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ALTERAÇÕES NOS TEORES DE COMPOSTOS BIOATIVOS, NA CAPACIDADE ANTIOXIDANTE E NA ATIVIDADE FENILALANINA AMÔNIA LIASE EM UVAS 'ISABEL' TRATADAS COM RADIAÇÃO UV-C

DISSERTAÇÃO DE MESTRADO

Luana Haselein Maurer

Santa Maria, RS, Brasil

2014

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Luana Haselein Maurer

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

Orientadora: Profª Drª Tatiana Emanuelli
Co-orientadora: Profª Drª Cláudia K. Sautter

Santa Maria, RS, Brasil

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Universidade Federal de Santa Maria
Centro de Ciências Rurais
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A Comissão Examinadora, abaixo assinada, aprova a Dissertação de Mestrado

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elaborada por

Luana Haselein Maurer

como requisito parcial para obtenção do grau de mestre em Ciência e Tecnologia dos Alimentos.

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DEDICATÓRIA

À minha mãe Maristella e ao meu noivo Michel,
por todo apoio nos momentos difíceis,
incentivo aos estudos e confiança na minha capacidade.
Eu não teria conseguido sem vocês!

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EPÍGRAFE

"Nunca deixe que lhe digam
que não vale a pena acreditar no sonho que se tem
Ou que seus planos nunca vão dar certo
Ou que você nunca vai ser alguém
Tem gente que machuca os outros
Tem gente que não sabe amar
Mas eu sei que um dia a gente aprende
Se você quiser alguém em quem confiar
Confie em si mesmo
Quem acredita sempre alcança"

Renato Russo

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos
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ALTERAÇÕES NOS TEORES DE COMPOSTOS BIOATIVOS, NA CAPACIDADE ANTIOXIDANTE E NA ATIVIDADE FENILALANINA AMÔNIA LIASE EM UVAS 'ISABEL' TRATADAS COM RADIAÇÃO UV-C

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Data e Local da Defesa: Santa Maria, 26 de fevereiro de 2014.

A radiação ultravioleta do tipo C (UV-C) é uma tecnologia não-térmica que está sendo usada como um estressor abiótico para retardar o crescimento microbiano e aumentar o teor de fitoquímicos em culturas de alimentos. No entanto, seus efeitos podem variar dependendo da fruta, da cultivar, da dose hormética e do tempo após a exposição. A 'Isabel' é uma cultivar híbrida de uva (*Vitis labrusca L.*) que representa cerca de 50% da produção brasileira de uvas e é amplamente usada para consumo *in natura*. Foram avaliados os efeitos da irradiação com UV-C (0.5, 1.0, 2.0 e 4.0 kJ m^{-2}) seguida pelo armazenamento a 20°C (1, 3 e 5 dias) sobre indicadores de qualidade, compostos bioativos, capacidade antioxidante e atividade da fenilalanina amônia liase (FAL) de uvas 'Isabel'. As maiores doses (2.0 e 4.0 kJ m^{-2}) levaram a um rápido aumento dos sólidos solúveis totais (SST) (cerca de 10%) e a um decréscimo dos valores de pH (p<0,05). Todas as amostras apresentaram um aumento no teor de fenólicos totais (813,6 ± 99,6 no 1° dia *vs.* 1016,4 ± 79,6 mg ácido gálico 100 g^{-1} casca em peso fresco no 5° dia), flavonois (351,1 ± 58,4 no 1° dia *vs.* 496,9 ± 83,1 mg quercetina-3-rutinosídeo 100 g^{-1} casca em peso fresco no 5° dia) e antocianinas (215,3 ± 35,4 no 1° dia *vs.* 272,2 ± 43,5 mg malvidina-3-glicosídeo 100 g^{-1} casca em peso fresco no 5° dia) ao longo do armazenamento (p<0,05). Os compostos fenólicos totais aumentaram após a irradiação com UV-C nas doses de 1.0 e 2.0 kJ m^{-2}, enquanto o teor de antocianinas aumentou após irradiação com as doses 0,5, 1.0 e 2.0 kJ m^{-2} (p<0,05). Os teores de ácido ascórbico (AA) e vitamina C total diminuíram em todas as amostras durante o armazenamento (p<0,05). O tratamento com UV-C não teve efeito sobre o teor de flavonois, mas diminuiu o teor de AA e vitamina C total (0,5-2.0 kJ m^{-2}, p<0,05). A atividade da FAL diminuiu ao longo do armazenamento em todas as amostras (47,7 ± 3,6 no 1° dia *vs.* 38,4 ± 2,7 e 32,4 ± 2,1 10^4 EAU min^{-1} mg $proteína^{-1}$ no 3° e 5° dias após o tratamento, p<0,05). A radiação UV-C (0,5-2.0 kJ m^{-2}) aumentou a atividade da enzima FAL em 2 vezes em relação ao controle. As uvas tratadas com UV-C tiveram maior capacidade antioxidante nos ensaios do poder antioxidante de redução do ferro (FRAP) e de remoção do radical 2,2-difenil-1-picril-hidrazil (DPPH) que as uvas controle (p<0,05). Além disso, este efeito antioxidante foi positivamente correlacionado ao teor de compostos fenólicos totais e antocianinas e negativamente correlacionado ao teor de ácido ascórbico e vitamina C total (p<0,05). Assim, a radiação UV-C pode ser usada para aumentar o teor de compostos bioativos e a capacidade antioxidante de uvas 'Isabel', sendo 1.0 kJ m^{-2} a dose mais apropriada nas condições estudadas. Os efeitos da UV-C apareceram imediatamente após a irradiação e diminuíram gradualmente ao longo do armazenamento.

Palavras-chave

UV-C, uva, pós-colheita, estresse abiótico, antocianinas, compostos bioativos.

ABSTRACT

Master Dissertation
Graduate Program in Food Science and Technology
Federal University of Santa Maria

CHANGES IN LEVELS OF BIOACTIVE COMPOUNDS, ANTIOXIDANT CAPACITY AND FENILALANINE AMMONIA LYASE ACTIVITY IN 'ISABELLA' GRAPES TREATED WITH UV-C RADIATION

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Date and Defense place: Santa Maria, February 26, 2014.

Ultraviolet light type C (UV-C) is a non-thermal technology that has been used as an abiotic stressor to delay microbial growth and enhance the phytochemical content of food crops. However, its effects may vary depending on the cultivar, fruit, hormetic dose and time after exposure. 'Isabella' is a hybrid grape cultivar (*Vitis labrusca L.*) that represents about 50% of Brazilian grape production and is commonly consumed *in natura*. The effects of UV-C irradiation (0.5, 1.0, 2.0, and 4.0 kJ m^{-2}) followed by storage at 20°C (1, 3, and 5 days) were evaluated on quality indicators, bioactive compounds, antioxidant capacity and phenylalanine ammonia lyase (PAL) activity of 'Isabella' grapes. The highest UV-C doses (2.0 and 4.0 kJ m^{-2}) led to a rapid increase (about 10%) of total soluble solids (TSS) and decrease of pH values ($p<0.05$). All samples showed an increase on total phenolics (813.6 ± 99.6 in the 1st day *vs*. 1016.4 ± 79.6 mg gallic acid equivalents 100 g^{-1} skin FW in the 5th day), flavonols (351.1 ± 58.4 in the 1st day *vs*. 496.9 ± 83.1 mg quercetin-3-rutinoside equivalents 100 g^{-1} skin FW in the 5th day), and anthocyanins (215.3 ± 35.4 in the 1st day *vs*. 272.2 ± 43.5 mg malvidin-3-glucoside equivalents 100 g^{-1} skin FW in the 5th day) content along the storage ($p<0.05$). Total phenolic compounds increased after UV-C at 1.0 and 2.0 kJ m^{-2}, whereas anthocyanin content increased after UV-C at 0.5, 1.0, and 2.0 kJ m^{-2} ($p<0.05$). The levels of ascorbic acid (AA) and total vitamin C decreased in all samples during the storage ($p<0.05$). UV-C treatment had no effect on the flavonol content, but decreased AA and total vitamin C content (0.5 - 2.0 kJ m^{-2}, $p<0.05$). PAL activity decreased along storage time in all samples (47.7 ± 3.6 in the 1st day *vs*. 38.4 ± 2.7 and 32.4 ± 2.1 10^4 EAU min^{-1} mg $protein^{-1}$ in 3rd and 5th days after treatment, $p<0.05$). UV-C radiation (0.5-2.0 kJ m^{-2}) increased PAL enzyme activity in two-fold relation to control. UV-C treated grapes had higher antioxidant capacity than control grapes in the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays ($p<0.05$). Indeed, this effect was positively correlated to the content of total phenolic and anthocyanin compounds and negatively correlated to ascorbic acid and vitamin C contents ($p<0.05$). Thus, UV-C radiation can be used to increase bioactive compounds and antioxidant capacity of 'Isabella' grapes, being 1.0 kJ m^{-2} the most appropriate dose for the studied conditions. UV-C effects appeared immediately after the irradiation and gradually decreased along the storage.

Keywords

UV-C, grape, postharvest, abiotic stress, anthocyanins, bioactive compounds.

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LISTA DE ABREVIATURAS

UV = ultravioleta

UV-A = ultravioleta tipo A

UV-B = ultravioleta tipo B

UV-C = ultravioleta tipo C

nm = nanômetro

W = watt

m^{-2} = metro quadrado

k = quilo

J = Joule

min = minuto

° Brix = graus Brix

FRAP = ferric reducing antioxidant power

DPPH = 2,2-difenil-1-picril-hidrazil

DNA = ácido desoxirribonucleico

EROs = espécies reativas de oxigênio

GSH = glutationa

GSSG = glutationa oxidada

FAL / PAL = fenilalanina amônia liase / phenylalanine ammonia lyase

CHS = chalcona sintase

Co-A = coenzima A

EAU = unidade de atividade enzimática

FW = peso fresco

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APRESENTAÇÃO

A presente Dissertação de Mestrado encontra-se organizada em quatro partes:

A primeira parte representa a **Introdução**, a qual visa situar o leitor a respeito do tema que será tratado nesta Dissertação, referenciando o que existe na literatura sobre o assunto e servindo de suporte para a construção das hipóteses investigadas.

A seguir, os **Objetivos** (Geral e Específicos) trazem o principal questionamento do trabalho e as questões específicas que nortearam a realização desta Dissertação.

A terceira parte contém os **Resultados** que fazem parte deste trabalho, apresentados sob a forma de um **artigo científico**, a ser submetido para publicação. As seções Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas citadas no artigo encontram-se no final do próprio artigo.

A quarta parte abrange a **Conclusão**, que contém um resumo dos principais achados desta Dissertação.

A seção **Referências Bibliográficas** apresenta a bibliografia citada na seção de Introdução desta Dissertação.

1 INTRODUÇÃO

Os consumidores estão cada vez mais preocupados em buscar alimentos que não sejam apenas seguros e forneçam os nutrientes básicos, mas que tragam algum benefício à sua saúde (CRUPI et al., 2013). Nesse sentido, as frutas, em especial as uvas e os produtos à base de uvas, se destacam como fontes importantes de compostos fenólicos e antocianinas, os quais são potentes antioxidantes que têm sido associados à redução do risco de diversas doenças (XIA et al., 2010).

A Isabel é uma cultivar de uva não-vinífera comumente encontrada ao alcance do público consumidor e possui uma capacidade antioxidante inferior às uvas viníferas usadas na fabricação de vinhos finos (ROCKENBACH et al., 2011) e não disponíveis para o consumidor na forma *in natura.*

A irradiação com luz UV-C vem firmando-se como uma das tecnologias de maior aplicação no futuro por ser uma opção que não deixa resíduo e que pode ser usada em alimentos com várias finalidades, inclusive aliada a outras tecnologias como baixas temperaturas e atmosfera modificada (KEYSER et al., 2008; MARTÍNEZ-HERNÁNDEZ et al., 2013). Esta tecnologia tem sido frequentemente empregada para retardar a deterioração microbiana e controlar o aparecimento de doenças pós-colheita em frutas e hortaliças. (ERKAN; WANG; WANG, 2008; TIECHER et al., 2010). Por outro lado, a aplicação pós-colheita deste estressor abiótico pode induzir a produção e incrementar a síntese de metabólitos secundários com propriedades nutracêuticas em diversas frutas, como os flavonoides e as antocianinas (CISNEROS-ZEVALLOS, 2003).

Por esta razão, diversos estudos têm examinado a importância da radiação UV-C com relação a indicadores de qualidade de frutas, avaliando os possíveis efeitos sobre suas propriedades funcionais (BRAVO et al., 2012; GONZÁLEZ-AGUILAR et al., 2001). No entanto, os efeitos da UV-C sobre a síntese de compostos antioxidantes e enzimas pode variar dependendo da dose hormética, do tempo após exposição e do tipo de fruta e da cultivar a ser tratada (ERKAN; WANG; WANG, 2008; GONZÁLEZ-AGUILAR et al., 2010).

1.1 Uva

O Rio Grande do Sul, maior produtor de uvas e vinhos no Brasil, é responsável por cerca de 55% da produção de uvas do país e, na safra de 2012, registrou um aumento de quase 82% na produção de uvas em relação ao ano de 2003. As cultivares *Vitis labrusca* (também chamadas de americanas ou comuns), como Bordô, Niágara rosada e Isabel, foram as principais responsáveis pelo aumento, uma vez que essas uvas representaram quase 90% da produção total de uvas em 2012. Produtos derivados dessas uvas comuns, tais como os vinhos de mesa aumentaram 32% em 2011, enquanto o suco de uva apresentou um aumento de 31% (MELLO, 2012; UVIBRA, 2013). O suco de uva pode ser elaborado tanto com uvas viníferas quanto não-viníferas, no entanto o suco produzido no Brasil é elaborado principalmente com uvas não-viníferas, sendo que Isabel (Figura 1), Bordô e Concord são as cultivares mais utilizadas (TERRA et al., 2001).

