentou conteúdo mensurável deste composto. Beveridge et al. (2005) relataram presença de α-tocoferol em óleos de diferentes cultivares, mesmo em pequenas quantidades de óleo (de 3,58 a 30,9 mg.100 g^{-1}).

Em relação aos carotenoides, o β-caroteno é um dos mais importantes e mais estudados, sendo utilizado na indústria de alimentos como corante e fonte de pró-vitamina A (Zeb & Murkovic, 2013), além de ser hidrofóbico e desenvolver um papel importante na promoção de saúde devido às propriedades antioxidantes e protetores contra os efeitos causados pelos radicais livres às células e tecidos humanos (Black et al., 2000).

Segundo Zeb (2012), o β-caroteno está presente em quase todos os óleos vegetais, incluindo os de milho, canola, linhaça, oliva, de girassol e de palma. O β-caroteno também protege lipídeos da auto-oxidação causada pela reação de radicais livres com radicais peroxil, inibindo a propagação da cadeia de reação de oxidação.

Quimicamente, carotenoides são compostos poli-isoprenoides e podem ser divididos em dois grupos principais: carotenos e xantofilas, que são derivados de hidrocarbonetos oxigenados como grupos hidroxi, ceto e epoxi ácidos, relatados como efetivos sequestradores de radicais livres (Zeb & Murkovic, 2011).

Espécies reativas de oxigênio e nitrogênio são gerados durante o matbolismo aeróbico e processos patológicos, podendo causar danos biologicamente importantes à moléculas como lipídeos, DNA ou proteínas, além de estarem envolvias no desenvolvimento de doenças degenerativas (Stahl & Sies, 2005).

Entre os vários radicais livres formados no organismo por danos oxidativos, os carotenoides apresentam ação mais eficiente contra os radicais peroxil. Geralmente, os radicais peroxil são gerados no processo de peroxidação lipídica, sendo que o sequestro desta espécie interrompe a sequência de reação que pode levar a danos nos compartimentos lipofílicos. Devido à lipofilicidade e propriedades específicas de sequestrar radicais peroxil, os carotenoides desempenham um papel importante na proteção de membranas celulares e lipoproteínas contra danos oxidados (Stahl & Sies, 2003).

Devido ao alto grau de insaturação, carotenoides estão susceptíveis à degradação durante processamento e armazenamento de alimentos (Rodriguez-Amaya, 2010). Na natureza, carotenoides são encontrados na forma *all-trans*, enquanto apenas uma pequena fração de cis-isômeros está presente. A isomerização é uma das primeiras mudanças que ocorrem na estrutura de carotenoides durante processos como aquecimento (Achir et al.,

2010), podendo levar à redução ou perda da capacidade de sequestrar radicais livres, ou seja, da capacidade antioxidante.

Óleos vegetais possuem diferentes susceptibilidades frente à degradação oxidativa devido às diferenças na composição de ácidos graxos insaturados e conteúdos de antioxidantes. Neste contexto, os tocoferois são os antioxidantes naturais de maior importância em óleos vegetais (Kamal-Eldin & Appelqvist, 1996). Em baixas temperaturas, estes compostos têm sido associados como facilitadores da transferência do radical hidrogênio dos polifenois ao radical peroxil. A transferência de hidrogênio produz um radical tocoperoxil, que deve ser combinado com outro radical peroxil lipídico em uma série de reações, gerando produtos não-radicais (Verleyen et al.,, 2001).

Tocoferois representam uma importante classe de antioxidantes, que podem proteger membranas celulares de danos oxidativos. Estruturalmente, todos os tocoferois conhecidos consistem de um anel cromanol ligados a uma cadeia fitol, diferindo apenas no número e posição de grupos metil no anel cromanol (Della Penna & Pogson, 2006). Diversos estudos tem relatado que estes metabólitos previnem o aparecimento de produtos de radicais livres, seqüestrando ou promovendo sua decomposição (Nasri et al., 2013).

O conteúdo de tocoferol de um óleo é fator dependente do genótipo da planta, condições climáticas de plantio e colheita, processamento e armazenamento. Em óleos de sementes, tocoferois são encontrados nas formas α, β, γ e δ-tocoferol, sendo o γ e δ-tocoferois os mais antioxidantes (Tasan & Demirci, 2005).

Neste contexto, os resíduos obtidos a partir do processamento de uvas podem fornecer importantes produtos e contituintes químicos para uso na alimentação humana. Os lipídeos obtidos a partir de tais produtos podem desempenhar um importante papel no corpo humano, não apenas em estruturas de membranas celulares, mas também como componentes do plasma e tecidos, como reflexo do tipo e quantidade de ingestão pela dieta (Mohammed et al.,

2002). Distúrbios no metabolismo de lípidos têm sido associados a doenças neurodegenerativas, como a doença de Alzheimer e doença de Parkinson (Hirsch-Reinshagen et al., 2009). Além disso, os lipídeos têm sido intimamente associados à geração de espécies reativas de oxigênio e nitrogênio (ROS e RNS, respectivamente) como substratos para reações oxidativas (Catalfo et al., 2013).

1.4. Agricultura orgânica

A população atual, de um modo geral, além da busca pelo consumo de alimentos mais saudáveis e que possuam uma alegação funcional, tem demonstrado preferência pelo cultivo orgânico, principalmente devido à ausência de contaminantes no processo de produção (Pussemier et al., 2006). Consequentemente, o mercado está se voltando aos produtos orgânicos frente a evidências de que esse tipo de cultivo origina alimentos com maior valor nutricional e menor conteúdo de nitrato, além de uma melhor qualidade organoléptica (Siderer et al. 2005).

Corrales et al. (2010) estudando possíveis diferenças químicas entre extratos de cascas de uvas orgânicas e convencionais, relataram que os extratos de uvas orgânicas contêm maiores níveis de quercetina e derivados em comparação com o convencional, e seus conteúdos totais de fenólicos também foram maiores. Porém, quanto à capacidade antioxidante, os extratos de uvas de cultivo convencional demonstraram níveis mais elevados.

Como a escolha pelos alimentos orgânicos é uma tendência mundial, uma melhor compreensão da influência das práticas de cultivo nas propriedades biológicas do alimento pode levar ao desenvolvimento de estratégias de marketing e tomadas de decisão. Dados obtidos até o momento sugerem que o modo de cultivo pode influenciar o conteúdo de flavonoides e outros metabólitos secundários em frutos, mas ainda há estudos controversos.

Malusa et al. (2004) comparando os efeitos dos cultivos orgânicos e convencionais sob a atividade antioxidante de uvas “Grignolino” (*Vitis vinifera*) relataram resultados favoráveis ao cultivo convencional, assim como Vian et al. (2006) em seus estudos sobre a influência das diferentes práticas de cultivo no acúmulo de antocianinas em cascas de uvas “Syrah” (*Vitis vinífera*) em diferentes estágios de amadurecimento.

1.5. Óleos vegetais virgens e refinados

A produção de óleos comestíveis aumentou no último século devido à maior busca, consumo e disponibilidade de processos e equipamentos tecnológicos, segundo a FAO (2011). A composição química de óleos virgens como os de oliva apresenta diversos compostos como fenois hidrofílicos, que contribuem para propriedades sensoriais e benéficas à saúde. Os antioxidantes mais encontrados são carotenos e compostos fenólicos, além de fenóis lipofílicos como os tocoferóis (Servili et al., 2004).

Um dos processos considerados críticos na produção de óleos vegetais é o refino, que remove ácidos graxos livres, os quais em alta concentração podem levar à rancidez do óleo (Carmona et al., 2010), além de outros compostos como fosfolipídeos, pigmentos, proteínas e possíveis resíduos de solventes utilizados na extração. As operações principais envolvidas no processo convencional de refino são a degomagem, neutralização, branqueamento e desodorização. Segundo Landucci et al. (2013), a realização destes processos é crucial à qualidade do produto final, portanto, a fim de melhorar a palatabilidade e qualidade e reduzir a acidez, utiliza-se o refino físico ou químico (Zeb & Murkovic, 2011).

As principais etapas do refino do óleo são: degomagem, que remove fosfatídeos e materiais mucilaginosos; neutralização, para eliminação de ácidos graxos livres; branqueamento, para remoção de pigmentos como clorofilas e carotenoides; e desodorização,

para remoção de substâncias voláteis (Caponio et al., 2013). O óleo obtido é caracterizado por uma baixa degradação oxidativa e hidrolítica, mas apenas os compostos de degradação de baixo peso molecular e ácidos graxos livres são quase completamente removidos, enquanto compostos passíveis de oxidação como triacilglicerois e diacilglicerois continuam presentes no óleo (Kamal-Eldin & Appelqvist, 1996).

Contudo, apesar de o objetivo do refino ser estender a vida de prateleira de óleos pela remoção de materiais indesejáveis, o processo pode conduzir a perda de antioxidantes naturais, fitoesterois (Alpaslan et al., 2001) e tocoferois, bem como à formação de ácidos graxos trans, podendo afetar a estabilidade oxidativa de óleos vegetais (Tasan & Demirci, 2005).

No refino químico e físico, a desodorização é a principal responsável pela diminuição do conteúdo de tocoferois em óleos, acarretando na perda de estabilidade oxidativa. Os teores de tocoferois são afetados pela temperatura, tempo de exposição à temperatura e pressão. Além disso, são sensíveis à luz, álcalis e metais como ferro e cobre (Tasan & Demirci, 2005).

1.6. Qualidade e estabilidade de óleos vegetais

A degradação de óleos depende da maior ou menor presença de ácidos graxos insaturados em sua composição. Óleos vegetais que possuem uma grande quantidade de ácidos graxos poliinsaturados estão mais sujeitos à oxidação do que óleos que possuem maior quantidade de ácidos graxos saturados (Del Ré & Jorge, 2006).

Os óleos vegetais podem ser hidrolisados, formando ácidos graxos livres, monoacilglicerol e diacilglicerol, e/ou oxidados, formando peróxidos, hidroperóxidos, dienos conjugados, epóxidos, hidróxidos e cetonas. Podem, ainda, ser decompostos em pequenos fragmentos ou

permanecer na molécula do triacilglicerol, e se associarem, conduzindo a triacilgliceróis diméricos e poliméricos (Takeoka et al., 1997).

O nível de alteração depende, sobretudo, das características do alimento, da absorção de ar e da temperatura utilizada e, como conseqüência, a degradação será tanto maior quanto mais prolongado for o período de utilização do óleo ou gordura e quanto maior sua insaturação (Dobarganes et al., 1989). Com o decorrer das alterações, as qualidades funcionais, sensoriais e nutricionais dos óleos se modificam, podendo chegar a níveis em que não se consegue mais obter alimentos de qualidade (Del Ré & Jorge, 2006).

Na perda da estabilidade de um óleo a oxidação é um processo degradativo dos lipídeos que ocorre quando o oxigênio atmosférico ou aquele que está dissolvido no óleo reage com ácidos graxos insaturados presentes. As reações químicas envolvidas no processo de oxidação são muito complexas e geram, em seus estágios mais avançados, produtos sensorialmente inaceitáveis (Lima & Gonçalves, 1995).

O processo de oxidação, de acordo com Nawar (1996), pode ser acelerado por meio da presença de contaminantes, como: metais que apresentam mais de um estado de valência (cobalto, cobre, ferro, manganês e níquel), encontrados na maioria dos óleos comestíveis, originários da própria terra, onde suas sementes foram cultivadas ou por meio de equipamentos utilizados no processo de refino, de estocagem ou de cocção.

No aquecimento de um óleo, o tempo influencia na quantidade de compostos de alteração formados. Em temperatura elevada, as reações ocorrem fundamentalmente na superfície de contato com o ar; enquanto durante o resfriamento, ao diminuir a velocidade das reações e aumentar a solubilidade do ar, há o favorecimento de entrada de ar na massa, produzindo grande quantidade de hidroperóxidos e radicais livres (Lima & Gonçalves, 1995).

A temperatura é também importante em relação aos efeitos da pressão parcial do oxigênio na taxa de oxidação. Com o aumento da temperatura, a taxa de concentração de

oxigênio torna-se menos influente, pois o oxigênio é menos solúvel em temperatura elevada. A taxa de oxidação aumenta de acordo com a área superficial, onde o óleo ou a gordura são expostos ao contato com o ar. Entretanto, quando a relação entre volume e superfície aumenta, reduzindo a pressão parcial do oxigênio, a taxa de oxidação se torna menor (Sanibal & Mancini-Filho, 2002).

A oxidação lipídica é um problema constante na indústria de alimentos quando os substratos lipídicos são compostos de ácidos graxos insaturados ou poliinsaturados (PUFA), podendo desenvolver sabores e aromas indesejados e levar à formação de compostos tóxicos (Ramadan, 2013). A efetividade dos antioxidantes depende da sua reatividade química (como seqüestradores de radicais e quelantes de metais), interação com os compostos do alimento, condições ambientais e localização do antioxidante na matriz alimentícia (Lucas et al., 2010).

Apenas a composição de ácidos graxos não explica adequadamente a estabilidade de óleos, segundo Normand et al. (2001). Óleos virgens contêm uma variedade de compostos como hidrocarbonetos, esteróis, tocoferois, polifenois, pigmentos e traços de metais. Alguns destes compostos, como tocoferois e carotenoides são benéficos à estabilidade durante o aquecimento (Rossi et al., 2007).

A estrutura lipídica, como saturação e triglicerídeos mono ou poli insaturados são os principais indicadores da estabilidade oxidativa. Além disso, outro fator importante é o conteúdo de antioxidantes de ocorrência natural em óleos, como os tocoferóis, dentre os quais o α-tocoferol é considerado predominante (Jerzykiewicz et al., 2013).

O óleo de semente de uva é composto de aproximadamente 90 % de ácidos graxos poli e monoinsaturados, que são responsáveis pelo seu valor nutritivo, principalmente o ácido linoléico e oléico, além de uma pequena quantidade de ácidos graxos saturados. Óleos virgens contêm compostos bioativos que incluem tocoferois e inúmeros compostos fenólicos,

consistindo de fenólicos de baixo e alto pesos moleculares que contribuem com os efeitos benéficos dos óleos vegetais (Bail et al., 2008).

Determinar a vida de prateleira de óleos vegetais consome muito tempo quando em temperatura ambiente, então é necessário um método que obtenha a estabilidade oxidativa de maneira mais rápida a altas temperaturas. A auto-oxidação é a principal causa de deterioração da qualidade de óleos, mas a reação é lenta até que haja um acúmulo de espécies reativas que promovam maior taxa de reação. O tempo necessário para atingir este acúmulo é definido como o período de indução (Fashoosh et al., 2007a).

O método mais conhecido que determina o período de indução é o Rancimat, que é baseado nas alterações da condutibilidade da água deionizada após recolher os ácidos orgânicos voláteis produzidos pela oxidação do óleo (Méndez et al., 1996). O tempo requerido para produzir o aumento da condutividade devido à formação de ácidos voláteis determina o índice de estabilidade oxidativa (OSI), que pode ser definido como uma medida da resistência à oxidação de uma gordura ou óleo (Farhoosh, 2007a, 2007b). O fluxo de ar, peso da amostra e temperatura são os parâmetros operacionais que podem afetar a determinação da OSI (García-Moreno et al., 2013).

É necessário entender as mudanças e as alterações que os óleos vegetais sofrem durante períodos longos de aquecimento e buscar critérios objetivos para definir quando os óleos devem ser descartados, garantindo produtos de melhor qualidade nutricional (Corsini & Jorge, 2006).

CAPÍTULO 2:

CAROTENOID CONTENT IN AND ANTIOXIDANT ACTIVITY OF WASTE FROM ORGANIC AND CONVENTIONAL GRAPE JUICE PROCESSING

The article was formatted according to the Journal of the Science of Food and Agriculture.

