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CAROLINA FAGUNDES ASSUMPÇÃO

RADIAÇÃO UV-B SUPLEMENTAR: FERRAMENTA PARA MODULAÇÃO DE COMPOSTOS BIOATIVOS EM FRUTAS E HORTALIÇAS

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RADIAÇÃO UV-B SUPLEMENTAR: FERRAMENTA PARA MODULAÇÃO DE COMPOSTOS BIOATIVOS EM FRUTAS E HORTALIÇAS

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos como requisito parcial para a obtenção de grau de Doutor em Ciência e Tecnologia de Alimentos.

Orientador: Prof. Dr. Alessandro de Oliveira Rios

Co-orientador: Profª. Drª. Simone Hickmann Flôres

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TESE

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RESUMO

As diferentes intensidades de luz ou até mesmo a sua qualidade podem desempenhar um papel importante em algumas das principais vias metabólicas envolvidas na síntese de compostos bioativos. A radiação ultravioleta B (UV-B), além de influenciar mudanças no DNA, na atividade fotossintética e no crescimento das plantas, pode induzir a síntese e o acúmulo de metabólitos secundários. Assim, para investigar a efetividade da radiação UV-B suplementar na pós-colheita, cáqui (*Diospyros kaki*) e goiaba (*Psidium guajava*) foram submetidos a 48 horas de tratamento e posteriormente analisados em relação ao seu conteúdo de carotenoides. O acúmulo de carotenoides ocorreu de forma significativa para ambas as frutas, porém em momentos diferentes. A fim de entender os efeitos exercidos pela radiação UV-B suplementar em alimentos fontes de outros compostos bioativos, maçãs (*Malus domestic*a) foram submetidas a 36 horas de tratamento e acompanhadas por 21 dias de armazenamento. Os parâmetros de qualidade durante o armazenamento das frutas não foram influenciados pela radiação UV-B, ocorrendo apenas perda de firmeza e de peso em todas as frutas. As diferentes classes de compostos fenólicos identificados e quantificados por HPLC-MS apresentaram comportamentos diversos após o tratamento. Ácidos hidroxicinâmicos e antocianinas foram positivamente afetados pela suplementação de radiação UV-B. Para avaliar os efeitos da radiação UV-B suplementar sobre os compostos bioativos durante a pré-colheita de alimentos, alfaces verdes e roxas (*Lactuca sativa*) foram submetidas a 1 hora de tratamento por dia durante duas semanas. O conteúdo de carotenoides nas alfaces verdes e de compostos fenólicos nas alfaces roxas foi significativamente maior após o tratamento com radiação suplementar. Neste contexto, a radiação UV-B pode ser considerada uma tecnologia promissora no que diz respeito à modulação de compostos bioativos em alimentos, tanto durante o cultivo quanto após a colheita.

Palavras-chave: radiação UV-B suplementar; carotenoides, compostos fenólicos, HPLC-MS

ABSTRACT

Different light intensities or even their quality may play an important role in some of the major metabolic pathways involved in the synthesis of bioactive compounds. In addition to influencing changes in DNA, photosynthetic activity and plant growth, ultraviolet B radiation (UV-B) may induce the synthesis and accumulation of secondary metabolites. Therefore, to investigate the effectiveness of post-harvest UV-B radiation, kaki (*Diospyros kaki*) and guava (*Psidium guajava*) were submitted to 48 hours of treatment and then analyzed for their carotenoid content. The accumulation of carotenoids occurred in a significant way for both fruits, but at different times. In order to understand the effects exerted by supplemental UV-B radiation on food sources of other bioactive compounds, apples (*Malus domestica*) were subjected to 36 hours of treatment and accompanied by 21 days of storage. The quality parameters during fruit storage were not influenced by UV-B radiation, with only loss of firmness and weight occurring in all fruits. The different classes of phenolic compounds identified and quantified by HPLC-MS showed different behavior after treatment. Hydroxycinnamic acids and anthocyanins were positively affected by the supplementation of UV-B radiation. To evaluate the effects of supplemental UV-B radiation on bioactive compounds during food cultivation, green and red lettuces (*Lactuca sativa*) were subjected to 1 hour of treatment per day for two weeks. The carotenoid content in green lettuce and phenolic compounds in red lettuce was significantly higher after treatment with supplementary radiation. In this context, UV-B radiation can be considered a promising technology for the modulation of bioactive compounds in food, both during and after harvest.

Keywords: supplemental UV-B radiation; carotenoids, phenolic compounds, HPLC-MS

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CAPÍTULO 1

1. INTRODUÇÃO

Estudos epidemiológicos estimam que o consumo frequente de frutas e hortaliças pode reduzir de forma significativa o risco de doenças cardiovasculares e cânceres devido ao alto conteúdo de compostos bioativos encontrados nesses alimentos. Assim, cada vez mais as atenções se voltam à métodos que possam preservar ou até mesmo aumentar a concentração de fitoquímicos de frutas e hortaliças por ferramentas moleculares e não moleculares.

Entre as ferramentas não moleculares, os tratamentos realizados na pós-colheita de frutos com indutores; baixa ou alta temperatura; fito-hormônios e radiação ultravioleta, podem levar ao acúmulo de nutracêuticos nestes alimentos vegetais (SCHREINER e HUYSKENS-KEIL, 2006). Neste contexto, a modificação da intensidade da luz e/ou de sua qualidade é particularmente promissora devido ao papel desempenhado por esta sobre algumas das principais vias metabólicas envolvidas na síntese de compostos bioativos (CASTAGNA *et al.*, 2013). A radiação que excede a habilidade fotossintética da planta pode induzir a síntese e o acúmulo de metabólitos secundários (TROJAK e SKOWRON, 2017).

A radiação ultravioleta B é capaz de induzir mudanças complexas da capacidade de reparação do DNA, na atividade fotossintética, no crescimento e morfologia da planta, bem como na expressão gênica, concentração de compostos nutracêuticos e resistência a fungos (FROHNMEYER e STAIGER, 2003). Dessa forma, a aplicação de radiação UV-B em frutos e hortaliças é particularmente interessante, podendo viabilizar o aumento da concentração de compostos de interesse e produzir alimentos mais ricos (nutricionalmente) e de melhor qualidade.

Na indústria de alimentos, um dos compostos bioativos passivos de incremento via radiação UV-B são os carotenoides. Estes corantes alimentares naturais possuem importantes funções de promoção da saúde, com ação antioxidante e por ser precursor da vitamina A no organismo humano, com aumento da atividade do sistema imunológico e diminuição do risco de doenças degenerativas (STRATI e OREOPOULOU, 2011).

Outro grupo de compostos bioativos que podem ser afetados positivamente pela suplementação de radiação UV-B são os compostos fenólicos. Dentre eles, as antocianinas destacam-se por responderem bem a esse tipo de tratamento e serem as responsáveis pela maioria das cores que varia entre vermelho, azul e roxo de frutas, hortaliças e flores. Além da

sua função como corante estes pigmentos são importantes na prevenção de doenças cardiovasculares, atuam como antioxidantes e anticancerígenos (JIANG *et al.*, 2016). Deste modo, este trabalho torna-se relevante por investigar o efeito de ferramentas não moleculares como a luz e a radiação UV-B sobre os compostos bioativos em frutas e hortaliças durante ao pré e pós-colheita.

1.2. OBJETIVOS

1.2.1. OBJETIVO GERAL

Avaliar os efeitos da radiação UV-B suplementar no conteúdo de compostos bioativos de caqui, goiaba e maçã na pós-colheita e de alfaces durante o cultivo.

1.2.2. OBJETIVOS ESPECÍFICOS

- Avaliar a qualidade pós-colheita das frutas de cáqui (*Diospyros kaki*) e de goiaba (*Psidium guajava*) submetidos à radiação UV-B suplementar, além de identificar e quantificar carotenoides por Cromatografia Líquida de Alta Eficiência (CLAE);
- Avaliar a qualidade pós-colheita de cascas de maçãs (M*alus domestica*) submetidas à radiação UV-B suplementar, identificar e quantificar compostos fenólicos e atividade antioxidante durante o armazenamento;
- Avaliar os efeitos da radiação UV-B suplementar sobre os compostos bioativos de alfaces verdes e roxas (*Lactuca sativa*) na pré-colheita, bem como avaliar parâmetros de crescimento e desenvolvimento.

CAPÍTULO 2

2 REVISÃO BIBLIOGRÁFICA

2.1 COMPOSTOS BIOATIVOS X RADICAIS LIVRES

Frutas e hortaliças são ricos em compostos funcionais, como os compostos fenólicos, antioxidantes e carotenoides (AMODIO *et al.*, 2014). Acredita-se que um aumento no consumo desses alimentos possa ter impactos benéficos sob a saúde humana, uma vez que a relação positiva entre seu consumo e a redução de riscos de doenças cardiovasculares e doenças crônicas já foi relatada previamente em diversos estudos (DUTHIE *et al.*, 2003; WHO, 2003; SCALBERT *et al.*, 2005; HE *et al.*, 2007; BAE *et al.*, 2008; WRIGHT *et al.*, 2008).

Segundo Wootton-Beard e Ryan (2011), os compostos antioxidantes atuam no sequestro dos radicais livres e reduzem a incidência de patologias degenerativas, sendo que a capacidade antioxidante das frutas varia dependendo do seu conteúdo de vitaminas C e E, carotenoides, flavonoides e outros polifenois. Contudo, sugere-se que as contribuições dos compostos fenólicos às atividades antioxidantes sejam muito maiores que às da vitamina C, por exemplo (SCALBERT *et al.*, 2005). Tem sido demonstrado que a cooperação de antioxidantes com diferentes propriedades químicas, vitaminas, minerais, fenólicos e fibras pode exercer um efeito sinérgico, protegendo as células de danos como os ataques de espécies reativas de oxigênio (ROS) e de nitrogênio (RNS) (SAURA-CALIXTO e GOÑI, 2006; CONTRERAS-CALDERÓN *et al.*, 2011).

Dados prévios indicam que metabólitos de plantas, incluindo polifenois, flavonoides e terpenos, exercem ação antioxidante, sendo relatados como inibidores da propagação das reações de radicais livres (ROS) e, assim, como protetores do organismo humano contra doenças (MOON *et al.*, 2013). Polifenois, por exemplo, podem sequestrar H_2O_2 e reduzir os danos oxidativos ao DNA ou reparar rapidamente o dano resultante (TAN *et al.*, 2009).

Geralmente os antioxidantes protegem as células das plantas dos danos causados pelos radicais livres, que se desenvolvem com o metabolismo celular normal ou devem-se a eventos de stress, como o excesso de luz e poluição. As propriedades dos compostos fenólicos podem estar envolvidas na desativação de ROS e de RNS, inibindo sua formação pela

eliminação de enzimas ou metais associados à produção dos radicais livres (TRIVELLINI *et al.*, 2016).

Em baixas concentrações, ROS cumprem funções fisiológicas cruciais em uma planta, como a manutenção da concentração de peróxido de hidrogênio (H_2O_2), a qual é utilizada na biossíntese da parede celular e na defesa contra patógenos. A altas concentrações, entretanto, ROS podem ter efeitos prejudiciais pelo dano a membranas, proteínas, clorofilas e ácidos nucléicos. Estresse ambiental e biótico como sais, radiação ultravioleta (UV), seca, danos físicos e ataques de patógenos são conhecidos como causadores de aumento da produção de ROS nas células (TANG *et al.*, 2010).

A produção de ROS e outros radicais livres é normalmente compensada por um sistema antioxidante endógeno complexo. Entretanto, devido a fatores ambientais e patológicos, um excesso de radicais pode se acumular nas células e resultar em estresse oxidativo. Este excesso de ROS é nocivo devido à sua alta reatividade, uma vez que atacam macromoléculas biológicas que induzem à oxidação e causam danos à membrana e ao DNA, além de inativação enzimática (MOON *et al.*, 2013).

A habilidade de plantas de sequestrar ou inativar espécies reativas tóxicas de oxigênio por meio do sistema antioxidante endógeno parece ser um importante fator determinante da sua tolerância ao estresse ambiental. Para prevenir injúrias, as plantas têm desenvolvido diferentes mecanismos pelos quais podem converter ROS em produtos menos tóxicos. Esses mecanismos de defesa são baseados em compostos metabólicos e enzimas, dentre os quais estão os metabólitos com propriedades antioxidantes ascorbato e glutationa (TANG *et al.*, 2010).

A intensidade da resposta da planta aos fatores de estresse pode variar dependendo da sua tolerância e capacidade antioxidante. Problemas hídricos, intensidade de radiação ultravioleta e a presença de patógenos podem causar estresse oxidativo pela geração de ROS e RNS (SANDRE *et al.*, 2014).

Um fato importante a se considerar é que a exposição da planta a altas concentrações de ozônio pode levar à degradação da clorofila, bem como a formação de necrose e acúmulo de metabólitos secundários (HEATH *et al.*, 2009). Um mecanismo antioxidante tem sido bem descrito como essencial no sequestro do excesso de ROS nas células, incluindo antioxidantes enzimáticos, como superóxido dismutase (SOD), catalase (CAT) e peroxidase (POD), o que torna essas enzimas importantes para a defesa antioxidante da planta (BOOKER *et al.*, 2012).

Substâncias fenólicas, como antocianinas e taninos, são capazes também de agir como antioxidantes não enzimáticos (POLLASTRI e TATTINI, 2011). A alta capacidade

antioxidante desses flavonoides deve-se à sua capacidade de sequestrar radicais livres pela doação de um átomo de hidrogênio da hidroxila fenólica para estabilizar o radical formado (BOOKER *et al.*, 2012). Compostos fenólicos como flavonoides se situam geralmente em vacúolos na forma de glicosídeos ou glutationa conjugados. Além disso, no apoplasto compostos fenólicos podem participar como doadores de elétrons, decompondo ozônio (O_3) produzindo radical hidroxila (OH^-) e oxigênio (O_2) (SANDRE *et al.*, 2014).

Segundo Gülçin (2006), os processos de peroxidação lipídica, que podem causar danos aos ácidos graxos e tendem a diminuir a fluidez da membrana, podem ser reduzidos pelos fenólicos encontrados em plantas – que são conhecidos por desativar espécies reativas. Os polifenois como antioxidantes podem exercer efeitos benéficos no sistema vascular como observado em estudos *in vitro* e *in vivo* de Vauzour *et al.*(2010). O consumo regular de polifenois por meio da dieta também tem sido associado à redução de demência e ao atraso no desenvolvimento de Alzheimer e Parkinson, mostrando-se ótimos candidatos à neuroprotetores (TRIVELLINI *et al.*, 2016).

2.1.1. COMPOSTOS FENÓLICOS EM FRUTAS E HORTALIÇAS

Os metabólitos secundários são um grupo de compostos orgânicos que são produzidos pelas plantas para facilitar a interação com o meio ambiente e estabelecer um mecanismo de defesa. Muitos deles são terpenos, fenólicos e alcaloides classificados de acordo com sua origem biossintética, possuem diferentes atividades biológicas e são usados como aditivos, pesticidas e corantes (MURTHY *et al.*, 2014).

Os compostos fenólicos dividem-se em várias classes, segundo o esqueleto carbônico dos fitoquímicos, dentre as quais se destacam a dos ácidos fenólicos e a dos flavonoides. A capacidade antioxidante dos compostos fenólicos deve-se, principalmente, as suas propriedades redutoras, que dependem, fundamentalmente, do número e posição de hidroxilas presentes na molécula (RICE-EVANS *et al.*, 2002). As frutas, principais fontes dietéticas de polifenois, em função de fatores intrínsecos (cultivar, variedade, estádio de maturação) e extrínsecos (condições climáticas e edáficas) apresentam, em termos quantitativos e qualitativos, composição variada desses constituintes. A eficácia da ação antioxidante destes componentes depende não só de sua estrutura química, como também da concentração destes fitoquímicos no alimento (MELO *et al.*, 2008).

Os compostos fenólicos são sintetizados pelas plantas durante o desenvolvimento e em resposta a condições de stress, como infecções e radiação ultravioleta, entre outras

(BECKMAN, 2000). Estes compostos são um grupo muito diversificado de fitoquímicos derivados da fenilalanina e tirosina (SHAHIDI e NACZK, 2004).

Os fenólicos das plantas incluem fenóis simples, ácidos fenólicos (benzóicos, cinâmicos e derivados), cumarinas, flavonoides, estilbenos, taninos hidrolizáveis e condensados, lignanas e ligninas. Em plantas, além da ação antioxidante, os fenólicos podem atuar como fitoalexinas, atraidores de polinizadores, contribuidores para a pigmentação da planta e agentes preventivos contra a luz UV (SHAHIDI e NACZK, 2004). Em alimentos, os fenólicos podem contribuir para a adstringência, amargor, cor, sabor, odor e estabilidade oxidativa dos produtos. Além disso, a capacidade de proteger a saúde de algumas propriedades antinutricionais de outros fenólicos é de grande importância aos produtores, processadores e consumidores (SHAHIDI e NACZK, 2004).

Os fenólicos não são distribuídos uniformemente nas plantas a níveis tecidual, celular e subcelular, sendo os insolúveis componentes das paredes celulares, enquanto os fenólicos solúveis são os que estão compartimentalizados nos vacúolos celulares. A nível tecidual, as camadas mais externas das plantas contêm altos níveis de fenólicos em relação as partes mais internas. Os fenólicos da parede celular, como as ligninas (polímeros de monolignol) e ácidos hidroxicinâmicos são ligados a vários componentes celulares (BAUCHER *et al.*, 1998). Estes compostos contribuem para a resistência mecânica das paredes celulares e desempenham um papel regulatório no crescimento celular, na morfogênese e na resposta celular ao stress e patógenos (NACZK e SHAHIDI, 2004).

Flavonoides diglicosilados são também bastante encontrados, sendo que os açúcares são geralmente substituídos mais tarde por resíduos acil como malonato ou acetato. Flavonoides são tidos como glicosídeos quando contêm um ou mais grupos de açúcar (ou glucosídeos), e como agliconas quando nenhum açúcar está presente. Considera-se a existência de mais de 4000 flavonoides conhecidos divididos em 12 subclasses, incluindo mais de 3000 flavonas e mais de 700 isoflavonas em plantas. Consequentemente, a separação, identificação e determinação de flavonoides é um desafio. Eles recebem atenção considerável na literatura, especialmente pela sua importância biológica e fisiológica. Os flavonoides de plantas estão envolvidos na resposta aos mecanismos de stress, como os causados pela elevada radicação UV-B, infecções por microrganismos, entre outros (RIJKE *et al.*, 2006).

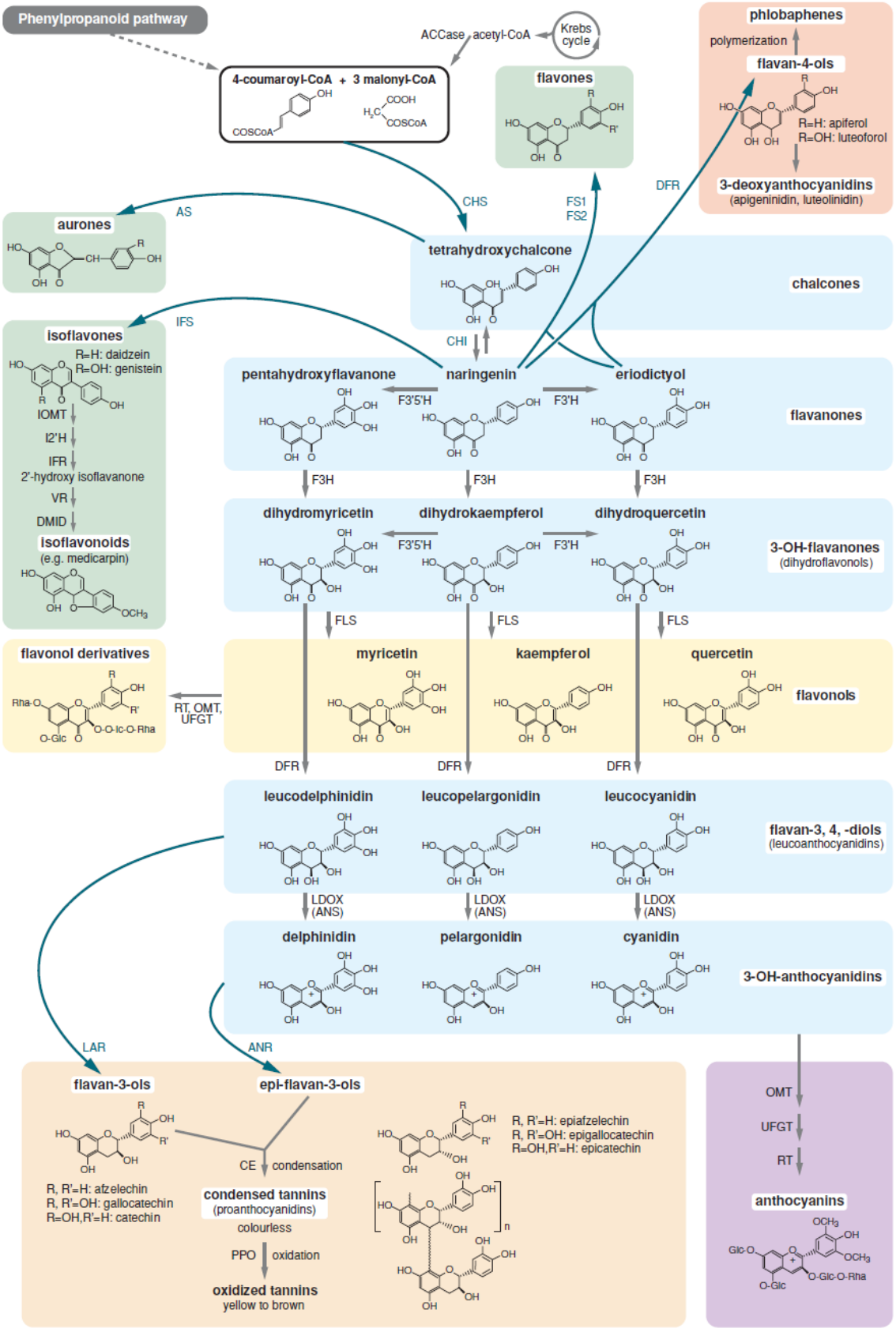

Figura 1. Via de biossíntese dos flavonoides.