Figura 1. Uva 'Isabel' (Fonte: Arquivo pessoal).

Essas cultivares híbridas geralmente são mais rústicas e mais produtivas que as *Vitis vinifera*, usadas para a elaboração de vinhos finos. Dentre as *Vitis labrusca*, a cultivar Isabel figura como a responsável pelo maior volume de suco produzido no Brasil, devido principalmente à sua fácil adaptação às condições edafoclimáticas e à sua elevada produtividade, que culminam em uma grande disponibilidade de matéria-prima (RIZZON; MIELE, 2006; ROMBALDI et al., 2004). Conforme Rizzon e Miele (2006), as cultivares híbridas ou americanas representam cerca de 85% das videiras na Serra Gaúcha, com

predominância da cultivar Isabel (*Vitis labrusca*), que representa aproximadamente 45% da área plantada.

Um grande número de estudos epidemiológicos têm associado o consumo de uvas e seus derivados à redução do risco de doenças cardiovasculares, à uma menor incidência de doenças degenerativas e, inclusive, de certos tipos de câncer (HOGAN et al., 2009; XIA et al., 2010). Estes benefícios à saúde trazidos pelas uvas são atribuídos principalmente às diversas atividades biológicas dos seus componentes funcionais, que incluem atividade antioxidante, hipolipidêmica, antiinflamatória, antienvelhecimento e antimicrobiana (HOGAN et al., 2011; SHANMUGANAYAGAM et al., 2012; TENORE et al., 2012).

A uva e seus derivados são caracterizados por altas concentrações e por uma grande variedade de compostos fenólicos, principalmente antocianinas e resveratrol. Estes compostos são extraídos tanto no processo de vinificação, quanto durante a fabricação de sucos, já que ambos processos envolvem a participação das cascas. Uma vez que as uvas tintas são ricas em antocianinas, isso faz com que a uva seja considerada uma das mais importantes fontes de antocianinas da nossa dieta (DANI et al., 2007; FRANKEL et al., 1998). Além disso, existe um grande interesse da indústria farmacêutica e alimentícia em extratos de frutas e plantas ricas em compostos fenólicos, já que, além dos benefícios que trazem à saúde humana, esses compostos podem ser úteis no retardo da oxidação lipídica, no aumento do teor de compostos bioativos nos alimentos e no fornecimento de matéria-prima para a produção de suplementos dietéticos com propriedades antioxidantes (MOURE et al., 2001).

1.1.1 Compostos fenólicos

As plantas, como organismos imóveis que são, não possuem mobilidade para escapar do ataque de patógenos, predadores ou mesmo de doenças e, então, desenvolveram mecanismos químicos para combatê-los. Assim, passaram a sintetizar uma grande variedade de compostos relacionados à sua defesa, os metabólitos secundários, que são classificados em diferentes grupos de acordo com suas rotas de síntese e características estruturais. Dentre os metabólitos secundários, os compostos fenólicos são os mais amplamente distribuídos no reino vegetal e apresentam estruturas que variam desde moléculas simples como os ácidos fenólicos até compostos altamente polimerizados como as proantocianidinas (LATTANZIO et al., 2008).

Os compostos fenólicos da uva, mais concentrados nas cascas e sementes, são classificados em dois grupos: os flavonoides, tais como as antocianinas, flavonois, flavanois e proantocianidinas; e os não-flavonoides, como os derivados do ácido hidroxicinâmico, derivados do ácido hidroxibenzoico e estilbenos (Figura 2) (IACOPINI et al., 2008).

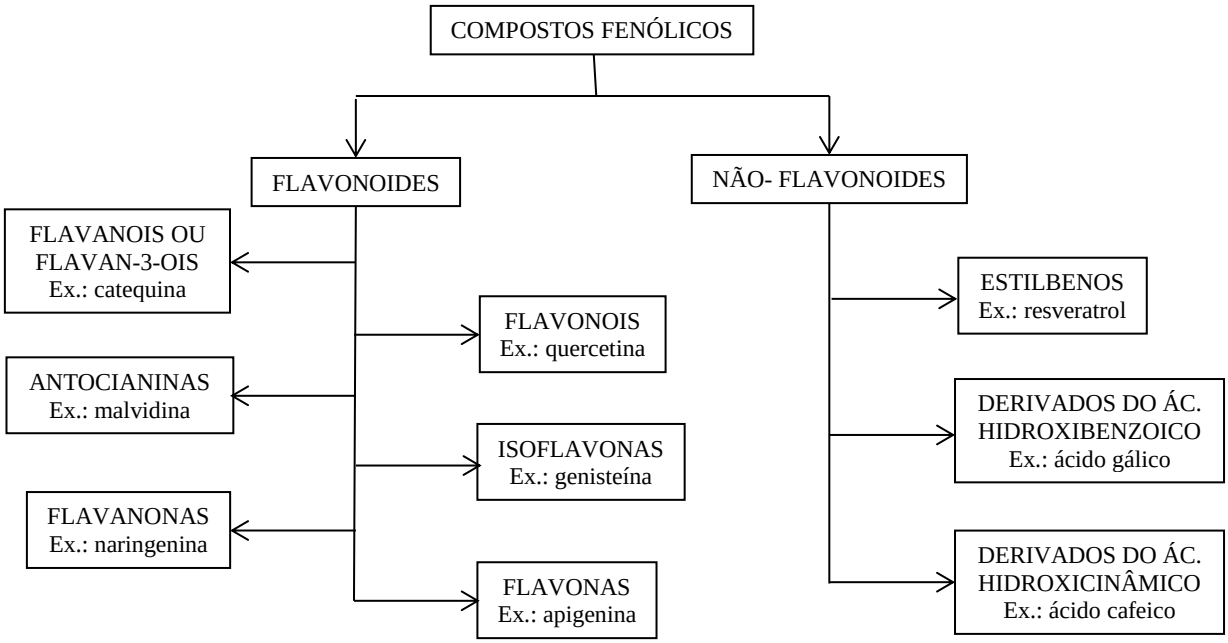

Figura 2. Esquema de classificação dos compostos fenólicos e exemplos das principais classes.

Alguns estudos têm mostrado que o metabolismo fenólico não seria apenas um mecanismo protetor contra formas de estresse bióticas ou abióticas, mas também seria parte de uma programação molecular que contribui para o desenvolvimento normal da planta. Assim, além de seu papel nas relações animal-planta, microrganismo-planta e planta-planta, os fenólicos também possuem papel fundamental na pigmentação, na atividade antioxidante e quelante de metais e na filtração da luz ultravioleta (LATTANZIO et al., 2008).

Na uva, as antocianinas estão localizadas principalmente na casca e são responsáveis pela coloração vermelha, azul e roxa que se acumula durante o amadurecimento das uvas tintas. Os flavonois são pigmentos amarelados sintetizados na casca das uvas durante dois períodos distintos: da floração até o estádio prematuro de desenvolvimento da baga e durante o amadurecimento. Já os flavan-3-ois, como catequina e epicatequina, estão presentes tanto nas cascas quanto nas sementes e são encontrados em maior extensão nas uvas brancas. As

proantocianidinas (ou taninos condensados) são polímeros de unidades de flavan-3-ois e representam uma das mais abundantes classes de fenólicos em uvas, sendo responsáveis pelas propriedades adstringentes e de amargor do vinho. Sua biossíntese ocorre primariamente no desenvolvimento da baga e é completada próximo ao *veraison*, que é o estádio de desenvolvimento da baga onde inicia-se a síntese de antocianinas (IACOPINI et al., 2008; LATTANZIO et al., 2008; YANG; MARTINSON; LIU, 2009).

Além do seu importante papel na defesa das plantas, os compostos fenólicos são relevantes também pelas qualidades organolépticas que conferem às frutas frescas, aos sucos e ao vinho, e ainda pelos benefícios que trazem à saúde humana devido seu potencial antioxidante e antimicrobiano (KOYAMA et al., 2012; ZHANG et al., 2012). Devido a isso, alguns estudos estão centrando-se em formas de aumentar os fenólicos em uvas (CRUPI et al., 2013; ZHANG et al., 2012).

1.2 Tratamentos pós-colheita

O principal objetivo dos tratamentos pós-colheita é a preservação da qualidade e do frescor de produtos vegetais bem como o combate ou retardo do desenvolvimento de microrganismos e doenças. No entanto, tem sido observada uma melhora no sistema antioxidante como uma resposta secundária a certas condições de estresse usadas como tratamento pós-colheita (GONZÁLEZ-AGUILAR et al., 2010).

A partir disso, tratamentos pós-colheita que induzem estresse têm sido desenvolvidos para preservar frutas. Esse estresse pode levar à ativação de sistemas antioxidantes enzimáticos e/ou não enzimáticos no produto fresco e contribuir, assim, para um processo de adaptação às condições de estresse e para a manutenção da qualidade da fruta, além de melhorar o potencial antioxidante (GONZÁLEZ-AGUILAR et al., 2010).

1.2.1 Elicitores abióticos

Elicitores podem ser definidos como tratamentos físicos ou químicos capazes de induzir a síntese de fitoquímicos nas plantas e podem ser classificados em bióticos, quando impostos por outro organismo, ou abióticos, quando decorrentes de uma situação de déficit ou de excesso de fatores ambientais químicos ou físicos (APEL; HIRT, 2004; SCHREINER; HUYSKENS-KEIL, 2006).

Em plantas, a aplicação de doses baixas de um tratamento biótico ou abiótico potencialmente danoso que induz respostas positivas ou negativas nos tecidos contra este estresse é um fenômeno chamado hormese (SHAMA; ALDERSON, 2005). Segundo Calabrese (2002), o termo hormese refere-se a uma resposta adaptativa com características diferenciáveis pela sua relação dose-resposta, que é induzida por um processo de ação direta ou de sobre-estimulação a doses baixas. As respostas horméticas podem ser em formato de "U" invertido ou em formato de "J" (Figura 3), onde baixas doses causam efeitos estimulatórios e altas doses causam efeitos deletérios e/ou inibitórios (CALABRESE; BLAIN, 2009).

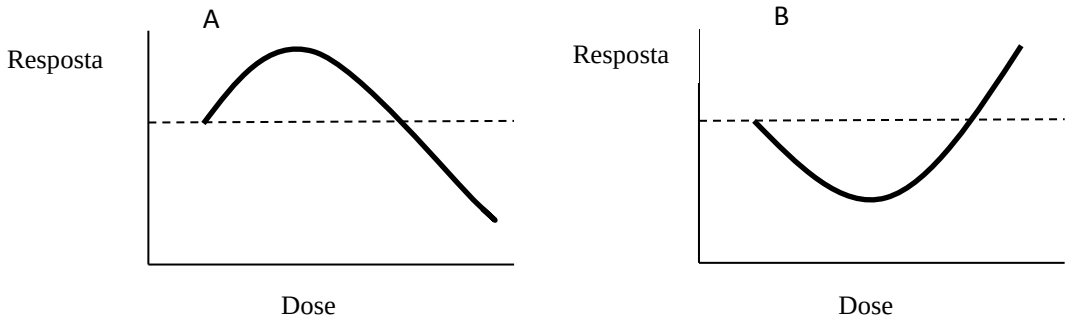

Figura 3. Curvas características de efeito hormético em formato de "U" invertido (A) ou em formato de "J" (B). (Adaptado de CALABRESE; BLAIN, 2009).

Conforme Ayala-Zavala et al. (2004), o interesse no papel exercido pelos compostos bioativos na saúde humana tem estimulado pesquisas a fim de avaliar e determinar como o seu teor e/ou atividade podem ser mantidos ou mesmo melhorados. Para isso, várias estratégias agronômicas de manejo e de processamento pós-colheita, como o uso de elicitores, que incluem tratamentos com altas/baixas temperaturas, aplicação de fitormônios e radiação ultravioleta, têm sido usadas para estimular a biossíntese e o acúmulo de compostos funcionais em alimentos de origem vegetal (SCHREINER; HUYSKENS-KEIL, 2006; TREUTTER, 2010).

Alterações osmóticas, abundância e privação de nutrientes ou alterações no fotoperíodo, na intensidade e qualidade da luz também são fatores abióticos que podem levar ao estresse (APEL; HIRT, 2004). De acordo com Castagna et al. (2013), a modificação da intensidade e/ou qualidade da luz é uma forma de estresse particularmente promissora porque parece existir um papel central da luz em alguns processos metabólicos envolvidos na biossíntese de compostos fitoquímicos.

1.2.2 Radiação ultravioleta

Em função da intensidade e do comprimento de onda, a radiação ultravioleta (UV) pode induzir diferentes estresses biológicos nas plantas e ativar mecanismos de defesa dos tecidos vegetais com a consequente produção de metabólitos secundários e, por isso, pode ser considerada um elicitor abiótico (RIVERA-PASTRANA et al., 2007).

De acordo com Bintsis, Litopoulou-Tzanetaki & Robinson (2000), a luz UV ocupa uma ampla faixa de comprimentos de onda na região não-ionizante do espectro eletromagnético, situando-se entre os raios-X (200 nm) e a luz visível (400 nm), podendo ser subdividida em três regiões:

- UV de onda curta (UV-C) com comprimentos de onda entre 200 e 280 nm;
- UV de onda média (UV-B) com comprimentos de onda entre 280 e 320 nm;
- UV de onda longa (UV-A) com comprimentos de onda entre 320 e 400 nm.

Determinadas doses de radiação UV-A, UV-B ou UV-C podem produzir diferentes efeitos indutivos na atividade fisiológica da planta. A UV-A é a menos prejudicial dentre os três tipos de UV, mas não é atenuada pelo ozônio atmosférico, executando um papel importante na fotomorfogênese. Já a UV-B é potencialmente prejudicial, mas é absorvida pelo ozônio atmosférico, enquanto uma pequena quantidade pode ainda penetrar a atmosfera, atingir a superfície e induzir uma variedade de efeitos danosos em plantas. Altamente energética, a UV-C é extremamente danosa aos microrganismos, mas insignificante sob condições naturais da irradiação solar, uma vez que é absorvida pelo ozônio e não chega à superfície terrestre (HOLLÓSY, 2002; ZHANG et al., 2012).