ABSTRACT

Background: The grapes used in the wine and juice agribusinesses generate large amounts of solid wastes rich in bioactive compounds, such as seeds. Oil can be extracted from this solid wastes, and this process yields residual material. Numerous studies have suggested that the cultivation method can influence the content of secondary metabolites in food. Therefore, this study investigated the influence of the cultivation method on the presence of carotenoids in and the antioxidant activity of grape seeds cultivated by conventional and organic farming (*Vitis labrusca*) and their residues after processing. *Results*: OR and OS presented more saturated and lighter color parameters with a greater tendency toward yellow and brown colors (lower h*). OR and CR showed higher levels of total carotenoids, provitamin A, and antioxidant activity by DPPH. CR exhibited higher antioxidant activity, as determined quantitatively and qualitatively by TRAP, and higher levels of carotenoids in the extract. *Conclusions*: The results from this study suggest that food produced by organic farming is not different and can present even lower levels of carotenoids than that produced through conventional methods.

Keywords List: lutein, carotene, bioactive compounds, antioxidant potential, color analysis.

INTRODUCTION

Agroindustrial wastes may represent a source of natural low-cost antioxidants that can be used in the pharmaceutical and food industries. Grapes are one of the most consumed fruits in the world, and the processing of grapes results in the production of large quantities of solid wastes rich in bioactive compounds. [1]

The beneficial health effects of grape seed are being intensively studied. Recent reports indicate a variety of biological activities, such as antihyperglycemic effects, antioxidants, protective effects against oxidative damage to the brain cells of animals [2], and anti-inflammatory effects [3], due to the presence of bioactive compounds.

Compounds with antioxidant activity can preserve flavor and color, avoid the destruction of vitamins in foods, and protect living systems from oxidative damage. [4] Numerous studies have proven the ability of antioxidants to protect cells from damage caused by free radicals, prevent cardiovascular diseases, and exert anticarcinogenic effects. [5]

Of these compounds, carotenoids have been shown to protect against various types of diseases both *in vitro* and *in vivo*. [6] The most common defense strategy of carotenoids is the scavenging of two species of free radicals, namely singlet oxygen ($^1O_2$) and peroxyl radicals. The interest in these pigments from the nutritional point of view has increased significantly due to their health benefits. [7]

The defense system of the body works involves a complex network of enzymatic and non-enzymatic antioxidants. [8] It has been suggested that interactions between compounds with different structural properties and varied antioxidant activities provide additional protection against increased oxidative stress. [9]

In vivo and *in vitro* studies have demonstrated that carotenoids may protect against several types of cancers. These findings are consistent with epidemiological studies, which

have shown that an increase in the consumption of a diet rich in carotenoids is associated with a decreased risk of cancer. [9]

Carotenoids have also been found to exhibit functional properties, including the prevention of cardiovascular disease and macular degeneration, due to the activity of pro-vitamin A. These properties make them ideal compounds for promoting the increased consumption of natural products that contain these compounds. [7]

The distribution of bioactive compounds in grapes can demonstrate significant differences depending on various factors, such as variety, location, culture, and processing. [10] In this regard, experimental data suggest that the mode of cultivation can influence the content of flavonoids and other secondary metabolites in fruits. [11] However, the available data are contradictory. Malusa et al. [12] reported a high content of polyphenols in organic compared with conventional grapes, whereas Vian et al. [13] found a higher content of anthocyanins in grapes grown in a conventional manner compared with organic grapes.

Organic agriculture is a production system that promotes biodiversity, biological cycles, and soil biological activity. [14] The primary goal of organic farming is to optimize the health and productivity of interdependent communities [15] and thereby provide sustainable integration between soil, plants, animals, and people.

Because there is a tendency to consume organic produce, a better understanding of the influence of agricultural practices on the functional properties of food can benefit marketing strategies and decision making. [11] Therefore, this study aimed to investigate the influence of the mode of cultivation (conventional and organic farming) on the presence of carotenoids in and the antioxidant activity of grape seeds and the waste products obtained by extracting oil from grape seeds (*Vitis labrusca*, variety Bordô).

EXPERIMENTAL

Raw Material

The organic grape seeds (*Vitis labrusca* Bordô, vintage 2013) were supplied by Econatura Company, and the conventional ones were provided by Co-op Garibaldi (Rio Grande do Sul, Brazil). The seeds are part of the waste arising from the processing of integral grape juice produced by the companies (vintage 2013). The seeds were dried in a rotary dryer with airflow for 4 h (70°C) and separated by ventilation. Part of the seeds were sealed in polyethylene bags *in vacuo* and transported to the Laboratory of Bioactive Compounds (UFRGS). To obtain the residue of grape seed oil, the other seeds were subjected to cold pressing extraction. Because the abrasion of seeds results in the dissipation of energy as heat, we used a refrigeration system to maintain the bioactive compounds; thus, the temperature did not exceed 50°C. The resulting press residues were sealed in polyethylene bags *in vacuo* and maintained at 5°C until analysis. All of the seeds and residues were analyzed fresh for the determination of moisture. CS corresponds to conventional seeds, OS corresponds to organic seeds, CR corresponds to conventional residues, and OR corresponds to organic residues.

Cromatograph

The HPLC analysis was performed in an Agilent 1100 Series HPLC system equipped with a quaternary solvent pumping system (G1311A – DE14917573 Agilent 1100 Series) and a UV/Vis detector (G1314B - DE71358944 Agilent 1100 Series).

Reagents and standards

The chromatographic analyzes were performed using HPLC grade solvents and products: methanol, methyl-tert-butyl and acetonitrile (Panreac). The standards used for the

construction of calibration curves, namely: β-carotene (purity > 93%), α-carotene (purity > 95 %) and zeaxanthin (purity > 95%) were purchased from Sigma Chemical (USA). Lutein (purity > 95%) was purchased from Indofine Chemical Company Inc. Hillsborough (USA).

Color analysis

The color analysis was performed with a MINOLTA CR 310 187 colorimeter using the *L* a* b**, illuminant D65, and a factor observer angle of 10°. The readings were performed in triplicate.

Carotenoids and Vitamin A content

The carotenoid extract was prepared according to the method described by Mercadante and Rodriguez-Amaya. [16] The pigments were extracted with chilled acetone until discoloration occurred, and the extract was saponified overnight with 10 $g.L^{-1}$ KOH in a methanol solution at room temperature. The extract was then washed to remove the alkali and concentrated in a rotary evaporator (Fisatom 802 com Banho Ultratermostático Quimis 0214 M2). The concentrated extract was transferred to an amber flask, dried under a nitrogen stream and stored at −18 °C until it was analyzed through high performance liquid chromatography (HPLC). A 250 mm × 4.6 mm i.d., 3 μm, C30 reversed phase polymeric column was used (YMC, Japan). The wavelength was adjusted to 450 nm. The mobile phase was water:methanol:tert-methyl butyl ether (MTBE) (J.T. Baker – Mallinckrodt, EUA) starting at a ratio of 5:90:5 and reaching 0:95:5 in 12 min, 0:89:11 in 25 min, 0:75:25 in 40 min and 0:50:50 after a total of 60 min. The mobile phase flow rate was 1 $mL.min^{-1}$, the injection volumn was 5 μL and the injector temperature was 33 °C. [17] The carotenoids were quantified using the standard curves obtained for lutein (1–65 $\mu g.mL^{-1}$), zeaxanthin (1–40 $\mu g.mL^{-1}$), α-carotene (2-25 $\mu g.mL^{-1}$) and β-carotene (5–50 $\mu g.mL^{-1}$). The results were expressed in

micrograms per 100 g of sample. The limits of detection (LD) and quantification (LQ) were, respectively, $6.9x10^{-3}$ $mg.kg^{-1}$ and $1.15x10^{-2}$ $mg.kg^{-1}$ for lutein; $9.56x10^{-2}$ $mg.kg^{-1}$ and $1.59x10^{-2}$ $mg.kg^{-1}$ for zeaxanthin; $4.46x10^{-2}$ $mg.kg^{-1}$ and $7.43x10^{-2}$ $mg.kg^{-1}$ for β-carotene and $1.97x10^{-2}$ $mg.kg^{-1}$ and $3.28x10^{-2}$ $mg.kg^{-1}$ for α-carotene. The vitamin A activity was calculated assuming the bioconversion factor proposed by Guilland et al. [18], which shows that 13 mg of β-carotene is equal to 1 mg of retinol.

Antioxidant Activity

A methodology based on the sequestering of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the antioxidant activity. The extract was obtained from 0.5 g of sample ground in methanol at different dilutions. The fresh samples were weighed in centrifuge tubes and extracted sequentially with 10 mL of methanol in a vortex at room temperature for 5 min. The tubes were centrifuged at 1922 g for 10 min, and the supernatant was recovered. This procedure was performed three times, and the extracts were combined and used to determine the antioxidant capacity. For the DPPH method, a 0.1 mL aliquot of each dilution of the extract was reacted with 3.9 mL of the DPPH radical. The readings were made in a spectrophotometer at 515 nm after 30 min. The results are expressed as the concentration of antioxidant required to reduce the original amount of free radicals by 50 % (EC50), and the values are expressed as grams of sample per gram of DPPH.

The TRAP and TAR were measured and calculated as previously described by Dresch et al. [19] The methanolic extract concentrate was diluted with water and DMSO. Briefly, the reaction mixture, which contained AAPH (10 mM) and luminol (4 mM) in glycine buffer (0.1 M, pH 8.6), was incubated at 21 °C for 2 h. AAPH is a source of peroxyl radicals that react with luminol to yield chemiluminescence (CL). The system was calibrated using Trolox. After 2 h of incubation, 180 μL of the mixture was placed in a 96-well plate, and 10 μL of the

sample (final concentration of 1 μM), Trolox, or vehicle (95 % water/10 % DMSO), which represents the maximum radical generation, was added. The results were transformed into a percentile rank, and the area under the curve (AUC) was calculated using the GraphPad software (San Diego, CA, USA). A smaller AUC (in comparison to the system) shows that the sample has a higher total reactive antioxidant potential. The TAR index was determined by measuring the initial decrease in luminol luminescence, which was calculated as the I_0/I ratio, where (I_0) is the initial emission of CL (before the addition of the antioxidant) and (I) is the instantaneous CL intensity after addition of an aliquot of the sample or the reference compound (Trolox). Each determination was performed in triplicate.

Carotenoid antioxidant potential

The same extract described in the "Antioxidant Activity" section was injected into and HPLC system using the same analysis parameters described in the "Carotenoids" section to investigate the presence and/or antioxidant potential of the carotenoids present in this type of extract and to analyze the influence of the extractive stages on the analysis of carotenoids.

Statistical analysis

The results were analyzed by ANOVA and Tukey's comparison test at a significance level of 5 %, using the Statistica 11.0 and GraphPad Prism 6 software programs.

RESULTS AND DISCUSSION

Color analysis

Table 1 shows that all of the samples differ significantly in the color parameters analyzed. The L* parameter reached values of 32.82 (CR) to 38.81 (OS). The same trend was

observed for C*, which exhibited values ranging from 12.64 (CR) to 16.40 (OS). However, the parameter h* values were higher for CS and OS compared with CR and OR.

The C* parameter indicates the contribution of a* (red color) and b* (yellow color) to the total color, whereas [20] h* indicates the tonality and varies from violet (h = 315 °) to pink (h = 0 °), and the parameter L* indicates the lightness of the sample. [21] The results show that the seed samples (organic and conventional) had the highest values for the parameters analyzed and that the residue samples (organic and conventional) had the lowest values, i.e., lower lightness (L*), lower saturation (C*), and brown color (h*).

Through a study of the evolution of color and phenolic compounds in grapes during the drying process without controlled conditions, Figueiredo-González [22] reported that the pressing of the grapes can cause damage and structural changes to the seed, resulting in cell rupture and thereby facilitating contact between the enzymes and phenolic substrates. The low values of L*, C*, and h* reported in this study may be due to the enzymatic browning described by Figueiredo-González. [22] Because the temperatures used in both studies did not exceed 50 ºC, the occurrence of enzymatic browning is more likely than the browning enzymatic system due to the extraction of grape seed oil, which results in the loss of color and sensory attributes in the residue.

Through an investigation of the changes in color and phenolic compounds that occur during the drying of *Vitis labrusca* grapes (variety Pedro Ximenes) in the sun, Serratosa et al. [23] found results similar to those found in this study with decreases in h* and increases in L*. These authors suggest that low- and medium-molecular-weight compounds are responsible for the color changes observed in musts.

The changes in the color of grapes during the drying process at moderate temperatures, such as during exposure to sunlight, are the results of reactions associated with enzymatic and non-enzymatic browning [24], including the pigment formation from phenolic substrates

(particularly hydroxycinnamic acids) under the action of polyphenol oxidases (PPOs). In healthy grapes, PPOs are found separate from polyphenols; however, the physical damages observed in grape tissues as a result of the drying process can bring the enzymes into contact with their substrates and thereby initiate browning reactions. However, at higher temperatures, browning can result in a Maillard reaction, which is a non-enzymatic process that yields colored products known as melanoidins from free amino and carbonyl groups. [25] The presence of amino acids and monosaccharides in grapes and high concentrations of these compounds resulting from the drying process facilitate the development of the Maillard reaction. [26]

Table 1. Color parameters of CS, OS, CR and OR.

Sample	**L***	**C***	**h***
CS	36.59 ± 0.03^{b}	13.99 ± 0.02^{c}	55.73 ± 0.07^{a}
OS	38.81 ± 0.07^{a}	16.40 ± 0.02^{a}	49.70 ± 0.14^{b}
CR	32.82 ± 0.03^{d}	12.64 ± 0.02^{d}	46.76 ± 0.13^{c}
OR	35.22 ± 0.02^{c}	14.03 ± 0.02^{b}	44.01 ± 0.02^{d}

All of the results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same column represent significant difference ($P < 0.05$).

By studying the influence of different solvents and temperatures on the antioxidant activity of, color of, and extraction of phenolic compounds from grape seeds *Vitis vinifera* variety "Frankovka", Bucic-Kokic et al. [27] observed that the color characteristics may result from the extraction of pigments (carotenoids or anthocyanins) and non-pigmented compounds (flavonoids).

Organic farming generally results in more saturated (higher C*) and lighter (higher L*) colors and a greater tendency to yellow and brown colors with lower values for the

parameter h*. In this study, the lowest value for h* was obtained for OR, which clearly shows a tendency toward red because this sample had high amounts of lutein and significant amounts of β-carotene and zeaxanthin, as observed in Table 2.

Ribeiro et al. assessed the stability of pequi oil and reported an L* value of 28, which indicates browning and the loss of red intensity. Thus, the lower L* values obtained for some of the samples may be related to higher amounts of carotenoids, which would present a darker color.

Carotenoid and Vitamin A content

OR and CR showed significantly higher levels of all of the examined carotenoids and provitamin A than the seeds (Table 2). There was no α-carotene in the organic samples and no zeaxanthin in the conventional seeds. β-carotene was the carotenoid present in the highest amounts in all of the samples.

The residue from the extraction of oil is a byproduct that is often overlooked and destined for animal feed. Similar to the results of this study, which showed that organic and conventional waste had significantly higher levels of carotenoids compared with seeds, Maier et al. [3] demonstrated that this type of sample is a byproduct rich in polyphenols with high antioxidant activity. These authors even suggest the extraction of polyphenols for use as ingredients in functional foods or other enriched foods.

The higher levels of carotenoids assigned to the residues investigated in this study may be related to the effect caused by the extraction of oil from the seed because pressing can lead to structural damage and facilitate access to compounds such as carotenoids. The temperature was not changed in the extraction, but an increase of up to 50 °C is expected from the abrasion of seeds into the extractor.