Fonte: Lepiniec et al. (2006).

Os ácidos fenólicos são diferentes dos outros compostos fenólicos pelo seu comportamento e propriedades ácidas, provenientes da presença de grupo ácido carboxílico. Devido a presença deste grupamento, bem como de grupos hidroxila nas suas estruturas, os ácidos fenólicos são capazes de formar ligações éster e éter com outros compostos, o que conduz à formação de ligações com polissacarídeos de parede celular. Os ácidos fenólicos podem também estar esterificados com moléculas pequenas como álcoois, outros ácidos fenólicos, fenóis e alcaloides (MADHUJITH e SHAHIDI, 2009)

Entre os compostos fenólicos, as antocianinas destacam-se por conferirem coloração às plantas. Estes são os pigmentos mais importantes de plantas vasculares e possuem significativa atividade antioxidante, que desempenha um papel importante na prevenção de doenças neuronais e cardiovasculares, câncer e diabetes (KONCZAK e ZHANG, 2004). Há vários relatos sobre o efeito das antocianinas no tratamento do câncer (LULE e XIA, 2005; NICHENAMETLA *et al.*, 2006), nutrição humana (STINTZING e CARLE, 2004) e sua atividade biológica (KONG *et al.*, 2003).

As antocianidinas (ou agliconas) são as estruturas básicas das antocianinas e consistem de um anel aromático ligado a um anel heterocíclico que contém oxigênio e é ligado a um terceiro anel aromático (KONCZAK e ZHANG, 2004). Quando as antocianidinas são encontradas na sua forma glicosilada (ligada a uma molécula de açúcar) elas são conhecidas como antocianinas.

Há uma grande variedade de antocianinas espalhadas na natureza e as principais diferenças entre elas são o número de grupos hidroxilados, a natureza e o número de açúcares ligados à sua estrutura, bem como a posição destas ligações (KONG *et al.*, 2003). Sugere-se até o momento a existência de mais de 500 antocianinas diferentes e 23 antocianidinas das quais apenas seis são mais comuns em plantas vasculares: pelargonidina, peonidina, cianidina, malvidina, petunidina e delfinidina (ANDERSEN e JORDHEIM, 2006).

Os glicosídeos derivados das três antocianidinas não metiladas (cianidina, delfinidina e pelargonidina) são os mais comuns na natureza, sendo encontrados em 80 % das folhas pigmentadas, 69 % das frutas e 50 % das flores. As antocianinas isoladas são altamente instáveis e muito susceptíveis à degradação (GIUSTI e WROLSTAD, 2003). Sua estabilidade é afetada por vários fatores como pH, temperatura, estrutura química, concentração, luz, oxigênio, solventes, presença de enzimas, flavonoides, proteínas e íons metálicos (REIN, 2005).

A estabilidade das antocianidinas é influenciada pelos substituintes do anel B e pela presença adicional de hidroxilas ou metoxilas que diminuem a estabilidade da aglicona em meio neutro; portanto a pelargonidina é a antocianidina mais estável. Ao contrário das agliconas, os derivados de monoglicosídeos e de diglicosídeos são mais estáveis em condições neutras de pH. Este comportamento pode ser explicado pelo fato de as moléculas de açúcar evitarem a degradação de intermediários instáveis em ácidos fenólicos e aldeídos (FLESCHHUT *et al.*, 2006).

Investigações sobre a estabilidade de antocianinas e a variação de cor com o pH levam a crer que as mudanças de cor destes compostos são mais significativas na região alcalina devido à sua instabilidade (CABRITA *et al.*, 2000).

Os compostos que são mais fáceis de sofrer oxidação são, geralmente, os melhores antioxidantes (moléculas que podem doar um elétron livre ou átomos de hidrogênio a radicais livres reativos). Muitos estudos têm sugerido que o conteúdo de antocianinas e sua atividade antioxidante correspondente contribuem para o efeito protetor da ingestão de frutas e vegetais contra doenças degenerativas e crônicas. Além disso, alguns extratos de plantas e frutas com altos teores de compostos fenólicos têm sido relatados como agentes inibidores da mutagênese e da carcinogênese (CASTAÑEDA-OVANDO *et al.*, 2009).

A síntese de antocianinas está intimamente relacionada à via metabólica dos flavonoides, transformação do aminoácido fenilalanina com as vias do ácido chiquímico e malônico. O primeiro, envolvido na biossíntese de aminoácidos é responsável pela estrutura do anel B e a formação da ponte tricarbono (Figura 1). A via do ácido malônico está relacionada à síntese do anel A (STRZAŁKA, 2002; PETRONI e TONELLI, 2011).

Plantas como arabidopsis, milho e petúnia, são modelos para estudo da biossíntese de antocianinas e das enzimas envolvidas na via. Há seis enzimas principais na via biossintética das antocianinas: chalcona sintase (CHS), chalcona isomerase (CHI), flavanona 3-hidroxilase (F3H), flavonoide 3’-hidroxilase (F3'H), dihidroflavonol 4-redutase (DFR), e antocianidina sintase (ANS), e são responsáveis por sintetizar antocianinas de diferentes cores (ZHANG *et al.*, 2016).

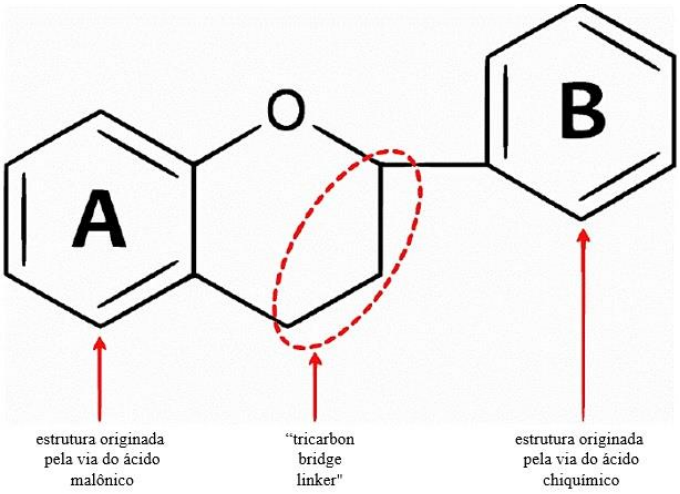

Figura 2. Estrutura básica dos flavonoides.

Fonte: modificado de Strzałka (2002).

A abundância de energia solar que atinge a Terra é a principal força que sustenta quase todas as formas de vida. Não mais de 4,5-5 % da energia disponível pode ser armazenada em produtos fotossintéticos (STRZAŁKA, 2002). Entretanto, mesmo pequenas quantidades podem ser excessivas para a planta e causar danos ao aparato fotossintético. A maioria das plantas absorve mais luz do que o necessário e esse excesso de luz resulta no aumento da disparidade entre energia gerada e usada e finalmente inibe a fotossíntese (fotoinibição) (LONG *et al.*, 1994). Se essa fotoinibição for durarouda, a produtividade da planta cai e inicia-se a geração de ROS. Portanto, a síntese de antocianinas faz parte de uma série de mecanismos protetores desenvolvidos pelas plantas para limitar a intensidade da luz que atinge as moléculas de cloforila (WEGER *et al.*, 1993; SMILLIE e HETHERINGTON, 1999).

Além da luz, o aumento do acúmulo de antocianinas notado como a pigmentação vermelha é também observado em reação à exposição à baixa (TROJAK e SKOWRON, 2017).

2.1.2. CAROTENOIDES EM FRUTAS E HORTALIÇAS

Os carotenoides estão entre os pigmentos naturais que conferem coloração amarela, laranja e vermelha às plantas. Estes compostos bioativos destacam-se pela presença de ligações duplas conjugadas que lhes conferem a capacidade de prover essas cores, além das suas propriedades antioxidantes (FRASER e BRAMLEY, 2004). Os carotenoides também

possuem outras ações relacionadas à promoção de saúde, como sua atividade pró-vitamínica A, o aumento da imunidade e a redução do risco de doenças degenerativas, como câncer e doenças cardiovasculares (STRATI e OREOPOULOU, 2011).

Substâncias naturais são geralmente adicionadas a muitos alimentos para conferir um apelo saudável. Entretanto, a incorporação do β-caroteno em alimentos apresenta alguns inconvenientes, como o fato de sua atividade antioxidante ser sensível a vários fatores, incluindo pH, temperatura e oxigênio. A luz, especialmente a UV (em comprimentos de onda abaixo de 425 nm), também desempenha um papel importante na degradação do β-caroteno, uma vez que esta é uma molécula fotossensível (GARCIA-FUENTES *et al.*, 2003). Quando a luz é absorvida, o sensitizador é convertido em um estado tripleto excitado que pode facilmente interagir com outra molécula e produzir radicais livres ou oxigênio singleto altamente reativo (LÓPEZ-RUBIO e LAGARON, 2011).

Carotenoides existem em todos os organismos fotossintetizadores, sendo componentes de complexos pigmento-proteína no aparato fotossintético onde estão intimamente associados com a clorofila. A sua função principal é captar a luz e transferir a energia de excitação via clorofilas para o fotossistema. Além disso, são fotoprotetores por quelar o estado tripleto da clorofila e dissipar a energia em forma de calor. Essa função previne a formação de oxigênio singleto, que é formado pela interação de um sensitizador tripleto excitado com outra molécula celular (LÓPEZ-RUBIO e LAGARON, 2011).

Os carotenoides são também capazes de inativar os demais radicais derivados de oxigênio (DAMBECK e SANDMANN, 2014), podendo sequestrar outras ROS, como ânions superóxidos, radicais hidroxila ou peróxido de hidrogênio. Sob certas circunstâncias, entretanto, como por exemplo, em altas pressões parciais de oxigênio, os carotenoides podem agir como pró-oxidantes (GORALCZYK e WERTZ, 2009).

Os carotenoides mais abundantes no organismo humano devido a ingestão de frutas e hortaliças são β-caroteno, α-caroteno e licopeno, bem como as xantofilas luteína, zeaxantina e α- e β-criptoxantina. Os carotenoides são transportados para a pele e acumulados principalmente nas camadas epidérmicas. A quantidade destes pigmentos depositada na pele está relacionada à dieta e à biodisponibilidade da fonte alimentar. Após a absorção, os carotenoides são transportados pela corrente sanguínea pelas lipoproteínas a vários tecidos alvo. Transportadores de colesterol como receptores sequestrantes classe B membro 1 (SR-B1) e grupos de diferenciação 36 (CD 36) foram relatados como facilitadores da absorção dos carotenoides no intestino (VAN BENNEKUM *et al.*, 2005). Dessa maneira, os carotenoides

seriam captados por esses transportadores na epiderme, que é um sítio ativo do acúmulo de colesterol para a manutenção da permeabilidade da barreira. SR-B1, por exemplo, é expresso na epiderme humana, predominantemente nas camadas basais (FERNÁNDEZ-GARCÍA, 2014).

2.2. CARACTERÍSTICAS DAS FRUTAS E HORTALIÇAS EM ESTUDO

Em um primeiro momento, este trabalho priorizou o estudo de frutas climatéricas. Estas frutas são caracterizadas por amadurecimento acompanhado de um distinto aumento na atividade respiratória. Uma série de mudanças bioquímicas iniciadas com a produção autocatalítica de etileno determina mudanças desde o crescimento à senescência e envolve o aumento da respiração levando ao amadurecimento (LADANIYA, 2008).

2.2.1. CAQUIS

Entre as frutas mais consumidas na região sul do Brasil e boa fonte de carotenoides, está o caqui (*Diospyros kaki* L.). Os caquis pertencem à família Ebenaceae e são nativos da Ásia, especialmente da China (TESSMER *et al.*, 2014), que tem a maior produção mundial dessa fruta: 3988957 toneladas em 2016 (FAOSTAT, 2017).

A casca dos caquis é fonte de polifenois e carotenoides que contribuem para a coloração alaranjada da fruta madura. Altas concentrações de carotenoides não só representam uma propriedade biológica importante como também favorecem a perspectiva mercadológica quanto aos alimentos ricos em compostos bioativos (VEBERIC *et al.*, 2010).

Como uma boa fonte de compostos bioativos, os caquis são ricos em taninos condensados (proantocianidinas), que tem a habilidade de formar complexos estáveis com metais e proteínas sendo responsáveis pela adstringência típica da fruta (VÁZQUEZ-GUTIERREZ *et al.*, 2011). Os cultivares adstringentes contêm taninos solúveis que ocorrem em altas concentrações até a maturação. Após essa etapa, o sabor doce predomina com os baixos níveis de taninos (LI *et al.*, 2011)

Zeaxantina, β-criptoxantina, α-caroteno e β-caroteno são os carotenoides de maior presença na casca e polpa de diversos cultivares de caquis. O carotenoide majoritário da casca de caquis é o β-caroteno, conforme dados de Daood *et al.* (1992), considerando apenas moléculas livres. Quando formas esterificadas são consideradas, o carotenoide mais expressivo pertence às xantofilas (VEBERIC *et al.*, 2010), como a β-criptoxantina. Na polpa

da fruta, o conteúdo de carotenoides é muito menor em relação à casca, apesar de o perfil ser o mesmo (Chitarra e Chitarra 2005).

2.2.2. GOIABA

A goiaba (*Psidium guajava*) é uma das frutas comerciais mais importantes nos países tropicais e subtropicais. Em países latino-americanos, na África e Ásia as goiabas possuem importância econômica. A Índia, China e Tailândia lideram a produção (ROJAS-GARBANZO *et al.*, 2017). Da família Myrtaceae e considerada a "maçã dos trópicos", a goiaba está associada a um alto valor nutritivo e é rica em antioxidantes como polifenois, ácido ascórbico e carotenoides. A quantidade e perfil destes compostos depende da variedade, maturação e ambiente em que a planta está inserida (MUSA *et al.*, 2015).

Os pigmentos presentes na coloração rosa da goiaba foram investigados por Mercadante *et al.*(1999), por Padula e Rodríguez-Amaya (1986) em goiabas brasileiras e, mais recentemente, por González *et al.* (2011) em frutas colombianas. De acordo com estes pesquisadores, o carotenoide majoritário na goiaba madura é o licopeno, seguido de β-caroteno e outros pigmentos em menores quantidades.

Vasconcelos *et al.* (2017) observaram que o extrato rico em licopeno obtido da goiaba tem efeitos benéficos na inflamação, oferecendo proteção contra as consequências do estresse oxidativo por diminuir os níveis de mediadores inflamatórios e inibir a expressão do gene envolvido na inflamação.

2.2.3. MAÇÃ

A maçã (*Malus domestica*) é uma das frutas mais consumidas no mundo, com uma produção de quase 90 milhões de toneladas em 2016 (FAOSTAT, 2017). Desde 2002 a produção mundial de maçãs tem aumentado, porém os países produtores variam bastante. A China, por exemplo, aumentou a produção de maçã em quase 100 %, junto à países como Chile, Brasil e África do Sul que são grandes exportadores para a Europa e Estados Unidos. O crescimento da produção está associado ao aumento do rendimento, uma vez que as áreas de produção permanecem praticamente inalteradas. Algumas variedades são cultivadas praticamente em um só lugar, como a Elstar na Alemanha (21 %), a Champion na Polônia (13 %) e a Red Delicious na Itália (12,5 %) (FAOSTAT, 2017). A maçã Red Delicious é uma das mais consumidas na Itália, representando uma fatia de mais de 2,4 milhões de toneladas de maçãs em 2016 (FAOSTAT, 2017).

As maçãs são fontes de compostos fenólicos, especialmente flavonoides, que variam suas concentrações dependendo do cultivar, estádio de maturação, condições ambientais e parte da fruta. Os principais flavonoides da maçã são a cianidina 3-galactosídeo (antocianina) e a quercetina 3-galactosídeo. Sua cor vermelha é estabelecida pela cianidina co-pigmentada pela quercetina e proantocianidinas (BAKHSHI e ARAKAWA, 2006).

Em 1992, Lancaster e Dougall já haviam observado que as quantidades de clorofila e carotenoides nas cascas de maçã eram fatores importantes na determinação da coloração final dessas frutas. Apesar de as antocianinas serem os pigmentos majoritários da casca da maçã, interações complexas ditam a coloração final percebida pelos olhos humanos (LANCASTER e DOUGALL, 1992).

2.2.4. ALFACE

Alimento de produção e consumo mundial, a alface (*Lactuca sativa* L.) está disponível em diversos cultivares com diferentes cores, incluindo desde o verde (escuro) até o roxo (HEO *et al.*, 2012). Sua coloração roxa, assim como o vermelho na maçã, deve-se às antocianinas.

As alfaces são geralmente consumidas cruas, sem restrições quanto à ingestão diária (KOSMA *et al.*, 2013) e contém um número significativo de fitonutrientes (MARTIN *et al.*, 2011). A quantidade de antioxidantes nas alfaces é bastante susceptível à variação de acordo com o cultivar, condições de cultivo (*indoor* ou *outdoor*) e estresse do meio ambiente (ILIĆ *et al.*, 2017).

Por ser um dos alimentos mais produzidos durante o ano todo em casas de vegetação, mas também em campo aberto, a alface torna-se um ótimo modelo para estudos de resposta à luz e temperatura (KIM *et al.*, 2004).

Apesar de ser uma das hortaliças mais consumidas no mundo todo, seu valor nutricional ainda é subestimado. Além de ser um alimento com baixo valor calórico, é uma boa fonte de fibra, ferro, folato, bem como de compostos bioativos potencialmente benéficos à saúde (KIM *et al.*, 2016). Alguns dos compostos que contribuem para as propriedades promotoras de saúde na alface são as antocianinas e as clorofilas (LI *et al.*, 2010).

Entre os carotenoides presentes nas alfaces, por exemplo, estão β-caroteno, luteína, lactucaxantina, neoxantina e violaxantina (BASLAM *et al.*, 2013), que são tetraterpenoides lipossolúveis presentes principalmente nos plastídeos. Além disso, são as xantofilas majoritárias do complexo fotossintético das alfaces (BECKER *et al.*, 2015).

2.3. LUZ E RADIAÇÃO ULTRAVIOLETA

A indústria de alimentos utiliza uma variedade de métodos de preservação para estender a vida de prateleira de frutas e hortaliças, principalmente baseado na aplicação de técnicas convencionais como processos térmicos, uso do frio, desidratação ou com agentes de conservação como ácidos e solutos. Entretanto, estes processos causam perdas de compostos bioativos e a demanda dos consumidores por frutas e hortaliças pouco processadas tem resultado no desenvolvimento de novos métodos de preservação não térmicos, para minimizar os efeitos negativos dos tratamentos nestes compostos (BARRETT e LLOYD, 2012). Entre essas técnicas, o uso da luz e a aplicação de radiação ultravioleta suplementar (100-400nm) têm atraído atenção como uma tecnologia promissora e *eco-friendly* para a preservação de alimentos líquidos e matérias-primas frescas (CASTAGNA *et al.*, 2014).

Neste contexto, maior atenção tem sido dispensada à possibilidade de preservar, ou até mesmo aumentar, a concentração de fitoquímicos ou compostos bioativos promotores de saúde através de ferramentas moleculares ou não moleculares. Entre as ferramentas não moleculares, tratamentos específicos de pós-colheita com elicitores, como tratamentos com baixa ou alta temperatura, aplicação de fitormônios, radiação ultravioleta, podem causar o aumento do acúmulo de compostos bioativos em plantas (SCHREINER e HUYSKENS-KEIL, 2006). A modificação da intensidade da luz e/ou da qualidade é promissora devido ao papel fundamental desempenhado pela luz em alguns dos processos metabólicos mais importantes envolvidos na biossíntese de fitoquímicos (CASTAGNA *et al.*, 2013).