A intensidade da luz UV que atinge a superfície terrestre depende da atenuação pela atmosfera e pode ser expressa como irradiância ou intensidade de fluxo (W m^{-2}). Já a dose,

sendo uma relação da intensidade em função do tempo de exposição, é expressa como exposição radiante (J m^{-2}) (BINTSIS; LITOPOULOU-TZANETAKI; ROBINSON, 2000).

1.2.2.1 Radiação ultravioleta do tipo C (UV-C)

Como já mencionado, a UV-C é uma radiação de baixo comprimento de onda e alta energia que é completamente absorvida pela camada de ozônio e que não chega à superfície terrestre. No entanto, pode ser encontrada de forma artificial em lâmpadas de mercúrio de UV de onda curta, que produzem energia na região germicida (254 nm), a qual é letal à maioria dos microrganismos (BINTSIS; LITOPOULOU-TZANETAKI; ROBINSON, 2000; LI et al., 2008). Essa capacidade germicida faz com que a UV-C seja usada com sucesso na indústria para a desinfecção de superfícies, esterilização do ar e de líquidos. Sua principal limitação é, no entanto, sua reduzida penetração, que faz com que seja adequada apenas para superfícies (BINTSIS; LITOPOULOU-TZANETAKI; ROBINSON, 2000; MANZOCCO et al., 2011).

Por ser um potente estressor abiótico, a radiação UV-C pode levar à ativação de mecanismos de defesa dos tecidos vegetais, especialmente a síntese de compostos fenólicos, carotenoides e fitoalexinas (RIVERA-PASTRANA et al., 2007).

Fitoalexinas são compostos oriundos do metabolismo secundário das plantas que podem ser sintetizadas *de novo* ou acumuladas em resposta à diferentes fatores de estresse abióticos (como a luz UV) ou bióticos (como os fungos) capazes de estimular a planta a produzir ou a liberar maiores concentrações desses compostos (BOUE et al., 2009; CANTOS; ESPÍN; TOMÁS-BARBERÁN, 2002; GUERRERO et al., 2010).

O acúmulo destes compostos pode ser acompanhado pela indução de outros sistemas de defesa, como a modificação da parede celular, síntese de enzimas do sistema antioxidante e, inclusive, morte celular (RIVERA-PASTRANA et al., 2007). Tudo isso a fim de proteger as células e os compartimentos subcelulares dos efeitos citotóxicos das espécies reativas de oxigênio (EROs) geradas durante o processo de irradiação (ALOTHMAN; BHAT; KARIM, 2009a; GONZÁLEZ-AGUILAR; ZAVALETA-GATICA; TIZNADO-HERNÁNDEZ, 2007; GONZÁLEZ-AGUILAR et al., 2010).

Outras vantagens citadas para o uso da UV-C é que este método não deixa resíduo e não afeta as características sensoriais (sabor e aroma) do produto, além de poder ser aplicado

em conjunto com outras tecnologias, como refrigeração e atmosfera modificada (KEYSER et al., 2008; RIVERA-PASTRANA et al., 2007).

A radiação UV-C geralmente tem ação danosa sobre a vida das plantas, uma vez que gera radicais livres e pode modificar a estrutura do DNA ao induzir a formação de dímeros de timina e citosina, levando a um bloqueio da transcrição e da replicação (SASTRY; DATTA; WOROBO, 2000). No entanto, baixas doses desse agente estressor podem estimular respostas benéficas e, assim, o uso de uma dose hormética de UV-C como um método elicitor pós-colheita tornou-se uma área de interesse para pesquisadores do setor hortícola que buscam culturas com alta resistência a doenças, vida de prateleira estendida e alto teor de compostos bioativos (ROHANIE; AYOUB, 2012).

Efeitos da UV-C em frutas e hortaliças

A radiação UV pode causar alterações em processos bioquímicos e fisiológicos, bem como na morfologia das plantas e, dependendo de fatores como espécie, cultivar e condições de crescimento, pode levar a diferentes efeitos (HOLLÓSY, 2002).

Além disso, uma vez que a exposição de tecidos vegetais a baixas doses de UV-C pode induzir a produção de compostos fungicidas, como as fitoalexinas, e retardar processos de maturação e senescência, tem sido avaliado seu potencial em reduzir as perdas ocasionadas por desordens fisiológicas, ataque de fitopatógenos, danos mecânicos e perda de firmeza (SHAMA, 2007; STEVENS et al., 1998).

Há evidências do efeito da UV-C no aumento da resistência a doenças e ao ataque de microrganismos em uvas (NIGRO; IPPOLITO; LIMA, 1998; ROMANAZZI; GABLER; SMILANICK, 2006), tomates (CHARLES et al., 2008), maçãs (SAUTTER; STORCK; RIZZATTI, 2008), morangos (NIGRO et al., 2000) e pêssegos (EL GHAOUTH; WILSON; CALLAHAN, 2003). A exposição a doses baixas de UV-C também retardou a maturação e senescência em maçã (LIU *et al*., 1991), manga (GONZÁLEZ-AGUILAR; ZAVALETA-GATICA; TIZNADO-HERNÁNDEZ, 2007) e tomates (MAHARAJ; ARUL; NADEAU, 2010; TIECHER et al., 2013).

Outra vantagem reconhecida do tratamento com UV-C é a melhora das propriedades funcionais e da capacidade antioxidante dos vegetais através do estímulo da biossíntese de metabólitos secundários (GONZÁLEZ-BARRIO et al., 2009; JAGADEESH et al., 2011). O

tratamento de goiaba e banana com UV-C aumentou o teor de fenólicos e de flavonoides, enquanto que em abacaxi ocorreu aumento de flavonoides, mas não houve efeito sobre o teor de fenólicos totais (ALOTHMAN; BHAT; KARIM, 2009b). Pêssegos tratados com UV-C também apresentaram maior teor de compostos fenólicos e maior capacidade antioxidante quando comparados a pêssegos não submetidos à radiação UV-C (TIECHER et al., 2010). Perkins-Veazie, Collins, & Howard (2008) ao estudarem a aplicação de UV-C em mirtilos, encontraram um aumento no teor de fenólicos, antocianinas e capacidade antioxidante (pelo método FRAP) usando doses de 1 kJ m^{-2} na variedade Collins. No entanto, ao usarem doses de 2 e 4 kJ m^2 na variedade Bluecrop não observaram aumento no teor de fenólicos, apenas nas antocianinas e na capacidade antioxidante. Em morangos, o tratamento com radiação UV-C aumentou os teores de antocianinas (BAKA et al., 1999), compostos fenólicos e incrementou a capacidade antioxidante (ERKAN; WANG; WANG, 2008). Crizel (2012) observou que a aplicação de UV-C durante o cultivo de morangueiros levou à obtenção de morangos com maior teor de ácido ascórbico e antocianinas. Da mesma forma, a exposição à radiação UV-C levou a um aumento do teor de ácido ascórbico e fenólicos totais em tomates, além de promover o retardo da maturação e senescência (JAGADEESH et al., 2011; MAHARAJ; ARUL; NADEAU, 2010). Jiang et al. (2010) também observaram um incremento na síntese de flavonoides e ácido ascórbico em cogumelos submetidos à radiação UV-C.

Aprovada em 2002 pela *Food and Drug Administration* para uso como tecnologia descontaminante de superfícies de alimentos, a UV-C pode ser aplicável tanto a frutas e hortaliças inteiras quanto minimamente processadas, como uma forma de estender sua vida útil através da preservação de sua aparência e frescor e da prevenção do desenvolvimento microbiano (ALOTHMAN; BHAT; KARIM, 2009b; MANZOCCO; DA PIEVE; MAIFRENI, 2011; UNITED STATES - FOOD AND DRUG ADMINISTRATION, 2013; LEMOINE, M.L.; CHAVES, A.R.;MARTÍNEZ, 2010). Além disso, é uma tecnologia de custo relativamente baixo e que pode ser útil na redução do uso de fungicidas em frutas e hortaliças, o que certamente faz com que o produto seja mais valorizado pelo consumidor (SAUTTER, 2003; SHAMA, 2007).

Efeitos da UV-C em uvas

Diversas pesquisas têm sido feitas baseadas na aplicação de UV-C em uvas, tanto no controle de doenças (CIA et al., 2009; NIGRO; IPPOLITO; LIMA, 1998; ROMANAZZI; GABLER; SMILANICK, 2006), quanto na síntese de fitoquímicos (CANTOS; ESPÍN; TOMÁS-BARBERÁN, 2002; CRUPI et al., 2013; GONZÁLEZ-BARRIO et al., 2006; LI et al., 2008; SAUTTER, 2008) e compostos voláteis (GINDRI, 2013; KOBAYASHI et al., 2011; TREPTOW, 2012). A maioria desses estudos, no entanto, foram focados basicamente na produção de estilbenos e de antocianinas, em diversos tecidos de *Vitis* (LIU et al., 2010; WANG et al., 2010; ZHANG et al., 2012).

Uvas viníferas das cultivares Tempranillo, Cabernet sauvignon, Merlot, Syrah, Monastrell, Garnacha e Cariñena, tratadas com UV-C após serem colhidas, tiveram seu teor de estilbenos (trans-resveratrol, trans-piceatanol e viniferinas) aumentado 6 dias após o tratamento (CANTOS et al., 2003a). Os mesmos autores também encontraram aumento no teor de resveratrol em uvas de mesa 'Napoleon' após o tratamento com pulsos de radiação UV-C quando comparadas a uvas não tratadas e sugeriram que a UV-C poderia ser usada como forma de criar uvas "funcionais" e enriquecidas em resveratrol (CANTOS; ESPÍN; TOMÁS-BARBERAN, 2001). Nesse estudo, também foi avaliado o teor de ácido ascórbico, que permaneceu inalterado após uma semana de armazenamento. Cultivares de uvas de mesa tintas (Flame, Red Globe, Crimson e Napoleon) e brancas (Superior, Dominga e Moscatel Italica) estudadas por Cantos et al. (2002) também tiveram o teor de estilbenos aumentado após tratamento com pulsos de UV-C e foram sugeridas como "frutas funcionais". Em outro estudo, Cantos et al. (2003b) avaliaram o teor de resveratrol e parâmetros enológicos de vinhos produzidos a partir de uvas 'Monastrell' tratadas com UV-C e também obtiveram resultados positivos para o teor de estilbenos. No entanto, a irradiação com UV-C não modificou o padrão de compostos fenólicos (incluindo antocianinas e flavonóis) dessas uvas. Da mesma forma, Cantos, Espín e Tomás-Barberán (2002) e Cantos et al. (2003a) não observaram aumento no teor de compostos fenólicos não-estilbenos (antocianinas, flavonois e flavan-3-ois) em vinhos e uvas de mesa, mas apenas em compostos da classe dos estilbenos, como resveratrol e viniferinas.

Guerrero et al. (2010) observaram um aumento na síntese de estilbenos (trans-resveratrol) na casca de diferentes espécies híbridas e viníferas de *Vitis* após o tratamento com UV-C e o maior aumento foi observado entre 5 e 7 dias após o tratamento. Além disso, as variedades com maior teor inicial foram as que mais responderam à UV-C e produziram mais resveratrol. Da mesma forma, Li et al. (2008) estudaram as cultivares de uva Takasuma, Carigane e Tano Red e observaram que o aumento de resveratrol foi dependente da dose, da

cultivar e do tipo de radiação UV aplicado, sendo que a UV-C teve maior efeito sobre a síntese de resveratrol que a UV-B.

Sautter (2003) verificou que sucos produzidos a partir de uvas ‘Isabel’ e ‘Seibel 10.096’ submetidas à UV-C tiveram seu teor de antocianinas e de trans-resveratrol aumentado de forma significativa. Esses sucos apresentaram um teor de trans-resveratrol de 3 a 16 vezes maior quando comparado a amostras de suco comercial integral.

Treptow (2012), ao avaliar o teor de fenólicos totais e a evolução dos compostos voláteis de bagas de uvas 'Isabel' irradiadas com UV-C e armazenadas a 20°C, observou que tanto o teor de fenólicos totais quanto o teor de ésteres e aldeídos foram intensificados com o uso de uma dose de 3 kJ m^{-2}. Além disso, verificou-se que os sucos provenientes de uvas tratadas com UV-C apresentaram maior intensidade de aroma e foram significativamente diferenciados dos sucos provenientes de uvas não irradiadas quando avaliados sensorialmente.

Gindri (2013) avaliou o teor de fenólicos totais, antocianinas e a capacidade antioxidante de vinhos produzidos a partir de uvas viníferas 'Merlot' e 'Cabernet sauvignon' submetidas à UV-C, não obtendo diferença significativa frente a vinhos elaborados com uvas não irradiadas. Neste mesmo estudo também foi avaliada a composição volátil dos vinhos, onde o tratamento com UV-C levou a um aumento na concentração de vários ésteres e álcoois. As doses horméticas em relação aos diferentes parâmetros de análise dos vinhos foram estabelecidas em 3 kJ m^{-2} para a cv. Merlot e 6 kJ m^{-2} para a cv. Cabernet sauvignon.

A maioria dos trabalhos existentes na literatura reportando os efeitos da UV-C em uvas foram feitos em cultivares viníferas e avaliaram principalmente a produção de resveratrol e antocianinas, sendo poucos os estudos voltados para as uvas híbridas, como a ‘Isabel’. Além disso, o efeito da UV-C sobre outras classes de compostos bioativos, seu efeito sobre a capacidade antioxidante e sobre a atividade de enzimas envolvidas na síntese de compostos fenólicos de uvas ‘Isabel’ não foi plenamente estudado.