A study that evaluated the heating of grape seed (*Vitis vinifera*, variety Campbell) showed a release of phenolic compounds that resulted in increases in the amounts of catechins and caffeine in the extract. The heating conditions and the physical form of the grape seed affected the antioxidant activity of the extract, as reported by So-Young et al. [29]

According to Pellegrini et al., [30] who studied vegetables belonging to the Brassica genus, some processing, such as boiling, microwave, and steam, can lead to disruption of the food matrix, increasing the bioaccessibility of various phytochemicals and improving the nutritional quality of vegetables.

Through an investigation of the evolution of anthocyanins during the controlled drying of red "Merlot" and "Tempranillo" grapes, Marquez et al. [26] found that drying in a chamber maintained at 40 °C increased the concentration of five glycosidic anthocyanins during the first 24 hours. These data are likely due to the diffusion of anthocyanins from the grape skins to the pulp as a result of cell damage induced by the high temperature.

Table 2. Content of carotenoids (μg.100 g $^{-1}$ in d.b.) and pro-vitamina A (mg retinol.100 g $^{-1}$ in d.b.) in CS, OS, CR and OR.

Analyses	CS	OS	CR	OR
Lutein	33.95 ± 1.71^{c}	56.31 ± 0.11^{b}	87.41 ± 0.47^{a}	84.06 ± 3.29^{a}
Zeaxathin	nd	0.50 ± 0.18^{c}	13.32 ± 0.37^{a}	5.14 ± 0.93^{b}
α-carotene	6.16 ± 0.92^{b}	nd	14.23 ± 0.02^{a}	nd
β-carotene	236.51 ± 18.52^{b}	204.06 ± 2.99^{c}	479.44 ± 2.99^{a}	259.64 ± 8.02^{b}
Total	276.62 ± 21.14^{c}	260.86 ± 2.70^{c}	593.07 ± 5.25^{a}	348.83 ± 11.83^{b}
Pro-vitamin A	0.0182 ± 0.00^{b}	0.0157 ± 0.00^{b}	0.0369 ± 0.00^{a}	0.0199 ± 0.00^{b}

All of the results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same row represent a significant difference ($P < 0.05$).

The values observed in this study are similar to those reported by Perry et al., [31] who studied the carotenoid content in foods that are frequently consumed by the American population. The lutein contents were similar in various foods, such as artichoke (95 μg.100 g^{-1}), white grape (53 μg.100 g^{-1}), and tomato (32 μg.100 g^{-1}). The levels of zeaxanthin were similar to those found in various foods, such as artichokes (18 μg.100 g^{-1}) and lettuce (12 μg.100 g^{-1}). The levels of α-carotene were similar to those found in mango (14 μg.100 g^{-1}) and yellow pepper (17 μg 100 g^{-1}), and the values of β-carotene are comparable to the levels found in foods such as cooked broccoli (405 μg.100 g^{-1}), zucchini (357 μg.100 g^{-1}), and red pepper (354 μg.100 g^{-1}).

Only the results found for lutein and α-carotene showed a higher content in the conventional samples. This type of result is common in studies that explore the effect of the cultivation mode on food.

Park et al. [32] investigated possible differences between different cultivars of organic and conventional kiwi and obtained favorable results for the variety "Bidan" and the opposite results for the variety "Hayward". Several authors argue that the impact of the cropping system on the content of carotenoids, such as β-carotene, is not yet clear. Hallmann et al. [33] studied the juices of organic and conventional tomatoes and reported a higher content of β-carotene and a lower lycopene content in the organic juices. In contrast, Kaack et al. [34] described a lower content of β-carotene in organic carrots, i.e., the opposite of the previously discussed results.

Through a study of flour seeds of different varieties of conventional grapes (Chardonnay, Concord, Muscadine, and Ruby), Lutterodt et al. [35] reported the presence of lutein (from 39.7 to 950 μg.g^{-1}), zeaxanthin (from nd to 157 μg.g^{-1}), β-carotene (from nd to 4490 μg.g^{-1}), and cryptoxanthin (from nd to 34.4 μg.g^{-1}).

The content of vitamin A was calculated based on the β-carotene content analyzed because carotenoids may also be divided into provitamin A compounds and non-provitamin A compounds. [36] The provitamin A carotenoid that is most widely available in the Western diet is β-carotene, but α-carotene and β-cryptoxanthin may also prevent deficiencies. Vitamin A is essential for the promotion of growth, embryonic development, and visual function. The contribution of provitamin A carotenoids to the daily intake of vitamin A depends on the eating habits and available sources. [37] It has been estimated that carotenoids from fruits and vegetables provide more than 70 % of the vitamin A intake in third-world countries, whereas the corresponding contribution in Western countries is much lower. [9]

β-carotene is one of the most common carotenes and contains specific end groups that act as provitamin A. [38] According to Hiranvarachat et al. [39], β-carotene has high antioxidant activity, i.e., it is able to scavenge peroxyl radicals that are formed through oxidation reactions, especially at low oxygen partial pressures.

Antioxidant activity

The analysis of the DPPH revealed better results for the organic seeds, followed by the samples of organic and conventional waste, which did not differ, as shown in Table 4.

Table 3. DPPH results of CS, OS, CR and OR.

Sample	**DPPH** (g sample.g DPPH $^{-1}$ d.b.)
CS	6933.84 ± 209.95^{a}
OS	2684.81 ± 5.17^{c}
CR	3159.74 ± 84.73^{bc}
OR	3445.35 ± 61.72^{b}

All of the results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same column represent a significant difference ($P < 0.05$).

All of the extracts were able to reduce the chemiluminescence, i.e., all showed antioxidant capacity. Of the extracts, the CR and OS extracts showed the greatest capacity to reduce chemiluminescence, and no significant difference was found between them (Figure 1).

The TRAP is based on the extinction of luminol chemiluminescence. This assay is considered to be simple, sensitive, and reproducible and can be used to determine the antioxidant capacity of complex mixtures such as extracts of plants. In contrast, the TAR [40] is given by the initial decrease in luminescence associated with the incorporation of the sample by the system and thus indicates the initial reactivity of the sample compared with other compounds, such as Trolox. [41]

The antioxidant activity of individual compounds, mixtures, or body fluids has been widely investigated, and many methods, including chemiluminescence-based assays, have been developed. [42] This type of test is based on the reaction of oxygen radicals with the analyzed compounds to produce excited-state species that emit chemiluminescence (chemically induced light), [43] and TRAP is one of the most widely used tests for the determination of antioxidant activity. The parameter analyzed is the area under the curve (AUC), which indicates the amount of antioxidants in the extract analyzed. In contrast, the total antioxidant reactivity (TAR) indicates the quality (given by the reactivity) of these

extracts with antioxidant activity. The TRAP and TAR cannot be proportional because these ratios depend on the presence or absence of efficient antioxidants in the extract, and sometimes there are antioxidants with relative low reactivity. [44]

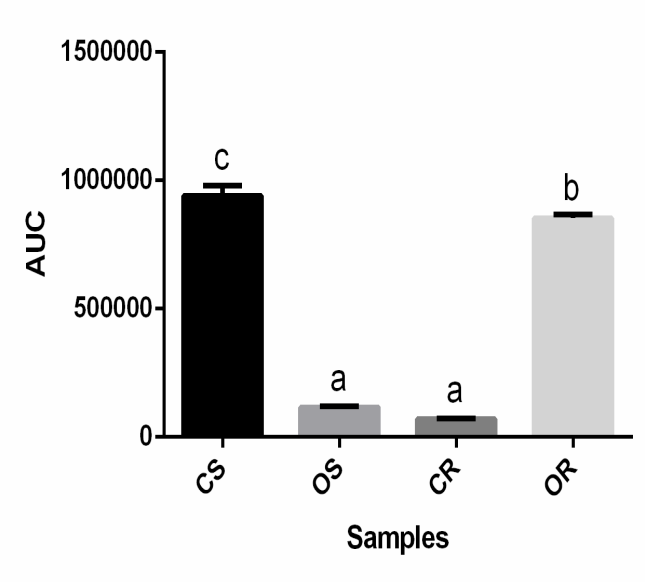

Figure 1. AUC values of CS, OS, CR and OR.

Different letters indicate a significant difference ($P < 0.05$).

Similarly to the results of the DPPH analysis, CR and OS showed the most significant results, and no difference was found between these samples. In contrast, although CR exhibited significantly higher levels of lutein, zeaxanthin, and α and β-carotene, the same trend was not observed for OS.

OS revealed significant higher levels of antioxidant compounds compared with CS. Studies on organic and conventional agriculture have contradictory data. For example, Vian et al. [13] investigated the composition of anthocyanins in conventional and organic "Syrah" grapes (*Vitis vinifera*) during ripening and obtained favorable results with organic farming. However, Dani et al., [45] who investigated the antioxidant activity of different types of

conventional, organic, white, and red grape juices, reported that samples of conventional grape juice “Bordô” do not differ significantly from samples of organic grape juice “Bordô”.

OS showed significantly higher levels than OR, whereas CS showed significantly lower levels compared with CR. Initially, OS exhibited the highest antioxidant activity of the tested samples, but the antioxidant activity of OR was significantly decreased after the oil was extracted, suggesting that the compounds present in high amounts prior to extraction were degraded and/or eliminated with the oil through the extraction process.

In contrast, the CS showed the lowest antioxidant activity. However, the extraction of the oil, which resulted in the breakage of the structures of the conventional seeds, may have promoted an increased release of antioxidant compounds into the food matrix, significantly raising the antioxidant activity in the residue.

As discussed in this article, the heating to which the seeds are subjected during oil extraction can lead to a significant increase in bioactive compounds. CR exhibited significant higher levels compared with CS, thereby confirming this hypothesis.

By investigating a heat treatment for the acceleration of oxidative aging in sweet wines “Pedro Ximenez”, López de Lerma et al. [46] reported an increase in antioxidant activity due to the content of phenolic acids, phenolic compounds, and procyanidins, suggesting that an increase in temperature can result in increased levels of antioxidants.

By studying the residues obtained from the extraction of oil from different varieties of grapes, Maier et al. [3] reported that the phenolic acid profiles of the residues are more complex than those of the seeds. Caffeic acid, p-coumaric acid, and ferulic acid were present in the "Lemberger" waste and were not present in the seeds. These compounds may originate from the degradation of high-molecular-weight phenolic compounds that were not identified by HPLC. [3]

At present, it is not possible to give an exact answer regarding the various factors that influence the contents of bioactive compounds in foods grown through different cultivation modes. For example, Corrales et al. [11] reported higher antioxidant activity by ABTS in white grape skins grown conventionally compared with those grown organically. However, the analysis of phenolic compounds revealed that the organic grapes exhibited higher levels.

By studying the effects of cultivation type on the contents of various fruits and vegetables, Faller and Fialho [47] reported different profiles depending on the sample. In general, the skin of fruits, such as banana, papaya, mango, and tangerine, exhibited higher antioxidant activity when grown organically. However, the pulps of these fruits often showed the opposite results. The analysis of vegetables, such as potatoes, broccoli, carrots, cauliflowers, tomatoes, and onions, showed that only the latter exhibited favorable results with conventional farming. All of the other samples did not exhibit differences based on the type of cultivation. These authors suggest that the modulation of these parameters due to the type of cultivation is unique for each species and cultivar.

The TAR index is calculated by the instantaneous decrease in luminescence associated with the incorporation of the extract into the medium. Although the measurement of TRAP indicates the amount of antioxidants present in the extract, TAR is determined to measure the quality (given by the reactivity) of the same extracts. [48]

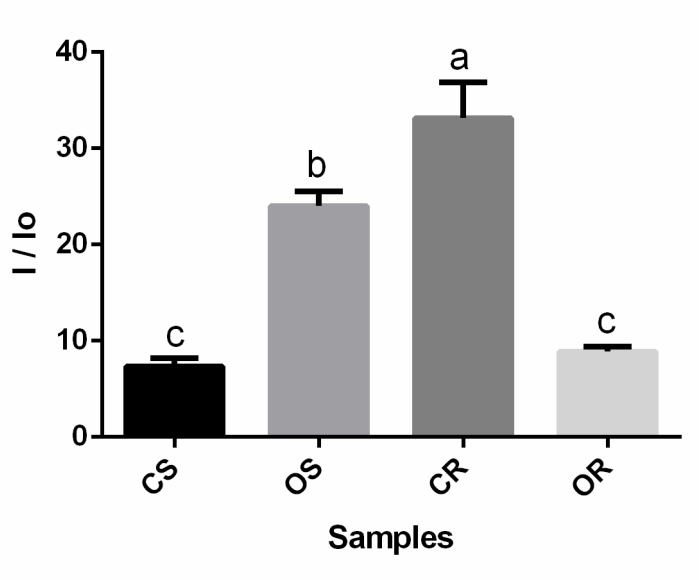

Figure 2. TAR values of CS, OS, CR and OR.

Different letters indicate a significant difference ($P < 0.05$).

The TAR results indicate that all of the extracts have antioxidant reactivity, but the same extracts that showed better activity in the quantitative analysis of TRAP showed a higher TAR.

Carotenoid antioxidant potential

To correlate the antioxidant activity with the presence and/or content of carotenoids and to evaluate the effects of the extraction steps on the bioactive compound profile, the methanol extracts used for the determination of antioxidant activity (DPPH, TRAP, and TAR) were analyzed to identify and quantify their carotenoid contents by HPLC (Table 5). The results of the analysis of the extract antioxidant activity by HPLC are shown in Table 5.

The OR and OS samples did not exhibit significantly different contents of lutein and β-carotene. CR had the highest amount of lutein; however, because the contents of β-carotene in CR and CS were not significantly different, both of these samples were found to have the highest amounts of this compound. Traces of zeaxanthin were found in all of samples, and a-

carotene was found in the CS and CR samples, but it was not possible to quantify the levels of these two compounds.

Table 4. Carotenoids in the CS, OS, CR and OR methanol extracts.

Sample	Lutein	α-Carotene	β-Carotene	Total
CS	133.43 ± 6.57^{c}	tr	382.44 ± 6.66^{a}	516.67 ± 7.12^{ab}
OS	140.47 ± 0.88^{b}	nd	333.42 ± 14.93^{b}	486.28 ± 0.64^{b}
CR	164.33 ± 10.39^{a}	tr	379.78 ± 24.32^{a}	538.86 ± 29.14^{a}
OR	122.00 ± 0.66^{bc}	nd	316.26 ± 4.20^{b}	438.62 ± 3.54^{b}

All of the results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same column represent significant difference ($P < 0.05$). The results are expressed as µg/100 g sample d.b; nd, not detected; tr, trace.

The comparison of the lutein contents with the antioxidant activity (DPPH and TRAP) showed that CR and OS exhibited the most significant levels, as well as significant contents of β-carotene and the highest concentrations of total carotenoids. Therefore, the results suggest that the carotenoids present in these samples exhibit stronger antioxidant activity even if found in small quantities.

A higher content of β-carotene and total carotenoids was obtained in the conventional samples (CS and CR), and CS exhibited the highest content of β-carotene.

The extraction of carotenoids for quantitative analysis includes a saponification step due to the complexity of the samples. [49] Because carotenoids may be esterified with fatty acids, many authors choose to use a saponification step for the removal of interfering lipids, chlorophylls, and carotenoids to only analyze free units of carotenoids. [50] However, there is controversial data regarding the losses caused by saponification.

In this study, the extract obtained for antioxidant activity analysis did not undergo the process of saponification. The analysis of lutein and β-carotene revealed that the not-saponified samples exhibited much higher values compared with the saponified samples (with the exception of the β-carotene content in the CR samples). Nevertheless, the not-saponified samples showed no appreciable amount of zeaxanthin and α-carotene, which is different from the results obtained with the saponified samples.