A luz é tanto uma fonte de energia através da fotossíntese como um regulador de desenvolvimento por meio da ativação de fitocromos e outros fotorreceptores, de tal modo que influencia tamanho, forma e a fisiologia das plantas. Variações nos padrões de crescimento e desenvolvimento têm sido observados quando plantas são cultivadas sob diferentes fotoperíodos e níveis de radiação (CRAKER e SEIBERT, 1983).

Mudanças no tipo da luz têm um potencial de alterar processos fisiológicos e bioquímicos, o perfil de metabólitos e a qualidade de frutas e hortaliças. Diferentes níveis de intensidade resultam em características morfológicas e fisiológicas diferentes, além e afetar a produção de metabólitos secundários como os compostos fenólicos (NTSOANE *et al.*, 2016;

ILIĆ *et al.*, 2017). Entre os fatores que podem ser controlados, destacam-se o comprimento de onda, intensidade e fotoperíodo, segundo (KAWKA *et al.*, 2017).

Apesar de a luz ser crucial para a fotossíntese e crescimento da planta, seus efeitos são complexos. Dois fotorreceptores – fitocromos (absorvem vermelho/"far-red") e criptocromos (absorvem azul/luz ultravioleta A) – são responsáveis pelas mudanças morfológicas e de desenvolvimento nas plantas (DENG e QUAIL, 1999). Diversos estudos têm demonstrado que uma quantidade controlada de luz aumenta a qualidade e da vida de prateleira de frutas e hortaliças ao induzir a produção de nutrientes e compostos bioativos (HASAN *et al.*, 2017).

Anteriormente falava-se na hipótese de que o aumento do acúmulo de metabólitos primários em alimentos poderia ocorrer devido à inibição da translocação de produtos fotossintéticos, causado pelas lâmpadas de LED. O aumento do acúmulo de metabólitos secundários em resposta à luz, incluindo luz UV, pode ser uma resposta de stress e/ou um efeito de proteção solar (PARK *et al.*, 2012). A luz também afeta as vias de transdução que incluem enzimas, metabólitos e mensageiros secundários. Evidências sugerem que a luz pode ser usada para a produção de metabólitos secundários importantes para a saúde humana. Entretanto, os efeitos de diferentes espectros de luz podem variar de acordo com a planta e o cultivar. Para o aumento de valores nutricionais de alimentos, o uso de LEDs vermelhos e azuis tem apresentado bom resultados, por exemplo (HASAN *et al.*, 2017).

Comprimentos de onda acima de 280nm são um componente ubíquo da radiação solar, mas seus níveis variam consideravelmente na biosfera de forma espacial e temporal. Durante a última parte do século 20, a média de radiação ultravioleta B (UVB) na biosfera aumentou principalmente devido a compostos contendo clorina e bromina que foram liberados na atmosfera por atividades antropogênicas (INTERDONATO *et al.*, 2011)

A UV-B (280-320 nm), dependendo da intensidade aplicada e da sensibilidade da planta, pode favorecer o acúmulo de moléculas antioxidantes e UV-protetoras (JANSEN *et al.*, 2008). O mecanismo de percepção e transdução de sinal do UV-B tem sido elucidado em Arabdopsis e envolve inúmeros fatores de transcrição e complexos multiprotéicos (FAVORY *et al.*, 2009; RIZZINI *et al.*, 2011).

A radiação UV-B não alimenta a fotossíntese, mas pode representar uma ameaça à integridade da planta. Para se aclimatar aos efeitos nocivos do UV-B, as plantas usam o fotorreceptor UV RESISTANCE LOCUS8 (UVR8) para desencadear grandes mudanças na expressão gênica, levando a adaptações morfológicas e à produção de flavonois que atuam

como proteção à UV-B. Além disso, o UVR8 media o fototropismo, o movimento estomático e o relógio circadiano (GALVÃO e FRANKHAUSER, 2015).

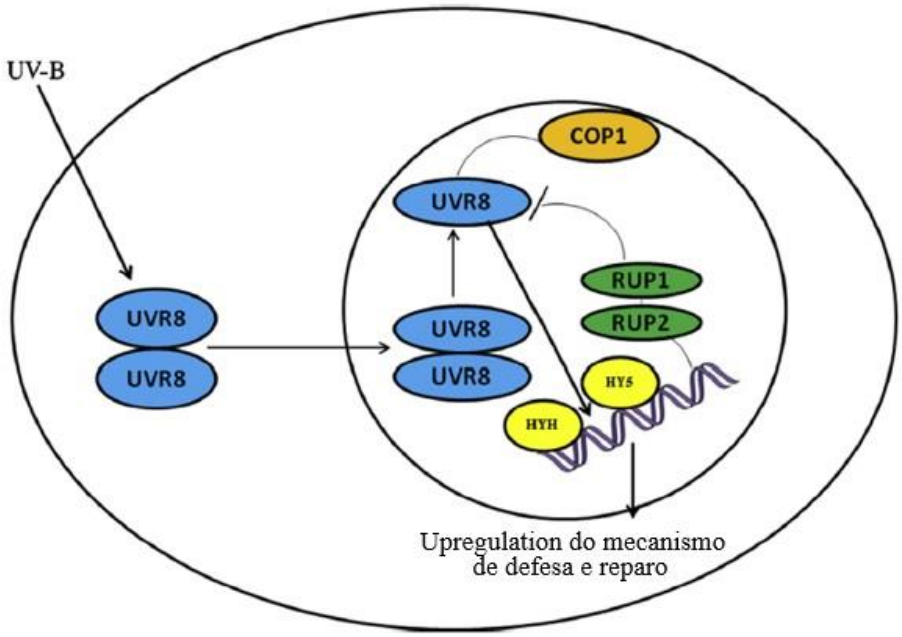

Figura 3. Apresentação esquemática da cascata de sinalização mediada pelo UVR8 na indução pela radiação UV-B.

Fonte: Adaptado de Singh *et al*. (2014).

A radiação UV-B pode funcionar tanto como um stress prejudicial quanto como um sinal para superar este stress, dependendo da intensidade. Especificamente, altas intensidades de UV-B sinalizam danos ao DNA, proteínas, membranas e lipídeos, produzem ROS e podem levar à morte celular. Para se protegerem contra os efeitos do stress causado pelas altas intensidades de UV-B, as plantas desenvolveram diversos sistemas como os compostos fenólicos que absorvem o UV-B, sistemas de reparo ao DNA e sistemas antioxidantes (LEE, 2016).

Os efeitos do UV-B incluem mudanças complexas na capacidade de reparação do DNA, atividade fotossintética, crescimento, morfologia da planta, expressão gênica, resistência a patógenos e metabólitos secundários (FROHNMEYER e STAIGER, 2003). Os tecidos das plantas respondem à UV-B pela indução de processos protetores celulares que incluem mudanças no metabolismo dos fenilpropanoides com incremento da síntese de compostos que absorvem o UV-B, sendo na maioria flavonoides e outros fenólicos relacionados (TEGELBERG *et al.*, 2001). Embora compostos UV-absorventes primariamente

protejam a molécula de DNA, eles também desempenham um papel importante na defesa antioxidante da planta e contra patógenos ou ataques de herbívoros (HAGEN *et al.*, 2007).

Na biossíntese de compostos fenólicos, várias enzimas desempenham um papel importante. A via dos fenilpropanoides é iniciada pela enzima chave fenilalanina amônia-liase (PAL, EC 4.3.1.5). Como uma enzima essencial na defesa da planta, a PAL pode ser ativada por vários estressores e desencadear a biossíntese de compostos fenólicos (DIXON e PAIVA, 1995). Além disso, assume-se que as enzimas envolvidas nessa via de síntese tenham propriedades antioxidantes, uma característica que é influenciada pelo estresse como a radiação UV. Jansen *et al.* (2001) encontraram que um aumento na atividade da peroxidase estava altamente correlacionado com a tolerância à radiação UV. Li *et al.* (2010) em acordo com essa afirmação, relataram a habilidade da peroxidase em sequestrar radicais livres e aliviar o stress causado pela UV.

A localização e a disponibilidade dos fotoassimiladores são parâmetros importantes na determinação das respostas da planta à radiação UV-B (GWYNN-JONES, 2001). A radiação UV-B está ligada à assimilação de CO_2 pela taxa com a qual o carbono é desviado do metabolismo primário para o secundário (KASIGE e TAKASHI, 2009). Portanto, fatores que afetam o metabolismo de carboidratos também irão afetar a produção de compostos UVB-absorventes, parcialmente como resultado do fornecimento de substratos (carboidratos) e/ou intermediários (por exemplo, ácidos cinâmicos), e parcialmente da ação enzimática limitante. Neste contexto, a radiação UV-B pode agir como um regulador da assimilação de CO_2 e do metabolismo de carboidratos. Dessa maneira, as relações induzidas pela UV-B entre carboidratos e metabólitos secundários são esperadas em plantas (INTERDONATO *et al.*, 2011).

Há várias maneiras de relatar as consequências potenciais da radiação UV-B em plantas, mas há um conhecimento limitado sobre o efeito nos metabólitos secundários, como compostos fenólicos e voláteis, por exemplo. Sabe-se que apenas uma pequena porção do total do espectro solar possa induzir estresse fotobiológico, ativar o sistema de defesa da planta e levar a um acúmulo de metabólitos secundários no tecido (TERAMURA, 2006). Além disso, a aplicação da radiação ultravioleta (especialmente UV-C) para fins de sanitização e prevenção de doenças na pós-colheita já foi discutida em muitos estudos (TERRY e JOYCE, 2004; CHARLES *et al.*, 2008). Uma vez que os compostos secundários das plantas são influenciados pelos tratamentos também com UV-B na pós-colheita, essa pode ser uma ferramenta interessante para aumentar os benefícios à saúde que as frutas e os vegetais oferecem (EICHHOLZ *et al.*, 2011)

Informações interessantes sobre os efeitos da radiação na pós-colheita em fenólicos e flavonoides de tomates (cv Zhenfen 202) expostos a diferentes doses de radiação UV-B ou UV-C, seguidas de armazenamento no escuro (14 °C por 37 dias) foram apresentados nos trabalhos de Liu *et al.* (2011; 2012). Ambos os tratamentos foram efetivos, em doses apropriadas, ao promover o acúmulo de fenólicos e flavonoides.

As baixas taxas de UV-B presentes na luz do sol constituem um fator ambiental importante ao promover mudanças metabólicas e de desenvolvimento, como a transcrição de genes envolvidos na síntese de flavonoides, consequentemente, como estímulo para sua síntese e podem promover, junto a outros compostos fenólicos, a proteção contra UV no tecido da epiderme (WINKEL-SHIRLEY, 2001). As baixas taxas de incidência de UV-B também ativam uma gama de genes envolvidos na resposta ao estresse oxidativo e ao reparo de danos de DNA (BROWN *et al.*, 2005) e tem papel fotomorfogênico como regulador de crescimento, expansão do cotilédone e de folhas e respostas fototrópicas (SHINKLE *et al.*, 2004). Altos níveis de UV-B estimulam a expressão de um número de genes envolvidos na percepção e sinalização do estresse, respostas a danos e de defesa (A-H-MACKERNESS *et al.*, 2001; STRATMANN, 2003).

A radiação ultravioleta, especialmente na região B tem se tornado uma fonte potencial de sérios danos aos organismos vivos. Suesslin e Frohnmeyer (2003) descobriram um fotorreceptor de UV-B ou, ao menos, um componente importante da sinalização de transdução do sinal de UV-B. Essa descoberta contribui, junto com outros dados importantes (ORAVECZ *et al.*, 2006), para melhor explicar a existência de uma via de sinalização de UV-B estresse-dependente. Apesar disso, é estabelecido que os fitocromos e criptocromos da planta não são fotorreceptores de UVB, mesmo que UV-A, elicitor destes receptores, possa desempenhar um papel importante na transdução do UV-B (BOVY *et al.*, 1995).

Estudos recentes relataram efeitos da radiação UV-B como a geração de mudanças distintas no metabolismo secundário da planta. Essa resposta particularmente resulta no acúmulo de compostos fenólicos (TREUTTER, 2010). Baseado na sua estrutura química, os compostos fenólicos agem como sequestradores de radicais livres como ROS, que são muito produzidas sob estresse oxidativo (EICHHOLZ *et al.*, 2012).

Alguns estudos têm explorado o efeito da radiação UV-B durante o amadurecimento de tomate antes da colheita, o que indica como o tratamento pode afetar significativamente os teores de ascorbato, carotenoides, flavonoides e ácido hidroxicinâmico, sendo este efeito genótipo-dependente e os resultados têm sido diferentes entre casca e polpa (GIUNTINI *et al.*, 2005; GIUNTINI *et al.*, 2008; CALVENZANI *et al.*, 2010; LAZZERI *et al.*, 2012).

Apesar de menos informação estar disponível sobre o efeito da irradiação UV-B na pós-colheita de frutos de tomate, alguns estudos já demonstraram, por exemplo, que essa radiação promoveu o acúmulo de fenois totais e flavonoides, com aumento também na capacidade antioxidante quando doses de 20 ou 40 $kJ.m^{-2}$ de irradiação foram aplicadas no início do armazenamento do tomate (LIU *et al.*, 2011).

Mais recentemente, Castagna *et al.* (2014) relataram que os tratamentos com UV-B em diferentes estágios (verde maturo e *turning*) afetaram a qualidade de frutos de tomate *Money Maker* com aumento na concentração de ácido ascórbico e carotenoides em polpa e casca, mas também causaram perda de firmeza.

As mudanças induzidas pela radiação UV no metabolismo dos polifenois dependem da dose e da duração da exposição. Além disso, essas mudanças também são afetadas pela idade fisiológica do tecido, sendo que todas essas diferenças influenciam uma grande variabilidade nas respostas ao UV-B (EICHHOLZ *et al.*, 2012).

Os flavonoides são uma classe de compostos que estão envolvidos na resposta de plantas ao estresse, o que inclui o dano foto-oxidativo por UV, devido à sua habilidade de absorver luz na região do ultravioleta e às suas propriedades antioxidantes (WINKEL-SHIRLEY, 2000). Além disso, tais compostos acumulam-se nos vacúolos das células epidérmicas, mais provavelmente agindo como um escudo de proteção às camadas celulares interiores. Não obstante, o UV-B tem se mostrado um importante indutor de duas enzimas de síntese de flavonoides, a chalcona sintase (CHS) e a chalcona isomerase (CHI) (BROSCHÉ e STRID, 2003). Flavonoides não são os únicos compostos envolvidos na resposta das plantas à radiação UV-B; outros fenólicos, como os ácidos hidroxicinâmicos também são conhecidos por contribuir com a proteção natural da planta (GIUNTINI *et al.*, 2008).

CAPÍTULO 4

DIFFERENT CAROTENOID BEHAVIOR IN TWO CLIMACTERIC FRUITS AFTER POST-HARVEST UV-B TREATMENT

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Abstract

Background: Ultraviolet B (UV-B) radiation is a promising and environmentally friendly technique, which in low flow rate, can induce bioactive compound synthesis. This work aimed at evaluating the effectiveness of post-harvest UV-B treatment in order to improve carotenoid content in climacteric fruits like persimmon and guava fruits. Method: The fruits were harvested at commercial maturity and placed into climatic chambers equipped with UV-B lamps. This radiation was applied during 48 hours and fruit were sampled at 25, 30 and 48 hours of each treatment. HPLC analysis was performed to separate and identify carotenoid compounds from fruit skin after a saponification process. Results: Fruit from 30 hours treatment began to present a carotenoid accumulation since the majority of analyzed compounds exhibited its synthesis stimulated from this time on. In persimmon skin, it was observed that the maximum content was reached after 48 hours of UV-B treatment. Conclusion: These results suggest that this post-harvest UV-B treatment can be an innovative and a viable method to induce beneficial effects on persimmon fruit.

Keywords: climacteric fruits, Psidium guajava, Diospyros kaki, UV-B radiation, postharvest, carotenoid.

Introduction

There is a positive relation between consumption of fruit and vegetables and the reduction of cardiovascular diseases risks [1]. Carotenoids may react with free radicals, resulting in less reactive and harmful species. Cell damage caused by free radicals contributes greatly to chronic diseases such as cardiovascular diseases and stroke in humans. Free radicals normally result from oxidation reactions, and their unpaired electrons capture electrons from other substances, which stabilizes in turn the free radicals. Unpaired electrons present along the conjugated chain of carotenoid radicals can react with functional groups from other chemicals, terminating oxidation chain reactions by removing the free radical [2]. Like other antioxidants, carotenoids prevent oxidative stress by protecting against lipid peroxidation and subsequent damage to proteins and DNA by triggering genes responsible for regulating enzymes, including superoxide dismutase (SOD), catalase and lipid peroxidase [3].

In this context, persimmon (*Diospyros kaki*) and guava (*Psidium guajava*) stand out between climacteric fruits since they are considered a good source of carotenoids. Considering its valuable health effects, scientific interest in these fruits has increased in recent years. Persimmon and guava fruits are rich in β-cryptoxanthin and β-carotene, and both of them are considered precursors of vitamin A. In addition to their beneficial health effects, these bioactive compounds can also influence fruit appearance and astringency quality [4].

Concerning to the plant foods quality, it is also receiving increasing attention on techniques to extend shelf life of plant products preserving organoleptic and nutraceutical properties. Among postharvest treatments with elicitors, such as low or high temperature treatments, application of phytohormones and γ radiation, the ultraviolet light B (UV-B) may enhance nutraceutical accumulation in plant food as reported by many authors [5, 6]. UV-B radiation, depending on dose and plant sensitivity, may provide accumulation of antioxidant and UV-protective molecules [7]. Many authors reported increasing in phenol concentrations by applying UV-B treatment in different kind of fruit, such as apples [8, 9], tomatoes [10], white asparagus [11] and lemons [12]. On the other hand, information about the influence of UV-B on carotenoid content is scarce.

This technique has attracted attention as an environmental friendly technology and a promising approach to maintain or to enhance bioprotective molecules of plant food also through molecular-based researches. Indeed, some authors [13] suggest that low flow rates of UV-B can activate genes involved in response to oxidative stress, such as those involved in the synthesis of some bioactive compounds. As few information is available in literature on the evaluation of the effectiveness of post-harvest UV-B radiation on the improving secondary metabolite content in climacteric fruits, the aim of this research was to study the

behavior of carotenoids in persimmon and guava climacteric fruits in a time course experiment.

Material and Methods

1. Plant material and UV-B treatment

Persimmon fruit of cultivar "Kyoto" and guava fruits of cultivar "Pedro Sato" were harvested at commercial maturity from an orchard in Porto Alegre during season 2014 (May/Jun). Fruit without defects, at physiological maturity and selected by uniform size, color and appearance were collected and transported to Institute of Food Science and Technology (ICTA), at University of Rio Grande do Sul (Brazil).

A group of fruit was immediately sampled representing the first time (zero) of the experiment. The remaining fruits were distributed into climatic chambers (Novatecnica NT718) at 18 °C with 75 % humidity, equipped with four UV-B lamps (Philips Ultraviolet B, TL 20 W/12RS, k4), which provided a radiation of 1.69 $W \cdot m^{-2}$. To ensure a uniform UV-B dose, fruit were aligned in front of lamp tubes and placed with their peduncle facing down; approximately 0.45 m from the lamps, then only one part of each fruit was irradiated and analyzed.

The UV-B radiation was applied for uninterrupted 48 hours (292 $kJ \cdot m^{-2}$) and fruit were sampled at 25, 30 and 48 hours. Fruit from control treatment underwent the same conditions, but UV-B lamps were covered by benzophenone-treated polyethylene film, known to block UV-B radiation. In all treatments, fruits were carefully peeled using a scalpel, and only the skins of both fruits were immediately frozen in liquid nitrogen and stored at -80 °C for analyses.

2. Extraction and quantification of carotenoids by high-performance liquid chromatography (HPLC)

The high-performance liquid chromatography (HPLC) analysis was performed on 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), using HPLC grade solvents and products: methanol, methyl-tert-butyl ether and acetonitrile (Panreac).

The carotenoid extraction was performed according to the method described by Mercadante and Rodriguez-Amaya [14]. The main steps were the extraction of pigments with chilled acetone until discoloration of the sample, followed by saponification with 10 % KOH in methanol overnight at room temperature. After removing of alkali, the extract was concentrated in a rotary evaporator (Fisatom Quimis 0214 M2) (T < 35 °C). The concentrated extract was transferred to an amber flask, dried in a nitrogen flow, and stored in freezer (-18 ºC) for subsequent quantitation by 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 ratio of 5:90:5 and reaching 0:95:5 in 12 minutes, 0:89:11 in 25 minutes, 0:75:25 in 40 minutes and 0:50:50 after a total of 60 minutes. The mobile phase flow rate was 1 mL.min-1, injection volume was 5 μL and the injector temperature was 33 °C.