Mecanismo de ação da UV-C

No que ser refere à sua ação germicida, sabe-se que a UV-C pode atuar tanto diretamente sobre os microrganismos, através do dano ao DNA, como indiretamente pela estimulação de mecanismos de defesa e síntese de fitoalexinas (ESCALONA et al., 2010). Apesar desse efeito parecer não depender da temperatura, a efetividade da UV-C depende da

incidência determinada pela estrutura e topografia da superfície do produto tratado, da dose (J m^{-2}) e da distância entre a fonte de energia e a amostra (BINTSIS; LITOPOULOU-TZANETAKI; ROBINSON, 2000; SHAMA, 2007).

Já os mecanismos pelos quais ocorrem as alterações causadas pela UV-C, como o aumento do potencial antioxidante, ainda não estão completamente elucidados. No entanto, sabe-se que o metabolismo secundário é ativado junto com o sistema antioxidante enzimático e que haveria a formação de espécies reativas de oxigênio (EROs), como oxigênio singlete ($^1O_2$), radical superóxido ($O_2^{\cdot-}$), peróxido de hidrogênio (H_2O_2) e radical hidroxil ($^{\cdot}OH$). (GONZÁLEZ-AGUILAR et al., 2010; KOVÁCS; KERESZTES, 2002).

Shama & Alderson (2005) sugerem que tratamentos utilizando UV promovem reações de foto-oxidação nas plantas através da produção de EROs, que fazem com que as plantas reajam e estimulem seus mecanismos de defesa. Quando uma dose hormética de radiação é absorvida por um material biológico, ela pode interagir com átomos e moléculas, principalmente a água, produzindo as EROs pela redução univalente do oxigênio de uma maneira rápida e controlada (GONZÁLEZ-AGUILAR et al., 2010).

1.3 Defesa contra a radiação UV

A sensibilidade das células aos efeitos prejudiciais da radiação será determinada pelo balanço entre o dano causado à célula e pela eficiência dos processos de reparo para restabelecer as funções que foram prejudicadas (KOYAMA et al., 2012).

De acordo com Hollósy (2002), a epiderme é a primeira camada celular a interceptar a radiação UV e representa uma importante barreira de proteção, uma vez que contém compostos capazes de absorver essa energia. Uma estratégia efetiva é, então, o acúmulo de compostos fenólicos que seletivamente absorvem a radiação UV na cutícula e epiderme da planta (KOYAMA et al., 2012).

Em resposta a elevadas doses de radiação UV e ao estresse oxidativo gerado por esta exposição, as células das plantas desenvolveram formas de proteção que envolvem mecanismos de defesa enzimáticos (superóxido dismutase, catalase, ascorbato peroxidase, glutationa redutase) e não-enzimáticos, como alguns metabólitos secundários, que convertem

as EROs até produtos menos tóxicos (GONZÁLEZ-AGUILAR et al., 2010; HOLLÓSY, 2002; KOYAMA et al., 2012).

Os mecanismos não-enzimáticos de remoção de radicais livres incluem os compostos antioxidantes como o alfa-tocoferol, o beta-caroteno, o ascorbato e os flavonoides (FOYER; NOCTOR, 2005; HOLLÓSY, 2002). Tem sido proposto que os flavonoides seriam um mecanismo adaptativo de proteção contra a radiação UV, já que absorvem fortemente nesta faixa de energia. Os flavonoides podem ser sintetizados em reações catalisadas por enzimas da via dos fenilpropanoides, como a fenilalanina amônia liase (FAL), juntamente com enzimas da via dos flavonoides, como a chalcona sintase (CHS) (LATTANZIO; CARDINALI; LINSALATA, 2012; LATTANZIO et al., 2008). O acúmulo destes compostos é regulado pela isomerização *cis-trans* do ácido cinâmico, catalisada pela luz com incidência de 360nm e, assim, a radiação UV produz uma mistura equilibrada dos dois isômeros (CZELUSNIAK et al., 2012).

Outro mecanismo não-enzimático inclui a glutationa (GSH), que pode ser oxidada (GSSG) por diversos fatores de estresse. A GSH pode atuar diretamente como antioxidante reduzindo EROs, pode entrar no ciclo GSH-ascorbato para a redução do ácido deidroascórbico, contribuir na remoção do peróxido de hidrogênio via glutationa peroxidase (GILL; TUTEJA, 2010) e ainda funcionar como um possível marcador de estresse na via dos flavonoides, capaz de induzir a transcrição de genes de enzimas de defesa como a CHS e a FAL (NOCTOR; FOYER, 1998).

Assim como em qualquer outro organismo de metabolismo aeróbico, as plantas também produzem continuamente EROs e essa produção ocorre principalmente nos cloroplastos, nas mitocôndrias e nos peroxissomos. A produção e a remoção das EROs é estritamente controlada. No entanto, esse equilíbrio pode ser perturbado por diversos fatores de estresse abióticos, como temperaturas extremas e alta exposição à luz (APEL; HIRT, 2004; WRZACZEK et al., 2011). Essencialmente todos os estresses abióticos levam à produção de EROs, embora em diferentes formas e em diferentes compartimentos subcelulares (JASPERS; KANGASJÄRVI, 2010).

Em altas concentrações, as EROs podem ter efeitos prejudiciais sobre o metabolismo normal em razão de oxidar ácidos nucleicos, proteínas, lipídios ou carboidratos, afetar a integridade das membranas celulares e inativar funções celulares-chave, podendo, inclusive, levar à aceleração da senescência (GILL; TUTEJA, 2010). O impacto comercial destes efeitos deve ser minimizado, ou então eliminado, pelo controle cuidadoso da dose máxima de UV que pode ser aplicada às frutas (SHAMA, 2007).

No entanto, sugere-se que o acúmulo rápido, mas moderado, de produtos da foto-oxidação em resposta ao tratamento com doses horméticas de UV poderia estimular a defesa antioxidante (MAHARAJ; ARUL; NADEAU, 2010). Assim, em contraste ao seu conhecido papel como agentes prejudiciais às células, as EROs atuariam também como moléculas sinalizadoras, com papel essencial na regulação do desenvolvimento e da adaptação ao estresse e no estímulo da produção de compostos antioxidantes (APEL; HIRT, 2004; GONZÁLEZ-AGUILAR et al., 2010; JASPERS; KANGASJÄRVI, 2010).

Geralmente, o estresse, como qualquer outro estímulo, pode ser percebido de uma maneira direta ou indireta. Na percepção direta, o agente causador do estresse é percebido através de um receptor, enquanto na percepção indireta são percebidos os efeitos causados pelo agente estressor. Evidências sugerem que as plantas usam ambos os modelos em paralelo na percepção de estresses abióticos, sendo que as EROs são frequentemente usadas como moléculas sinalizadoras na percepção indireta (WRZACZEK et al., 2011).

1.4 Metabolismo secundário

O metabolismo primário é uma importante fonte de precursores para as vias de biossíntese do metabolismo secundário e sabe-se que a via do ácido chiquímico (Figura 4) é a responsável por mediar o fluxo de carbono do metabolismo de carboidratos (metabolismo primário) até a biossíntese de compostos aromáticos (metabolismo secundário) em microrganismos e plantas (LATTANZIO; CARDINALI; LINSALATA, 2012; TREUTTER, 2010; ZHANG et al., 2012).

No entanto, ao contrário do metabolismo primário, que se refere a processos catabólicos e anabólicos voltados à manutenção e à proliferação celular, o metabolismo secundário envolve a síntese de compostos que não são diretamente essenciais para o metabolismo básico, mas que são necessários para a sobrevivência das plantas no ambiente (KLIEBENSTEIN, 2004; LATTANZIO et al., 2008; TREUTTER, 2010).

O metabolismo secundário das plantas constitui uma grande reserva de diversos metabólitos e enzimas e um amplo espectro de mecanismos de regulação genética e de transporte, que podem ser induzidos tanto por condições ambientais como podem ser geneticamente controlados (LATTANZIO et al., 2008).

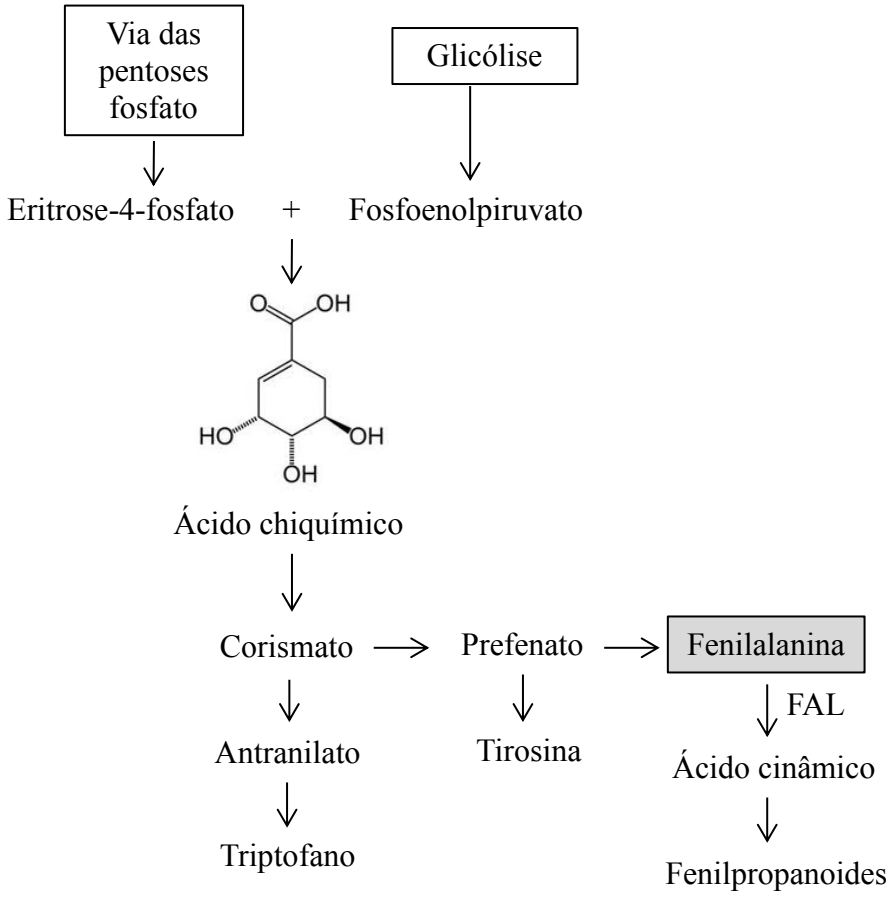

Figura 4. Via do ácido chiquímico (Adaptado de TAIZ; ZEIGER, 2009).

Apesar de haver uma enorme variedade de metabólitos secundários, o número de vias biossintéticas é restrito e distinto. Os precursores geralmente são provenientes de vias metabólicas básicas como ciclo de Krebs, glicólise e via do ácido chiquímico (Figura 5). Exemplos de metabólitos secundários incluem os alcaloides, terpenos, flavonoides, taninos, fenilpropanoides, lignina, lignanas, glucosinolatos, entre outros. Alguns podem ser produzidos em todos os tecidos, mas os sítios de biossíntese geralmente são compartimentalizados na célula vegetal e localizados principalmente no citoplasma, retículo endoplasmático ou em organelas. Compostos de caráter hidrossolúvel são armazenados nos vacúolos, enquanto substâncias de caráter hidrofóbico são armazenadas nos pelos glandulares, tricomas, membranas tilacoides ou cutícula (PETERSEN; HANS; MATERN, 2010).

Esses compostos são necessários para as plantas no que se refere a crescimento, reprodução e resistência, devido, principalmente, às suas propriedades antibióticas, antinutricionais e antipalatáveis (LATTANZIO et al., 2008). Além do seu papel protetor

contra herbívoros, microrganismos, vírus, patógenos e plantas competidoras, os metabólitos secundários também atuam como compostos sinalizadores (na atração de polinizadores e dispersores de sementes) e como protetores da planta contra agentes oxidantes, radiação UV e outros estresses físicos (LATTANZIO et al., 2008; PETERSEN; HANS; MATERN, 2010).

Flores, frutos e sementes são órgãos envolvidos com a reprodução e continuação da espécie e, por isso, são especialmente ricos em metabólitos secundários. No entanto, o perfil desses metabólitos é variável de acordo com o tempo, ambiente e estado de desenvolvimento do tecido vegetal (PETERSEN; HANS; MATERN, 2010).