The influence of saponification on the esterified forms of carotenoids has been previously discussed in fruits and vegetables, but a conclusion has not been reached. However, in the case of seeds, the extraction methods have not been widely discussed. Investigating this relationship in three species of *Passiflora*, Wondracek et al. [51] reported higher amounts of carotenoids in the not-saponified samples of only one species (commercial *P. edulis*), whereas the analysis of the other species (*P. setacea*) revealed that saponification was beneficial and increased the levels of the compounds examined.

A saponification step should be included in the analytical procedure only when necessary, according to Rodriguez-Amaya. [52] It is unnecessary, for example, in the analysis of leafy vegetables, tomatoes, carrots, and all foods containing a low lipid content and therefore free esters of carotenoids.

The study of the composition of carotenoids in natural matrices can be affected by isomerizations and rearrangements and thus result in the underestimation of some carotenoids; thus, saponification is generally used to release the carotenoids in their free form to simplify the analysis. Therefore, a better approach for the analysis of the content of carotenoids may involve the classification of plants depending on their free or esterified xanthophyll profile. Moreover, the xanthophylls that are usually included in the diet are esterified with fatty acids. [7]

By studying the influence of the saponification of carotenoids on the analysis of five different varieties of Italian raspberry, Carvalho et al. [49] reported significant differences between the extracts. After saponification, these authors observed the disappearance of chlorophyll and its derivatives in the immature samples. The authors emphasize that the saponification process was gentle but did obtain a decrease in the content of some carotenoids, such as lutein. In the mature samples, the chromatograms showed peaks that eluted later and disappeared after saponification. Based on the similarity of the spectra, these peaks were hypothesized to be lutein esters.

CONCLUSIONS

OS showed clearer and more saturated color parameters, higher antioxidant activity, and a high content of lutein in the extract than CS. The analysis of the waste residues revealed that CR exhibits better results for most of the parameters analyzed, such as the color parameters, total carotenoids, TRAP, TAR and content of antioxidant carotenoids present in the extract. This work demonstrates that grapes produced through organic farming is not different and can present even lower antioxidant activity than that produced by conventional cultivation.

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CAPÍTULO 3:

BIOACTIVE COMPOUNDS AND STABILITY OF ORGANIC AND CONVENTIONAL *VITIS LABRUSCA* GRAPE SEED OILS

The article was formatted according to the Journal of Agricultural and Food Chemistry

Abstract

The aim of this study was to determine the differences between organic and conventional grape seed oils extracted from different grapes (Bordô and Isabel). The physicochemical quality, bioactive compounds and oxidative stability of the oils were investigated. The organic samples exhibited the best color parameters, and all samples were within the limits established by the Codex Alimentarius regarding their quality parameters. Only Bordô grape seed oils presented lutein and the best results regarding α- and β-carotene and α-tocopherol contents. All samples exhibited the same antioxidant activity results, but the Bordô ones exhibited higher oxidative stability. Overall, the results from this study suggest no differences between organic and conventional grape seed oils but between the grape varieties.

Keywords: carotenoids, tocopherol, color, phenolic compounds, antioxidant activity

1. Introduction

Grape seed is an important part of the pomace, representing 38 to 52 % of the dry matter thereof[1], which makes it a significant residue of agribusiness juices and wines[2]. Grape seed may contain 7 to 20 % oil[3]; thus, its extraction may represent a good option for adding value to a product.

In addition to the basic function of providing nutrition, vegetable oils contribute to the palatability of food, act as a vehicle for fat-soluble vitamins and are sources of essential fatty acids such as linoleic, linolenic and arachidonic acids[4].

Virgin oils contain bioactive compounds, including carotenoids, tocopherols and phenolic compounds of numerous low and high molecular weights, that may offer beneficial effects to human health[5]. These compounds have been of great interest to the food and pharmaceutical industries due to their anti-inflammatory, anticarcinogenic and antimutagenic effects as well as their association with a decreased risk of cardiovascular disease[6].

Some carotenoids are valued for their pro-vitamin A activity (β-carotene) or their protection against age-related macular degeneration (lutein), and numerous studies have been performed to evaluate these compounds in foods[7]. These carotenoids have also been related to important functional properties, especially antioxidant activity, and have been implicated in preventing cardiovascular disease. These properties render the compounds ideal for promoting the consumption of natural products containing them[8].

Tocopherols are important inhibitors of lipid oxidation in food and biological systems[9]. Each type of oil exhibits a characteristic tocopherol content, which also depends on plant genotype, climatic conditions and crop growth, content of polyunsaturated fatty acids and processing and storage conditions[10]. In addition to exhibiting vitamin E activity, tocopherols occur in seed oils in different forms: α, β, γ and δ-tocopherol, where γ-tocopherol is reported as one of the most highly antioxidant forms[11]. Because of their fat-soluble antioxidant properties, these compounds inhibit the peroxidation processes of polyunsaturated fatty acids and other compounds that affect the cell membrane[12], as well as prevent the rancidity of oils during storage[13].

An increasingly large portion of the population prefers to consume products of organic farming, mainly due to the absence of contaminants in the production process[14]. The number

of farms dedicated to organic agriculture has increased worldwide. Consequently, the market is shifting toward the consumption of organic products, and numerous studies have been conducted in this area; however, little attention has been paid to the quality of these food products[15]. Many studies have reported that organic foods provide higher nutritional value, exhibiting a lower nitrate content and better organoleptic quality[16].

The aim of this study was to evaluate the quality of seed oils of organic and conventional grapes (*Vitis labrusca* cv. Bordô and Isabel) for the identification and quantification of bioactive compounds and to determine the oils' physicochemical characteristics and oxidative stability.

2. Material and Methods

2.1. Raw Material

The processed residual material (pomace) of the production of Bordô and Isabel (organic and conventional, respectively) grape juices (*Vitis labrusca*, vintage 2013) was dried in a horizontal rotary dryer at 70 °C for 4 hours until the moisture content was approximately 7 %, avoiding rancidity. A ventilation process was used to separate the seeds from the rest of the waste, and grape seed oil extraction was performed by cold extraction using a worm-type extractor composed of plated carbon steel. The temperature was controlled such that it did not exceed 50 ± 1 °C, preserving the quality of the oil obtained as well as allowing for the greatest possible amount of bioactive compounds to be obtained. The oils were transported to the Laboratory of Bioactive Compounds of the Federal University of Rio Grande do Sul (UFRGS) and were stored in amber vials at room temperature (25 ± 1 °C). The oils were decanted for 72 hours to remove the residual sludge formed naturally in crude oils. After this period, the oils were refrigerated (5 ± 1 °C) until analysis. The equipment used in obtaining

the raw material stage were designed and built by Econatura Company. The following oils were obtained for analysis: Bordô Conventional Oil (BCO); Bordô Organic Oil (BOO); Isabel Conventional Oil (ICO) and Isabel Organic Oil (IOO).

2.2. Reagents and standards

The chromatographic analyzes were performed using solvents of HPLC grade: methanol, methyl tert-butyl and acetonitrile (Panreac). The standards used for the construction of calibration curves were: (±)-α-Tocopherol (99.5 % HPLC, SULPECO), (+)-γ-Tocopherol (≥ 96 % HPLC, Sigma-Aldrich). The carotenoids α-carotene (purity > 93 %), β-carotene (purity > 95 %) and zeaxanthin (purity > 95 %) were purchased from Sigma Chemical (USA). Lutein (purity > 95 %) was purchased from Indofine Chemical Company Inc. Hillsborough (USA). All the analysis were performed in triplicate.

2.3. Cromatograph

The HPLC analysis was performed in an Agilent 1100 Series HPLC system equipped with a quaternary solvent pumping system (G1311A – DE14917573 Agilent 1100 Series, Waldbronn, Germany) and a UV/Vis detector (G1314B - DE71358944 Agilent 1100 Series, Waldbronn, Germany).

2.4. Color analysis

Color analysis was performed via a MINOLTA CR 310 187 colorimeter using the color parameters *L* a* b**, Illuminant D65 and a factor observer angle of 10°.

2.5. Physicochemical quality analyses

The refraction and acidity indices of the oils were determine according to the methodology of the Instituto Adolfo Lutz[17]. The peroxide value and unsaponifiable matter were determined by the Cd 8-53 and Ca 6a-40 methods according to AOCS[18], respectively.

2.6. Carotenoids

The carotenoid extract was prepared according to the method described by Mercadante & Rodriguez-Amaya[19]. A 250 mm × 4.6 mm i.d., 3 μm, C30 reversed phase polymeric column was used (YMC, Japan) in HPLC and the wavelength was adjusted to 450 nm. The mobile phase used was water:methanol:tert-methyl butyl ether (MTBE) (J.T. Baker – Mallinckrodt, EUA) starting at a ratio of 5:90:5 and reaching 0:95:5 in 12 min, 0:89:11 in 25 min, 0:75:25 in 40 min and 0:50:50 after a total of 60 min, with a flow rate of 1 $mL.min^{-1}$ and a injection volumn of 5 μL at 33 °C[20]. The carotenoids were quantified using standard curves of lutein (1–65 $\mu g.mL^{-1}$), β-carotene (5–50 $\mu g.mL^{-1}$) and α-carotene (2-25 $\mu g.mL^{-1}$). The limits of detection (LD) and quantification (LQ) were, respectively, $6.9x10^{-3}$ $mg.kg^{-1}$ and $1.15x10^{-2}$ $mg.kg^{-1}$ for lutein; $4.46x10^{-2}$ $mg.kg^{-1}$ and $7.43x10^{-2}$ $mg.kg^{-1}$ for β-carotene, and $1.97.10^{-2}$ $mg.kg^{-1}$ and $3.28.10^{-2}$ $mg.kg^{-1}$ for α-carotene. The results were expressed in micrograms per 100 g of sample.

Qualification analysis was assured taking into consideration the following criteria: (1) elution order in reverse HPLC of each carotenoid standard in the established conditions of chromatographic analysis; (2) retention time based on the average of three different measurements of all the commercial standards comparing with the retention time of all the coincident peaks in the sample and in its duplicate; and (3) comparison with commercial standards acquired.

The carotenoids were quantified using standard curves of lutein (1–65 $\mu g.mL^{-1}$), β-carotene (5–50 $\mu g.mL^{-1}$) and α-carotene (2-25 $\mu g.mL^{-1}$). The limits of detection (LD) and quantification (LQ) were, respectively, $6.9x10^{-3}$ $mg.kg^{-1}$ and $1.15x10^{-2}$ $mg.kg^{-1}$ for lutein; $4.46x10^{-2}$ $mg.kg^{-1}$ and $7.43x10^{-2}$ $mg.kg^{-1}$ for β-carotene, and $1.97.10^{-2}$ $mg.kg^{-1}$ and $3.28.10^{-2}$ $mg.kg^{-1}$ for α-carotene. The results were expressed in micrograms per 100 g of sample.

2.7. α and γ-tocopherol

The extraction of oils tocopherols was carried out with methanol at room temperature. For chromatographic separation, a polymeric Vydac C18 column (218TP54) (250 mm x 4.6 mm id) containing 5 mM methanol was used at a wavelength of 292 nm. The analysis was performed in isocratic mode, and a methanol/water solution (96:4, v/v) was used as the mobile phase at a flow rate of 1 $mL.min^{-1}$ for 10 min. The column temperature was maintained at 30 °C, and an injection volume of 10 µL was used. For the quantification of α-tocopherol, an external standard method involving the construction of a calibration curve determined by individual diluted solutions of α-tocopherol in methanol (from 2 to 303 $mg.L^{-1}$) was employed. The limits of detection and quantification were, respectively, 0.09 and 0.17 $mg.kg^{-1}$. The quantification of γ-tocopherol was carried out in the same way but with individual solutions of γ-tocopherol diluted in methanol (from 7062 to 12600 $mg.L^{-1}$). The limits of detection and quantification were, respectively, 0.10 and 0.18 $mg.kg^{-1}$. The results were expressed in micrograms per 100 g of sample.

2.8. Phenolic compounds and antioxidant activity

The determination of the total phenolic compounds of the grape seed oils was performed according to the method used by Capannesi et al.[21]. Quantification was based on a calibration

curve using gallic acid dissolved in methanol as a standard. The results were expressed in mg gallic acid.g oil^{-1}. The TRAP and TAR were measured and calculated as previously described by Dresch et al.[22] with a methanolic extract diluted with water and DMSO. The results were converted to percentile ranks, and the area under the curve (AUC) was calculated by utilizing the GraphPad software program (San Diego, CA, USA). The smaller the AUC is (relative to that of the system), the higher the total reactive antioxidant potential of a sample becomes. The TAR index was determined by measuring the initial decrease in luminol luminescence, calculated as the ratio I0/I, where (I0) is the initial emission of CL (before the addition of the antioxidant) and (I) is the instantaneous CL intensity after the addition of an aliquot of the sample or the reference compound (Trolox).

2.9. Oxidative stability index

Three grams of grape seed oil was used placed in a Rancimat 743 (Metrohm AG, Switzerland) instrument to determine the oxidative stability of the samples. The oxidation process was assessed by response surface methodology (RSM). A full-rotational 2^2 experimental design with 4 axial points and 3 central points was used. The variables studied were temperature (T from 50 to 210 ºC) and flow rate (F from 8 to 22 L.h^{-1}). At each time point, eight oil samples were analyzed simultaneously by the equipment. Each sample was analyzed in duplicate. Statistical analysis was performed using the software Statistica 12.0 (Statisoft Inc.), allowing for the evaluation of the experimental data fitting to mathematical models obtained by the F test and the determination of the response surface as a function of the variables.

3. Results and Discussion

3.1. Color analysis

Table 1 shows the results of the colorimetric analysis and shows that all samples presented color angles (h) smaller than 90 °, suggesting a tendency toward yellow and brown colors.

Table 1

Color parameters of conventional and organic grape seed oils Bordô and Isabel[a].

sample	L	a*	b*	C	h
BCO	30.52 ± 0.03 d	4.10 ± 0.03 a	12.04 ± 0.06 c	12.71 ± 0.06 c	71.20 ± 0.16 c
BOO	32.30 ± 0.04 c	3.32 ± 0.02 c	14.82 ± 0.04 b	15.18 ± 0.04 b	77.40 ± 0.09 b
ICO	35.93 ± 0.0 b	2.78 ± 0.04 d	5.65 ± 0.02 d	6.29 ± 0.02 d	63.80 ± 0.35 d
IOO	47.61 ± 0.08 a	3.45 ± 0.02 b	26.85 ± 0.11 a	27.07 ± 0.11 a	82.69 ± 0.02 a

[a]All of the results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same column represent a significant difference ($P < 0.05$).

IOO showed high rates for L, b*, C and h as the lightest sample (high L*); although more saturated (higher C*), on the other hand, ICO showed lower levels for a, b*, C* and h. With respect to BCO, BOO also showed more significant results, suggesting that the organic samples exhibited more favorable color parameters.

The color measurement of a sample is of great importance to food production due to the relationship between sensory attributes and the acceptability of a product for consumers[23]. Lee et al.[24] observed values similar to those obtained in this work for parameters L* and b* in studying ginseng oils, and the extraction method (pressing, solvent or supercritical fluid) did not affect these characteristics of the samples.

Investigating eight varietal cultivars of conventional Spanish olive oils, Moyano et al. [25] observed values ranging from 61.94 to 99.28 for L*, from -14.96 to 9.96 for a*, from 11.98

to 128.68 to b*; 12.20 to 128.96 for C* and from 85.03 to 100.84 to h*. Although similar to those explored in this study, the cultivars studied by Moyano[25] exhibited very different values for the same parameter, indicating a tendency toward lighter and more saturated samples with less reddish colors.