The standards used for calibration curves construction were: β-carotene (purity > 93 %; $5x10^{-6}$–$50x10^{-5}$ kg·L^{-1}), zeaxanthin (purity > 95 %; $1x10^{-6}$–$40x10^{-5}$ kg·L^{-1}), β-cryptoxanthin (purity > 95 %; $4x10^{-6}$-$100x10^{-4}$ kg·L^{-1}) and lycopene (purity > 90 %; $1x10^{-5}$-$1x10^{-4}$ kg·L^{-1}) were purchased from Sigma Chemical (USA). Lutein (purity > 95 %; $1x10^{-6}$–$65x10^{-5}$ kg·L^{-1}) was purchased from Indofine Chemical Company Inc. Hillsborough (USA).

Results were expressed in micrograms per g of sample and the limits of detection (LOD) and quantification (LOQ) were, respectively, $3.51x10^{-2}$ mg·kg^{-1} and $2.11x10^{-2}$ mg·kg^{-1} for β-cryptoxanthin; $6.9x10^{-3}$ 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 $7.43x\ 10^{-2}$ mg·kg^{-1} for β-carotene; and $1.44x10^{-2}$ mg·kg^{-1} and $4.4x10^{-2}$ mg·kg^{-1} for lycopene.

3. Statistical analysis

The results were analyzed by Anova and Tukey's test at a significance level of 5% or 10%, using Statistica 11.0.

Results and Discussion

No significant difference was shown between UV-B-treated and untreated persimmon fruits regarding for β-carotene level until 30 hours. From this time, β-carotene increased in both samples, reaching at 48 hours of treatment a higher (+15.85 %) value in UV-B irradiated samples in comparison to the controls (Fig. 1a).

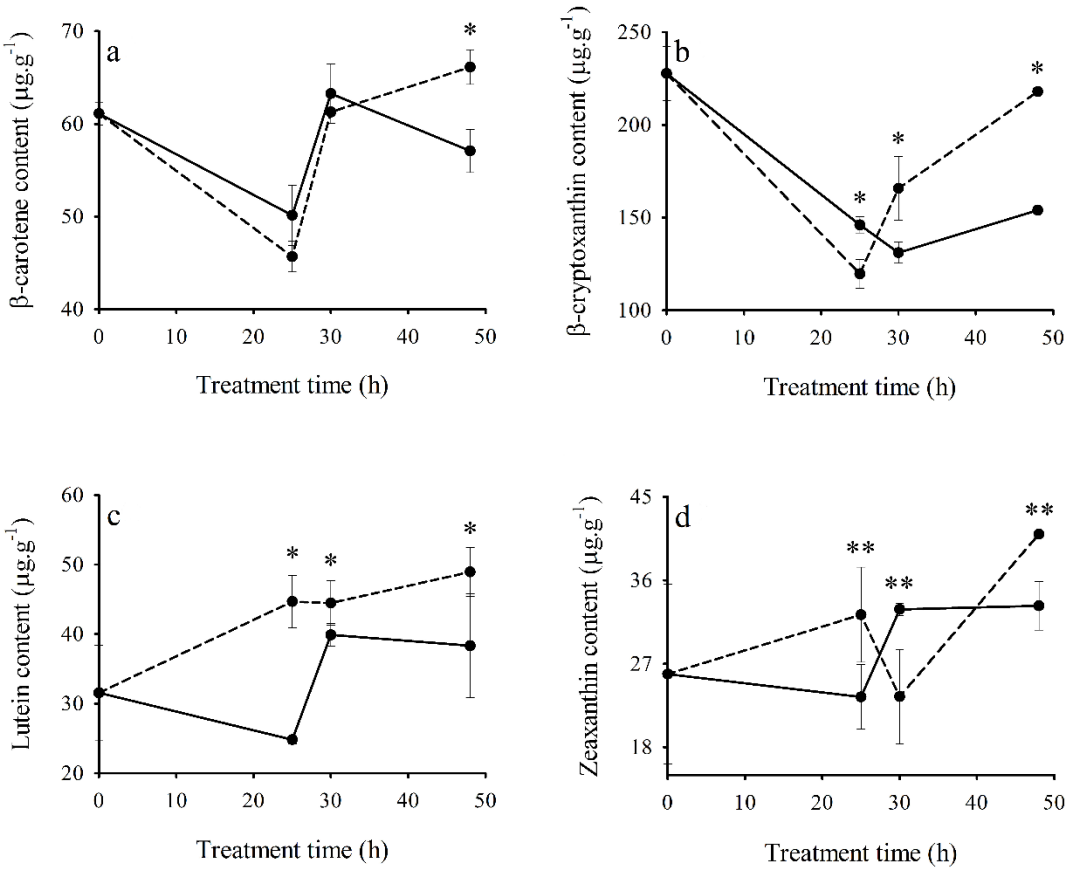

Fig. 1 Carotenoid content analyzed by HPLC in persimmon skin submitted (dotted line) or not (continuous line) to UV-B radiation (in $\mu g \cdot g^{-1}$ dry matter ± SE). Significant differences between UV-B and control are indicated with * ($p \leq 0.05$) or ** ($p \leq 0.10$), according to one-way ANOVA followed by Tukey's test.

After 48 hours of treatment, irradiated fruit has enriched the compound contents compared to control (Fig. 1b). Respecting to these results, which showed that β-cryptoxanthin starts to increase in from 30 hours and β-carotene shows an accentuated increment only after 48 hours, the behavior of these two compounds is coherent with the conversion of β-carotene in β-cryptoxanthin along the carotenoid biosynthetic pathway in persimmon.

The content of β-carotene (Fig. 2a) and lycopene (Fig. 2c) of guava increased progressively until 30 h of exposure. Control and UV-B differed statistically regarding the content of β-carotene and lycopene. The irradiated guava presented an increase of more than

18 % of β-carotene and lycopene contents after 30 h of radiation exposure. However, after 48 h of treatment its content decreased in control and treated guavas. This result indicates that UV-B radiation should stimulate the synthesis of carotenoids in guava in the firsts hours, probably exhausting its biosynthetic pathway or suggesting an oxidation of these molecules by the UV-B radiation accumulated during the experiment.

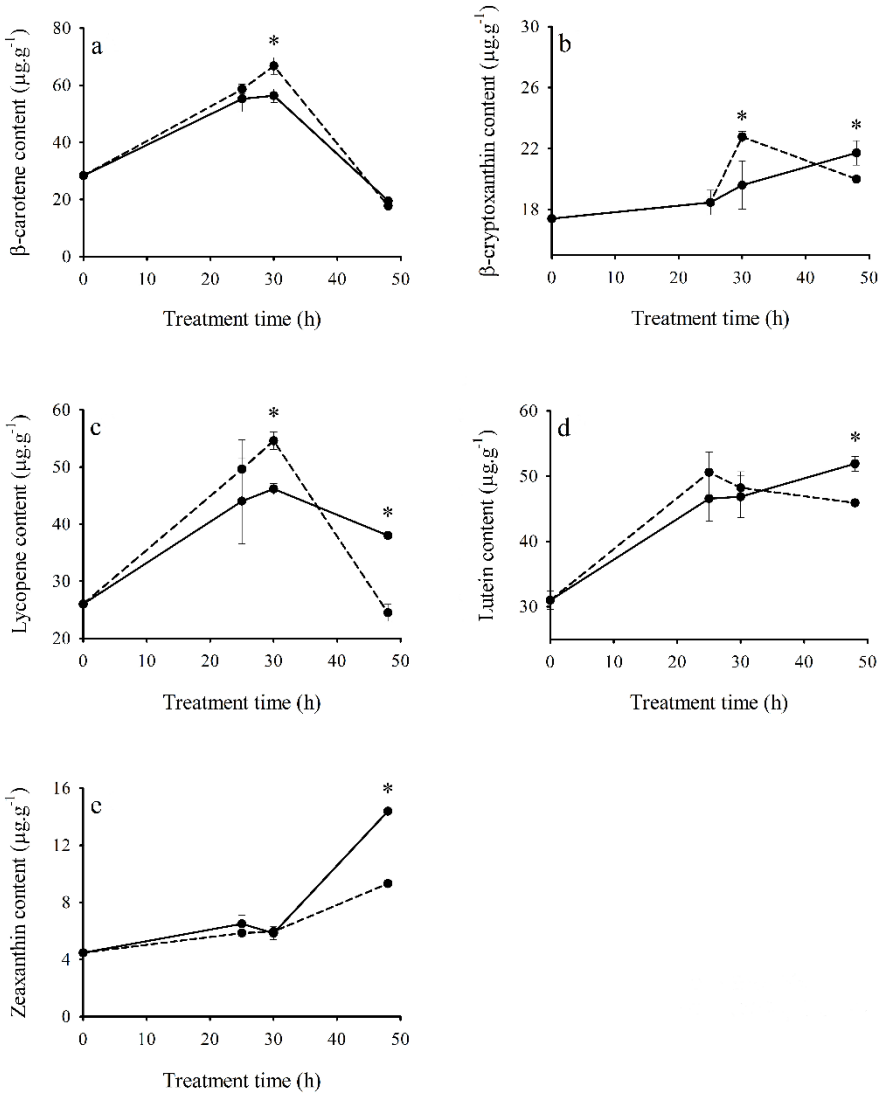

Fig. 2 Carotenoid content analyzed by HPLC in guava skin submitted (dotted line) or not (continuous line) to UV-B radiation (in $\mu g \cdot g^{-1}$ DW ± SE). Significant differences between UV-B and control are indicated with * ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated fruit; UV-B radiation means UV-B-treated fruits.

Persimmon and guava are sources of β-carotene, a compound that plays a protective role in the human body and it is the most important carotenoid in human nutrition, being the

major precursor of vitamin A and other retinoids present in fruit and vegetables. Furthermore, it exhibits a radical scavenging property and, consequently, antioxidant power [15]. β-carotene has been shown as lipid protector from free radical autoxidation and also an effective quenching of singlet oxygen.

It is suggested that the simultaneous presence and synergic action of lycopene, β-carotene, and other antioxidant compounds are required for the prevention of degenerative diseases [16]. Some authors have shown anticarcinogenic effects in the synergic action of carotenoids that indicates that these compounds can contribute to prevention of certain types of cancer [17-19].

Persimmon from no UV-B treatment (control) presented a linear decrease in β-cryptoxanthin content in its skin over time and reached after 48 hours of treatment a value 32.37 % lower than at time zero. For the other hand, UV-B treated samples, after a decrease at 25 hours, recovered the level of β-cryptoxanthin reaching the same value of time zero (Fig. 1b) with an increase of 41.56 % compared to control treatment at 48 hours. For this compound, both time and treatment factors were statistically significant ($p<0.05$) by two-way analysis (ANOVA). The β-cryptoxanthin is the major carotenoid in persimmon and its trend, under UV-B radiation, allows suggesting that its accumulation has been more intensively stimulated via metabolic pathway after first 25 hours of treatment.

The content of β-cryptoxanthin (Fig. 2b) in guava (control and UV-B) did not show a linear trend over time, however when the plant material was maintained for a period longer than 25 hours under UV-B radiation could be observed a greater accumulation of β-cryptoxanthin than in the control samples at 30 h. The same behavior profile was observed for β-carotene and lycopene in UV-B treated guava, which compounds also presented a decreased after 48 h of treatment.

β-cryptoxanthin is the major carotenoid in persimmon and its decrease in both treated and untreated fruits, together with β-carotene, until 25 hours of storage under chamber conditions, allows us to hypothesize an alteration in physiological responses towards an enhancement of cell oxidative metabolism during storage. The decrease of antioxidants metabolites, such as β-carotene and β-cryptoxanthin, under these conditions suggests their consumption to counteract oxidative damage. However, both samples react after 25 hours accumulating antioxidant compounds, but UV-B irradiation stimulates much more their synthesis.

β-cryptoxanthin is an antioxidant which protects organs and tissues from oxidative damage, as well as it plays role in cell-to-cell communication and may have unique functions in bone health. However, the most important and well-established function of β-cryptoxanthin is a precursor of vitamin A activity [20]. In humans, vitamin A is important for good fetal development, maintaining healthy immune function, epithelial cell and red blood cell production as well. Its deficiency is considered a health problem in developing countries, and despite recent progress, it remains the leading cause of blindness and childhood mortality. Provitamin A carotenoids, especially β-carotene, α-carotene and β-cryptoxanthin, are important sources of vitamin A, mainly for those who live in regions where its deficiency is common. Among these compounds, β−cryptoxanthin is a better source of vitamin A in the human diet when comparing to others such as β-carotene e.g. [21].

The fruit carotenoids conversion to vitamin A tends to be better than those from vegetables, once in fruit carotenoid are dissolved in oil droplets in chromoplasts, being absorbed easily by the human digestive system than carotenoid bounded in the thylakoids in chloroplasts of vegetables leafy green [22]. Thus, β-cryptoxanthin absorption might be greater than β-carotene and α-carotene since its most common sources are fruit, while the other ones are vegetables.

In all sampled times, the concentration of lutein in persimmon was positively affected by UV-B. The analysis of times, treatment and the interaction between them were affected significantly. Differently, from other carotenoids, lutein in irradiated fruit increased at 25 hours maintaining its levels higher than control treatment over time. The increment reached 27.68 % after 48 hours of UV-B radiation (Fig. 1c).

Comparing UV-B treated and untreated guava, it was observed that the highest lutein content occurred on fruit submitted to UV-B light for 25 hours, which present an increase of 40.06 % of this compound (Fig. 2d) compared to time zero. However as other carotenoids presented in guava, lutein also had a decrease in its content after peak accumulation.

In recent years, some researches have found protective effects of lutein against eye diseases. Some of these studies report the correlation of age-related macular degeneration, with antioxidant property of lutein [23], which include its strong ROS (reactive oxygen species) scavenger capacity [24] and prevention of lipid peroxidation [25].

The zeaxanthin levels in persimmon from control treatment followed the same trend of β-carotene during all experiment. Regarding samples from UV-B, a significant difference among times of analysis was evidenced ($p<0.10$) (Fig. 1d). The zeaxanthin level in

persimmon treated for 48 hours increased in comparison with the control (+23.19 %). This fact seems to be sustained by an increase of its precursors in the biosynthetic pathway (β-carotene) starting at 25 hours of treatment.

A gradual increase of zeaxanthin was verified along the treatment for the control guava fruits and those irradiated with UV-B (Fig. 2e). On the other hand, at the end of storage of fruits (48 h), a difference of 54.37 % of zeaxanthin content was observed in the control samples over the treated samples. In addition to directly quenching reactive oxygen species, zeaxanthin may prevent oxidative damage to protein, lipid, and DNA by regulating other cellular antioxidant systems [24].

Fruit carotenoid biosynthesis and accumulation occur in chloroplasts and chromoplasts. There are different features during ripening period due to different roles played by these compounds in the fruit [26]. Ibañez et al. [27] investigated variations in photosynthetic pigments, in UV-B-absorbing compounds, anthocyanins contents and growth parameters on citrus leaves (*Citrus aurantifolia*) with or without UV-B radiation. They concluded that leaves exhibited a different sensibility to solar UV radiation according to the development stage.

The intensity and period of treatment, ripening stage of fruit and enrichment of carotenoid content is also dependent on species and cultivar used for experiment under UV-B radiation. In contrast with results presented in this study about positive effects played by UV-B radiation on carotenoid accumulation in persimmon, Du et al. [28] observed that total carotenoid content did not enhance in tested samples, and instead, there was a slight decrease in different commercial fresh-cut carrot products (baby carrots, carrot sticks, shredded carrots, crinkle cut coins and carrots chips).

Gonzales et al. [29] studied two different varieties of quinoa (*Chenopodium quinoa*) under UV-B conditions and suggested that responses observed could not be correlated between analyzed varieties. They consider that variations in such parameters cannot be used to predict the quinoa varieties sensitivity to solar UV–B radiation.

On evaluating tomato ethylene mutants, Becatti et al. [30] reported that UV-B effects on carotenoid metabolism occur through both ethylene-dependent and ethylene-independent mechanisms, which seem to act in an antagonistic way. Solovchenko and Schmitz-Eiberger [31] observed that the influence of UV-B radiation on fruit carotenoid content seems to be also cultivar-dependent in apple fruit.

The total carotenoid content in persimmon on present study varied from 346.28 in control fruit at time zero to 374 $\mu g \cdot g^{-1}$ after 48 hours of UV-B radiation; and from 107.26 in

control fruit at time zero to 117.42 $\mu g \cdot g^{-1}$ after 48 hours of UV-B radiation in guava. This result demonstrates that all individual carotenoid analyzed, as well as the total carotenoid content, showed a similar trend with an increase in carotenoid content in persimmon, reaching a maximum level after 48 hours of treatment. This increase is of great importance since several evidences have demonstrated the ability of carotenoids to act as antioxidants in the human body [24].

On the other hand, the carotenoids from guava did not present the same profile of persimmon after 48 hours of treatment. Different species, cultivar and fruit ripening stage may respond differently to UV-B intensity and period of radiation, showing a diverse profile of each carotenoid compound. The experiment presented for guava require more attention and studies to find the best condition to improve its carotenoid content, once it was demonstrated an increase of these compounds after 30 hours of UV-B exposure.

From a carotenoid amount of 107.26 $\mu g \cdot g^{-1}$ in control guava fruit at time zero, after 48 hours of UV-B radiation the fruits reached an amount of 117.42 $\mu g \cdot g^{-1}$. The behavior of carotenoids in the time course experiment did not present the same profile of persimmon fruits. Indeed, the highest content was reached at 30 hours of treatment suggesting that the concentration of UV-B was suitable to stimulate the carotenoid accumulation. On the other hand, after this time point the increased quantity of radiations (in UV-B treated samples) or the more prolonged shelf life (in the untreated samples) seems to induce damage to carotenoid synthetic machinery.

Conclusions

A modulation of environmental parameters in post-harvest may represent a valid technique to stimulate biosynthetic pathways leading to bioactive compounds production. Data presented in this study demonstrate that UV-B radiation is a valid approach to obtain carotenoid-enriched climateric fruit as persimmon and guava. The carotenoid accumulation was evident after 30 hours, since the compounds analyzed had its synthesis stimulated from this time. These results suggest that UV-B post-harvest treatment can induce carotenoid synthesis in climateric fruit, providing beneficial effects for human health and improving its market value. Future research would be important to indicate exposure time and radiation

intensity appropriate to different fruit, or even to different cultivars, and also best fruit maturity stage to be exposed to this radiation and improve their antioxidants levels.

References

[1] Sgherri, C.; Pinzino, C.; Quartacci, M. F. Reactive oxygen species and photosynthetic functioning: past and present. In Reactive oxygen species in plants: boon or bane? Revisiting the Role of ROS, Singh, V. P.; Singh, S.; Tripathi, D. K.; Prasad, S. M.; Chauhan, D. K.; Eds. J. Wiley & Sons: New York (USA), 2017; pp. 137-155.

[2] Raposo, M.; de Morais, A.; de Morais, R. Carotenoids from Marine Microalgae: A Valuable Natural Source for the Prevention of Chronic Diseases. Mar Drugs, 2015, 13 (8), 5128-5155.

[3] Cuvelier, M.-E.; Bondet, V.; Berset, C. Behavior of phenolic antioxidants in a partitioned medium: structure—Activity relationship. J Am Oil Chem Soc, 2000, 77 (8), 819.

[4] Lin-Wang, K.; Micheletti, D.; Palmer, J.; Volz, R.; Lozano, L.; Espley, R.; Hellens, R. P.; Chagnè, D.; Rowan, D. D.; Troggio, M.; Iglesias, I.; Allan, A. C. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ., 2011, 34, 1176-1190.

[5] Schreiner, M.; Huyskens-Keil, S. Phytochemicals in Fruit and Vegetables: Health Promotion and Postharvest Elicitors. Critical Reviews in Plant Sciences, 2006, 25 (3), 267-278.

[6] Castagna, A.; Chiavaro, E.; Dall'Asta, C.; Rinaldi, M.; Galaverna, G.; Ranieri, A. Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. Food Chemistry, 2013, 137 (1), 151-158.

[7] Jansen, M. A. K.; Hectors, K.; O'Brien, N. M.; Guisez, Y.; Potters, G. Plant stress and human health: Do human consumers benefit from UV-B acclimated crops? Plant Sci, 2008, 175 (4), 449-458.

[8] Hagen, S. F.; Borge, G. I. A.; Bengtsson, G. B.; Bilger, W.; Berge, A.; Haffner, K.; Solhaug, K. A. Phenolic contents and other health and sensory related properties of apple fruit

(Malus domestica Borkh, cv. Aroma): Effect of postharvest UV-B irradiation. Postharvest Biology and Technology, 2007, 45, 1-10.