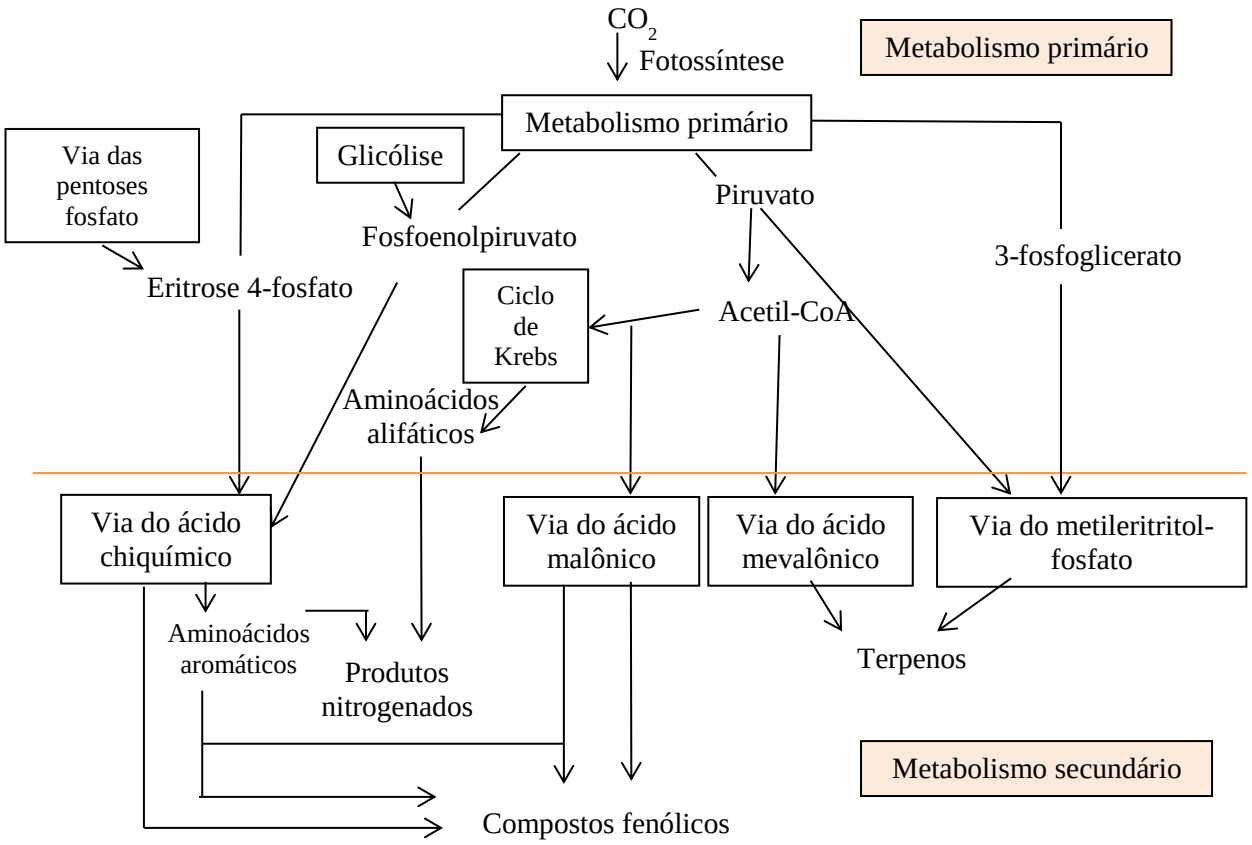

Figura 5. Esquema simplificado das principais vias de biossíntese envolvidas no metabolismo secundário e suas inter-relações com o metabolismo primário (Adaptado de TAIZ; ZEIGER, 2009).

A principal via para a formação dos compostos fenólicos inicia-se com os aminoácidos aromáticos L-fenilalanina e, em menor extensão, L-tirosina. A fenilalanina liberada como produto da via do ácido chiquímico entra como precursor na via dos fenilpropanoides para dar origem a diversos compostos fenólicos (Figura 7), que incluem os flavonoides, isoflavonas,

estilbenos, cumarinas, chalconas, lignanas e lignina (KOYAMA et al., 2012; TREUTTER, 2010; ZHANG et al., 2012).

A transferência dos aminoácidos do metabolismo primário para o secundário é realizada pela atividade das enzimas fenilalanina/tirosina amônia liase (FAL/TAL), que transformam estes aminoácidos em ácido cinâmico, posteriormente metabolizado até 4-coumaroil-CoA por outras enzimas da via dos fenilpropanoides, como a ácido cinâmico 4-hidroxilase e a 4-coumarato CoA ligase (PETERSEN; HANS; MATERN, 2010).

1.5 Fenilalanina amônia liase (FAL)

A FAL (EC 4.3.1.5) é uma enzima que catalisa a entrada da fenilalanina na via dos fenilpropanoides pela remoção do nitrogênio na forma de amônia enquanto introduz uma dupla ligação entre os carbonos 7 e 8 (Figura 6).

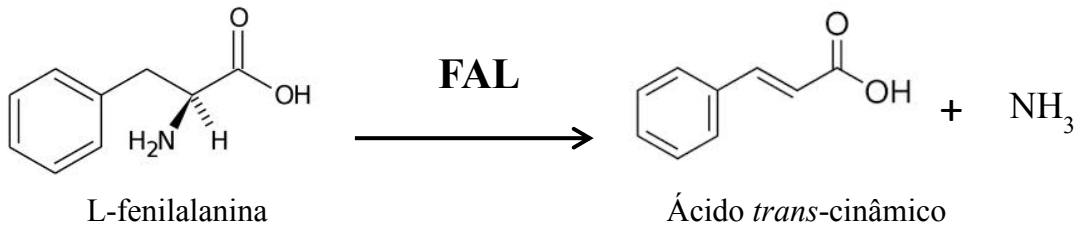

Figura 6. Reação catalisada pela enzima fenilalanina amônia liase (FAL). Baseada em (PETERSEN; HANS; MATERN, 2010).

Descrita pela primeira vez em 1961, a FAL é uma das enzimas mais bem estudadas do metabolismo secundário vegetal e em muitas plantas as isoformas da FAL são codificadas por famílias multigênicas. Sua forma ativa é uma proteína homotetramérica e que não requer co-fator. Apesar de ser considerada uma enzima solúvel, as diferentes isoformas variam na sua localização subcelular, podendo ser encontradas no citoplasma ou ligadas às membranas (PETERSEN; HANS; MATERN, 2010).

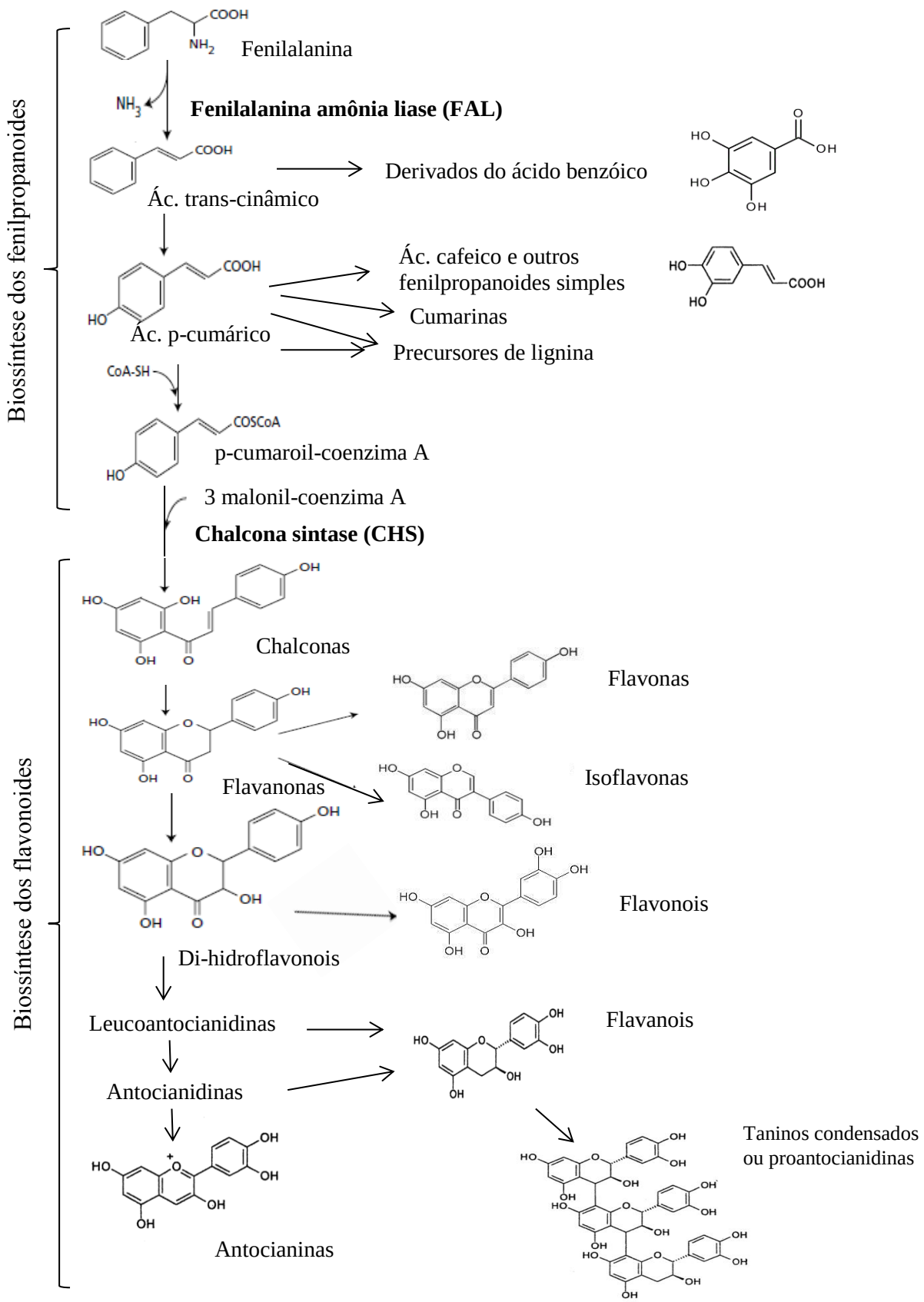

Figura 7. Síntese dos compostos fenólicos a partir da fenilalanina (Adaptado de KANAZAWA et al., 2012; KOYAMA et al., 2012; TAIZ; ZEIGER, 2009).

Esta enzima pode ser induzida junto com outros genes e enzimas do metabolismo fenólico por fatores ambientais, como ataque de patógenos ou luz UV, e, assim, diferentes rotas de produção de compostos podem ser ativadas em detrimento de outras dependendo da planta (PETERSEN; HANS; MATERN, 2010).

O aumento na atividade da FAL em situações de estresse pode ocorrer tanto pela síntese *de novo* da enzima, como pela liberação da FAL de um complexo enzima-inibidor pré-existente, mas inativo. Cabe destacar que a produção de etileno em tecidos vegetais submetidos a um estresse ocorre quase ao mesmo tempo em que ocorre o aumento da atividade da FAL, mostrando que ambos os metabolismos estão interligados (LATTANZIO; CARDINALI; LINSALATA, 2012). Tiecher et al. (2013), por exemplo, observou que o tratamento de tomates com UV-C estimulou a produção de etileno e sugeriu que a indução do acúmulo de carotenoides totais pela UV-C seria parcialmente dependente do etileno produzido, uma vez que a aplicação apenas de 1-metilciclopropeno (1-MCP), um inibidor da síntese de etileno, inibiu esse acúmulo e a aplicação apenas de UV-C induziu esse acúmulo.

A indução da atividade da FAL também pode ser dependente da temperatura, já que o estresse causado pela baixa temperatura pode não levar a um aumento correspondente na produção de fenólicos, sendo possível que etapas subsequentes da biossíntese de compostos fenólicos limitem sua formação (LATTANZIO; CARDINALI; LINSALATA, 2012).

1.6 Compostos fenólicos como agentes filtrantes da radiação UV

Diversos estudos têm demonstrado que os fenólicos são substâncias capazes de absorver a luz UV e que uma radiação UV suplementar poderia induzir a produção desses compostos nas plantas, em particular as antocianinas (ZHANG et al., 2012). Além disso, um excesso de radiação UV levaria a mudanças na composição dos flavonoides e à ativação de genes envolvidos em sua biossíntese. Ryan et al. (2001) observaram que mutantes de *Arabidopsis thaliana* que tiveram a síntese de flavonoides bloqueada apresentaram hipersensibilidade à radiação UV e sugeriram que outros fenólicos podem ser tão importantes quanto os flavonoides na proteção contra a radiação UV. Kliebenstein (2004) observou que mutantes deficientes na rota dos fenilpropanoides e expostos à UV-B foram mais sensíveis à radiação e sugeriu que tanto ácidos hidroxicinâmicos pré-formados e flavonois-induzidos

atuariam como protetores contra a UV-B e que a importância deles estaria diretamente relacionada às suas concentrações (LATTANZIO et al., 2008).

2 OBJETIVOS

O objetivo geral desta dissertação foi avaliar o efeito da radiação UV-C e do tempo de armazenagem após a irradiação sobre o teor de compostos bioativos e a capacidade antioxidante de uvas ‘Isabel’, determinando a dose hormética de UV-C.

Os objetivos específicos foram:

- Avaliar indicadores de qualidade, como o teor de sólidos solúveis totais e pH das uvas;
- Avaliar o teor de diferentes classes de compostos bioativos (fenólicos totais, antocianinas totais, flavonois totais) na casca das uvas;
- Avaliar o teor de ácido ascórbico na uva inteira;
- Avaliar a atividade da enzima fenilalanina amônia liase;
- Avaliar a capacidade antioxidante total da casca das uvas.

3 ARTIGO CIENTÍFICO

Os dados resultantes desta dissertação estão em fase final de revisão pelos autores e serão apresentados na forma de artigo científico. O artigo será submetido à revista *International Journal of Food Science and Technology* e está configurado de acordo com as normas desta, as quais estão listadas no anexo A.

Postharvest UV-C radiation increases bioactive compounds, antioxidant capacity and phenylalanine ammonia lyase activity in 'Isabella' grapes (*Vitis labrusca* L.)

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Abstract

Ultraviolet light type C (UV-C) has been used to enhance the phytochemical content in food crops. However, its effects may vary depending on the fruit cultivar, hormetic dose, and time after exposure. The effects of UV-C irradiation (0.5, 1.0, 2.0 and 4.0 kJ m^{-2}) followed by storage at 20 ± 1°C (1, 3 and 5 days) were evaluated on the quality parameters, bioactive compounds, antioxidant capacity, and phenylalanine ammonia lyase (PAL) activity of 'Isabella' grapes. UV-C effects appeared immediately after the irradiation and gradually decreased along the storage. The highest UV-C doses (2.0 and 4.0 kJ m^{-2}) resulted in a rapid increase of total soluble solids (TSS) and a decrease of pH values ($p<0.05$). Total phenolic compounds increased after irradiation at 1.0 and 2.0 kJ m^{-2}, whereas anthocyanin content increased after irradiation at 0.5, 1.0, and 2.0 kJ m^{-2} ($p<0.05$). UV-C treatment had no effect on the flavonol content but decreased ascorbic acid (AA) and total vitamin C content (0.5-2.0 kJ m^{-2}, $p<0.05$). UV-C irradiation (0.5-2.0 kJ m^{-2}) increased PAL activity. UV-C treated grapes had higher antioxidant capacity than control in the ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays ($p<0.05$). Thus, UV-C irradiation can be used to increase bioactive compounds and antioxidant activity of 'Isabella' grapes and 1.0 kJ m^{-2} was considered the hormetic dose in the studied conditions.