The color parameters of plant products can provide indications of quality and can be determined in terms of sensory and physicochemical analyses. The values of the parameter a* may decrease during storage, tending toward green (- a*), whereas b* values may decrease at temperatures near 20 °C, showing a greater tendency toward yellow (+ b*). The possible variations of these parameters can be related to the reactions of lipid oxidation and decomposition of antioxidants, which are common during the storage of oils[26].

3.2. Physicochemical quality analyses

The results of physicochemical analyses of the conventional and organic grape seed oils Bordô and Isabel are shown in Table 2.

The physicochemical properties of the oils are related to preservation and quality by parameters such as acidity and peroxide values, which depend on the nature and quality of the raw material. The results indicate the level of conservation of the oils considering effects of deterioration by light, temperature and oxygen, which accelerate the decomposition of glycerides, the development of rancidity and the release of fatty acids[26].

Table 2

Acidity index refractive index, peroxide value and unsaponifiable matter of conventional and organic grape seed oils Bordô and Isabel[a].

sample	acidity index[b]	refractive index	peroxide value[c]	unsaponifiable matter[d]
BCO	1.54 ± 0.15 b	1.47 ± 0.0 a	0.31 ± 0.03 c	0.09 ± 0.03 a
BOO	2.19 ± 0.01 a	1.47 ± 0.0 a	0.39 ± 0.03 bc	0.04 ± 0.0 a
ICO	1.26 ± 0.14 b	1.47 ± 0.0 a	0.58 ± 0.03 a	0.07 ± 0.01 a
IOO	0.90 ± 0.16 c	1.47 ± 0.0 a	0.46 ± 0.03 b	0.05 ± 0.0 a

[a]All results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same column represent significant difference ($P < 0.05$). [b]Expressed as mg $KOH.g^{-1}$. [c]Expressed as meq $O_2.kg^{-1}$. [d]Expressed as $g.kg^{-1}$.

IOO showed the lowest and therefore best acidity index among the analyzed samples, followed by ICO, BCO and BOO.

With respect to the refractive index, all oils showed equal values within the limits established by the Codex Stan (1.467 to 1.477).

The peroxide value is one of the main parameters in the quality analysis of oils because it indicates the oxidation state of a sample. BCO and BOO showed the best results. The peroxide value of an oil is an empirical measure of oxidation that is useful for samples that are oxidized at low levels (indices lower than 50) and exposed to sufficiently mild conditions such that the decomposition of hydroperoxides is not significant[27]. During auto-oxidation, the peroxide value can reach its maximum, decrease during later stages and varying according to the fatty acid composition of an oil and the oxidation conditions[28].

Lipid oxidation is an important reaction of deterioration that has significant implications in terms of the quality of fats and oils, specifically in relation to off-flavors that develop as a result of auto-oxidation. During the initial stages of the oxidation process, hydroperoxides accumulate as primary oxidation products, subsequently breaking down

constituents in the form of low-molecular-weight oxygenates such as alcohols, aldehydes, ketones and free fatty acids, leading to rancidity. The accumulation of hydroperoxides is monitored using the peroxide value. Together with the acidity index, which is a measure of hydrolytic rancidity, the two indices are most often determined during the production, storage and sale of oils[29].

The oils did not differ significantly in the analysis of unsaponifiable matter, all being within the standard set of values indicated by Codex Stan (< 20 $g.kg^{-1}$). The saponification reaction can help establish the degree of deterioration and stability of oils, verify that the properties of the oils are in accordance with specifications and identify potential fraud and adulteration[30]. Waxes, sterols and hydrocarbons in oils are generally determined as unsaponifiable matter. Water contributes to the hydrolysis of oils during the various handling and processing steps that generate products such as free fatty acids and glycerol[28].

3.3. Carotenoids

BCO and BOO showed values that were statistically equivalent to the content of lutein. The presence of lutein was not detected in ICO or IOO (Table 3).

Table 3

Content of carotenoids, tocopherols and total polyphenols of conventional and organic grape seed oils Bordô and Isabel[a].

analyses	BCO	BOO	ICO	IOO
lutein[b]	12.93 ± 1.65 a	14.99 ± 0,06 a	nd	nd
α-carotene[b]	10.62 ± 1.52 a	3.21 ± 0.23 b	8.72 ± 0.46 a	9.46 ± 0.21 a
β-carotene[b]	299.30 ± 0.76 a	153.42 ± 9.27 c	241.84 ± 26.94 b	230.14 ± 12.21 b
α-tocopherol[b]	1.76 ± 0.05 c	2.03 ± 0.06 a	1.91 ± 0.05 b	1.74 ± 0.0 c
γ-tocopherol[b]	0.25 ± 0.03 d	1.19 ± 0.06 a	0.57 ± 0.05 b	0.41 ± 0.04 c
total polyphenols[c]	853.29 ± 18.63 a	809.74 ± 27.60 a	676.67 ± 2.01 b	858.62 ± 28.61 a

[a]All results are reported as the means of three replicates ± standard deviation (n = 3). nd means not detected. Different letters in the same row represent significant difference ($P < 0.05$). [b]Expressed as μg.100 g^{-1}). [c]Expressed as mg of galic acid.g^{-1}.

BCO presented the most significant contents of β-carotene, followed by ICO and IOO, which showed no significant difference in content.

β-carotene is particularly interesting due to its pro-vitamin A structure of, providing 100% activity[31]. Lutein is an important component of the human retina and is associated with a reduction in age-related macular degeneration[32]. These properties, combined with an attractive color, have led to the increased use and demand for β-carotene, lutein and zeaxanthin in particular as supplements and food additives[33].

The occurrence of a specific profile of pigments in oils could be used to ensure the genuineness of a product according Giuffrida et al.[34] because the quality control of food provides knowledge of the composition of the original product.

Quantifying chlorophylls and carotenoids in varietal conventional olive oil of two consecutive harvests, Criado et al.[35] state that during the extraction of oil, mass transfer

phenomena occur and determine the distribution of pigment between the olive solid (pomace) and liquid phase (oil) and wastewater. The lipophilic nature of chloroplasts can determine their affinity for the oil phase, and the more hydrophilic nature of anthocyanins determines their retention in pomace and wastewater. Thus, the chloroplast pigments (chlorophyll and carotenoids) are primarily responsible for the color of virgin olive oil, which ranges from yellow to green.

The ingestion of oils and fats is known to increase the bioavailability of lipophilic vitamins. Many studies have clearly demonstrated that incorporating these substances into the diet increases the bioavailability of plant-derived carotenoids in human subjects[36,37]. The effects of oils and fats in the diet are manifested by the dispersion of carotenoids in the digestive tract or the indirect promotion of pancreatic juice secretion. Moreover, the secretion of chylomicrons promoted by oils and fats facilitate the transfer of lymph micellar carotenoids. The effects of free oils and fats, unsaponifiable matter and other classes of lipids on bioavailability fatty acids have not been fully elucidated[38].

The red palm oil obtained from the fruit of palm (*Elaeis guineensis*) also has a high content of carotenoids and is one of the richest natural sources of β-carotene, containing approximately 550 $\mu g.g^{-1}$ of total carotenes and 375 $\mu g.g^{-1}$ of β-carotene[39].

Investigating carotenoids, polyphenols and antioxidant activity in organic and conventional grapes (*Vitis vinifera*), Bunea et al.[40] reported more significant levels of lutein in organic grapes compared to those observed in conventionally cultivated grapes; however, the latter showed more favorable results regarding β-carotene content. Despite studying six different cultivars, the authors noted that it was not possible to discern any specific trend in the results obtained for the samples, i.e., a cultivar may have higher levels of a carotenoid under organic farming, but the opposite behavior for another carotenoid may occur within the same farming unit.

Studying the effects of type of cultivation in carotenoids, antioxidant activity and vitamin C in organic and conventional Brazilian fruits, Cardoso et al.[41] reported statistically equivalent contents of lycopene and β-carotene between organic and conventional khaki crops. In acerola fruit, conventional cultivation showed more significant results for levels of β-carotene. In strawberry fruits, organic farming did not differ significantly from conventional cultivation with respect to the content of β-carotene. According to the authors, organic farming has environmental and social impacts related to the health of workers and consumers, increasing socioeconomic viability when compared to conventional farming, but does not necessarily imply a better nutritional value of food.

3.4. α and γ-tocopherol

Regarding the content of α and γ-tocopherol, BOO showed the highest level, followed by ICO (Table 3).

Blekas et al.[42], studying the role of α-tocopherol in olive oil and its relation to the amount and composition of phenolic compounds, observed that phenolic compounds inhibit the auto-oxidation at an early stage in oils, whereas α-tocopherol is behaves effectively when the product reaches a critical concentration of compounds of auto-oxidation.

Investigating the extraction of grape seed oil with conventional pressurized liquid, Freitas et al.[13] reported less than 1 mg of α-tocopherol in 100 g of oil in Isabel grapes. The authors stated that Isabel, Herbemont and Seibel grape cultivars are unsatisfactory for producing quality wine but are widely consumed fresh in Brazil and are therefore used for the production of juices.

Studying 10 different conventional varieties of grape seed oils from Portuguese, Fernandes et al.[2] reported values above those observed in this study: 85.5 to 244 $mg.kg^{-1}$ of

oil for α-tocopherol and 2.50 to 45.0 mg.kg^{-1} of oil for γ-tocopherol. It is noteworthy that *Vitis vinifera* grapes were used, whose seeds may contain more bioactive compounds by having undergone fermentation in the winemaking process.

Analyzing seven conventional flaxseed oils marketed in New Zealand, Choo et al.[28] observed very significant levels of tocopherols. The amounts of α-tocopherol varied from 9.11 mg.kg^{-1} of oil and those of γ-tocopherol varied from 10.56 to 15.0 mg.kg^{-1} of oil.

Studying quality parameters of organic and conventional extra virgin olive oils for three consecutive years, Ninfali et al.[43] reported that it was not possible to observe a trend in the results obtained between organic and conventional crops with respect to tocopherol content, for example. The authors state that the differences observed in one year are not guaranteed to occur in the coming years. Moreover, they suggest that differences between the cultures could not be observed or proved inconsistent over the analysis period due to factors such as cultivar type and conditions and storage time.

3.5. Phenolic compounds and antioxidant activity

IOO, BCO and BOO showed no significant differences in the content of total polyphenols (Table 3).

Organic farming is generally characterized by the absence of pesticides and synthetic fertilizers during the cultivation period. The literature suggests that organic agriculture could result in foods with high levels of polyphenols, mainly due to two reasons. The first is that the use of synthetic fertilizers could provide more bioavailable nitrogen sources, accelerating plant growth and thereby leading to a reduction in resources used in the production of secondary metabolites. The second reason is the absence of synthetic pesticides that could result in greater exposure of the plant to stress, leading to a natural increase in the amount of defense substances such as phenolic compounds[16]. Both cases result in foods with high

antioxidant capacity as a result of a higher amount of polyphenols. The literature reports mixed results regarding the antioxidant activity and phytochemicals of organic and conventional vegetable composition, which vary according to the bioactive compounds and the type of food analyzed[44].

Bail et al.[5] studied conventional grape seed oils (Welschriesling, Chardonnay, Schilcher, Merlot, Cabernet-Sauvignon, Zweigelt and unknown cultivars) derived from wine production, reporting total polyphenol contents that ranged from 69.5 to 115.5 μg.g of oil^{-1}. These oils were obtained by cold pressing, and the highest content was observed in red grapes.

Characterizing grape seed oils obtained by the cold pressing of different conventional cultivars of Turkey (IFTA, Mazruna, Black Kerküş, Zeyti, Verdani, Karfoki, and Kerküş), Demirtas et al.[45] reported phenolic compound contents ranging from 2.19 to 4.70 mg GAE.100 g of oil^{-1}.

Lutterodt et al.[46] studied the antioxidant properties of oils of different cultivars of conventional grapes from the wine industry (Chardonnay, Muscadine, Ruby Red and Concord) and reported phenolic compound contents between 0.16 and 0.80 mg GAE.g of oil^{-1}. The authors attribute the low values to the low solubility of low-molecular-weight polyphenols in the oil.

Comparing conventional and organic peaches and pears, Carbonaro et al. [47] observed a higher content of polyphenols in both organic fruits. The authors suggest that changes occur in the metabolism of polyphenols as a result of the practice of organic farming but reported that the total content of polyphenols, as well as the proportion of several food components, are subject to variations according to the variety and species of plants studied.

Investigating the phenolic compound content and antioxidant activity of organic and conventional grape juices, Bordô and Niagara, Dani et al.[48] observed higher levels of

polyphenols in organic juices. The authors reported that the choice of method of organic farming resulted in different levels of resveratrol, anthocyanins and tannins in the juices.

Organic farming does not use chemical substances, making plants more susceptible to pathogens, which can cause the production of significant amounts of phenolics as a defense mechanism[49]. However, according to Dangour et al.[50], although the differences in composition between organic and conventional foods are biologically plausible, there is no evidence that supports the consumption of these foods over conventional foods to increase the intake of specific nutrients or bioactive substances.

Antioxidant compounds suppress oxidation reactions and quench light emission; therefore, it is possible for chemiluminescence to be applied during the analysis of antioxidant activity. The extracts of the oils analyzed were able to reduce chemiluminescence, i.e., the extracts showed antioxidant activity, but the activities were not significantly different (Figures 1 and 2). The measurement of chemiluminescence in the gradual extinction of the consumption of antioxidants depends on the reactivity of a substance toward the concentration of free radicals[51].

According to Desmarchelier et al.[52], the ability of antioxidant compounds to scavenge free radicals relative to that of synthetic standards, e.g., Trolox, allows for the determination of the antioxidant activity of substances such as plant extracts.

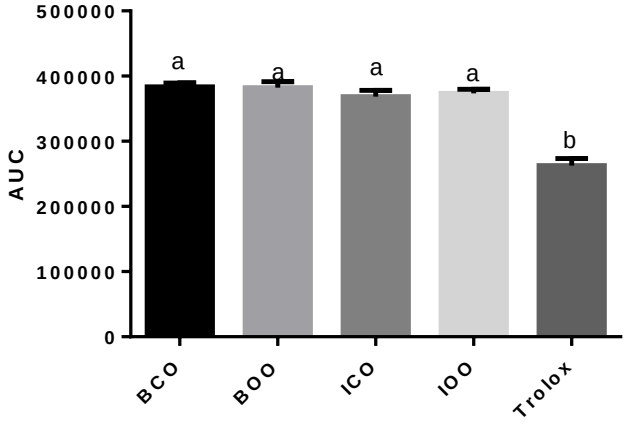

Fig. 1. AUC values of BCO, BOO, ICO, IOO and Trolox*.

*Different letters indicate significant difference ($P < 0.05$).

The antioxidant content of a food is an important parameter with respect to the relationship between nutrition and human health, in addition to affecting the shelf life of a product[47].

The harmful effects caused by oxidative stress may be delayed or reversed by increased levels of antioxidants, particularly phytochemicals such as polyphenols[53]. Therefore, many studies have suggested an inverse relationship between the consumption of foods rich in polyphenols and the risk of degenerative diseases, cancers and cardiovascular diseases[54].

Faller & Fialho[44], studying the relationship between phenolics and antioxidant activity of six vegetables and six different organic and conventional fruits, reported that it was not possible to discern a trend for the types of cultivation studied. The authors observed that in this food, the high content of soluble polyphenols observed in onions did not manifest as high an antioxidant activity. Furthermore, the authors suggest that other components can be attributed antioxidant capacity, such as vitamin C, carotenoids and glucosinolates.