[9] Scattino, C.; Castagna, A.; Neugart, S.; Chan, H. M.; Schreiner, M.; Crisosto, C. H.; Tonutti, P.; Ranieri, A. Post-harvest UV-B irradiation induces changes of phenol contents and corresponding biosynthetic gene expression in peaches and nectarines. Food Chemistry, 2014, 163, 51-60.

[10] Liu, C.; Han, X.; Cai, L.; Lu, X.; Ying, T.; Jiang, Z. Postharvest UV-B irradiation maintains sensory qualities and enhances antioxidant capacity in tomato fruit during storage. Postharvest Biology and Technology, 2011, 59, 232–237.

[11] Eichholz, I.; Rohn, S.; Gamm, A.; Beesk, N.; Herppich, W. B.; Kroh, L. W.; Ulrichs, C.; Huyskens-Keil, S. UV-B-mediated flavonoid synthesis in white asparagus (*Asparagus officinalis* L.). Food Res Int, 2012, 48 (1), 196-201.

[12] Interdonato, R.; Rosa, M.; Nieva, C. B.; González, J. A.; Hilal, M.; Prado, F. E. Effects of low UV-B doses on the accumulation of UV-B absorbing compounds and total phenolics and carbohydrate metabolism in the peel of harvested lemons. Environ Exp Bot, 2011, 70 (2), 204-211.

[13] Calvenzani, V.; Martinelli, M.; Lazzeri, V.; Giuntini, D.; Dall'Asta, C.; Galaverna, G.; Tonelli, C.; Ranieri, A.; Petroni, K. Response of wild-type and high pigment-1 tomato fruit to UV-B depletion: flavonoid profiling and gene expression. Planta, 2010, 231 (3), 755-765.

[14] Mercadante, A. Z.; Rodriguez-Amaya, D. B. Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango. J Agric Food Chem, 1998, 46 (1), 128-130.

[15] Cao-Hoang, L.; Fougère, R.; Waché, Y. Increase in stability and change in supramolecular structure of β-carotene through encapsulation into polylactic acid nanoparticles. Food Chem, 2011, 124 (1), 42-49.

[16] Giuntini, D.; Graziani, G.; Lercari, B.; Fogliano, V.; Soldatini, G. F.; Ranieri, A. Changes in carotenoid and ascorbic acid contents in fruits of different tomato genotypes related to the depletion of UV-B radiation. J Agric Food Chem, 2005, 53 (8), 3174-3181.

[17] Calvenzani, V.; Martinelli, M.; Lazzeri, V.; Giuntini, D.; Dall'Asta, C.; Galaverna, G.; Tonelli, C.; Ranieri, A. P., K. Response of wild-type and high pigment-1 tomato fruit to UV-B depletion: flavonoid profiling and gene expression. Planta, 2010, 231, 755-765.

[18] Shklar, G.; Schwartz, J.; Trickier, D.; Cheverie, S. R. The effectiveness of a mixture of β-carotene, α-tocopherol, glutathione, and ascorbic acid for cancer prevention. Nutr Cancer, 1993, 20 (2), 145-151.

[19] Toma, S.; Losardo, P. L.; Vincent, M.; Palumbo, R. Effectiveness of beta-carotene in cancer chemoprevention. Eur J Cancer Prev, 1995, 4 (3), 213-24.

[20] Turner, T.; Burri, B. J.; Jamil, K. M.; Jamil, M. The effects of daily consumption of β-cryptoxanthin–rich tangerines and β-carotene–rich sweet potatoes on vitamin A and carotenoid concentrations in plasma and breast milk of Bangladeshi women with low vitamin A status in a randomized controlled trial. Am J Clin Nutr, 2013, 98 (5), 1200-1208.

[21] Sommer, A.; Vyas, K. S. A global clinical view on vitamin A and carotenoids. Am J Clin Nutr, 2012, 96 (5), 1204S-1206S.

[22] Burri, B. J. Beta-cryptoxanthin as a source of vitamin A. J Sci Food Agr, 2015, 95 (9), 1786-1794.

[23] Serpeloni, J. M.; Cólus, I. M. d. S.; de Oliveira, F. S.; Aissa, A. F.; Mercadante, A. Z.; Bianchi, M. L. P.; Antunes, L. M. G. Diet carotenoid lutein modulates the expression of genes related to oxygen transporters and decreases DNA damage and oxidative stress in mice. Food Chem Toxicol, 2014, 70, 205-213.

[24] Gammone, M. A.; Riccioni, G.; D'Orazio, N. Carotenoids: potential allies of cardiovascular health? Food Nutr Res, 2015, 59.

[25] Chiu, C.-J.; Taylor, A. Nutritional antioxidants and age-related cataract and maculopathy. Exp Eye Res, 2007, 84 (2), 229-245.

[26] Lazzeri, V.; Calvenzani, V.; Petroni, K.; Tonelli, C.; Castagna, A.; Ranieri, A. Carotenoid Profiling and Biosynthetic Gene Expression in Flesh and Peel of Wild-Type and hp-1 Tomato Fruit under UV-B Depletion. J Agric Food Chem, 2012, 60 (19), 4960-4969.

[27] Ibañez, S.; Rosa, M.; Hilal, M.; González, J. A.; Prado, F. E. Leaves of Citrus aurantifolia exhibit a different sensibility to solar UV-B radiation according to development stage in relation to photosynthetic pigments and UV-B absorbing compounds production. J Photochem Photobiol B, 2008, 90 (3), 163-169.

[28] Du, W.-X.; Avena-Bustillos, R. J.; Breksa, A. P.; McHugh, T. H. Effect of UV-B light and different cutting styles on antioxidant enhancement of commercial fresh-cut carrot products. Food Chem, 2012, 134 (4), 1862-1869.

[29] González, J. A.; Rosa, M.; Parrado, M. F.; Hilal, M.; Prado, F. E. Morphological and physiological responses of two varieties of a highland species (*Chenopodium quinoa* Willd.) growing under near-ambient and strongly reduced solar UV–B in a lowland location. J Photochem Photobiol B, 2009, 96 (2), 144-151.

[30] Becatti, E., Chkaiban, L., Tonutti, P., Forcato, C., Bonghi, C., Ranieri, A.M., 2010. Short-term postharvest carbon dioxide treatments induce selective molecular and metabolic changes in grape berries. J. Agric. Food Chem 58, 8012-8020.

[31] Solovchenko, A.; Schmitz-Eiberger, M. Significance of skin flavonoids for UV-B-protection in apple fruits. J Exp Bot, 2003, 54 (389), 1977-1984.

CAPÍTULO 5

PHENOLIC ENRICHMENT IN APPLE SKIN FOLLOWING POST-HARVEST FRUIT UV-B TREATMENT

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Abstract

In apple fruit, phenolic compounds are the major sources of antioxidants, which are particularly concentrated in the skin. In the present experiment apples (cv. Red Delicious) were analyzed for their phenolic composition after the exposure to UV-B for 36 h (219 kJm−2) and during storage (7, 14 and 21 d after the end of the treatment) in order to assess if UV-B treatment could improve marketability of the products as well as shelf-life. Since UV-B irradiation is also known to induce the generation of reactive oxygen species (ROS), the spin-trapping technique was applied to monitor the generation of free radicals under UV-B. The UV-B for 36 h treatment induced the generation of carbon-centered radicals in the skin, the tissue more exposed to radiation, but fruit quality parameters were not affected. Even if firmness progressively decreased and an increasing weight loss occurred during storage, differences between treated and control fruit were not observed. The different phenolic classes of apple skin reacted differently to the UV-B for 36 h irradiation, hydroxycinnamic acids increasing and flavonols decreasing. However, during storage, hydroxycinnamic acids and anthocyanins increased in UV-B-treated samples, as well as flavonols at the end of the storage period. As a consequence, the fruit skin showed a higher antioxidant activity in all the treated samples during storage, increasing the healthy properties of the fruit. This suggests that UV-B technique results in a valid strategy to induce antioxidant production in apple, increasing their nutraceutical value, thus allowing the attainment of phenolic-enriched fruit.

Keywords: apple skin; UV-B radiation; phenolic compounds; anthocyanin; UPLC-MS

1 Introduction

Ultraviolet-B radiation (UV-B) is intrinsic to sunlight and reaches the earth's surface and has major biological effects on plant growth and development. In Arabidopsis, UV-B light regulates several important photo-morphogenic responses, including stomatal opening, phototropic curvature, and biosynthesis of anthocyanins and other flavonoids (Suesslin and Frohnmeyer, 2003). In addition to its effects on the model plant Arabidopsis, UV-B radiation can increase flower development and fruit color in many fruit trees, such as grape and apple (Zhao et al., 2016). The effectiveness of UV-B radiation has been demonstrated in stimulating secondary metabolism, which influences the nutraceutical value and sensorial quality of fruit (Castagna et al., 2013; Castagna et al., 2014; Liu et al., 2011; Scattino et al., 2014). However, at high doses, UV-B radiation causes similar conditions to oxidative stress, resulting from additional reactive oxygen species (ROS) generation (Hideg et al., 2013) and it was demonstrated that *Withania somnifera* plants experienced lipid peroxidation causing damages to the cell (Takshak and Agrawal, 2014). In fact, the level of carbon-centered free radicals is the result of an equilibrium between free radical production and their neutralization by antioxidants. In a previous paper carried out on peaches (Sgherri et al., 2015) authors demonstrated that UV-B technique is a good approach to induce antioxidant production in peach fruit, increasing their nutraceutical properties. Indeed, cyanidin-3-O-glucoside, the main cyanidin component, was capable of radicalization in the place of other organic molecules, thus protecting cells from oxidation.

Apple fruit is rich in health-promoting antioxidants such as anthocyanins and other phenolic compounds (Allan et al., 2008). It is reported that, due to their high antioxidant capacity, phenolics offer protection from cancer, cardiovascular conditions and some age-related diseases (Sun et al., 2014).

The red coloration of apple skin derives from anthocyanins, whose accumulation is influenced by light, temperature, nutrition as well as by genetic factors (Lin-Wang et al., 2011). The anthocyanins in apples are predominantly glycosylated cyanidin. According to Peng and Moriguchi (2013), cyanidin-3-O-glycosides (cy3-gly) are the main forms of

anthocyanins in apple skin, and cyanidin 3-O-galactoside (cy3-gal) covers 80 % of the total cy3-gly, being higher in red cultivars as Red Delicious.

Anthocyanin biosynthesis in apple fruit is developmentally regulated and occurs at two stages. The first peak of production occurs at the fruitlet stage in both red and non-red cultivars and it is not economically important (Lancaster and Dougall, 1992). The second peak occurs at the ripening fruit stage in red cultivars like Red Delicious. The anthocyanin accumulation at the second peak is affected by environmental factors, including temperature and light and impact greatly on the marketable value of the product (Ubi et al., 2006).

In the present paper, Red Delicious apple fruit was subjected to UV-B for 36 h in order to evaluate the ability of supplementary UV-B radiation to increase the health-promoting potential of apple tissues and, at the same time, to improve shelf-life and quality.

2 Material and Methods

2.1 Chemicals

Acetonitrile HPLC grade (assay 99.9 %) was purchased from Panreac Química S.A. (Barcelona, Spain); trifluoroacetic acid for HPLC (assay 99 %) and formic acid for HPLC (assay 98 %) were purchased from Sigma–Aldrich (Madrid, Spain). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA).

2.2 Plant material and treatment

Fruit of 'Red Delicious' apple cultivar were produced by local company (Illuminati Frutta Soc. Cons. a r.l., Civitella in Val di Chiana, Arezzo, Italy. Latitude: 43.2772° and longitude: 11.8294°) using integrated pest management practices. Apples were used at commercial maturity. Ninety fruit were selected for size and appearance and were transported to the laboratory at the Department of Agriculture, Food, and Environment, University of Pisa (Italy). The experiment was performed once.

A group of ten fruit were immediately sampled at the laboratory, representing the time zero (0 h) of the experiment. The remaining fruit was distributed into two climatic chambers (20 ºC; R.H. 85 %), each equipped with three UV-B lamp tubes (Philips Ultraviolet B, TL 20 W - 12RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands) which

provided 1.69 W m^{-2} at fruit height. The apples were placed with their peduncle facing up (approximately 0.40 m under the lamps) and were aligned parallel to the lamp tubes in order to ensure a uniform UV-B dose. The treatment lasted 36 h (219 kJ m^{-2}). Control fruit (Vis) were placed for the same time in the climatic chamber where UV-B lamps were screened by benzophenone-treated polyethylene film. This kind of compound is known to block UV-B radiation (Calvenzani et al., 2010). After the UV-B treatment, a group of ten apples was sampled (36 h) while the remaining fruit were stored at room temperature (20 °C) in the dark.

Groups of ten fruit from control and treated apples were sampled at day 7, 14 and 21 d. The apples were carefully peeled using a scalpel and skin samples (thickness of 0.2 mm, approximately) were immediately frozen in liquid nitrogen and stored at -80 ºC for further analyses.

2.3 Determination of fruit quality parameters

The texture of apple samples was analyzed on the equatorial of two opposite sides of each fruit after removing a small disc of skin using a penetrometer with 8 mm probe (Model 53205; TR, Forlì, Italy). Six measurements were carried out on each fruit. Values were expressed in Newton (N). Total soluble solid content (TSS) was measured in the apple juice by a digital refractometer (Digital Brix Refractometer DBR 35) and expressed as %. Titratable acidity (TA) was determined by titration of 0.01 L of juice with 0.1 mol L^{-1} NaOH to an endpoint of pH 8.2 by automatic titrator (Model T80/20; Schott, Mainz, Germany), and expressed as percentage of malic acid (%). The percentage of weight loss was calculated in comparison with initial weight. Ten fruit were tested for each group.

2.4 Phenolics extraction and determination of total phenols and flavonoids

Frozen dried samples of control and treated apple skin (2 10^{-4} kg dry weight) were ground with liquid nitrogen to a fine powder. The plant material was extracted in triplicate essentially following the method described by Becatti et al. (2010).

Total phenols were determined in control and treated samples of skin according to the Folin–Ciocalteu colorimetric method. Amounts of 1.85 mL of distilled water, 1.25 10^{-4} L of Folin–Ciocalteu reagent (Sigma-Aldrich Chemical Co., St. Louis, MO) and 0.5 mL of a 20 % sodium carbonate solution were added to 25 10^{-6} L of extract. The solution was homogenized and left to stand for 30 min. The total phenol content was expressed as g kg^{-1}

gallic acid dry weight (DW) (Sigma-Aldrich Chemical Co., St. Louis, MO). Absorbance was read at 750 nm at room temperature.

Total flavonoids were quantified following the method reported by Kim et al. (2003). Absorbance was read at 525 nm at room temperature. Results were expressed as g kg^{-1} catechin of DW. Both analysis were performed using an Ultrospec 2100 pro-UV-visible spectrophotometer (Amersham Biosciences).

2.5 Determination of antioxidant activity

A spectrophotometric analysis of antioxidant activity was performed following the method described by Pellegrini et al. (1999), using the discoloration of the radical cation 2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid, $ABTS^{+}$) by skin apple extracts. The antioxidant capacity was expressed as gmol kg^{-1} Trolox equivalent antioxidant capacity (TEAC) DW.

2.6 UPLC–MS analysis method

UPLC–MS analysis was carried out on phenolic extracts using an Agilent 1290 Infinity II LC system (Agilent Technologies Italia S.p.A., Cernusco Sul Naviglio, Italy) consisting of a degasser, a binary pump, an autosampler, a column oven and equipped with an Agilent 6495A triple quadrupole.

A C18 column, 2.1 x 50 mm, 1.8 μm (Agilent Zorbax Eclipse Plus, Santa Clara, CA, USA) was used for separation of phenolic compounds. Solvent A consisted of 0.2 % formic acid in water whereas solvent B was 0.2 % formic acid in acetonitrile. The elution gradient was: 6 % B (3 min), from 6 to 30 % B in 11 min, from 30 to 100 % B in 2 min, 100 % B (2 min). The column temperature was 35 ºC, the flow rate was 0.3 mL min^{-1}, and the injection volume was 2 10^{-6} L. Supplementary table reports m/z and Multiple Reaction Monitoring (MRM) transitions of polyphenolic compounds identified in apple skin. MS parameters employed were as follow in ESI(+): gas temp: 150 °C; gas flow: 13 L min^{-1}; nebulizer: 50 psi; sheath gas heater: 350 °C; sheath gas flow: 12 L min^{-1}; capillary: 3500 V, HPRF funnel: 120; LPRF funnel: 40; in ESI(-): gas temp: 150 °C; gas flow: 13 L min^{-1}; nebulizer: 50 psi; sheath gas heater: 350 °C; sheath gas flow: 12 L min^{-1}; capillary: 1500 V; HPRF funnel: 120; LPRF funnel: 80. For quantification, an external standard method was used. A calibration curve in at least five different concentrations from 1 to 500 μg L^{-1} was constructed for each compound

analyzed. From these concentrations, as equation of lines (y = a + bx) was constructed that allowed to quantify each compound. Data are expressed as g kg^{-1} DW. For a better understanding of the data, the compounds identified and quantified were separated into groups according to Wildman (2006).

2.7 Electron paramagnetic resonance (EPR) measurements: detection of stable free radicals

Water was Millipore MilliQ grade which was further distilled through a 1-m long Todd column and then saturated with dioxygen or argon at 20 °C. The use of diethylenetriaminepentaacetic acid (DTPA) as a chelating agent further minimizes the artifacts resulting from trace metal impurities in the buffers. The DEPMPO was used as a spin trapping agent. Spin trapping agents are diamagnetic compounds which rapidly scavenge transient and/or stable radicals to form stable paramagnetic spin adducts for different types of radicals (ROS, carbon-centered radicals, etc.). Because these are secondary radicals that retain an unpaired electron, they can be detected by EPR (Sgherri et al., 2017).

Spectra were recorded at room temperature (25 °C) using a Varian E112 spectrometer equipped with a Varian variable temperature accessory. The spectrometer was interfaced to a PC 486/100 via an acquisition board and a software package designed for EPR measurements (Pinzino and Forte, 1992). EPR assays were carried out in 1 mm quartz sample tubes sealed at one end. All experiments were repeated at least three times and in the dark. Computer-based simulations of EPR spectra were performed using the Winsim software (Duling, 1994).

The EPR parameters used were: microwave power, 20 mW; microwave frequency, 9.16 GHz; modulation amplitude, 2.5 Gauss; time constant, 0.125 s.

Freeze-dried control and treated samples of apple skin (ca 0.1 g) were extracted with 10 mM 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES)/KOH (pH 7.4) containing 0.05 10^{-3} M DTPA. Reaction mixtures contained 10 mM DEPMPO in ethanol and sample extracts, obtained from the skin of apples previously treated with UV-B for 36 h and analyzed during the storage at 7, 14 and 21 d from the end of the treatment. EPR spectra were also monitored in the absence of DEPMPO and registering the signal in the dark. A sample containing 2 mM H_2O_2, 2 mM DTPA and 10 mM DEPMPO was irradiated for 5 min to monitor the appearance of the hydroxyl radical (·OH). In fact, the ultraviolet (UV)/H_2O_2 system is an advanced oxidation process in which H_2O_2 is added in the presence of UV light to generate hydroxyl radicals (·OH) (Sgherri et al., 2015). This procedure was done to test the

efficacy of the spin trap for the measurements on biological samples. Three replicates were analyzed for each material and results were expressed as spin g^{-1} DW.

2.8 Statistical Analysis

Data were subjected to one-way ANOVA using Statistica 11.0 software. Significant differences between treated and control fruit at each sampling time were calculated using at least three replicates according to Tukey's test ($p \leq 0.05$).

3 Results and Discussion

3.1 Effects of UV-B on quality parameters

The analyses of fruit quality parameters, carried out on the whole fruit, showed unaltered behavior in UV-B for 36 h treated apples, in comparison to the controls, in relation to the firmness (Table 1). Following storage, firmness progressively decreased in both treated and control fruit whereas an increasing weight loss occurred (Table 1), the latter being significantly different after 14 d (- 20 % in treated samples compared to controls). The texture is a physical attribute resulting from the structural constituents of the product, providing an idea of the transformations in the cellular structure, cell cohesion and biochemical alterations (Chitarra and Chitarra, 2005).

Table 1. Quality parameters of of apple skin irradiated with UV-B for 36 hours and left to stand in the dark untill 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples.