Keywords

UV-C, grape, postharvest, abiotic stress, anthocyanin, bioactive compounds

1 Introduction

'Isabella' is a black hybrid grape cultivar (*Vitis labrusca L.*) that represents about 50% of Brazilian grape production and is largely used for fresh consumption and for the elaboration of table wine, juice, vinegar, sweets, and jams (Nixdorf & Hermosín-Gutiérrez 2010). However, even with the great commercial availability, this hybrid has lower antioxidant capacity than wine grape varieties (Rockenbach et al., 2011).

The consumption of fruits and vegetables has been associated to the reduction of the risk of cancer, cardiovascular and neurodegenerative diseases, and more recently, to diabetes (Scalbert et al. 2005). This protective effect has been attributed to the antioxidant effect and modulatory biological activities of phytochemicals, among them are the phenolic compounds (Wootton-Beard & Ryan 2011). Grape phenolic compounds, specially the anthocyanins, flavonols, and resveratrol, have antioxidant, cardioprotective, anticancer, anti-inflammatory, anti-aging, and antimicrobial activities (Xia et al., 2010).

Ultraviolet light type C (UV-C) is a non-ionizing radiation, with wavelengths ranging from 200 to 280 nm and maximum germicide effect at 254 nm. It has been used in the surfaces disinfection, liquids sterilization, and as an alternative to the use of fungicides, extending the shelf life of fresh products (Bintsis et al., 2000; González-Aguilar et al., 2001; Shama and Alderson, 2005; Alothman et al., 2009). As a physical, non-thermal method, UV-C treatment produces no toxic residues (Keyser et al. 2008) and can be easily associated with other preservation technologies, such as modified atmosphere and refrigeration.

Despite its germicide effects, UV-C is an abiotic stress that generally has deleterious effects on plant tissues due to the generation of reactive oxygen species

(ROS). However, the exposure to low levels of a stressor may stimulate beneficial physiological defence responses, which contrasts with the detrimental effects caused by the exposure to high levels. This phenomenon is called hormesis (Shama & Alderson, 2005; Calabrese and Blain, 2009) and has been explored to improve the nutraceutical potential of some horticultural products (Cisneros-Zevallos, 2003; Shama and Alderson, 2005).

Plants which are able to synthesize flavonoids and other phenolic compounds that absorb UV light are more tolerant to the UV irradiation than mutants in which this pathway was impaired (Gill & Tuteja 2010). Thus, UV-C irradiation may trigger a defence response that results in the accumulation of phenolic compounds in many fruits and vegetables, as occurred in tomatoes (Bravo et al. 2012), strawberries (Erkan et al., 2008), mangoes (González-Aguilar et al. 2007), and mushrooms (Jiang et al. 2010). The synthesis of defence compounds is initiated by UV treatment but continues to occur during the days after the irradiation (Shama & Alderson 2005). Therefore, the simultaneous postharvest UV-C and storage treatments in table grape varieties has been scarcely investigated (Crupi et al., 2013).

Phenylalanine ammonia lyase (PAL) catalyses the deamination of L-phenylalanine into trans-cinnamate, which is the first step in the biosynthesis of phenylpropanoid compounds, such as chlorogenic acid, flavonoids, and lignin monomers, and may be induced by abiotic stresses as UV light (Petersen, Hans, & Matern, 2010; MacDonald & D'Cunha, 2007).

Some works have investigated the effects of UV-C on the synthesis of anthocyanins in grapes (Zhang et al. 2012). However, the majority of studies primarily focused on the synthesis of stilbenes in various tissues of *Vitis* (Bertagnolli et al., 2007;Wang et al., 2010; Li et al., 2008; Guerrero et al., 2010). To our knowledge, the

antioxidant capacity and PAL activity in grapes treated with UV-C has not been described yet. Thus, the purpose of this work was to evaluate the changes in fruit quality, bioactive compounds levels, antioxidant capacity and PAL activity, along the storage after UV-C irradiation of 'Isabella' grapes.

2 Materials and methods

2.1 Chemicals

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin-3-rutinoside, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), polyvinylpyrrolidone (PVPP), and dithiothreitol (DTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The other reagents were of analytical grade.

2.2 Fruit samples

Grapes cv. Isabella (*Vitis labrusca L.*) were hand-harvested in January, 2013 at Itaara, RS, Brazil (29°35'55.39" S and 53°46'13.49" W) from different plants at commercially mature stage. Immediately after the harvest, grapes were taken to the laboratory. Ruptured, injured or unripe berries were discarded. 'Isabella' grape samples selected for this study presented the following initial quality parameters: pH was 3.32 ± 0.02; titratable acidity was 0.62 ± 0.03 mg tartaric acid 100 g^{-1} FW, and total soluble solids (TSS) was 14.2 ± 0.3 (°Brix). Grape berries were cut at level of stem and randomized one day before the treatment to evaluate the effects of UV-C on the postharvest fruit metabolism. Each treatment was composed by four replicates (approximately 300 g fruit each one) at each time point.

2.3 UV-C treatment

Fruits were placed in aluminum trays and subjected to UV-C irradiation (0.5, 1.0, 2.0, or 4.0 kJ m^{-2}) using a Philips® lamp (TUV-30W/G30T8) distant 30 ± 1 cm from samples. Non-irradiated grapes were considered as control. The radiation intensity of the lamp at 254 nm was determined with a radiometer (International Light® RPS900) and the UV-C irradiation doses were obtained by changing the exposure time. The doses of irradiation were chosen based on previous studies that applied UV-C in grapes (Li et al. 2008). After treatment, samples from each repetition and treatment were stored in tulle bags under natural light at 20 ± 1°C. After 1, 3, and 5 days in this storage conditions, treated and untreated (control) samples were immediately frozen in liquid nitrogen and kept at -20°C for further analyses.

2.4 Preparation of extracts

Grapes were peeled and 5 g (FW) of skin were grinded with 15 mL of water:ethanol:formic acid solution (27:70:3, v/v/v) for 2 min at 12,000 rpm using an ultra-turrax. The mixture was centrifuged at 2,300 *x g* for 10 min and the supernatant was stored in amber flasks at -20°C until analyses. This skin extract was used to determine the content of phenolics, flavonols, and anthocyanins and the antioxidant capacity. The remaining pulp was centrifuged at 2,300 *x* g for 10 min and total soluble solids (TSS) and pH were determined in the supernatant.

2.5 TSS and pH

The TSS was determined in a portable refractometer (Biobrix® mod. 103, Curitiba, PR, Brazil) and pH was evaluated using a pHmeter (Digimed® DM-22, São Paulo, SP, Brazil).

2.6 Determination of phenolic compounds, flavonol, and anthocyanin content

These assays were performed in the skin extract. The total content of phenolic compounds was evaluated using Folin-Ciocateau's reagent at 765 nm (Singleton & Rossi, 1965) and results were expressed as mg gallic acid equivalents 100 g^{-1} of skin (FW).

The total content of flavonols was measured by a colorimetric assay (Zhishen, Mengcheng & Jianming, 1999) and expressed as mg quercetin-3-rutinoside equivalents 100 g^{-1} of skin (FW). This method is based on the aluminum complexation by the phenolic compound, which must have a 4-keto group and at least one neighboring (3- or 5-) hydroxyl group. Flavones and flavanones, which are also detected by the method, are not common in grapes (Tenore et al. 2012).

Total monomeric anthocyanin content was evaluated using the pH-differential method (Wrolstad & Giusti 2001) and calculated as:

$A = (A_{535\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{535\ nm} - A_{700\ nm})_{pH\ 4.5}$ (1);

Total monomeric anthocyanin (mg malvidin-3-glucoside equivalents 100 g^{-1} skin (FW)) $= A * MW * DF * 1000 * 100 / \varepsilon * 1 * 333$ (2);

where: MW=molar weight of malvidin-3-glucoside (493.5); DF= dilution factor; ε= molar extinction coefficient absorption of malvidin-3-glucoside (29500).

2.7 Determination of ascorbic acid

Whole frozen grape berries without seeds were ground with pestle and mortar in liquid nitrogen until pulverization. The obtained power (1 g) was extracted with 1 mL of 4.5% meta-phosphoric acid and centrifuged at 23,145 *x* g at 4°C, for 15 min. The supernatant was filtered at 0.45 µm (Millipore® filter, Bedford, Md., USA) and stored up to seven days at -20°C prior to the analysis in a high performance liquid chromatography (HPLC) system (LC-20AT prominence) coupled to an UV-Vis detector (SPD-20AV prominence) and to a reverse phase Microsorb-MW® C-18 column (4.6 x 250 mm, particle size 5 µm). The mobile phase was sulfuric acid and ultrapure water at pH 2.6 ± 0.02 and the flow was maintained at 1 mL min^{-1}, as described by Sánchez-Mata, Cámara-Hurtado, Díez-Marqués, & Torija-Isasa (2000). AA was quantified at 245 nm with an eight-point calibration curve (0.03125 – 10 mg L^{-1}, R^2 = 0.9995). The detection and the quantification limits, calculated based on the standard deviation of the response and the slope of the calibration curve as described by Ribani et al. (2004), were 0.096 and 0.32 mg kg^{-1} fruit, respectively.

The total AA was determined after adding freshly prepared 10 mM DTT (final concentration) to the sample to reduce dehydroascorbic acid into AA (Hernandéz, Lobo & González, 2006), whereas reduced ascorbic acid was determined by injecting the sample with no reaction.

2.8 Antioxidant capacity

The antioxidant capacity assays were performed in the skin extracts. The DPPH radical scavenging assay was carried out as described by Brand-Williams et al. (1995), with some modifications. The antiradical power of the extracts was measured as the decrease of DPPH absorbance against a blank containing the extraction solution at 517 nm and calculated as percent of DPPH inhibition as follow:

$$\% \text{ inhibition} = ((Abs_{blank} - Abs_{sample})/Abs_{blank})*100 \quad (3)$$

Trolox, a hydrosoluble analog of alfa-tocopherol, was used as standard for the calibration curve and results were expressed as mmol Trolox equivalents 100 g^{-1} of skin (FW).

The ferric reducing antioxidant power (FRAP) assay was assessed at 593 nm based on the formation of a colored ferrous-TPTZ complex after the reduction of ferric to ferrous ion (Benzie and Strain, 1996). A curve of Trolox was constructed and results were expressed as mmol Trolox equivalents 100 g^{-1} of skin (FW).

2.9 Phenylalanine ammonia lyase activity (PAL)

Whole frozen grape berries without seeds were ground with pestle and mortar in liquid nitrogen until pulverization. The obtained power (0.5 g) was homogenized with 1.5 mL of cold extraction buffer (0.1 M sodium borate buffer, pH 8.8, containing 5 mM 2-mercaptoethanol, 2 mM EDTA, and 3% PVPP). After 30 min at 8°C, the mixture was centrifuged at 23,145 *x* g for 20 min at 4°C. The supernatant was used to determine PAL activity by assessing the production of cinnamate at 290 nm after 0, 0.5, 1, 1.5, 2, and 2.5 h of incubation with L-phenylalanine at 40 °C (Pombo, Rosli, Martínez & Civello, 2011). Results were expressed as enzyme activity units (EAU) min^{-1} mg^{-1} protein, where EAU was defined as the amount of enzyme that changes one unit of absorbance min^{-1}.

2.10 Protein determination

Protein content of the enzymatic extracts was determined by the Bradford method (Bradford 1976), using bovine serum albumin as standard protein.

2.11 Statistical analyses

All date were submitted to factorial analyses of variance (5 irradiation doses x 3 time points), followed by Tukey's HSD test, when appropriate. The data were expressed as mean ± standard error of four replicates for each treatment and time point. Results were considered significantly different when $p<0.05$. Analyses were carried out using Statistica 9.0® (StatSoft, Inc., Tulsa, OK, USA).

3 Results and discussion

3.1 Quality assessment

Although the UV-C has a limited penetration in the plant tissues (Jagadeesh et al. 2011), some pulp quality parameters of 'Isabella grapes' were affected by the irradiation (Table 1). There was a significant interaction effect between the radiation dose and the storage time after irradiation on the TSS content and pH values of 'Isabella' grapes. Grapes treated with 2.0 and 4.0 kJ m^{-2} had a rapid increase (about 10%) on TSS content at the first day after treatment when compared with control and the other doses ($p<0.05$). However, this increase was transitory so that at the 3rd day after irradiation no difference was observed among groups and at the 5th day, grapes treated with 2.0 and 4.0 kJ m^{-2} had lower TSS values than the control ($p<0.05$; Table 1). In contrast, the behavior of the lowest doses (0.5 and 1.0 kJ m^{-2}) was similar to that of the control, which had slow increase in TSS along the time, showing TSS values significantly higher at the 5th day compared to the previous days of storage.

Some studies found no effect of UV-C on the TSS, pH or acidity values of blueberry and starfruit (Perkins-Veazie, Collins, & Howard, 2008; Andrade-Cuvi, Moreno-Guerrero, Henríquez-Bucheli, Gómez-Gordillo & Concellón, 2010). However,

boysenberries and pears had increased TSS content after UV-C treatment (Vicente et al., 2004; Li et al., 2010), whereas strawberries had a rapid and transient increase in sugar levels (Baka, Mercier, Corcuff, Castaigne & Arul, 1999), as observed in the present study. The hydrolysis of cell wall polysaccharides also may contribute to this increase in TSS (Bolouri-Moghaddam et al., 2010). Soluble sugars do not act only as plant nutrients but also as signaling molecules and may be involved in mechanisms of ROS removal (Couée et al., 2006). The flux of soluble sugars through the oxidative pentose-phosphate pathway provides NADPH, which increase the cellular reducing and antioxidant potential (Bolouri-Moghaddam et al., 2010). Thus, the accumulation of soluble sugars may be related to the stress induced by UV-C irradiation in 'Isabella' grapes and may contribute to the antioxidant defence against this stress.