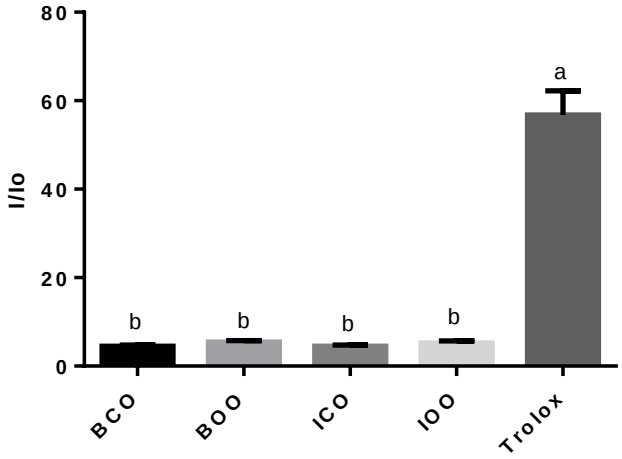

Fig. 2. TAR values of BCO, BOO, ICO, IOO and Trolox*.

*Different letters indicate significant difference ($P < 0.05$).

3.6. Oxidative stability index (OSI)

The results of the experimental design for the OSI are shown in Table 4 and Figure 3.

Table 4

Oxidative stability index (OSI) of BCO, BOO, ICO and IOO under the conditions of the experimental design[a].

experiment	T[b]	F[c]	OSI (h)			
			BCO	BOO	ICO	IOO
1	73	10	77.12±4.39 bB	86.45±0.81 bA	70.07±1.10 aC	70.30±0.79 aC
2	187	10	0.12±0.01 dA	0.12±0.01 dA	0.18±0.01 dA	0.16±0.0 dA
3	73	20	19.25±0.16 cA	20.52±2.94 cA	18.72±1.07 dA	18.17±0.23 cA
4	187	20	0.12±0.01 dA	0.15±0.02 dA	0.13±0.02 dA	0.12±0.01 dA
5	130	15	0.92±0.02 dA	1.13±0.01 dA	1.21±0.01 dA	0.89±0.08 dA
6	130	15	0.91±0.01 dA	1.07±0.02 dA	1.09±0.01 dA	1.13±0.01 dA
7	130	15	1.12±0.16 dA	1.10±0.01 dA	0.93±0.0 dA	1.11±0.01 dA
8	50	15	95.00±0 aA	95.0±0 aA	63.41±0.87 bB	62.98±0.90 bB
9	130	8	1.00±0.14 dA	0.92±0.0 dA	1.06±0.0 da	1.08±0.01 dA
10	210	15	0.05±0.01 dA	0.09±0.01 dA	0.07±0.04 dA	0.05±0.0 dA
11	130	22	1.05±0.10 dA	0.90±0.14 dA	1.30±0.02 dA	1.20±0.13 dA

[a]Different lower-case letters in the same column and different upper-case letters in the same row represent indicate differences ($p < 0.05$)*. [b]Expressed as C. [c]Expressed as $L.h^{-1}$.

The models were tested by analysis of variance (ANOVA) for a confidence level of 95 % ($p < 0.05$), yielding F values of 58.57 and 60.45 for BCO and BOO, respectively. For ICO and IOO, the calculated F values were 52.83 and 51.61, respectively. OSI is highly dependent on the linear and quadratic effects of temperature for both oils. The quadratic models adequately predict the influence of the experimental factors studied, with an R^2 value of 0.9482 for BCO, 0.9497 for BOO, 0.9429 for ICO and 0.9416 for IOO.

The experimental data were fitted to a full quadratic model. Polynomials fitted to the surface responses of BCO, BOO, ICO and IOO (Eqs. 1, 2, 3 and 4, respectively) were calculated by multiple regression:

Equation 1.

$$OSI = 0.9794 - 57.6757 \times T + 46.6887 \times T^2 - 14.4948 \times F - 0.0896 \times F^2 + 28.9350 \times T \times F$$

Equation 2.

$$OSI = 1.0853 - 60.3145 \times T + 47.9639 \times T^2 - 16.5312 \times F + 1.0523 \times F^2 + 32.9800 \times T \times F$$

Equation 3.

$$OSI = 1.0506 - 44.5812 \times T + 33.7478 \times T^2 - 12.8065 \times F + 3.0074 \times F^2 + 25.6475 \times T \times F$$

Equation 4.

$$OSI = 1.0188 - 44.3605 \times T + 33.5770 \times T^2 - 13.0415 \times F + 3.0227 \times F^2 + 26.0450 \times T \times F$$

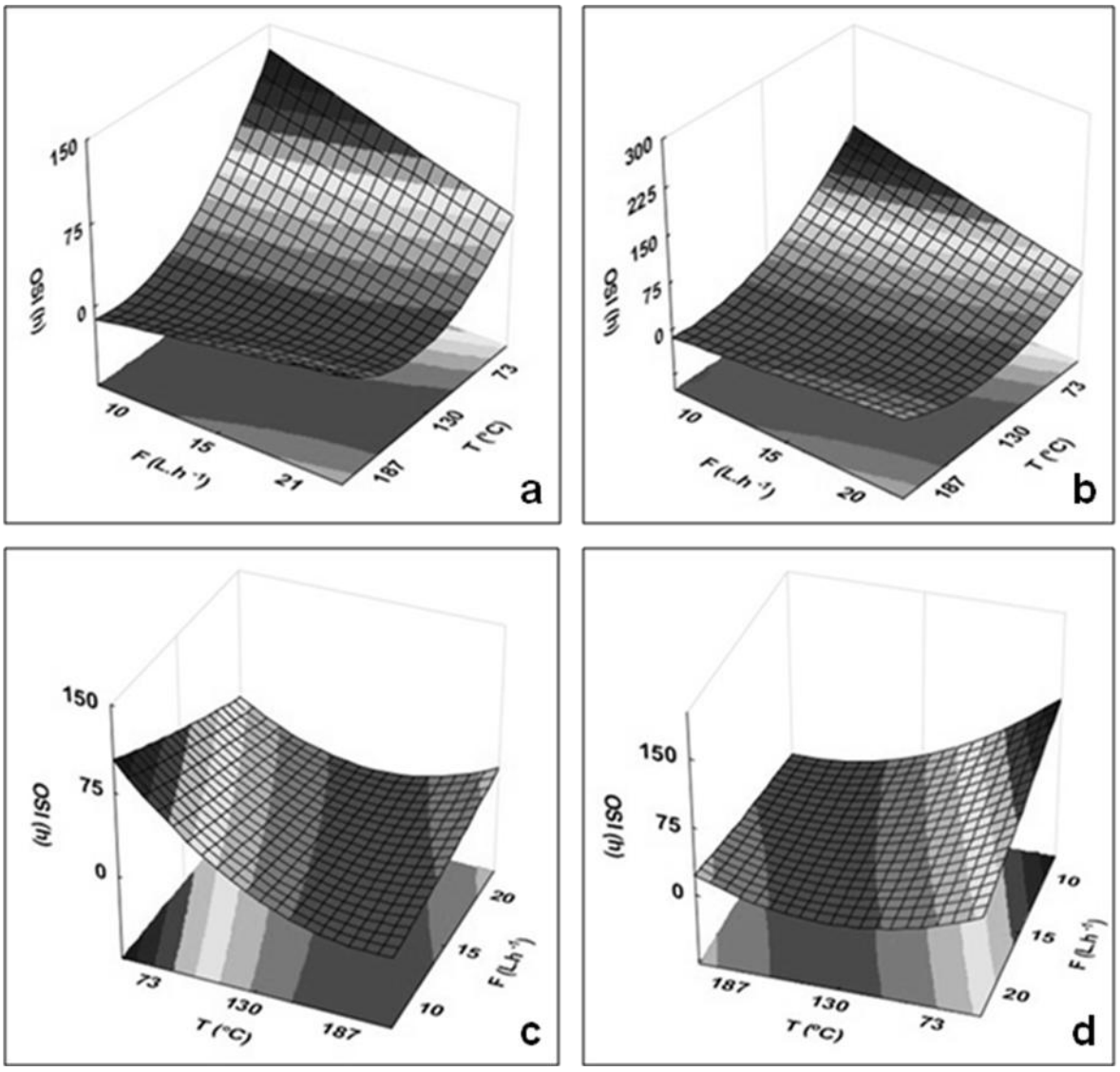

Fig. 3. Response surfaces for the oxidative stability index (OSI) of BCO (a), BOO (b), ICO (c) and IOO (d).

OSI decreased with increasing temperature, and air flow showed a linear effect on the stability index. BCO and BOO did not differ significantly at a temperature of 50 °C and air flow of 15 $L.h^{-1}$, yielding the same OSI of 95 h. At 73 °C and 20 $L.h^{-1}$, the samples showed no significant differences. At 73 °C and air flow of 10 $L.h^{-1}$, BOO may be considered more stable than BCO, obtaining a OSI of 86.45 h. In other experiments, BOO showed the same behavior as BCO, with no significant difference. Thus, it was observed that BCO and BOO

retained the same trend of decreasing OSI. At 73 °C, an increase of 10 $L.h^{-1}$ in airflow caused a drastic reduction in the OSIs of BCO and BOO, from 77.12 h to 19.25 h and from 86.45 h to 20.52 h, respectively. For ICO and IOO, these values decreased from 70.07 to 18.72 h and from 70.30 h to 18.77 h, respectively.

The optimal parameters determined by the experimental design for BCO and BOO are a temperature of 50 °C and an air flow of 15 $L.h^{-1}$. For IOO and ICO, the optimal conditions are 73 °C and 10 $L.h^{-1}$. Therefore, BOO and BCO are the most stable samples.

Investigating the oxidative stability of cranberry, carrot, cumin and flaxseed seed oils compared to that of soy and corn seed oils, Parker et al.[55] observed that cumin obtained the most significant OSI (151 h) using 4 mL of sample at 80 °C and 7 $L.h^{-1}$. The authors state that the Rancimat is a device widely used to automate the determination of OSI, which is directly related to the stability of oils.

According to Santos et al.[56], to compare the OSI results reported in other studies for seed oils can be difficult due to the different conditions analyzed and parameters employed, such as temperature, air flow, sample size and equipment, and other factors that are peculiar to each sample.

Studying the quality and oxidative stability of "Pará" nut obtained by supercritical fluid extraction, Santos et al.[26] obtained an OSI of 14.85 h using 5 g of sample at 100 °C and 10 $L.h^{-1}$. The oxidation curves obtained by the authors showed that the more the oil was oxidized, the greater the amount of volatile compounds derived from fatty acids was released and the shorter the induction period or the lower the OSI became.

Studying the lipidic characteristics, antioxidant activity and oxidative stability of seven genotypes of macadamia oils in two different crops, Wall[57] obtained OSIs of 6.82 to 10.08 h using 2.5 g of sample at 130 °C and 20 $L.h^{-1}$.

In determining the quality of virgin olive oil during the ripening of Arbequina olives, Benito et al.[58] obtained OSIs ranging from 1.7 to 2.3 h using 3 g of sample at 120 °C and 20 $L.h^{-1}$. The authors reported that, although the OSI is not considered a parameter indicative of quality, it is useful in providing information regarding the shelf life of an oil, demonstrating its resistance to oxidation, as characterized by reactions with free radicals.

Conclusions

Although the current literature suggests that exposure of food products to stress can lead to the synthesis of defense substances such as polyphenols, the benefits of organic farming, such as the increase in the amounts of this type of substance, were not observed in this study. IOO showed the most significant results for color analysis and acidity, whereas BCO and BOO showed the lowest values for peroxides. Regarding the analysis of carotenoids and α-tocopherol, BCO and BOO stood out by yielding the most significant results. When analyzing the content of polyphenols, IOO, BCO and BOO showed no significant differences, but when analyzing the antioxidant activity, all samples showed the same behavior. The determination of oxidative stability indicated better performance by BCO and BOO, which may be due to the greater amount of bioactive compounds present in these samples, such as lutein and α- and β-carotene and α-tocopherol. The results showed no significant differences between the types of cultivation but between different cultivars, where the Bordô oil exhibited the most significant amounts of bioactive compounds.

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CAPÍTULO 4:

THE QUALITY, STABILITY AND BIOACTIVE COMPOUND COMPOSITION OF VIRGIN AND REFINED ORGANIC GRAPE SEED OIL

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ABSTRACT

Samples of virgin and refined organic grape seed oil were studied for their physico-chemical quality, oxidative stability and the bioactive compounds they contained. All of the samples were within the limits established by the Codex Alimentarius with regard to their quality parameters. Lutein, zeaxanthin, β-carotene, α-tocopherol and catechin were the bioactive compounds analyzed, and the virgin oil obtained more significant results. No measurable amounts of zeaxanthin and β-carotene were observed in the refined oil, most likely due to the refining process that was carried out at high temperatures. The oxidative stability index (OSI) decreased with increasing temperature, whereas the air flow had no effect on the stability index. The optimal parameters for the OSI are 80 °C and a flow rate of 15 $L.h^{-1}$, and the virgin oil sample showed the best OSI, possibly because it was not subjected to any treatment after extraction. The results from this study suggest that it would be preferable to consume virgin instead of refined grape oil because it is a better source of bioactive compounds and has a higher stability when heated.

Keywords: carotenoids; tocopherol; catechin; oxidative stability index; refining process.

1. INTRODUCTION

A major concern of agribusiness producers of wines and juices is the generation of waste. The full use of grapes, including the pomace by-product, is an important aspect in reducing waste generation and in the generation of economic resources (Peschel et al., 2006).

Grape seed oil is one of these by-products and has been studied as a possible source of special lipids, such as linoleic acid, which is associated with the promotion of cardiovascular health (Wijendran & Hayes, 2004). In addition, grape seed oil has high levels of tocopherols, which confer considerable oxidative stability. These compounds contribute to grape seed oil being an anticholesterolemic food by reducing the levels of low-density lipoproteins (LDL) and increasing the levels of high-density lipoproteins (HDL) (Beveridge et al., 2005).

As part of the search for a healthier lifestyle by consuming foods rich in bioactive compounds, many studies have suggested the consumption of organic instead of conventional foods over the last decade. Organic farming can result in foods with high amounts of polyphenols due to the following two reasons: the use of organic fertilizers offers nitrogen sources with greater bioavailabilities, which accelerates the plant development and the production of secondary metabolites, and the absence of synthetic pesticides results in an increased exposure of the plant to situations of stress, which leads to an increased production of natural defense compounds such as phenolics (Winter & Davis, 2006). Both cases result in foods with greater antioxidant activities as a result of the content and the composition of the polyphenols (Faller & Fialho, 2010).

In this context, virgin vegetable oils stand out as sources of bioactive compounds, including tocopherols, sterols, polyphenols and carotenoids. Some of these compounds are beneficial to the stability of the oil during heat treatment (Rossi et al., 2007).

The compound α-tocopherol is considered to be the predominant antioxidant in olive and sunflower oils, while γ-tocopherol prevails in canola oil (Blekas et al, 1995). Tocopherols

have been studied for their ability to interfere in the initiation of oxidation reactions or as donors of hydrogen atoms to peroxyl radicals (Jerzykiewicz et al., 2013).

Carotenoids, due to their unsaturated structures, tend to degrade during processing and storage as a result of exposure to high temperature or light (Achir et al., 2010). The loss of these compounds in food processing has been reported by Aman et al. (2005) and Hiranvarachat et al. (2008).

The refinement of oil is widely used by the industry to remove impurities such as free fatty acids, phospholipids and pigments with the least possible damage to the beneficial constituents and the maximum yield (Karabulut et al., 2005), giving a better look to the oil and making it more acceptable to the senses. However, the refining process can result in a reduction in the tocopherol content of vegetable oils by 10 to 20 %, most likely due to its absorption into the soaps formed during the alkaline treatment (Karabulut et al., 2005).

In addition to the loss of bioactive compounds during the refining process, the stability of the vegetable oils is a critical factor for the food industry. As a result of its high degree of unsaturation, grape seed oil is very susceptible to oxidation. This process causes the development of off-flavors, which greatly reduces its use by the food industry (Jacobsen et al. 2008). Therefore, the determination of the oxidative stability of virgin organic grape seed oil is essential for the oil industry and for further research to control and optimize processes and predict its shelf-life.