		Treatment	Storage		
		36 h	7 days	14 days	21 days
Titratable Acidity	Control	0.1518 ± 0.01[a]	0.1281 ± 0.01[a]	0.1532 ± 0.01[a]	0.1560 ± 0.01[a]
	UV-B	0.1776 ± 0.01[a]	0.1382 ± 0.01[a]	0.1437 ± 0.01[a]	0.1664 ± 0.01[a]
TSS	Control	13.33 ± 0.04[a]	14.14 ± 0.25[a]	13,60 ± 0.35[a]	12,90 ± 0.21[a]
	UV-B	12.51 ± 0.07[b]	13.29 ± 0.13[b]	12,79 ± 0.20[a]	12,90 ± 0.19[a]
pH	Control	3.64 ± 0.04[a]	3.55 ± 0.07[a]	3,75 ± 0.06[a]	3,53 ± 0.04[a]
	UV-B	3.51 ± 0.02[b]	3.61 ± 0.05[a]	3.73 ± 0.05[a]	3.63 ± 0.06[a]
Firmness	Control	68.20 ± 3.62[a]	59.07 ± 2.12[a]	53.33 ± 3.12[a]	47.62 ± 1.90[a]
	UV-B	71.44 ± 4.17[a]	63.62 ± 2.24[a]	44.28 ± 4.60[a]	38.16 ± 6.73[a]
Weight loss	Control	0.63 ± 0.06[a]	2.39 ± 0.22[a]	4.05 ± 0.25[a]	5.08 ± 0.74[a]
	UV-B	0.72 ± 0.02[a]	2.76 ± 0.22[a]	3.24 ± 0.10[b]	5.66 ± 0.25[a]

Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples. Acidity as % of malic acid; TSS (Total Soluble Solids) as %; Firmness as Newton and Weight loss as %.

Titratable acidity was not affected by UV-B irradiation, each treated sample showing the same value as control during the whole storage period (Table 1). Conversely, both pH and TSS exhibited reduced values in the 36-h-treated samples in comparison with the controls, indicating an acceleration of the ripening process (Majidi et al., 2011). However, at the end of the storage period (21 d), no great difference in any qualitative attributes was shown between the treated and untreated samples, suggesting that the UV-B treatment did not negatively affect fruit shelf-life (Table 1).

Hagen et al. (2007) found that postharvest irradiation of *Malus domestica* cv. Aroma for ten days improved the apple skin colour without influencing the level of soluble solids or titratable acidity, thus indicating that application of UV-B irradiation could contribute to the maintenance of apple properties. However, the maintenance of firmness depends, for each

fruit considered, on the ripening stage, storage conditions as well as on the UV-B dose applied. In our conditions, Red Delicious apples were not affected when irradiated with 219 kJ m^{-2} whereas Liu et al. (2011) found that mature-green tomato fruit maintained a high level of firmness if exposed to 20 and 40 kJ m^{-2} UV-B and then stored in the dark at 14 °C for up to 37 d. However, the highest dose of 80 kJ m^{-2} resulted in higher bioactive compound content but showed negative effects on texture, color, and other antioxidants.

3.2 Effects of UV-B on total phenols, total flavonoids and antioxidant activity in apple skin

Total phenols (Figure 1A), as well as total flavonoids (Figure 1B) of apple skin, showed a significant reduction after UV-B treatment (Figure 1) even if at the end of the storage period (21 d) an accumulation of total phenols had occurred in comparison with the untreated sample. The lower content of total phenols and flavonoids after the 36-h-treatment could be explained by the increased generation of stable carbon radicals in the skin of apples (Figure 4), and then with their inactivation, which minimized the oxidative stress induced by UV-B in the fruit tissues (Hideg et al., 2013). In fact, it is well known that phenolic compounds are potent antioxidants, directly or indirectly removing ROS and free radical species (Rice-Evans et al., 1997).

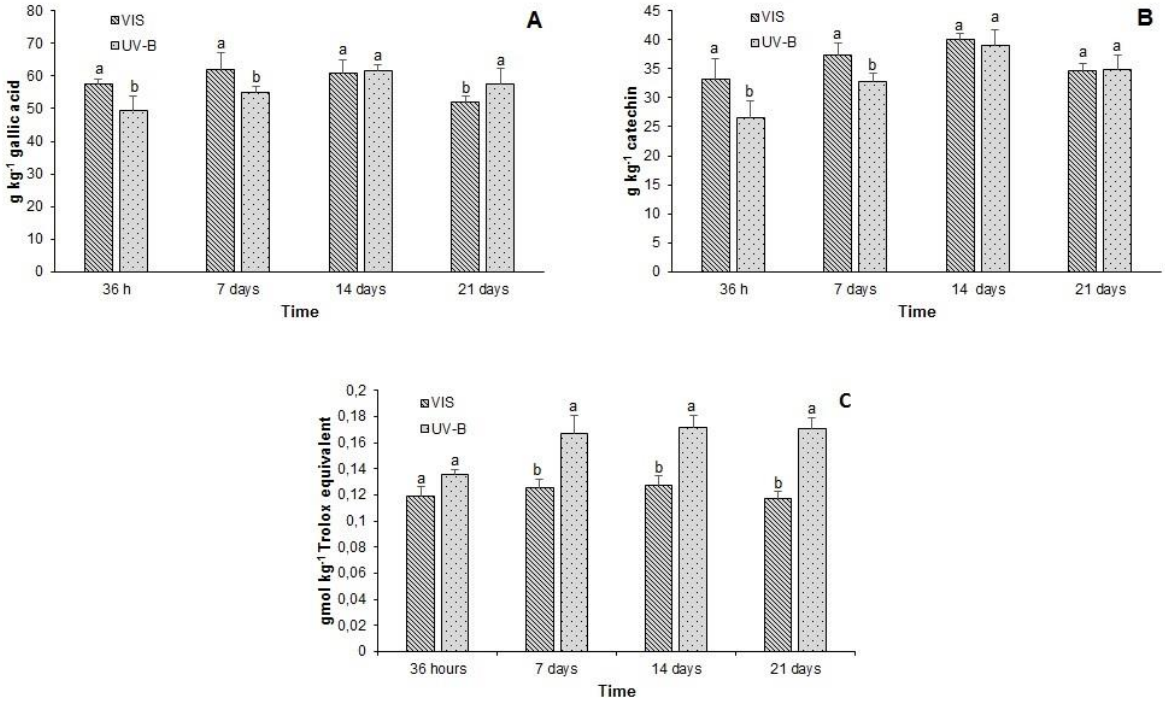

Figure 1. Total phenols, flavonoids (g kg-1 DW) and antioxidant activity (gmol kg-1 DW) of apple skin irradiated with UV-B for 36 hours and left to stand in the dark until 21 days. Data

represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples.

As phenolics biosynthesis varies according to the developmental stage, genotype, and environmental factors (Saure, 1990; Treutter, 2001), the increase in total phenols monitored in the treated apples at the end of the storage period could be ascribed to induction by UV-B treatment (Figure. 1). According to Du et al. (2014) stress-mediated changes in phenol content, as well as the physiological status of specialty crops, are dependent on the exposure (adaptation) time and in turn on the dose of UV.

In addition to their ability to provide human daily requirements of antioxidants from fresh consumption, phenolics present antimicrobial properties and color potential (in the case of anthocyanins). For all these characteristics, they appear very attractive as a dietary supplement, pharmaceutical components, and preservatives during food processing and conservation (Du et al., 2014).

In contrast with the decrease in total phenols after treatment for 36 h, antioxidant activity in apple skin extracts was not affected, but rather it increased in the treated samples during storage (Figure 1C). This discrepancy could be explained considering that each phenolic compound contributed to the total antioxidant activity differently, depending on the number of hydroxylations and methoxylations on their aromatic rings (Rice-Evans et al., 1996). Besides, another compound other than polyphenolics could be contributing to antioxidant capacity as ascorbic acid (Hernández-Herrero and Frutos, 2014).

3.3 Effects of UV-B on main polyphenolic classes in apple skin

Hydroxycinnamic acids (Figure 2A), flavan-3-ols (Figure 2B), flavonols (Figure 2C), anthocyanins (Figure 2D) and dihydrochalcones (Figure 2E) represent the main polyphenolic classes identified in apple skin after UPLC-MS analysis. Flavan-3-ols and flavonols were the most representative groups whereas the class of dihydrochalcones includes some compounds such as phloretin and phloridzin, typically found in apple skin (Treutter, 2001) (Table 2). Flavonoid-rich foods exert cardio- and cerebro-protective effects by decreasing oxidative damage to LDL and vascular cells (Lotito and Frei, 2004). Since apple skin is richer than flesh in these nutraceutical compounds, it is recommended that apple be eaten unpeeled, thus getting as many benefits as possible (Scattino et al., 2014).

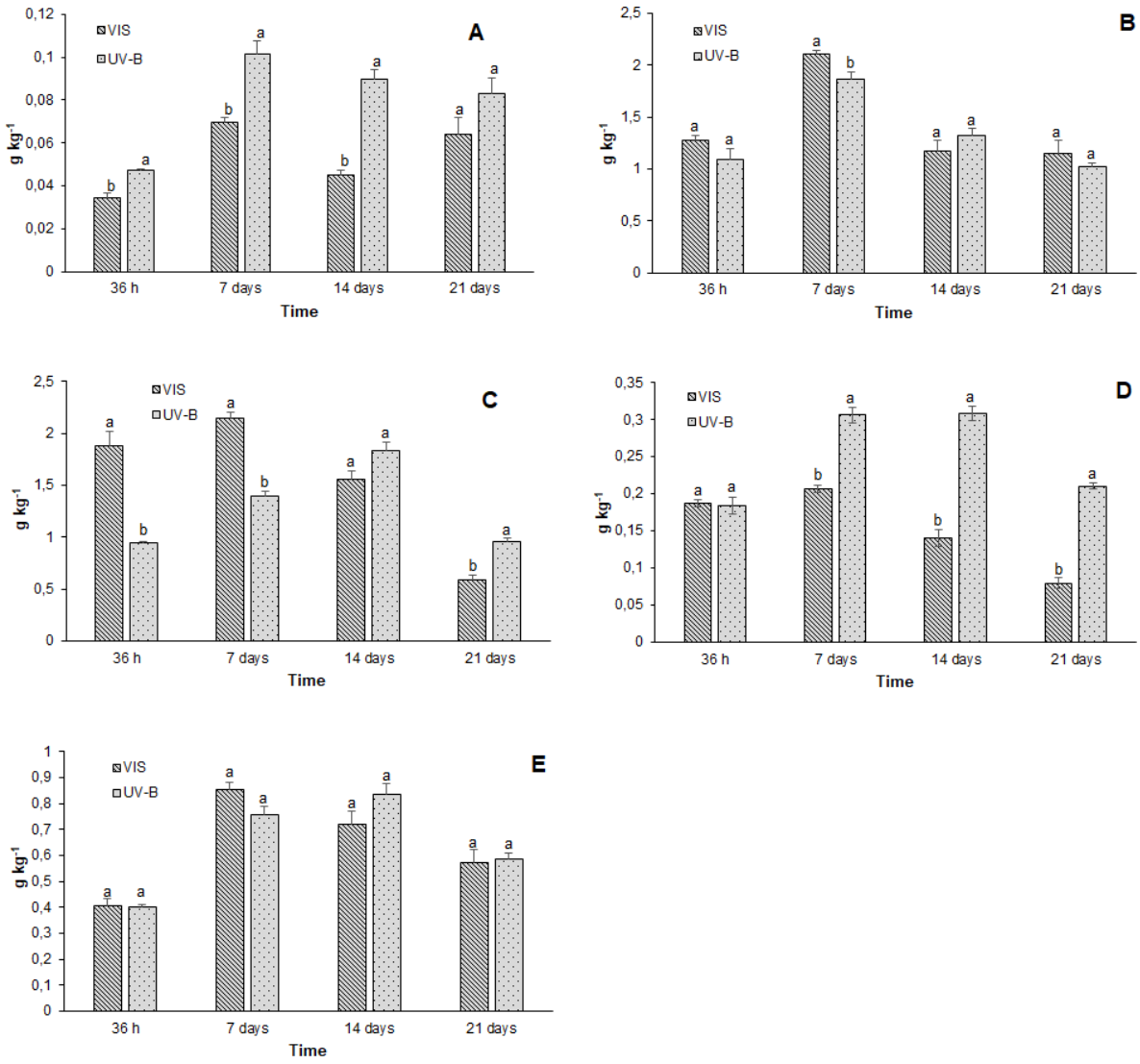

Figure 2. Total hydroxycinnamic acids (A), flavan-3-ols (B), flavonols (C), anthocyanins (D) and dihydrochalcones (E) (g kg^{-1} DW) of apple skin irradiated with UV-B for 36 hours and left to stand in the dark untill 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples.

Similarly to what was observed by Ryan et al.(2002) in the case of plants adaptation to strong sunlight, the different classes of phenolic compounds showed a different behavior when submitted to UV-B radiation (Figure 2). In particular, hydroxycinnamic acids showed an increase by 38 % following 36 h of treatment and maintained higher values in the treated samples during storage as well as anthocyanins (Figure 2D). Treutter (2001) also observed an

accumulation of anthocyanins in apples exposed to UV-B, demonstrating this enhancement to be related to an increased expression of biosynthetic anthocyanin genes.

On other hand, dihydrochalcones remained unaltered at any period analyzed (Figure 2E), while flavonols were negatively affected by the treatment, showing a reduced content at the end of the exposure (- 45 %) and after 7 d (- 31 %) in comparison with the respective control (Figure 2C). However, at the end of the period (21 d) flavonols were 64 % higher in the UV-B-treated sample than control level, suggesting that UV-B treatment slowed down flavonoid loss during storage (Figure 2). Moreover, reduced values were also observed after 7 d as in the case of flavan-3-ols.

3.4 Effects of UV-B on phenolic composition in apple skin

Twenty-four phenolic compounds were determined in apple skin extracts by UPLC–MS analysis and they are present as free or glycoside forms (Table 2). The identification of the molecules is reported in the Table 3. Most phenolic compounds are naturally present in food as conjugated forms. In fact, in higher plants, low molecular weight phenols occur as glycosides or esters with sugars or related compounds (Imeh and Khokhar, 2002).

The compounds that mostly contributed to the increase in hydroxycinnamic acids after 36 h of UV-B treatment was feruloyl glucoside, while cryptochlorogenic and chlorogenic acids showed an increment during storage, this latter phenol exhibiting values from 4.5 to 6.7-fold higher than the controls (Table 2).

Table 2. Phenolic compounds (g kg^{-1} DW) determined after UPLC–MS analysis of methanolic extracts from apple skin irradiated with UV-B for 36 hours and left to stand in the dark until 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples. irradiated with UV-B for 36 h and left to stand in the dark until 21 days.

Group of compounds	*Identified compounds*		*Treatment*	*Storage*		
			36 h	7 days	14 days	21 days
Hydroxycinnamic acids	Caffeoyl glucoside	Control	0.0018 ± 0.01[a]	0.0020 ± 0.01[a]	0.0010 ± 0.01[b]	0.0011 ± 0.01[a]
		UV-B	0.0015 ± 0.01[a]	0.0017 ± 0.01[a]	0.001.32 ± 0.01[a]	0.0008 ± 0.01[a]
	Chlorogenic Acid	Control	0.0031 ± 0.01[a]	0.0071 ± 0.01[b]	0.006.49 ± 0.01[b]	0.0046 ± 0.01[b]
		UV-B	0.0045 ± 0.01[a]	0.0326 ± 0.01[a]	0.034.76 ± 0.01[a]	0.0315 ± 0.01[a]
	Neochlorogenic acid	Control	0.0006 ± 0.01[a]	0.0011 ± 0.01[a]	0.0009 ± 0.01 [a]	0.0011 ± 0.01[a]
		UV-B	0.0004 ± 0.01[a]	0.0011 ± 0.01[a]	0.0007 ± 0.01 [b]	0.0006 ± 0.01[b]
	p-Coumaroyl glucose	Control	0.0017 ± 0.01[a]	0.0028 ± 0.01[a]	0.0013 ± 0.01[b]	0.0021 ± 0.01[a]
		UV-B	0.0013 ± 0.01[b]	0.0025 ± 0.01[a]	0.0025 ± 0.01[a]	0.0016 ± 0.01[a]
	p-Coumaroyl quinic acid	Control	0.0006 ± 0.01[a]	0.0015 ± 0.01[a]	0.0007 ± 0.01[a]	0.0013 ± 0.01[a]
		UV-B	0.0005 ± 0.01[a]	0.0013 ± 0.01[a]	0.0007 ± 0.01[a]	0.0010 ± 0.01[a]
	Protocatechuic acid	Control	0.0003 ± 0.01[a]	0.0004 ± 0.01[a]	0.0006 ± 0.01[a]	0.0002 ± 0.01[b]
		UV-B	0.0004 ± 0.01[a]	0.0004 ± 0.01[a]	0.0005 ± 0.01[a]	0.0005 ± 0.01[a]
	Cryptochlorogenic acid	Control	0.0002 ± 0.01[a]	0.0004 ± 0.01[b]	0.0004 ± 0.01[b]	0.0004 ± 0.01[b]
		UV-B	0.0004 ± 0.01[a]	0.0016 ± 0.01[a]	0.0013 ± 0.01[a]	0.0017 ± 0.01[a]
	Feruloyl Glucoside	Control	0.0257 ± 0.01[b]	0.0541 ± 0.01[a]	0.0335 ± 0.01[b]	0.0527 ± 0.01[a]
		UV-B	0.0378 ± 0.01[a]	0.0598 ± 0.01[a]	0.0478 ± 0.01[a]	0.0452 ± 0.017[a]

Flavan-3-ols	(+)Catechin	Control	0.0340 ± 0.01[a]	0.0445 ± 0.01[a]	0.0229 ± 0.01[a]	0.0283 ± 0.01[a]
		UV-B	0.0317 ± 0.01[a]	0.0405 ± 0.01[a]	0.0281 ± 0.01[a]	0.0200 ± 0.01[b]
	(-)Epicatechin	Control	0.8431 ± 0.02[a]	1.4051 ± 0.02[a]	0.8007 ± 0.06[a]	0.7645 ± 0.08
		UV-B	0.7244 ± 0.07[a]	1.2534 ± 0.05[a]	0.9028 ± 0.04[a]	0.6807 ± 0.02[a]
	Procyanidin B1	Control	0.0091 ± 0.01[a]	0.0153 ± 0.01[a]	0.0082 ± 0.01[a]	0.0107 ± 0.01[a]
		UV-B	0.0079 ± 0.01[a]	0.0141 ± 0.01[a]	0.0101 ± 0.01[a]	0.0071 ± 0.01[b]
	Procyanidin B2	Control	0.0828 ± 0.01[a]	0.1456 ± 0.01[a]	0.0798 ± 0.01[a]	0.0810 ± 0.01[a]
		UV-B	0.0737 ± 0.01[a]	0.1251 ± 0.01[b]	0.0876 ± 0.01[a]	0.0725 ± 0.01[a]
	Procyanidin B3	Control	0.3046 ± 0.01[a]	0.4877 ± 0.01[a]	0.2579 ± 0.01[a]	0.2556 ± 0.03[a]
		UV-B	0.2474 ± 0.02[a]	0.4169 ± 0.01[b]	0.2825 ± 0.01[a]	0.2327 ± 0.01[a]
	Procyanidin B4	Control	0.0039 ± 0.01[a]	0.0072 ± 0.01[a]	0.0059 ± 0.01[a]	0.0051 ± 0.01[a]
		UV-B	00032 ± 0.01[a]	0.0108 ± 0.01[a]	0.0055 ± 0.01[a]	0.0045 ± 0.01[a]
Flavonols	Quercetin 3-O-glucoside	Control	0.2967 ± 0.02[a]	0.2869 ± 0.01[a]	0.1891 ± 0.01[b]	0.0815 ± 0.01[b]
		UV-B	0.1201 ± 0.01[b]	0.2137 ± 0.01[b]	0.2540 ± 0.01[a]	0.1533 ± 0.01[a]
	Quercetin 3-O-galactoside	Control	0.7664 ± 0.05[a]	0.9774 ± 0.02.[a]	0.6825 ± 0.03[b]	0.2161 ± 0.01[b]
		UV-B	0.4199 ± 0.01[b]	0.6174 ± 0.01[b]	0.8481 ± 0.04[a]	0.4386 ± 0.01[a]
	Quercetin 3-O-rhamnoside	Control	0.1585 ± 0.01[a]	0.1575 ± 0.01[a]	0.1335 ± 0.01 [a]	0.0622 ± 0.01[a]
		UV-B	0.0755 ± 0.01[b]	0.0921 ± 0.01[b]	0.1397 ± 0.01 [a]	0.0636 ± 0.01[a]
	Quercetin 3-O-arabfuranoside	Control	0.3091 ± 0.02[a]	0.3226 ± 0.01[a]	0.2578 ± 0.01[a]	0.1058 ± 0.01[b]
		UV-B	0.1681 ± 0.01[b]	0.2363 ± 0.01[b]	0.2833 ± 0.01[a]	0.1478 ± 0.01[a]
	Quercetin 3-O-arabpiranoside	Control	0.2255 ± 0.01[a]	0.2442 ± 0.01[a]	0.1823 ± 0.01[a]	0.0728 ± 0.01[b]
		UV-B	0.1097 ± 0.01 [b]	0.1504 ± 0.01[b]	0.1941 ± 0.01[a]	0.0979 ± 0.01[a]

Anthocyanins	Quercetin	Control	0.1244 ± 0.11[a]	0.1593 ± 0.01[a]	0.1105 ± 0.01[a]	0.0444 ± 0.01[a]
		UV-B	0.0545 ± 0.01[b]	0.0841 ± 0.01[b]	0.1096 ± 0.01[a]	0.0586 ± 0.01[a]
	Cyanidin 3-O-galactoside	Control	0.1843 ± 0.01[a]	0.2042 ± 0.01[b]	0.1366 ± 0.01[b]	0.0784 ± 0.01[b]
		UV-B	0.1826 ± 0.01[a]	0.3050 ± 0.01[a]	0.3072 ± 0.01[a]	0.2091 ± 0.01[a]
	Peonidin 3-O-galactoside	Control	0.0026 ± 0.01[a]	0.00242 ± 0.01[a]	0.0031 ± 0.01[a]	0.0003 ± 0.01[b]
		UV-B	0.0007 ± 0.01[b]	0.00130 ± 0.01[b]	0.0012 ± 0.01[b]	0.0008 ± 0.01[a]
Dihydrochalcones	Phloridzin	Control	0.3291 ± 0.01[a]	0.6532 ± 0.01[a]	0.5677 ± 0.03[a]	0.43267 ± 0.03[a]
		UV-B	0.3149 ± 0.01[a]	0.5979 ± 0.02[a]	0.6538 ± 0.03[a]	0.4502 ± 0.01[a]
	Phloretin 2'-O-xyloside-glucoside	Control	0.0777 ± 0.01[a]	0.2002 ± 0.01[a]	0.1492 ± 0.01[a]	0.1388 ± 0.01[a]
		UV-B	0.0862 ± 0.01[a]	0.1587 ± 0.01[b]	0.1829 ± 0.01[a]	0.1369 ± 0.01[a]

An enhancement in chlorogenic acid amounts was also found by Lancaster et al. (2000) in UV-B exposed apples. Since this phenolic compound is known to have a high *in vitro* antioxidant activity, more than vitamin C and E (Rice-Evans et al., 1997), its behavior could explain, in part, the higher value of the antioxidant activity of treated samples during post-harvest (Table 2, Figure 1). It is worth noting that a positive correlation between DPPH• scavenging ability and the presence of chlorogenic acid has been demonstrated in lettuce (Złotek et al., 2014). For this reason, phenolic acids have attracted considerable interest in the past few years due to their potential health benefits (Mattila and Hellström, 2007), the antioxidant nature of phenolic acids being related to the number and kind of substituents on their aromatic rings (Rice-Evans et al., 1996).