The lowest UV-C doses had no effect on the pH values (Table 1). In contrast, the treatment with 2.0 and 4.0 kJ m^{-2} UV-C decreased pH values compared to the control ($p<0.05$; Table 1). This effect was observed for 4.0 kJ m^{-2} UV-C during all storage time and for 2.0 kJ m^{-2} UV-C after the 3rd day of storage.

Once UV-C can damage cell membrane, these results suggest a higher disruption of tissue and a major release of electrolytes (Martínez-Hernández et al. 2013), which would reduce the pH after exposure to the higher UV-C doses. A similar result was found for irradiated boysenberries (Vicente et al., 2004).

Table 1

Total soluble solids (TSS) and pH of ‘Isabella’ grapes after UV-C radiation.

UV-C (kJ m^{-2})	Day 1	Day 3	Day 5
		TSS (°Brix)	
Control	15.0 ± 0.3 ^{bB}	15.6 ± 0.3 ^{aB}	17.3 ± 0.2 ^{a,bA}
0.5	15.2 ± 0.1 ^{bB}	15.2 ± 0.2 ^{aB}	17.4 ± 0.5 ^{aA}
1.0	15.5 ± 0.1 ^{bB}	15.8 ± 0.2 ^{aB}	17.0 ± 0.4 ^{a,b,cA}
2.0	16.8 ± 0.2 ^{aA}	15.2 ± 0.2 ^{aB}	15.9 ± 0.1 ^{cB}
4.0	16.6 ± 0.3 ^{aA}	15.7 ± 0.1 ^{aA}	16.0 ± 0.2 ^{b,cA}
		pH	
Control	3.71 ± 0.04 ^{aA}	3.73 ± 0.02 ^{aA}	3.75 ± 0.06 ^{aA}
0.5	3.67 ± 0.04 ^{aA}	3.75 ± 0.04 ^{aA}	3.76 ± 0.02 ^{aA}
1.0	3.61 ± 0.05 ^{a,bB}	3.76 ± 0.01 ^{aA}	3.63 ± 0.03 ^{a,bA,B}
2.0	3.65 ± 0.02 ^{a,bA}	3.58 ± 0.04 ^{bA,B}	3.51 ± 0.02 ^{bB}
4.0	3.55 ± 0.02 ^{bA}	3.59 ± 0.04 ^{bA}	3.53 ± 0.03 ^{bA}

Results show the average of 4 replicates ± standard error. Means that have no common lower case letters within the same column and upper case letters within the same line are significantly different (p<0.05; Tukey’s HSD test).

3.2 Phenolic compounds

There was a significant effect of the storage time on the content of phenolic compounds. All samples showed an increase on total phenolics (813.6 ± 99.6 in the 1st day *vs*. 1016.4 ± 79.6 mg gallic acid equivalents 100 g^{-1} skin FW in the 5th day), flavonols (351.1 ± 58.4 in the 1st day *vs*. 496.9 ± 83.1 mg quercetin-3-rutinoside equivalents 100 g^{-1} skin FW in the 5th day) and anthocyanins (215.3 ± 35.4 in the 1st day *vs*. 272.2 ± 43.5 mg malvidin-3-glucoside equivalents 100 g^{-1} skin FW in the 5th day) content along the storage (p<0.05; Figure 1). In the same way, the anthocyanin content of 'Monastrell' wine grapes increased after 4 days of storage independent of UV-C treatment (Cantos et al., 2003).

UV light can induce the synthesis of UV-absorbing compounds, such as flavonoids and phenolic compounds, mainly in epidermal tissues of fruits (Bravo et al., 2012). There was a significant effect of the radiation dose on the total phenolic content of 'Isabella' grapes (p< 0.05; Figure 1A), but no interaction between the treatment and the storage time. Irradiation with 1.0 and 2.0 kJ UV-C m^{-2} enhanced the total phenolic content (~20%) of grape skin compared to the non-irradiated control samples (p<0.05), but 0.5 and 4.0 kJ m^{-2} had no effect (Figure 1A). This bell-shaped effect is similar to the results obtained for the phenolic and anthocyanin contents of blueberries and strawberries (Wang, Chen & Wang, 2009; Erkan, Wang, & Wang, 2008).

Flavonols are among the most abundant flavonoids in plants. They act as UV filters and can be accumulated in their glycosylated form after UV-B treatment (Gill & Tuteja 2010). However, UV-C treatment had no effect on the flavonol content of 'Isabella' grapes (Figure 1B), although there was a tendency of effect (p= 0.10).Similar results were found for red grape (*V. vinifera* × *V. amurensis* 'Beiquan') skin (Li et al. 2009), for some table grapes (Cantos et al., 2002) or in 'Monastrell' wine grapes

(Cantos et al. 2003). In contrast, the amount of quercetin derivatives in ‘Redglobe’ table grapes (*V. vinifera* L.) was increased by UV-C when grapes were stored at 4°C during 5 days, but not when grapes were stored at room temperature (Crupi et al., 2013).

Several studies agree that the induction of flavonoids is a specific acclimation response to different stress, supporting the hypothesis of the existence of different UV-signaling pathways in plant tissues (Jiang et al. 2010). Although our results do not support an effect of UV-C in the flavonol compounds content in ‘Isabella’ grapes, we cannot rule out that changes in some specific flavonol compounds could have been undetected during the assessment of total flavonol content.

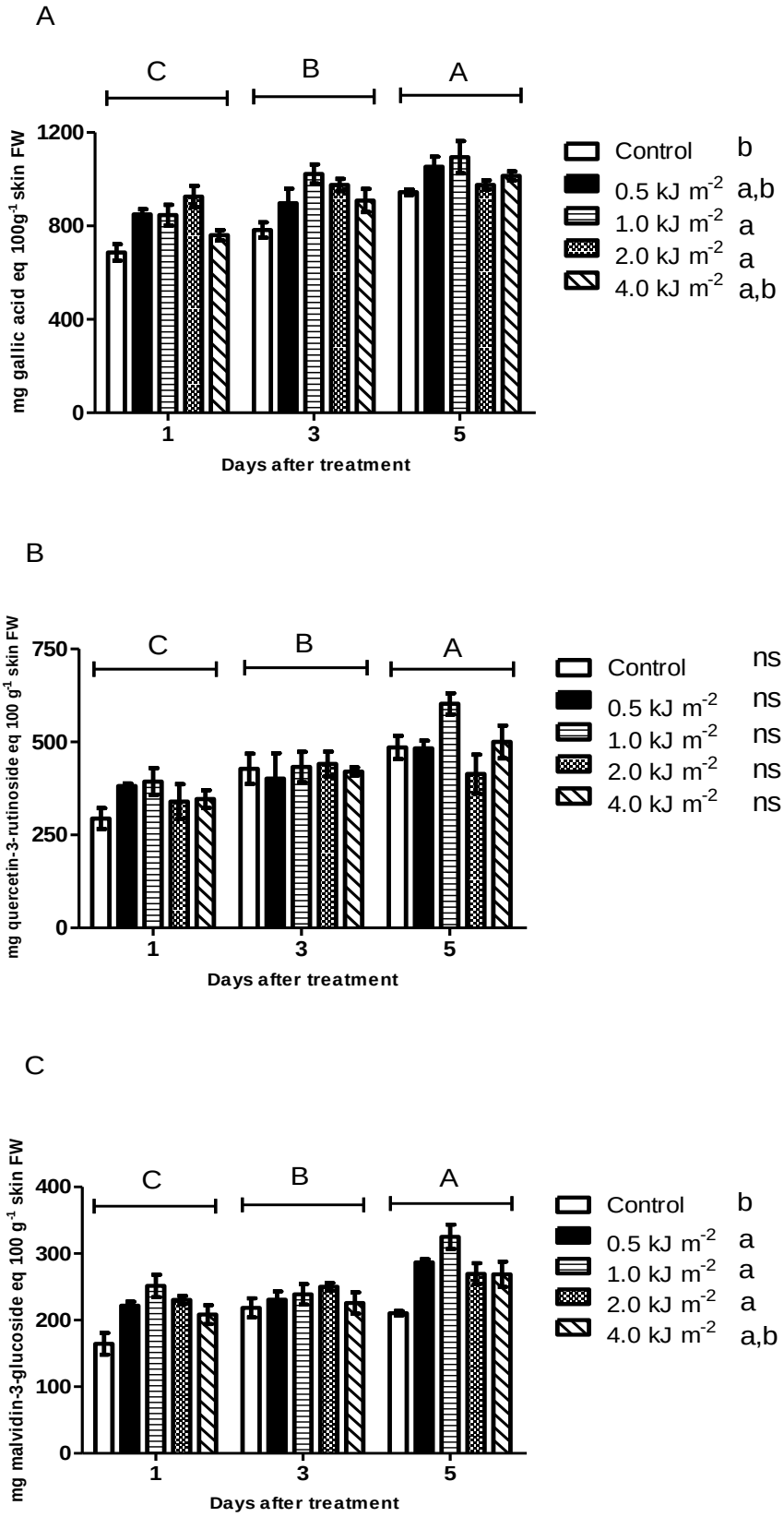

Figure 1. Effect of UV-C radiation on total phenolics (A), flavonols (B), and anthocyanin content (C) of 'Isabella' grapes. Results show the average of 4 replicates ± standard error. Capital letters above bars indicate significant differences among the days after treatment, whereas small letters in the right side of the treatment legend indicate significant differences among treatments ($p<0.05$; Tukey's HSD test).

The anthocyanin content of ‘Isabella’ grapes treated with all UV-C doses below 4.0 kJ m^{-2} was greater than control ($p<0.05$; Figure 1C). The average increase induced by these UV-C doses amounted to ~40% for the whole storage period. The accumulation of anthocyanin due to irradiation with UV-C exhibited a bell-shaped behavior in ‘Isabella’ grapes, because 1.0 kJ m^{-2} was the most effective dose. Previous studies in strawberries and blueberries using similar UV-C doses also showed a bell-shaped effect on the accumulation of anthocyanins (Erkan et al., 2008; Wang et al., 2009). According to the results obtained in this study the hormetic dose for 'Isabella' grape would be around 1.0 kJ m^{-2}, because higher doses had smaller or no effect on total phenolic and anthocyanin content.

In this study UV-C increased the content of total phenolics and anthocyanin compounds but not flavonols content in ‘Isabella’ grapes. The biosynthesis pathway of phenylpropanoids is common for anthocyanins and flavonols until the production of dihydroflavanols. Thus, we propose that this pathway may have diverted to the synthesis of anthocyanidins (by dihydroflavonol reductase and anthocyanidin synthase activities) after this step, since these compounds are abundant in grapes.

The effect of UV-C technology on the synthesis of antioxidant compounds and enzymes in fruits and vegetables is dependent on the cultivar (Charles & Arul 2007). UV-C treatment did not change non-stilbene phenolic compounds (anthocyanins, flavonols, and flavan-3-ols) in the skin of table (‘Flame’, ‘Red Globe’, ‘Crimson’, ‘Napoleon’, ‘Superior’, ‘Dominga’, ‘Moscatel Italica’) or wine (‘Monastrell’) grapes (Cantos et al., 2002; 2003). However, similar to our findings on ‘Isabella’ grapes, UV-C radiation increased the total content of anthocyanins in ‘Cabernet sauvignon’ grapes after 4-8 h of treatment and a similar increase with prolonging storage time after UV radiation was also observed (Zhang et al. 2012). In ‘Redglobe’ grapes the highest

concentrations of anthocyanins were observed after 24 hours of exposure to 4.1 kJ UV-C m^{-2} with no difference in storage at 4°C or at room temperature (Crupi et al., 2013).

3.3 Ascorbic acid (AA)

There was a significant interaction effect between the dose of radiation and the storage time after irradiation on the AA and the vitamin C contents. The level of these compounds decreased in all samples during the storage time ($p<0.05$; Table 2). At day 1, the AA content was lower in grape samples treated with 0.5 up to 2.0 kJ UV-C m^{-2}, whereas the vitamin C content was lower in samples treated with 0.5 and 1.0 kJ UV-C m^{-2} than in the control and 4.0 kJ m^{-2} treated samples ($p<0.05$; Table 2). However, this difference disappeared in the other storage times (Table 2).

The decrement in AA levels after UV-C irradiation of ‘Isabella’ grapes is associated to ascorbate degradation rather than to its oxidation, because vitamin C levels, which also include dehydroascorbate levels, also decreased. Results obtained by other studies for the content of AA in fruits after irradiation with UV-C are variable. Some studies found that UV-C had no effect (Artés-Hernández et al., 2010), retarded AA decrement (Lemoine, Chaves & Martínez, 2010; Jiang et al., 2010), increased (Li et al. 2010) or even decreased (González-Aguilar et al. 2007) the AA content along the storage.

Table 2

Ascorbic acid and total vitamin C content of ‘Isabella’ grapes after UV-C radiation.

UV-C dose ($kJ\ m^{-2}$)	Ascorbic Acid ($mg\ kg^{-1}$)			Total Vitamin C ($mg\ kg^{-1}$)		
	Day 1	**Day 3**	**Day 5**	**Day 1**	**Day 3**	**Day 5**
Control	0.81 ± 0.04^{aA}	0.62 ± 0.03^{aB}	0.50 ± 0.02^{aC}	0.92 ± 0.03^{abA}	$0.68 \pm 0.03^{a,bB}$	0.54 ± 0.02^{aC}
0.5	0.65 ± 0.02^{bA}	$0.58 \pm 0.02^{aA,B}$	0.50 ± 0.01^{aB}	0.70 ± 0.02^{cA}	$0.68 \pm 0.01^{a,bA}$	0.53 ± 0.01^{aB}
1.0	0.64 ± 0.03^{bA}	$0.58 \pm 0.02^{aA,B}$	0.50 ± 0.03^{aB}	0.70 ± 0.04^{cA}	$0.63 \pm 0.01^{bA,B}$	0.54 ± 0.04^{aB}
2.0	0.64 ± 0.03^{bA}	0.62 ± 0.01^{aA}	0.52 ± 0.03^{aB}	0.77 ± 0.05^{bcA}	$0.72 \pm 0.02^{a,bA}$	0.55 ± 0.03^{aB}
4.0	0.81 ± 0.03^{aA}	$0.68 \pm 0.05^{aA,B}$	0.56 ± 0.02^{aB}	0.95 ± 0.03^{aA}	0.77 ± 0.05^{aB}	0.62 ± 0.03^{aB}

Results show the average of 4 replicates ± standard error. Means that have no common lower case letters within the same column and upper case letters within the same line are significantly different ($p<0.05$; Tukey’s HSD test).