The aim of this study was to evaluate the quality of samples of virgin and refined organic grape seed oil by the identification and quantification of the bioactive compounds contained in the oils as well as the determination of their physicochemical characteristics and oxidative stabilities.

2. MATERIAL AND METHODS

2.1. Raw Material

During the production of grape juice (Bordô *Vitis labrusca*, vintage 2012), the Econatura Company, located in the mountain region in the state of Rio Grande do Sul (Brazil), obtained a mixture of residues that contained the following components: grape seeds, stalks and skins. The waste material was processed by drying it in a rotary horizontal dryer at 70 °C for 4 hours until a moisture content near 7% was obtained to avoid rancidity. The seeds were separated from the rest of the waste by the process of ventilation, and the extraction of the oil from the grape seeds was performed by cold extraction using a worm plated carbon steel extractor. The temperature was controlled so as not to exceed 50 ± 1 °C to preserve the quality of the oil obtained as well as the greatest possible amounts of the bioactive compounds. The oils were transported to the Laboratory of Bioactive Compounds at the Federal University of Rio Grande do Sul (UFRGS) and were stored in amber vials at room temperature (25 ± 1 °C) for 72 hours to remove the residual sludge naturally formed in virgin oils. After this period, part of the virgin oil (VO) was refined and both samples were refrigerated (5 ± 1 °C) until analysis. The equipment used in obtaining the raw materials was designed and built by the Econatura Company.

2.2. Oil refinement

The oil refinement process was carried out in three steps: degumming, neutralization and bleaching. The degumming was carried out by adding water (3 % of the total volume) in a thermostated oil bath (Dubnoff type with a DMG temperature controller N480 D-V3.2x Novus Automation) with stirring (300 rpm) for 20 minutes. After the completion of the hydration step, the oil was transferred into a separatory funnel and decanted for 24 hours to obtain the degummed oil. In the neutralization step, a concentrated solution of sodium

hydroxide (70 %) was added under heating (65 °C), and the sludge that formed was separated from the oil by centrifugation at 1922 g for 20 min (Sigma centrifuge 4K15). The neutralized oil was subjected to two washings using 20 % heated water and was centrifuged again. The bleaching stage consisted of oil filtration using the PA-activated carbon powder (Nuclear). Thus, the virgin oil (VO) and the refined oil (RO) samples were obtained for analysis

2.3. Reagents and standards

Chromatographic analyses were performed using solvents of HPLC grade, and the solvents used were as follows: methanol, tert-methyl butyl ether and acetonitrile (Panreac). The standards used for the construction of the calibration curves were (±)-catechin hydrate puriss (98.5 % HPLC, FLUKA) and (±)-α-tocopherol (99.5 % HPLC, SULPECO). The carotenoids β-carotene (purity > 93 %) and zeaxanthin (purity > 95 %) were purchased from Sigma Chemical (USA). Lutein (purity > 95 %) was purchased from the Indofine Chemical Company Inc. Hillsborough (USA).

2.4. Chromatography

High-performance liquid chromatography (HPLC) analysis was performed using an Agilent 1100 Series HPLC system equipped with a quaternary solvent pumping system (G1311A – DE14917573 Agilent 1100 Series, Waldbronn, Germany) and a UV/Vis detector (G1314B - DE71358944 Agilent 1100 Series, Waldbronn, Germany).

2.4. Physico-chemical quality analyses

The determination of the acid value of the oils was performed by titrating the sample using 1 N sodium hydroxide and phenolphthalein as an indicator. The index of refraction was determined by reading an Abbe refractometer according to the methodology of the Instituto

Adolfo Lutz (1985). The determination of the peroxide value was carried out by titrating the sample with 0.01 N sodium thiosulfate according to the AOCS method Cd 8-53 (1990). The content of unsaponifiable matter was determined by the weight difference after saponification of the sample with 0.5 N potassium hydroxide at 90 °C according to method Ca 6a-40 AOCS (1990).

2.5. Carotenoids

The carotenoid extract was prepared according to Mercadante & Rodriguez-Amaya (1998). The pigments were extracted with chilled acetone until discoloration occurred, and the extract was subjected to saponification overnight with 35 $g.L^{-1}$ KOH in a methanol solution at room temperature. Then, the extract was washed to remove the alkali and was concentrated in a rotary evaporator (Fisatom 802 com Banho Ultratermostático Quimis 0214M2). The concentrated extract was transferred to an amber flask, dried under a nitrogen stream and stored at −18 °C for further analysis using an HPLC instrument. A C30 reversed-phase polymeric column (250 mm × 4.6 mm; 3 μm i.d.; YMC, Japan) was used. The wavelength was adjusted to 450 nm. The mobile phase was water:methanol:tert-methyl butyl ether (MTBE) (J.T.Baker – Mallinckrodt, EUA) that started at a ratio of 5:90:5 and reached ratios of 0:95:5 in 12 min, 0:89:11 in 25 min, 0:75:25 in 40 min and finally 0:50:50 after a total of 60 min. The flow rate was 1 $mL.min^{-1}$, and an injection volume of 5 μL was used at 33 °C (Zanatta & Mercadante, 2007). The carotenoids were quantified using standard curves of lutein (1–65 $mg.L^{-1}$), zeaxanthin (1–40 $mg.L^{-1}$) and β-carotene (5–50 $mg.L^{-1}$). The results were expressed in micrograms per 100 g of sample. The limits of detection (LD) and quantification (LQ) were, respectively, 6.9×10^{-3} $mg.kg^{-1}$ and 1.15×10^{-2} $mg.kg^{-1}$ for lutein; 9.56×10^{-2} $mg.kg^{-1}$ and 1.59×10^{-2} $mg.kg^{-1}$ for zeaxantin; and 4.46×10^{-2} $mg.kg^{-1}$ and 7.43×10^{-2} $mg.kg^{-1}$ for β-carotene.

Qualification analysis was assured taking into consideration the following criteria: (1) the elution order in the reversed-phase HPLC of each carotenoid standard under the established conditions of the chromatographic analysis; (2) the determination of the retention time based on the average of three different measurements of all of the commercial standards and its comparison with the retention time of all of the coincident peaks in the sample and in its duplicate; and (3) comparison with the measurements on the commercial standards that were acquired.

2.6. α-Tocopherol

The extractions of tocopherol from the oils were performed with methanol at room temperature. The method is based on three extraction steps. In the first extraction, 10 g of sample was extracted through a portion of 25 mL of methanol for 10 minutes by ultrasonication (Unique USC 1400 ultrasonic cleaner). In the remaining steps, the residue from the previous step was extracted two more times with 25 mL portions of methanol by ultrasonication for 10 minutes. The extracts were combined, centrifuged at 1922 g for 15 min (Sigma 4K15 Centrifuge) and dried in a rotary evaporator at 40 °C (802 Fisatom with the Quimis 0214M2 ultrathermostatic bath). They were then dried under nitrogen and frozen for subsequent quantification by HPLC according to the method described by Freitas et al. (2008).

For the chromatographic separation, a polymeric Vydac C18 column (218TP54; 250 mm length; 4.6 mm diameter; and 5 mm particle size) was used, and a wavelength of 292 nm was used for detection. The analysis was performed in the isocratic mode for 10 min with a methanol/water solution (96:4, v/v) at flow rate of 1 $mL.min^{-1}$ as the mobile phase. The column temperature was maintained at 30 °C, and an injection volume of 10 μL was used. For quantification, an external standard method was used, and a calibration curve determined by

individual diluted solutions of α-tocopherol in methanol (from 2 to 303 $mg.L^{-1}$) was constructed. The limits of detection and quantification were 0.09 and 0.17 g $mg.100^{-1}$, respectively. The results were expressed in micrograms per 100 g of sample.

2.7. Catechin

The extraction of catechin was performed on samples of 20 g of oil in 30 mL of methanol by ultrasonication (Unique USC 1400 ultrasonic cleaner) for 30 minutes at room temperature. The extract was centrifuged (Sigma 4K15 centrifuge) at 1922 g for 15 min, the supernatant was reserved, and then another extraction was performed. The resulting extract was centrifuged again, evaporated in a rotary evaporator at 40 °C (802 Fisatom with a Quimis 0214M2 ultrathermostatic bath), dried under nitrogen and frozen until the time of analysis. The extract was dissolved in 2 mL of methanol for the chromatographic analysis. The HPLC analysis for the quantification of the catechins was proposed by Freitas (2007). The column used was a Vydac C18 polymer (218TP54), with a detection wavelength of 280 nm and a temperature of 30 °C. The injection volume that was used was 20 μL. The mobile phase consisted of methanol and water/acetic acid (97:3) starting at a 0:100 solution and increasing to 81.1:18.9 in 12 minutes at a flow rate of 1 $mL.min^{-1}$. The catechins were quantified using a catechin standard curve (14 to 81.7 $mg.L^{-1}$). The limit of detection was 1.40 $mg.100\ g^{-1}$, and the limit of quantification was 2.80 $mg.100\ g^{-1}$. The results were expressed in micrograms per 100 g of sample.

2.8. Oxidative stability index

The oxidative stabilities of the oil samples were determined with the Rancimat 743 (Metrohm AG, Switzerland). In brief, 3 g of the grape seed oil was weighed into the reaction vessel. The volatile products released during the oxidation process were collected in a flask containing

distilled water. The oxidation process was automatically recorded by measuring the change in the conductivity of the distilled water due to the formation of the volatile compounds and the oil stability index (OSI), which is expressed in hours (h) (Läubli & Bruttel, 1986) and was evaluated by the response surface methodology (RSM). A 2^2 rotational experimental design with 4 axial points and 3 central points was used. The variables studied were temperature (T in °C) and air flow (F in h^{-1}) (Table 1). At each time point, eight oil samples were analyzed simultaneously by the equipment. Each sample was analyzed in duplicate. Statistical analysis was performed using the Statistica 12.0 software (StatisoftInc.), which allowed the evaluation of the fit of the experimental data to mathematical models obtained by means of the F test and allowed response surfaces as functions of the variables studied to be obtained.

Table 1. Real and coded values of the experimental design (2^2) with axial points for the crude and refined seed oils of organic grapes (*Vitis labrusca*, variety Bordô).

Coded/real values	-1.41	-1	0	1	1.41
Temperature (°C)	80	92	120	148	160
Air flow ($L.h^{-1}$)	8	10	15	20	22

3. RESULTS AND DISCUSSION

3.1. Physicochemical quality analyses

The results of the chemical and physical analyses of the virgin and refined oil samples are shown in Table 2. Regarding the acidity index, there was no significant difference between the VO and RO, and the values are below the limits set by Codex Stan 210-1999 (Amendment 2013). The acidity is a key indicator of the oil quality parameters because it directly indicates the hydrolysis of the triacylglycerols catalyzed by light and heat, and lower acidity values

indicate oils of better quality. Values between 0.5 and 2.5 mg KOH.g^{-1} were previously reported by Choo et al. (2007) for different linseed oils grown by conventional methods. Farhoosh et al. (2009) reported a decrease in the acidity indices of rapeseed and soybean oil (from 3.22 to 0.14 mg KOH.g oil^{-1} for canola oil and from 2.09 to 0.09^{-1} mg KOH.g oil^{-1} for soybean oil) grown by conventional farming after the steps of degumming, neutralization and bleaching occurred.

The refractive index of an oil is a physical parameter that is directly proportional to the length of the hydrocarbon chain and the degree of unsaturation of the fatty acids of the triacylglycerols. Therefore, lower values have a higher acceptability, and the oils in this work are within the standard required by the Codex, which is between 1.466 and 1.477.

Table 2. Acidity index, refractive index, peroxide value and the unsaponifiable matter of VO and RO.

	Acidity index (mg KOH.g^{-1})	Refractive index (at 40 °C)	Peroxide value (meq O_2.kg^{-1})	Unsaponifiable matter (g.100 g^{-1})
VO	1.46 ± 0.16^{a}	$1.468 \pm 0,0^{a}$	0.23 ± 0.01^{b}	1.72 ± 0.02^{a}
RO	1.19 ± 0.15^{a}	$1.468 \pm 0,0^{a}$	0.77 ± 0.04^{a}	3.12 ± 0.7^{a}

All results are reported as the means of three replicates ± standard deviation (n = 3). Different letters in the same column represent significant difference ($P < 0.05$).

The peroxide indices of VO and RO were below the limit set by the Codex, but the RO peroxide value was higher than that of the VO, which suggests that the RO was more highly oxidized. A high amount of peroxides in a food can lead to nutritional losses, which can caused by refining processes during which temperatures above 65 ºC are used. However, a low peroxide index does not ensure a quality oil, it only indicates the current state of oxidation of an oil sample and indicates no potential for oxidation because there are other

important indices that can be evaluated such as the acidity and the refractive index (Frank et al., 2011).

Peroxide indices between 0.60 and 3.70 meq $O_2.kg^{-1}$ were reported by Adams et al. (2013) when analyzing crude palm oil. According to Jung et al. (1989), the increase in the peroxide value of refined oil depends on, among other factors, the material used in the bleaching stage. In this work, activated carbon was used when the refinement was carried out on a laboratory scale. Zacchi & Egger (2008) reported that the bleaching stage increases the peroxide value and that the effectiveness of the deodorization is affected by the intensity of the operating parameters, such as time and temperature.

The unsaponifiable matter in an oil is represented by the dissolved substances that do not saponify in the presence of sodium/potassium hydroxide and are, in other words, interfering substances. The unsaponifiable matter content did not differ significantly between the oils and remained within the values permitted by the Codex.

3.2. Carotenoids, α-tocopherol and catechin

The lutein, zeaxanthin, β-carotene, α-tocopherol and catechin contents are shown in Tables 3 and 4. The VO had higher lutein and catechin contents (24.17 and 1926.15 μg.100 g^{-1}, respectively). No measurable amounts of zeaxanthin and β-carotene were observed in the RO, most likely due to the refining process that was carried out at high temperatures (LD values of 9.56×10^{-2} for zeaxanthin and 4.46×10^{-2} $mg.kg^{-1}$ for β-carotene).

The intake of approximately 6 mg of lutein per day has been associated with a decreased risk of diseases such as cataracts and age-related macular degeneration (Seddon et al., 1994). The lutein content in the organic virgin grape seed oil is comparable to that of carrots, a good source of lutein. The ratio between lutein and zeaxanthin (1.41) in the VO is equivalent to that found in yellow fruits and oranges (Abdel-Aal et al., 2013).

Numerous studies report a relationship between the consumption of carotenoids in the diet and a reduced risk of diseases, particularly cancers (Johnson, 2002). Among the various defense strategies, carotenoids are involved in the scavenging of two reactive oxygen species, singlet molecular oxygen ($^1O_2$) and peroxyl radicals (Stahl & Sies, 2005). Along with vitamins C and E, carotenoids are important antioxidants due to their ability to affect cell differentiation or proliferation (Cramer et al., 1994).

Due to their high degrees of unsaturation, carotenoids are more susceptible to degradation during food processing, storage and heat treatment (Zeb & Murkovic, 2011), and isomerization is one of the first structural changes that occurs during heating (Achir et al., 2010).

The behavior of carotenoids in oils exposed to heat depends on the type of oil due to differences in the phenolic compounds, fatty acids and tocopherols (Zeb & Murkovic, 2011). In corn oil, decomposition products such as 5,8-epoxy-β-carotene showed greater amounts in the first hour of exposure to 110 °C and tended to disappear within 12 hours of heat exposure (Zeb & Murkovic, 2013). In olive oil, the same compound was present in small amounts during the first hour of heating, with its concentration increasing within the next 8 hours (Zeb & Murkovic, 2011).