The significant increases in chlorogenic, protocatechuic and cryptochlorogenic acid, observed in treated samples at the end of the storage period are in accordance with the accumulation of total phenolic compounds (Table 2, Figure 1). Conversely, in the earlier storage periods, these compounds exhibited a different trend of response to UV-B treatment in respect to total phenols, being importantly increased by the treatment despite decreased or unchanged phenolic levels. It should be noted, however, that, due to their low content in comparison to other phenolic classes (ranging from 0.1 % to 1.2 %), their contribution to changes in total phenols is low. It was not possible to establish a trend in relation to the response of neochlorogenic acid to UV-B radiation. This may be because of the small amount present in the samples.

Within the flavan-3-ols, catechin, epicatechin, and procyanidin B1-B4 were identified in apple skin (Table 2), the most representative compounds being epicatechin and procyanidin B3 (Table 2). None of these compounds were affected by the UV-B-treatment while a reduction by 29 and 33 % was observed at the end of the storage period in the levels of catechin and procyanidin B1, respectively (Table 2). In agreement with these results, both Lancaster et al. (2000) and Hagen et al. (2007) observed little or no effect of UV-B radiation on skin procyanidins of different apple cultivars. Moreover, the group of flavan-3-ols in apple skin of Red Delicious includes main phenolic compounds in the free state (Table 2), which could mostly contribute to the antioxidant capacity (Figure 1). In fact, aglycones have been demonstrated to be more potent antioxidants than their corresponding glycosides (Heim et al., 2002; Vinson et al., 2001) and this presence could have a particular biological significance in fruit (Vinson et al., 2001).

The lower content of quercetins after the treatment for 36 h is in agreement with the decrease of total flavonoids (Figure 1B). This could be related to the consumption of these

antioxidants by the cell to counteract the possible ROS generation due to increased oxidative metabolism (Hideg et al., 2013). In fact, the appearance of stable carbon-centered free radicals occurred after UV-B treatment (Figure 3 and 4) most likely as the result of Fenton-type reactions responsible for tissue damage including lipid peroxidation (Sgherri et al., 2015).

Besides quercetin, different conjugated forms of flavonols were identified in apple skin (Table 2), similarly with what was reported by (Boyer and Liu, 2004). Glycosylated forms of quercetin tended to decrease as the conservation time increased. The UV-B radiation negatively affected the concentration of these compounds immediately after treatment (36 h) and at day 7. However, at the end of the experimental time, there was a significant increase in quercetin 3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-arabfuranoside, and quercetin-3-O-arabpiranoside, respectively by 87, 103, 40 and 34 % (Table 2).

However, the increases of the most quercetin conjugated forms, at the end of storage, indicated that UV-B radiation induced the synthesis of this class of flavonoids, improving health properties of the fruit and contributing to extending their shelf-life. In agreement with these results, also Hagen et al. (2007) found increases in quercetins in the peel of shade-grown apples even if a different dose and duration of the UV-B treatment was applied.

Table 3. Polyphenolic compounds identified in apple skin by m/z, adduct and MRM transition in the HPLC-MS and MS/MS analysis. The details on the chromatographic conditions and mass spectrometric detection are described in Section 2.6.

Polyphenol group	Compound	Abbreviation	*m/z*	adduct	MRM transition
Anthocyanins	Cyanidin 3-O-galactoside	Cy-3gal	449.01	$[M+H]^+$	449.01→286.7
	Peonidin 3-O-galactoside	Pn-3gal	463.01	$[M+H]^+$	463.01→301.1
Dihydrochalcones	Phloritzin (Phloretin 2'-O-glucoside)*	Phlor	481.0	$[M+HCOO^-]^-$	481.0→434.9
	Phloretin 2'-O-xyloside-glucoside	Phlo-xyl	567.1	$[M-H]^-$	567.1→273.0
Flavan-3-ols	(+)Catechin*	Cat	289.0	$[M-H]^-$	289.0→203.1
	(-)Epicatechin*	Epi	289.0	$[M-H]^-$	289.0→245.0
	Procyanidin B1	ProB1	577.0	$[M-H]^-$	577.0→289.0
	Procyanidin B2	ProB2	577.0	$[M-H]^-$	577.0→289.0
	Procyanidin B3	ProB3	577.0	$[M-H]^-$	577.0→406.9
	Procyanidin B4	ProB4	577.0	$[M-H]^-$	577.0→289.0
Flavonols	Quercetin 3-O-glucoside*	Q-glu	463.0	$[M-H]^-$	463.0→301.1
	Quercetin 3-O-galactoside	Q-gal	463.0	$[M-H]^-$	463.0→301.1
	Quercetin 3-O-rhamnoside	Q-rha	447.1	$[M-H]^-$	447.1→301.1
	Quercetin 3-O-arabfuranoside	Q-arab-fur	433.0	$[M-H]^-$	433.0→301.1
	Quercetin 3-O-arabpiranoside	Q-arab-pyr	433.0	$[M-H]^-$	433.0→301.1
	Quercetin	Q	301.0	$[M-H]^-$	301.0→179.0
Hydroxycinnamic acids	Caffeoyl glucoside	Caf-glu	341.2	$[M-H]^-$	341.2→178.9
	Chlorogenic acid*	Clor	353.0	$[M-H]^-$	353.0→191.1
	Neochlorogenic acid	Neoclor	353.0	$[M-H]^-$	353.0→172.8
	p-Coumaroyl glucose	p-Cou-glu	325.1	$[M-H]^-$	325.1→264.8
	p-Coumaroyl quinic acid	p-Cou-qui	337.0	$[M-H]^-$	337.0→172.9
	Protocatechuic Acid	Procat	153.0	$[M-H]^-$	153.0→108.9
	Cryptochlorogenic Acid	CryptoClor	353.0	$[M-H]^-$	353.0→191.1
	Feruloyl glucoside	Fer-glu	355.3	$[M-H]^-$	355.3→193.1

Anthocyanins in apple skin are mainly represented by cyanidin-3-O-galactoside (Table 2), but peonidin-3-O-galactoside was also identified (Table 3). In contrast to the latter, the former was not affected by the treatment of UV-B for 36 h (Table 2). Similarly to flavonols during storage times, cyanidin-3-O-galactoside showed values about 1.5-2.7 higher in the treated samples compared to their controls (Table 2), which influenced the antioxidant activity of apple skin extracts (Figure 1). The importance of accumulation of this kind of molecules following UV-B treatment was previously demonstrated in peach skin where the capacity of cyanidin-3-O-glucoside radicalization was related to protection of other cell organic molecules from oxidation (Sgherri et al., 2015). This could represent a mechanism by which shelf life of UV-B irradiated fruit can be prolonged in the presence of anthocyanins.

The typical dihydrochalcones found in apple skin were phloretin 2'-O-xyloside-glucoside and phloridzin (Table 2), the former being a product of phloridzin oxidation by polyphenoloxidase in the presence of ascorbic acid (Treutter, 2001). No differences were shown between the treated and untreated samples, only phloretin glucoside exhibiting a reduction by 20 % in the UV-B treated apples during 7 d of storage (Table 2).

The differences observed in our study for each phenolic compound could be ascribed to the fact that in apple skin the different genes involved in the phenylpropanoid pathway respond to UV-B uncoordinatedly (Hagen et al., 2007). This uncoordinated synthesis has also been demonstrated regarding flavonoid accumulation in response to high sunlight (Solovchenko and Schmitz-Eiberger, 2003) as well as in the case of phloridzin, catechin and chlorogenic acid, which did not change between shade condition or exposure to sunlight (Awad et al., 2000). Moreover, some authors pointed out that different genotypes have a strong influence on the sensitivity of apple skin to UV-B radiation (Bakhshi and Arakawa, 2006; Glenn and Yuri, 2013).

3.5 Electron paramagnetic resonance (EPR) measurements

Apples exposed to UV-B for 36 h, presented in treated skin the occurrence of stable free radicals as shown in Figures 3 and 4. The addition of the spin trap DEPMPO to the sample skin extract borught to the generation of the spectrum reported in Figure 4. Following spectra simulation, two DEPMPO carbon-centered adducts can be identified. Similarly to what was previously reported for the peach skin (Sgherri et al., 2015) values for EPR hyperfine splitting constants of the first radical (74.5 %) were: aP: 46.57 G, aN, 14.39 G; aH:

20.85 G whereas those for the second one (25.5 %) were: aP: 45.39 G, aN, 14.24 G; aH: 22.53 G.

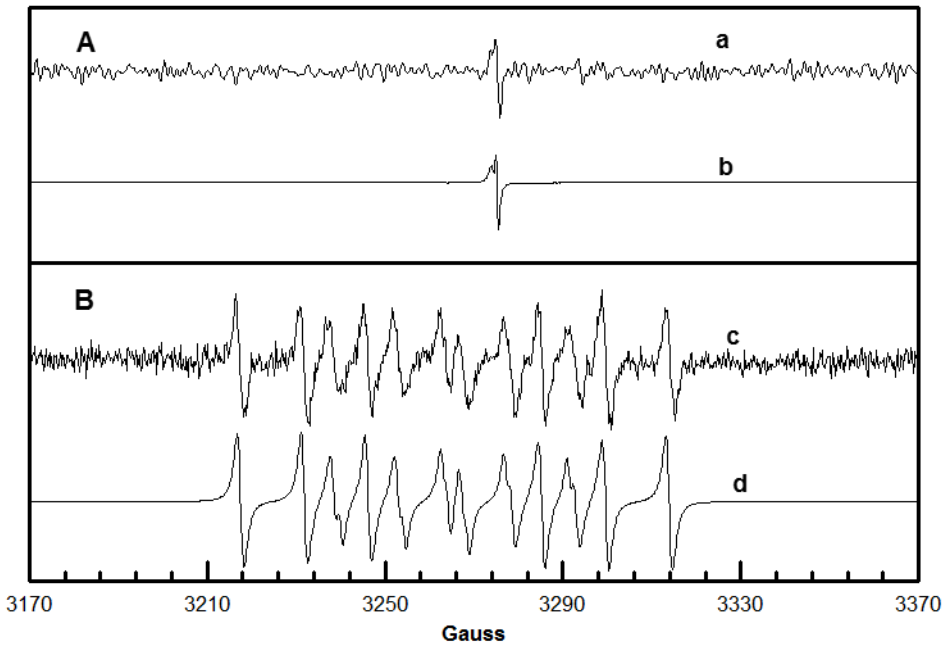

Figure 3. EPR spectra relative to the generation of carbon-centered free radicals following irradiation of apple skin with UV-B (A, before and B after the addition of DEPMPO); a, c experimental; b, d simulated. Two carbon-radicals can be recognized. The first (74.5 %) presents the following hyperfine splitting constants: aP: 46.57 G, aN, 14.39 G; aH: 20.85 G. The second one (25.5 %) presents the following hyperfine splitting constants: aP: 45.39 G, aN, 14.24 G; aH: 22.53 G.

Carbon-centered free radicals occurred in the tissues after 36 h of UV-B treatment as the result of oxidative reactions induced by ROS (Figure 4), in particular, hydroxyl radical, the most harmful species (Sgherri et al., 2015). In the presence of an efficient antioxidative system, tissue damage can be avoided, and apple skin is particularly enriched with antioxidants, mainly phenolic compounds such as quercetins, which are consumed following UV-B treatment (Table 2).

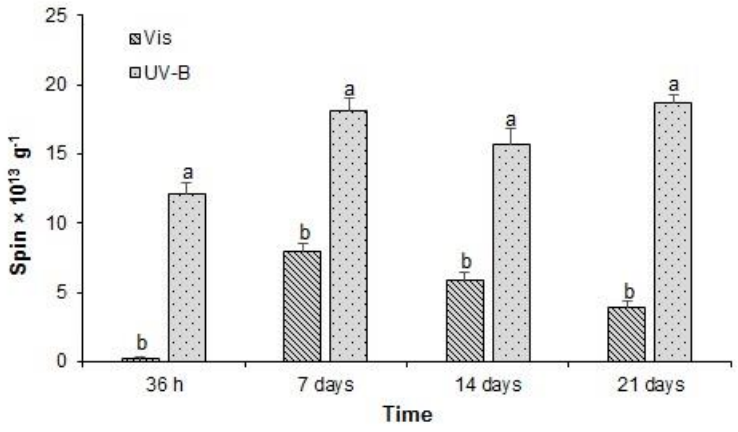

Figure 4. Stable carbon radicals (Spin × 1013 g-1 DW) generated in skin of apples irradiated for 36 hours with UV-B and left to stand in the dark until 21 days. Data represent the mean of 3 replicates ± SE. For each time, significant differences between UV-B and control are indicated with different letters ($p \leq 0.05$), according to one-way ANOVA followed by Tukey's test. Control means not-UV-B-treated apples; UV-B means UV-B-treated apples.

Following storage (7, 14 and 21 d) also control samples of apple skin showed the presence of stable radicals probably due to aging (Figure 4). A relationship between aging and free radical production has been well established in sunflower seeds and pigeonpea orthodox seeds (Bailly et al., 1996; Kalpana and Rao, 1994) as well as in Araucaria seeds (Francini et al., 2006), but evidence on fruits has not been shown yet.

The sample treated with UV-B for 36 h maintained all over the storage period values of carbon-centered radicals always higher than the relative controls. Moreover, the difference in concentration between treated and control fruit increased during the storage (+ 128 %, + 169 % and 382 % after 7, 14 and 21 d respectively) indicating increasing oxidative conditions (Figure 4). However, free radicals are known to act also as signal molecules (Schieber and Chandel, 2014), and the increase in some phenolic compounds in the skin of the apple following storage (Table 2) can be the result of the induction of some biosynthetic pathways by some radical species. In particular, synthesis appeared to be induced as regards hydroxycinnamic acids and anthocyanins, which responded positively to the dose of UV-B applied (Table 2). This could explain why, notwithstanding the free radical increase, tissue of apple skin was able to counteract oxidative damages, such as lipid peroxidation (Sgherri et al., 2017), with the result that main quality parameters of fruits were unaffected.

4 Conclusions

Post-harvest UV-B radiation is a promising tool to modulate the concentration of bioactive compounds in apple fruit. A UV-B for 36 h treatment induced the generation of carbon-centered radicals in the skin, the tissue more exposed to radiation, but quality parameters of the fruit were not affected. Even if firmness progressively decreased and an increasing weight loss occurred during storage, differences between treated and control fruit were not observed. Moreover, in the treated-apple skin, an increase in some important nutraceutical compounds, among which anthocyanins, was observed together with the enhancement in total phenolic compounds at the end of the storage period. Phenols in fruit and vegetables may have a diverse range of properties, and a higher presence of these compounds increases the marketability of the products. In fact, the fruit skin showed a higher antioxidant activity increasing the healthy properties of the fruit, thus demonstrating that UV-B radiation is a valid eco-friendly approach to obtain phenolic-enriched apple fruit.

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References

Allan, A.C., Hellens, R.P., Laing, W.A., 2008. MYB transcription factors that colour our fruit. Trends Plant Sci. 13, 99-102.

Awad, M.A., de Jager, A., van Westing, L.M., 2000. Flavonoid and chlorogenic acid levels in apple fruit: characterisation of variation. Sci. Hortic. 83, 249-263.

Bailly, C., Benamar, A., Corbineau, F., Come, D., 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiol. Plant 97, 104-110.

Bakhshi, D., Arakawa, O., 2006. Effects of UV-B irradiation on phenolic compound accumulation and antioxidant activity in 'Jonathan' apple influenced by bagging, temperature and maturation. J Food Agric Environ 4, 75-79.

Becatti, E., Chkaiban, L., Tonutti, P., Forcato, C., Bonghi, C., Ranieri, A.M., 2010. Short-term postharvest carbon dioxide treatments induce selective molecular and metabolic changes in grape berries. J. Agric. Food Chem 58, 8012-8020.

Boyer, J., Liu, R.H., 2004. Apple phytochemicals and their health benefits. Nutr J 3, 5.

Calvenzani, V., Martinelli, M., Lazzeri, V., Giuntini, D., Dall'Asta, C., Galaverna, G., Tonelli, C., Ranieri, A.P., K., 2010. Response of wild-type and *high pigment-1* tomato fruit to UV-B depletion: flavonoid profiling and gene expression. Planta 231, 755-765.

Castagna, A., Chiavaro, E., Dall'Asta, C., Rinaldi, M., Galaverna, G., Ranieri, A., 2013. Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. Food Chem 137, 151-158.

Castagna, A., Dall'Asta, C., Chiavaro, E., Galaverna, G., Ranieri, A., 2014. Effect of post-harvest UV-B irradiation on polyphenol profile and antioxidant activity in flesh and peel of tomato fruits. Food and Bioprocess Technology 7, 2241-2250.

Chitarra, M.I.F., Chitarra, A.B., 2005. Pós colheita de frutos e hortaliças: fisiologia e manuseio, 2nd ed. FAEPE, Lavras.

Du, W., Avena-Bustillos, R.J., Breksa III, A.P., McHugh, T.H., 2014. UV-B light as a factor affecting total soluble phenolic contents of various whole and fresh-cut specialty crops. Postharvest Biol Technol. 93, 72–82.

Duling, D.R., 1994. Simulation of multiple isotropic spin trap EPR spectra. J Magn Reson. 104, 105-110.

Francini, A., Galleschi, L., Saviozzi, F., Pinzino, C., Izzo, R., Sgherri, C., Navari-Izzo, F., 2006. Enzymatic and non-enzymatic protective mechanisms in recalcitrant seeds of *Araucaria bidwillii* subjected to desiccation. Plant Physiol Biochem. 44, 556-563.

Glenn, D.M., Yuri, J.A., 2013. Photosynthetically active radiation (PAR) × ultraviolet radiation (UV) interact to initiate solar injury in apple. Sci. Hortic. 162, 117–124.

Hagen, S.F., Borge, G.I.A., Bengtsson, G.B., Bilger, W., Berge, A., Haffner, K., Solhaug, K.A., 2007. Phenolic contents and other health and sensory related properties of apple fruit

(*Malus domestica* Borkh, cv. Aroma): Effect of postharvest UV-B irradiation. Postharvest Biol Technol. 45, 1-10.

Heim, K.E., Tagliaferro, A.R., Bobilya, D.J., 2002. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem 13, 572-584.

Hernández-Herrero, J.A., Frutos, M.J., 2014. Colour and antioxidant capacity stability in grape, strawberry and plum peel model juices at different pHs and temperatures. Food Chem 154, 199-204.