3.4 Phenylalanine ammonia lyase (PAL) activity

Figure 2 shows the effect of storage time and UV-C treatment on PAL activity. There was a significant effect of the storage time and UV-C radiation on PAL activity ($p<0.05$), but no interaction between these factors. PAL activity diminished along the storage in all samples (47.7 ± 3.6 in the 1st day *vs*. 38.4 ± 2.7 and 32.4 ± 2.1 10^4 EAU min^{-1} mg $protein^{-1}$ in 3rd and 5th days after treatment, $p<0.05$), but no difference was observed between the 3rd and the 5th day. The PAL activity of 'Isabella' grapes treated with all UV-C doses below 4.0 kJ m^{-2} was almost 2-fold greater than control ($p<0.05$). This enhanced PAL activity was parallel to the increase in total phenolic and anthocyanin content after UV-C treatment of 'Isabella' grapes and may be responsible for the accumulation of these phytochemicals, because PAL is key enzyme in the phenylpropanoid pathway (MacDonald & D'Cunha, 2007). Similarly, increased PAL expression and activity has been reported in strawberries and pears after exposure to UV-C (Nigro et al., 2000; Li et al., 2010).

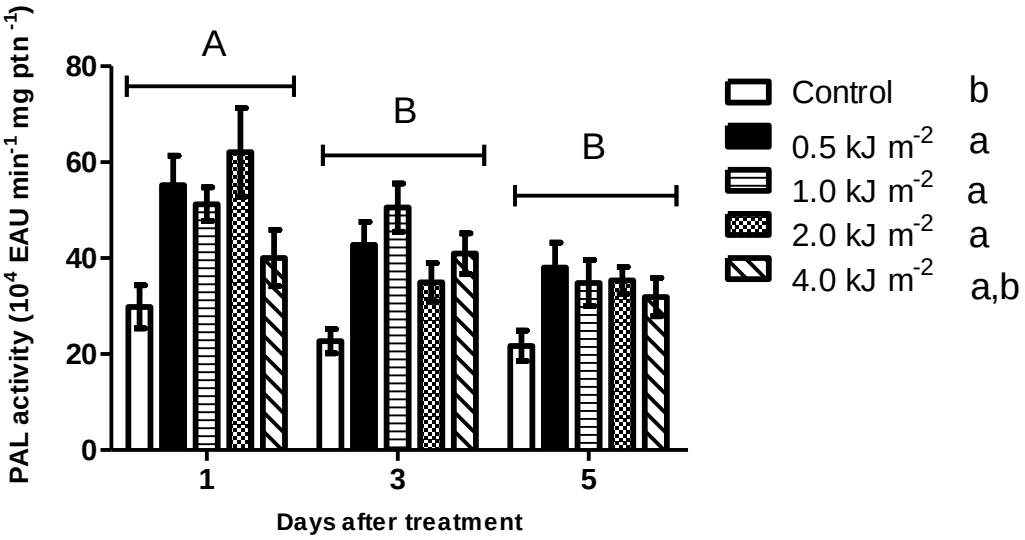

Figure 2. Effect of UV-C radiation on phenylalanine ammonia lyase (PAL) activity from 'Isabella' grapes. Results show the average of 4 replicates ± standard error. Capital letters above bars indicate significant differences among the days after treatment, whereas small letters in the right side of the treatment legend indicate significant differences among treatments ($p<0.05$; Tukey's HSD test).

3.5 Antioxidant capacity

There was significant interaction effect between the radiation dose and the storage time after irradiation on the antioxidant capacity of 'Isabella' grapes evaluated by FRAP and DPPH assays ($p<0.05$).

Except for grapes treated with a dose of 4.0 kJ m^{-2}, after one day of treatment, all treated grapes showed higher antioxidant capacity assessed by the DPPH method than the control ($p<0.05$; Figure 3A). No difference between control and UV-C-treated grapes was observed at the 3rd day after treatment, but at the 5th day after treatment only grapes treated with 1.0 kJ m^{-2} had higher DPPH antioxidant capacity than control ($p<0.05$).

In the 1st day, UV-C treated grapes showed higher antioxidant capacity assessed by the FRAP method than control ($p<0.05$; Figure 3B). In addition, the FRAP values of all samples increased along the storage time ($p<0.05$; Figure 3B). The lowest UV-C dose (0.5 kJ m^{-2}) improved the antioxidant capacity only at the 1st day, whereas the other doses maintained the increased FRAP values until the 3rd day after treatment ($p<0.05$; Figure 3B). Five days post-treatment no significant differences in the FRAP values were observed among samples (Figure 3B).

The effects of UV-C treatment and storage time on the antioxidant capacity were parallel to the increase in the content of total phenolic and anthocyanin compounds (Figure 1A and C). Thus, the accumulation of these phytochemicals may underlie the increased antioxidant capacity of 'Isabella' grapes. In fact, the antioxidant activity assessed by the DPPH and FRAP assays had a positive correlation with total phenolics (r= 0.74 and 0.87, respectively; $p<0.05$), anthocyanins (r= 0.77 and 0.73, respectively; $p<0.05$), and flavonols (r= 0.61 and 0.78, respectively; $p<0.05$). In contrast, ascorbic acid and total vitamin C content decreased after UV-C treatment (except 4.0 kJ m^{-2} dose) and along the storage, and therefore, ascorbic acid is unlikely to contribute to the UV-C-induced increase in the antioxidant

capacity of ‘Isabella’ grapes. Ascorbic acid and vitamin C were negatively correlated to FRAP values (r= -0.64 and -0.66, respectively; $p<0.05$) and did not present significant correlation with DPPH value. Previous studies have not investigated the effects of UV-C on the antioxidant capacity of grapes. However, irradiation with UV-C has been shown to enhance the antioxidant capacity assessed by the FRAP, DPPH, and ORAC methods in various fruits including blueberries (Perkins-Veazie et al., 2008), tomatoes (Bravo et al., 2012), mangoes (González-Aguilar et al., 2007), and strawberries (Erkan et al., 2008). In most studies, the effect of UV-C treatment was parallel to the increase in the content of phenolic compounds as observed in our study (total phenolic and anthocyanin compounds; Figure 1A and C).

The UV-C stress generates reactive oxygen species, which can activate some enzymatic and/or non-enzymatic antioxidant systems of fruits and contribute to an adaptation process to the stressful conditions and a better antioxidant potential (González-Aguilar et al. 2010). Thus, the increase in total phenolic compounds and anthocyanins reported in this work may be part of the defence mechanism of ‘Isabella’ grapes against the stress induced by irradiation with UV-C. This increase, which seems to be mediated by the increased activity of the PAL, may contribute to the increased antioxidant capacity of irradiated ‘Isabella’ grapes, because these compounds have reactive species scavenging capacity.

Most of the health benefits associated with the consumption of red berries has been attributed to their high content of anthocyanins and polyphenols (Crupi et al., 2013; Scalbert et al., 2005). Thus, methods able to enhance these bioactive compounds would be useful to improve the nutraceutical potential of fruits.

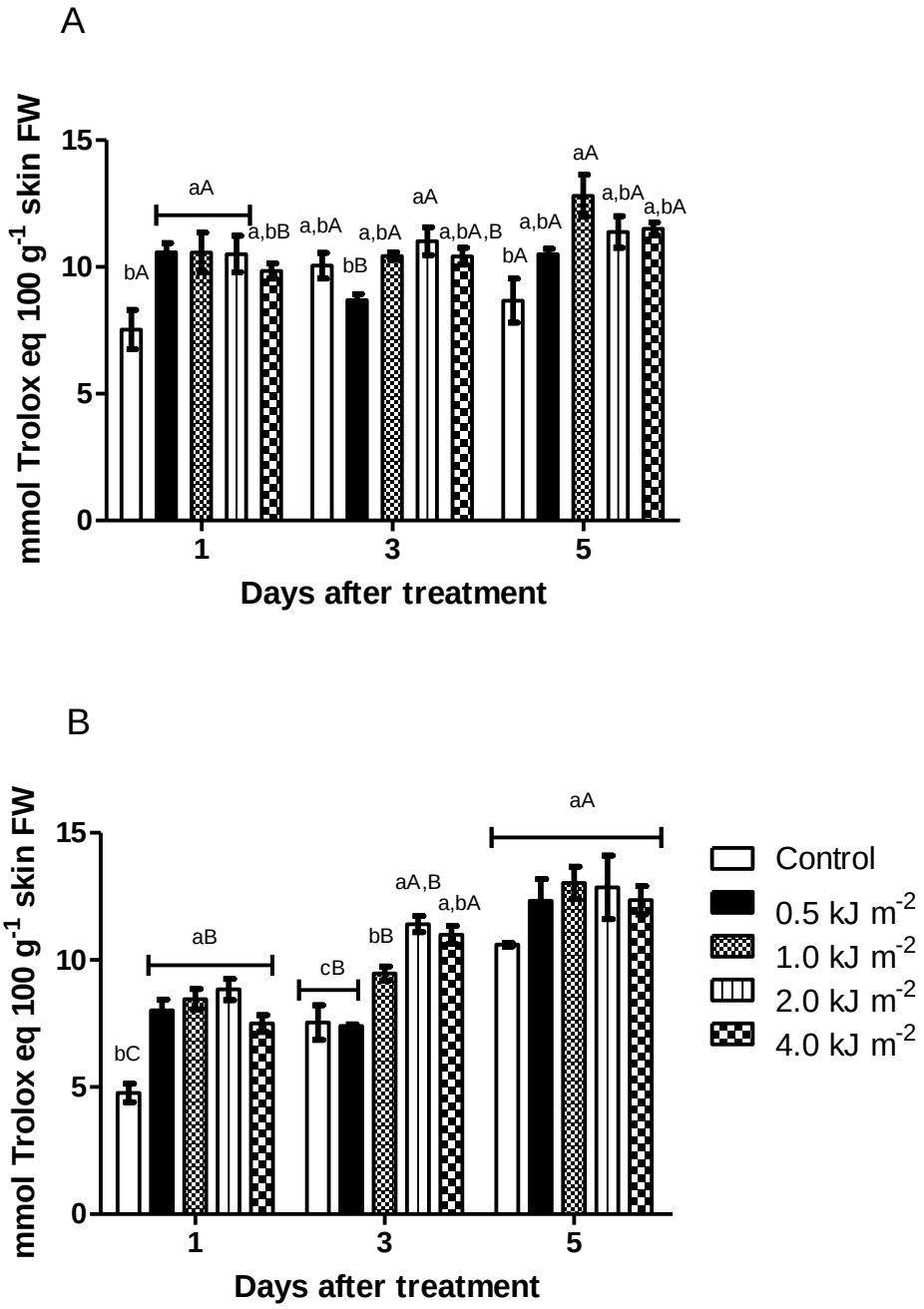

Figure 3. Effect of UV-C radiation on antioxidant capacity by DPPH (A) and FRAP (B) assays. Results are the average of 4 replicates ± standard error. Bars that have no common lower case letters within the same day after treatment and upper case letters within the same UV-C dose are significantly different ($p<0.05$; Tukey's HSD).

4 Conclusion

UV-C radiation was effective in increasing the synthesis of phenolic compounds and improving the antioxidant capacity in 'Isabella' grapes. The hormetic dose established in the studied conditions was 1.0 kJ m^{-2}. Thus, this technology can be an alternative to increase the content of phytochemicals with nutraceutical potential.

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4 CONCLUSÃO

A aplicação pós-colheita de radiação UV-C promoveu um aumento no teor de compostos fenólicos totais e antocianinas da casca de uvas ‘Isabel’, enquanto o teor de flavonois aumentou significativamente apenas em função do tempo de armazenagem. Os teores de ácido ascórbico e de vitamina C total diminuíram em função do tratamento com UV-C. As uvas submetidas à irradiação com UV-C também apresentaram um aumento significativo na atividade da enzima fenilalanina amônia liase e uma capacidade antioxidante superior logo após o tratamento. As maiores doses de UV-C levaram a um aumento no conteúdo de sólidos solúveis totais e a um decréscimo nos valores de pH. A dose hormética encontrada nas condições estudadas foi 1 kJ m^{-2}. A partir disso, pode-se concluir que a radiação UV-C pode ser usada com sucesso como um tratamento pós-colheita para aumentar o teor de fitoquímicos com propriedades funcionais em uvas ‘Isabel’.

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6 APÊNDICES

Apêndice A. Imagem de uvas ‘Isabel’ e das bagas dispostas em calhas de alumínio.

Apêndice B. Imagem da câmara de irradiação utilizada.

7 ANEXOS

Anexo A - Normas para a publicação de artigos científicos submetidos à revista *International Journal of Food Science and Technology*.

Author Guidelines
Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Submission of Manuscripts, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance.
Relevant Documents: Colour Work Agreement Form
Useful Websites: Submission Site, Author Services, Wiley's Ethical Guidelines, Guidelines for Figures

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The Editor welcomes the submission of original articles relevant to the science and technology of food and beverages. Contributions are accepted on the strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. Topics of only narrow local interest will not be accepted unless they have wider potential or consequences. If accepted, papers will become the copyright of the journal. Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in the *International Journal of Food Science & Technology*. Authors are encouraged to visit Wiley-Blackwell's Author Services for further information on the preparation and submission of articles and figures.

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