Studying the losses from the refining process, Szydłowska-Czerniak et al. (2011) reported a total carotenoid content between 32.7 and 45.8 mg.100 g^{-1} in crude palm oils. After these oils were refined, there was a loss of nearly 99 % of the total amount of carotenoids, of which 56 % of the loss occurred after the bleaching stage in which a blend of clay and silica was used. The authors emphasize that the loss of carotenoids is dependent upon the material used for bleaching (at 95 °C).

Table 3. Retention times and carotenoid contents of VO and RO in μg.100 g^{-1} of oil.

Carotenoid	t_R (min)	VO	RO
Lutein	19.9 – 20.1	24.17 ± 2.55[a]	16.64 ± 0.32[b]
Zeaxanthin	24.1	17.07 ± 0.02	Nd
β-Carotene	45.8	53.85 ± 1.17	Nd
Total		95.09	16.64

All results are reported as the mean of three replicates ± standard deviation (n = 3). nd means not detected. Different letters in the same line represent significant difference (P < 0.05).

The VO and RO did not significantly differ in their amounts of α-tocopherol (2.65 and 2.74 μg.100 g^{-1}, respectively). Lutterodt et al. (2011) reported the existence of α-tocopherol in only one of the four cultivars of grape seeds analyzed (from nd to 727 μg.g^{-1} in Concord grapes) by HPLC-MS with hexane used for the extraction of the compounds.

The content of the tocopherols in oils is a function of the climatic conditions, cultivation, processing and storage. Treatment with alkali in the refining of the oil affects the tocopherol content. During bleaching, the tocopherol content can be reduced due to adsorption to the bleach material (Tasan & Demirci, 2005). The tocopherol loss during the refining process may also be due to high temperature oxidation or the modification of tocopherol esters (Verleyen et al. 2001).

The beneficial effects of edible oils must not only include having a large ratio of unsaturated to saturated fatty acids but also include containing antioxidants such as vitamin E, carotenoids and phenolic compounds. The most active form of vitamin E *in vivo* is α-tocopherol, whereas β-carotene is the most important provitamin A source, and both are involved in the oxidative stability of the oil and offer protection against cardiovascular diseases and some cancers (Gimeno et al. 2002).

Table 4. VO and RO α-tocopherol and catechin contents and retention times in μg.100 g^{-1} of oil.

Compound	t_R (min)	VO	RO
α-Tocopherol	6.7 – 6.7	2.64 ± 0.47[a]	2.74 ± 0.02[a]
Catechin	13.9 – 16.0	1926.15 ± 68.79[a]	645.41 ± 60.38[b]

All results are reported as the mean of three replicates ± standard deviation (n = 3). nd means not detected. Different letters in the same line represent significant difference ($P < 0.05$).

The VO showed a higher catechin content than the RO. No previous studies of the quantification of catechin in grape seed oil were found, but a linear relationship between the flavonoids and the oxidative stability of extra virgin olive oil has been reported (Suliman et al., 2013).

Numerous studies have shown that these phenolics are potent inhibitors of LDL oxidation in vitro, which is linked to the formation of atherosclerotic plaques responsible for the development of cardiovascular diseases. The phenolics in olive oil have been beneficially related to the process that contributes to the pathogenesis of heart disease and cancers (Hayball & Tuck, 2002).

3.3. Oxidative stability index (OSI)

The results of the experimental design for studying the OSI are shown in Table 5 and Figure 1.

The experimental data were fitted to a full quadratic model. Polynomials for the response surfaces of the VO and the RO (Eqs. 1 and 2, respectively) were calculated by multiple regression analysis.

Equation 1.

$$OSI = 2.4476 - 12.93 \times T + 10.0234 \times T^2 - 0.0882 \times F - 1.1279 \times F^2 + 0.1887 \times T \times F$$

Equation 2.

$$OSI = 2.4750 - 4.8327 \times T + 2.7618 \times T^2 - 0.1463 \times F - 0.3139 \times F^2 - 0.0325 \times T \times F$$

The models were tested by analysis of variance (ANOVA) at a confidence level of 95 % ($p < 0.05$), and calculated F values of 34.33 and 58.11 for VO and RO, respectively, were obtained. The OSI for both oils is highly dependent on the linear and quadratic effects of temperature. The quadratic models adequately predict the influence of the experimental factors studied, with R^2 values of 0.9147 for the VO and 0.9484 for the RO.

The Rancimat method is a rapid technique that analyzes the oxidative stability of a sample, especially when the levels of the operating parameters are carefully chosen. This choice depends on the type of sample and can save time and costs in the food industry as well as in research laboratories (Farhoosh, 2007a).

The OSI decreased with increasing temperature, whereas the air flow had no effect on the stability index. The VO showed the highest OSI when the temperature was 80 °C and the flow rate was 15 $L.h^{-1}$, followed by the conditions of a temperature of 92 °C and air flows of 10 and 20 $L.h^{-1}$, which did not have significantly different results. At 120 °C, the OSI of the VO remained the same with different air flows. The RO showed the same behavior as the VO but with lower indices for temperatures of 80 and 92 °C. At temperatures from 120 to 160 °C, there were no significant differences in the OSI values of the RO at various air flows. Thus, it was found that the VO and the RO maintain the same trend of a decreasing OSI for temperatures up to 92 °C.

In the VO at a flow rate of 15 $L.h^{-1}$, the OSI decreased from 48 to 2.40 hours at temperatures of 80 to 120 °C, respectively. The same effect happened in the RO where the OSI decreased from 16.9 to 2.94 hours. This behavior can be attributed to the production of compounds from decomposition produced by the acceleration of oxidation. Furthermore, it is suggested that the flow does not have any influence due to the stability of the saturated air conditions and an insufficient sample size for oxidization to occur. However, the VO, at a temperature of 80 °C, obtained an induction time equal to 48.89 h, and the RO obtained an induction time of 16.9 for the same conditions, which suggests a change in the antioxidant content. The same trend was observed for temperatures equal to 92 °C with flows of 10 and 20 $L.h^{-1}$.

Table 5. Oxidative stability index (OSI) values of VO and RO under various experimental design conditions.

Experiment	T (°C)	Air flow ($L.h^{-1}$)	OSI (h)	
			VO	RO
1	92	10	18.18 ± 0.18^{bA}	8.0 ± 0.21^{bB}
2	148	10	0.5 ± 0.01^{dB}	0.53 ± 0.18^{eA}
3	92	20	17.4 ± 0.03^{bA}	8.13 ± 0.06^{bB}
4	148	20	0.48 ± 0^{dA}	0.53 ± 0.03^{eA}
5	120	15	2.36 ± 0.03^{cA}	2.70 ± 0.13^{cdA}
6	120	15	2.52 ± 0.01^{cA}	2.28 ± 0.02^{cdA}
7	120	15	2.40 ± 0.09^{cA}	2.43 ± 0.01^{cdA}
8	80	15	48.89 ± 0.69^{aA}	16.92 ± 0.56^{aB}
9	120	8	2.38 ± 0.04^{cA}	2.94 ± 0.01^{cA}
10	160	15	0.28 ± 0.01^{dA}	0.27 ± 0.01^{eA}
11	120	22	2.45 ± 0.10^{cA}	2.02 ± 0.41^{dA}

Different lowercase letters in the same column and different uppercase letters in the same line represent significant differences ($p < 0.05$).

The optimal parameters determined by the experimental design are a temperature of 80 °C and an air flow equal to 15 $L.h^{-1}$, and the VO sample showed the best results, possibly due to the fact that it did not undergo any treatment after extraction.

Investigating the oxidative stability of nine different brands of olive oil obtained directly from the local market, Farhoosh & Hoseini-Yazdi (2013) reported that the most stable sample had induction times of 55.1 h at 100 °C, 6.9 h at 110 °C, 24.2 h 120 °C and 5 h at 130 °C. The authors used 3 g of sample at an air flow rate of 25 $L.h^{-1}$. The authors suggest that

this sample obtained better results due to the high content of tocopherols and low acidity levels that were found by the analyses.

De Leonardis & Macciola (2012), studying extra virgin olive oils and blends of these oils with palm oil, reported that the addition of palm oil in percentages of 20, 40 and 60 % to the extra virgin olive oil increased the oxidative stability from 5.40 to 5.65 h, 6.03 h and 6.70 h, respectively. Rancimat was operated at 130 °C with 2.5 g samples at a stream rate of 20 $L.h^{-1}$.

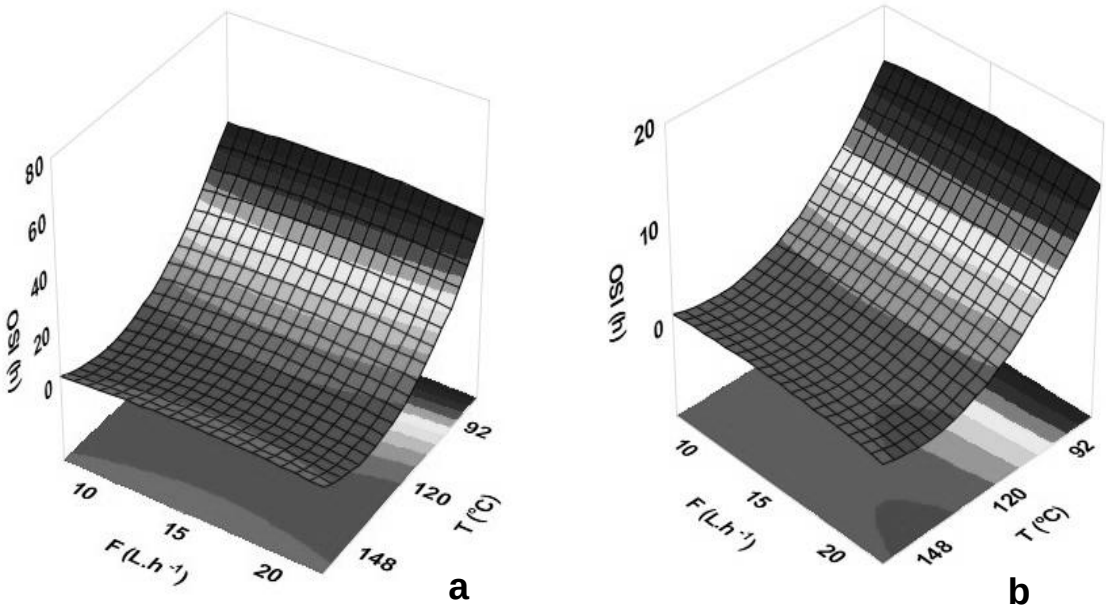

Figure 1. Response surfaces for the oxidative stability index (OSI) values of VO (a) and RO (b).

Studying the relationship of the parameters of Rancimat with the variation of the temperature of virgin olive oil, Mateos et al. (2006) suggested that for every 10 °C temperature increase, there is a 25 % decrease in the oxygen solubility in the oil, and they reported that air flows between 9 and 25 $L.h^{-1}$ do not influence the OSI.

Investigating the effect of the mass (g) of fish liver oil, air flow ($L.h^{-1}$) and temperature (°C) on the OSI, Garcia-Moreno et al. (2013) concluded that both the flow and the temperature influence the OSI and that the ideal parameters would be a mass of 6.91 g, a flow rate of 25 $L.h^{-1}$ and a temperature of 88.26 °C.

Studying the effects of the operating parameters of Rancimat in determining the OSI and the shelf-life of soybean oil, Farhoosh et al. (2007b) observed that for samples of 6 g of oil, the flow had no effect on the results and concluded that the OSI also decreases with increasing temperature. The best conditions presented in this study were 100 °C at 10 or 20 $L.h^{-1}$ with 6 g of oil, or 10 $L.h^{-1}$ with 3 g of oil.

4. CONCLUSIONS

The RO presented a higher peroxide value than the VO, and consequently, its oxidative stability was approximately three times smaller due to the temperature used in the refining process, which corroborates the OSI values obtained by Rancimat. The VO showed higher lutein and catechin contents and also showed the presence of β-carotene and zeaxanthin, carotenoids that were not found in the RO. The α-tocopherol contents of the oil samples did not differ significantly, most likely due to the high antioxidant protection conferred by this compound. According to the results, the use of virgin oil is suggested because it is a better source of bioactive compounds and has a greater stability against heating.

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CAPÍTULO 5:

DISCUSSÃO GERAL

Este trabalho foi desenvolvido com o objetivo de estudar o potencial funcional de resíduos oriundos da agroindústria de suco de uva, uma vez que representam danos ao meio ambiente, altos custos para a indústria, além de possibilidade de uso como ingredientes de alimentos.

No capítulo 2 foi investigado o teor de carotenoides e atividade antioxidante das sementes e dos resíduos da obtenção do óleo de semente de uvas orgânicas e convencionais. Foram determinados parâmetros de cor, carotenoides, vitamina A e atividade antioxidante por DPPH e TRAP. Os extratos utilizados nas metodologias de DPPH e TRAP foram analisados por cromatografia líquida de alta eficiência a fim de identificar e quantificar os carotenoides presentes, além de analisar a influência das etapas extrativas da análise destes compostos. As amostras foram divididas em semente convencional e orgânica (CS e OS, respectivamente) e resíduo convencional e orgânico (CR e OR, respectivamente). Apesar de os resíduos orgânicos e convencionais destacarem-se pelos teores significativos de carotenoides e pró-vitamina A, observou-se que apenas CR apresentou melhores resultados nos parâmetros de cor, teor total de carotenoides, TRAP, TAR e conteúdo de carotenoides presente no extrato antioxidante.

No capítulo 3 foi investigada a influência do modo de cultivo (orgânico ou convencional) na presença e quantidade de compostos bioativos e estabilidade de óleos de sementes de uvas Bordô e Isabel. Foram determinados os parâmetros de cor e de qualidade dos óleos, conteúdo de carotenoides, α e γ-tocoferol, compostos fenólicos, atividade antioxidante por TRAP e estabilidade oxidativa. As amostras foram divididas em BCO e

BOO (óleo convencional Bordô e óleo orgânico Bordô, respectivamente), e ICO e IOO (óleo convencional Isabel e óleo orgânico Isabel, respectivamente). Todos os óleos apresentaram ótimas características de qualidade, permanecendo de acordo com os limites estabelecidos pelo Codex Stan, além disso os óleos Bordô destacaram-se pelo teor significativo de carotenoides e α-tocoferol. ICO apresentou o melhor resultado para análise de compostos fenólicos, mas os óleos não diferiram significativamente quando à capacidade antioxidante analisada. Os óleos Bordô apresentaram melhor estabilidade oxidativa em relação aos Isabel, sugerindo que este resultado pode ser devido ao teor significativo de compostos bioativos determinados anteriormente.

Com estes resultados, o óleo de semente de uva orgânica Bordô foi refinado a fim de comparar suas propriedades de qualidade, estabilidade e compostos bioativos ao óleo virgem (Capítulo 4). Foram analisados o teor de carotenoides, α-tocoferol, catequina e estabilidade oxidativa. O óleo virgem (VO) destacou-se pelos conteúdos mais significativos de luteína, catequina, zeaxantina e β-caroteno, além de apresentar estabilidade cerca de três vezes maior do que a do óleo refinado (RO), no qual foi detectado maior índice de peróxidos.

De acordo com os resultados obtidos, sugere-se que os resíduos do processamento de suco de uva são fonte alternativa de compostos bioativos, uma vez que o resíduo da obtenção do óleo obteve resultados expressivos comparados à semente integral. Quanto aos diferentes tipos de óleo, não foi possível observar diferença entre os modos de cultivo, somente entre os cultivares, destacando-se o Bordô. Já quanto ao refino do óleo, recomenda-se a preferência pelo consumo do óleo virgem em relação ao refinado devido aos teores significativos de compostos bioativos e estabilidade analisados.

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