Hideg, É., Jansen, M.A.K., Strid, Å., 2013. UV-B exposure, ROS, and stress: inseparable companions or loosely linked associates? Trends Plant Sci. 18, 107-115.

Hooper, L., Cassidy, A., 2006. A review of the health care potential of bioactive compounds. J. Sci. Food Agric. 86, 1805-1813.

Imeh, U., Khokhar, S., 2002. Distribution of Conjugated and Free Phenols in Fruits: Antioxidant Activity and Cultivar Variations. J. Agric. Food Chem 50, 6301-6306.

Kalpana, R., Rao, K.V.M., 1994. Absence of the role of lipid peroxidation during accelerated ageing of seeds of pigeonpea *Cajanus cajan* (L.) Millsp. Seed Sci Technol 22, 253–260.

Kim, D.-O., Chun, O.K., Kim, Y.J., Moon, H.-Y., Lee, C.Y., 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. J. Agric. Food Chem 51, 6509-6515.

Lancaster, J.E., Dougall, D.K., 1992. Regulation of skin color in apples. CRC Crit. Rev. Plant. Sci. 10, 487-502.

Lancaster, J.E., Reay, P.F., Norris, J., Butler, R.C., 2000. Induction of flavonoids and phenolic acids in apple by UV-B and temperature. J. Hortic. Sci. Biotechnol. 75, 142-148.

Lin-Wang, K., Micheletti, D., Palmer, J., Volz, R., Lozano, L., Espley, R., Hellens, R.P., Chagnè, D., Rowan, D.D., Troggio, M., Iglesias, I., Allan, A.C., 2011. High temperature reduces apple fruit colour via modulation of the anthocyanin regulatory complex. Plant Cell Environ. 34, 1176-1190.

Liu, C., Han, X., Cai, L., Lu, X., Ying, T., Jiang, Z., 2011. Postharvest UV-B irradiation maintains sensory qualities and enhances antioxidant capacity in tomato fruit during storage. Postharvest Biol Technol. 59, 232–237.

Lotito, S.B., Frei, B., 2004. Relevance of apple polyphenols as antioxidants in human plasma: contrasting in vitro and in vivo effects. Free Radic Biol Med 36, 201–211.

Majidi, H., Minaei, S., Almasi, M., Mostofi, Y., 2011. Total soluble solids, titratable acidity and repining index of tomato in various storage conditions. Aust. J. Basic & Appl. Sci. 5, 1723-1726.

Mattila, P., Hellström, J., 2007. Phenolic acids in potatoes, vegetables, and some of their products. J Food Compost Anal. 20, 152−160.

Pellegrini, N., Re, R., Yang, M., Rice-Evans, C., 1999. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. Methods Enzymol. 299, 379–389.

Peng, T., Moriguchi, T., 2013. The molecular network regulating the coloration in apple. Sci. Hortic. 163, 1–9.

Pinzino, C., Forte, C., 1992. Esr-endor. Istituto di Chimica Quantistica ed Energetica Molecolare-CNR, Pisa, Italy.

Rice-Evans, C., Miller, N.J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2, 152−159.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20, 933-956.

Ryan, K.G., Swinny, E.E., Markham, K.R., Winefield, C., 2002. Flavonoid gene expression and UV photoprotection in transgenic and mutant Petunia leaves. Phytochemistry 59, 23-32.

Saure, M.C., 1990. External control of anthocyanin formation in apple. Sci. Hortic. 42, 181–218.

Scattino, C., Castagna, A., Neugart, S., Chan, H.M., Schreiner, M., Crisosto, C.H., Tonutti, P., Ranieri, A., 2014. Post-harvest UV-B irradiation induces changes of phenol contents and corresponding biosynthetic gene expression in peaches and nectarines. Food Chem 163, 51-60.

Schieber, M., Chandel, Navdeep S., 2014. ROS function in redox signaling and oxidative stress. Current Biology 24, 453-462.

Sgherri, C., Pinzino, C., Quartacci, M.F., 2017. Reactive oxygen species and photosynthetic functioning: past and present, In: Singh, V.P., Singh, S., Tripathi, D.K., Prasad, S.M., Chauhan, D.K. (Eds.), Reactive oxygen species in plants: boon or bane? Revisiting the Role of ROS. J. Wiley & Sons, New York (USA), pp. 137-155.

Sgherri, C., Scattino, C., Pinzino, C., Tonutti, P., Ranieri, A.M., 2015. Ultraviolet-B radiation applied to detached peach fruit: A study of free radical generation by EPR spin trapping. Plant Physiol Biochem. 96, 124-131.

Solovchenko, A., Schmitz-Eiberger, M., 2003. Significance of skin flavonoids for UV-B-protection in apple fruits. J. Exp. Bot. 54, 1977-1984.

Suesslin, C., Frohnmeyer, H., 2003. An Arabidopsis mutant defective in UV-B light-mediated responses. Plant J 33, 591-601.

Sun, S., Xin, L., Gao, H., Wang, J., Li, P., 2014. Response of phenolic compounds in 'Golden Delicious' and 'Red Delicious' apples peel to fruit bagging and subsequent sunlight re-exposure. Sci. Hortic. 168, 161–167.

Takshak, S., Agrawal, S.B., 2014. Effect of ultraviolet-B radiation on biomass production, lipid peroxidation, reactive oxygen species, and antioxidants in *Withania somnifera*. Biol Plant. 58, 328-334.

Treutter, D., 2001. Biosynthesis of phenolic compounds and its regulation in apple. Plant Growth Regul 34, 71-89.

Ubi, B.E., Honda, C., Bessho, H., Kondo, S., Wada, M., Kobayashi, S., Moriguchi, T., 2006. Expression analysis of anthocyanin biosynthetic genes in apple skin: Effect of UV-B and temperature. Plant Sci. 170, 571-578.

Vinson, J.A., Su, X., Zubik, L., Bose, P., 2001. Phenol antioxidant quantity and quality in foods: Fruits. J. Agric. Food Chem 49, 5315-5321.

Wildman, R.E.C., 2006. Handbook of nutraceuticals and functional foods, 2nd ed. CRC Press, Boca Raton.

Zhao, C., Mao, K., You, C.-X., Zhao, X.-Y., Wang, S.-H., Li, Y.-Y., Hao, Y.-J., 2016. Molecular cloning and functional analysis of a UV-B photoreceptor gene, MdUVR8 (UV Resistance Locus 8), from apple. Plant Sci. 247, 115-126.

Złotek, U., Świeca, M., Jakubczyk, A., 2014. Effect of abiotic elicitation on main health-promoting compounds, antioxidant activity and commercial quality of butter lettuce (*Lactuca sativa* L.). Food Chem 148, 253-260.

CAPÍTULO 6

APPLICATION OF SUPPLEMENTAL UV-B RADIATION IN PRE-HARVEST TO ENHANCE BIOACTIVE COMPOUNDS ACCUMULATION IN GREEN AND RED LETTUCE

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Abstract

In order to evaluate whether a daily supplemental UV-B radiation was effective in increasing the concentration of health-promoting compounds in green and red lettuce, carotenoids, chlorophylls and flavonoid compounds were quantified. The plants were exposed to daily doses of UV-B radiation for 1 hour fortwo weeks during growth. UV-B treated green lettuces at the second week presented a greater level of quercetin than control. Caffeoyltartaric acid, caffeoylquinic acid and caffeoylmalic acid also showed a significant accumulation in green lettuce after two weeks of UV-B treatment in comparison to those non-treated. Regarding carotenoid content, lutein, neoxanthin and V+A+Z showed significant increased values after two weeks of UV-B treatment in comparison to the non-treated green lettuces. However, chlorophylls and carotenoids content did not present significant difference between the analyzed weeks for the red lettuces. In the other hand, quercetins, as well as caffeoyltartaric acid, caffeoylquinic acid and cianidin of red lettuce showed a significant increase after UV-B treatment at both weeks in comparison with control red lettuces. The results suggest that it is possible to modulate the content of bioactive compounds of lettuces, mainly red ones, using supplemental UV-B radiation during growth.

Keywords: Chlorophylls; phenolic acids; flavonoids, carotenoids; HPLC-MS

1 Introduction

Lettuce (*Lactuca sativa*) is one of the most popular vegetables for salads that are consumed in increasing amounts due to their perception as “healthy” foods (DUPONT *et al.*, 2000). The health-promoting properties are attributed to a large amount to bioactive compounds (e.g., vitamins C and E, carotenoids, polyphenols) and fiber content (MULABAGAL *et al.*, 2010). Other phytochemicals that contribute to both the sensory and health-promoting properties of lettuce are anthocyanins and chlorophylls (LI *et al.*, 2010).

Within polyphenols, flavonoids are plant secondary metabolites that play multiple physiological roles in pigmentation, growth and reproduction. Besides, the plants use them as antioxidants or oxygen scavengers (MURATA *et al.*, 2007) representing a defence line against environmental factors, in particular biotic and abiotic stresses (NISHIYAMA *et al.*, 2001). Among the abiotic stresses, ultraviolet (UV) radiation has been reported to be the main factor able to induce phenolic accumulation (BASSMAN, 2004; ZHANG e BJÖRN, 2009).

Green leafy vegetables as lettuce are a major source of dietary carotenoids which can act as antioxidants and reduce the incidence of cataracts and macular degeneration (MOELLER *et al.*, 2000). According to Caldwell e Britz (2006), carotenoids and chlorophylls of vegetable leaf tissues are sensitive to plant growth conditions. Red lettuce is characterised by accumulation of anthocyanins, a class of flavonoids that have multiple biological functions, including visual signal for pollinating insects in plants (CHEYNIER *et al.*, 2013) and an important protecting role against a number of human diseases (SANTOS BUELGA *et al.*, 2014), like neuronal and cardiovascular illnesses; cancer and diabetes (YOUSUF *et al.*, 2016). Besides anthocyanins and carotenoids, lettuce is a good dietary source of several phenolic acids, such as caffeic acid derivatives, and flavonoid glycosides, such as quercetin and luteolin (MARIN *et al.*, 2015). A higher presence of phenolic compounds such as flavonoids may increase the marketability of fresh foods (ASSUMPÇÃO *et al.*, 2018), improving sensory quality and nutraceutical properties.

With rising consumer interest in healthy foods, attention has shifted to the enhancement of health-promoting phytochemicals. Therefore, to obtain fruit and vegetables enriched with phytochemicals, elicitor treatments might be used either singularly or in combination to trigger desired effects (SCHREINER e HUYSKENS-KEIL, 2006). In this context, during the last decades, several studies have shown promising results regarding the use of ultraviolet (UV) radiation as a non-molecular tool for the modification of the content of bioactive compounds in food (RODRIGUEZ *et al.*, 2014; SANKARI *et al.*, 2017; SHENG *et al.*, 2018).

UV-B radiation (280-315 nm) acts as an abiotic physical elicitor of resistance mechanisms, leading to a rapid increase of stress-response compounds such as phenols, flavonoids, and phytoalexins (SCHREINER e HUYSKENS-KEIL, 2006). UV-B radiation has diverse effects on plant developmental processes, growth, photosynthesis and secondary plant metabolites (NEUGART e SCHREINER, 2018).

The impact of UV-B on plants depends on the fluency rate, exposure time, wavelength of UV-B radiation and the amount of UV-B relative to photosynthetic active radiation (UV-B PAR ratio) (JANSEN *et al.*, 1998). The UV-B effect is assumedly due to the different applied doses of irradiation that commonly vary between 0.2 and 8 kJ m^{-2} (SCHREINER e HUYSKENS-KEIL, 2006).

Since carotenoids, chlorophylls and flavonoid compounds can be increased by ecologically relevant levels of UV-B in glasshouse production, this study was designed to verify whether supplemental UV-B radiation was effective in increasing the concentration of health-promoting compounds in green and red lettuce.

2 Material and Methods

2.1 Chemicals

Acetonitrile, acetone, methanol and ethy acetate HPLC grade (assay 99.9 %) were purchased from Panreac Química S.A. (Barcelona, Spain); trifluoroacetic acid for HPLC (assay 99 %) and formic acid for HPLC (assay 98 %) were purchased from Sigma–Aldrich (Madrid, Spain). Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). Water was purified by a Milli-Q water purification system from Millipore (Bedford, MA, USA).

2.2 Plant material and treatment

Green leaf and red leaf lettuce (*Lactuca sativa* L.) plantlets (cv. Salad Bowl) were purchased by an italian commercial grower (Pisa, Italy) and transplanted during the spring of 2016. Baby leaves at the commercial maturity stage (10−12 cm) were disposed in a greenhouse for two weeks before the beginning of the experiment (Temperature: 16 °C; humidity: 75 %). Twenty plants were transported to the laboratory at the Department of Agriculture, Food, and Environment, University of Pisa (Italy) for transplantation. The plants

were distributed into two climatic chambers (18 °C; R.H. 85 %), each equipped with three UV-B lamp tubes (Philips Ultraviolet B, TL 20W – 12RS, Koninklijke Philips Electronics, Eindhoven, The Netherlands) which provided 1.69 W m^{-2} at plant height. The plants were placed approximately 0.40 m under the lamps and were aligned parallel to the lamp tubes in order to ensure a uniform UV-B dose. The plants were exposed to daily doses of 0.5 kJ m^{-2} d^{-1} UV-B radiation for 1 h with acclimatization intervals of 23 h for two weeks during growth. Control plants were placed for the same time in the climatic chamber where UV-B lamps were screened by benzophenone-treated polyethylene film. This kind of compound is known to block UV-B radiation (CALVENZANI *et al.*, 2010). After the first week, a group of five plants was sampled (week 1) while the remaining plants were sampled at the end of the second week (week 2). The lettuces were immediately frozen in liquid nitrogen and stored at −80 °C for further analyses. Leaf disks were equally frozen for chlorophylls and carotenoid analysis.

2.3 Flavonoids glycosides extraction

Flavonoids were analyzed according to Schmidt *et al.* (2010) with slight modification. Lyophilized, ground material (0.02 g) was extracted with 600 µL of 60 % aqueous methanol on a magnetic stirrer plate for 40 min at 20 °C. The extract was centrifuged at 4500 rpm for 10 min at the same temperature, and the supernatant was collected in a reaction tube. This process was repeated twice with 300 µL of 60 % aqueous methanol for 20 min and 10 min, respectively; the three corresponding supernatants were combined. The extract was subsequently evaporated until it was dry and was then suspended in 200 µL of 10 % aqueous methanol. The extract was centrifuged at 3000 rpm for 5 min at 20 °C through a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma Aldrich Chemical Co., St. Louis, MO, USA) for the HPLC analysis. Each extraction was carried out in duplicate.

2.4 HPLC–MS analysis method for flavonoids

Flavonoid composition (including hydroxycinnamic acid derivatives and glycosides of flavonoids) and concentrations were determined from the filtrate using a series 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degaser, binary pump, autosampler, column oven, and photodiode array detector. An Ascentis® Express F5 column (150 mm × 4.6 mm, 5 µm, Supelco) was used to separate the compounds at a temperature of 25°C. Eluent A was 0.5 % acetic acid, and eluent B was 100 % acetonitrile. The gradient used

for eluent B was 5-12 % (0-3 min), 12-25 % (3-46 min), 25-90 % (46-49.5 min), 90 % isocratic (49.5-52 min), 90-5 % (52-52.7 min), and 5 % isocratic (52.7-59 min).

The determination was conducted at a flow rate of 0.85 mL min^{-1} and a wavelength of 280 nm, 320 nm, 330 nm, 370 nm and 520 nm. The hydroxycinnamic acid derivatives and glycosides of flavonoids were tentative identified as deprotonated molecular ions and characteristic mass fragment ions according to Schmidt *et al.* (2010) and Neugart *et al.* (2014) by HPLC-DAD-ESI-MS^n using an Bruker amazon SL ion trap mass spectrometer in negative ionisation mode. For the identification of the peaks the data were compared to the literature of the investigated species and their relatives. In the mass spectrometer nitrogen was used as the dry gas (10 L min^{-1}, 325 °C) and the nebulizer gas (40 psi) with a capillary voltage of -3500 V. Helium was used as the collision gas in the ion trap. The mass optimization for the ion optics of the mass spectrometer for quercetin was performed at m/z 301 or arbitrarily at m/z 1000. The MS^n experiments were performed in auto up to MS^3 in a scan from m/z 200-2000. Standards were used for external calibration curves in a semi-quantitative approach. Results are presented as mg g^{-1} dry weight.

2.5 Chlorophylls and carotenoids extraction

Carotenoids and chlorophylls were extracted and analysed according to the method reported by Castagna *et al.* (2001). Leaf disks were homogenized with 100 % HPLC-grade acetone, 1 mM sodium ascorbate under dimmed room light and filtered through 0.2 µm filters (Sartorius Stedim Biotech, Goettingen, Germany).

2.6 *HPLC–DAD analysis method for chlorophylls and carotenoids*

Extracts were immediately analysed by HPLC system (Spectra System P4000) equipped with a UV 6000 LP photodiode array detector (Thermo Fisher Scientific, Waltham, MA) using a Zorbax ODS column (5 µm particle size, 250 × 4.6 mm Ø, Agilent Technologies, Santa Clara, CA, USA). Pigments were eluted at a flow rate of 1 mL.min^{-1} using the following gradient of acetonitrile/methanol (85/15, solvent A) and methanol/ethyl acetate (68/32, solvent B): solvent A: 100 % (0-15 min), 100-0 % (15-17.5 min), 0 % (17.5-32 min), followed by 5 min re-equilibration in the initial condition before the next injection. Pigments were detected at 445 nm and quantified using calibration curves of commercial standards of chlorophyll *a*; chlorophyll *b*, lutein, neoxanthin, zeaxanthin, violaxanthin, antheraxanthin and β-carotene (Sigma-Aldrich, Milan, Italy). Results are presented as µg/cm^2.

2.7 Statistical Analysis

Data were subjected to one-way ANOVA using Statistica 11.0 software. Significant differences between treated and control lettuces at each sampling time were calculated using at least three replicates according to Tukey's test ($p \leq 0.05$).

3 Results and Discussion

3.1 Effects of UV-B on flavonoids in green lettuce

In the present work we investigated which role the UV-B treatment, carried out performing daily doses of 0.5 kJ m^{-2} for two weeks, plays on the accumulation of phenolic compounds in two different variety of lettuce characterized by different color and then in different profile of secondary metabolites.

In green lettuce, among the phenols, the main phenolic acids detected by HPLC-DAD-ESI-MS^n were caffeoyltartaric acid, caffeoylquinic acid and caffeoylmalic acid (Table 1). Regarding flavonoids the most represented was the flavonol quercetin that is present under different forms depending of its esterification with different sugars.

According to Table 1, during treatment time, the level of quercetin has diminished in treated and control samples. However, UV-B treated lettuces at the second week presented a greater level of quercetin than control lettuces. According to Goetz *et al.* (2010), mainly quercetin glycosides can be enhanced by UV-B radiation in Arabidopsis, the main UV-B studying model. Quercetin and cyanidin glycosides concentrations in lettuce was found to be highly sensitive to the transmission of UV light (ZIVCAK, 2017).

Table 1. Flavonoids determined after HPLC-UV-DAD analysis of green lettuce treated (UV-B) and non-treated (Control) for 1 and 2 weeks. Results expressed as $mg.g^{-1}$. Data represent the mean of 3 replicates ± SD.

Compounds		*Time of treatment*	
		Week 1	Week 2
Caffeoyltartaric acid	Control	0.65±0.12aA	0.05±0.01bB
	UV-B	0.33±0.07bB	0.59±0.01aA
Chlorogenic/Caffeoylquinic acid	Control	5.52±0.13aB	1.51±0.03bB
	UV-B	7.30±0.02aA	3.97±0.11bA
Caffeoylmalic acid	Control	1.44±0.07aA	0.51±0.06bB
	UV-B	1.45±0.01aA	1.29±0.12aA
Quercetin-3-*O*-(6‘‘-O-malonyl)-glucoside isomer 1	Control	2.11±0.11aB	0.52±0.07bB
	UV-B	2.84±0.01aA	1.78±0.01bA
Quercetin-3-*O*-(6‘‘-O-malonyl)-glucoside isomer 2	Control	0.10±0.01aA	0.03±0.01bA
	UV-B	0.10±0.01aA	0.09±0.01aA
Quercetin-3-*O*-glucoside/Quercetin-3-*O*-glucuronide	Control	1.07±0.03aA	0.34±0.02bB
	UV-B	1.33±0.01aA	0.73±0.07bA
Luteolin-3-glucoside	Control	4.87±0.16aB	0.57±0.05bB
	UV-B	5.68±0.02aA	1.53±0.07bA
Cyanidin-3-*O*-(6‘‘-O-malonyl)-glucoside	Control	nd	nd
	UV-B	nd	nd

Significant differences between UV-B and control are indicated with different uppercase letters at the same column ($p \leq 0.05$) and significant differences between weeks are indicated with different lowercase letters at the same line ($p \leq 0.05$).

Following the same trend, during treatment time, the level of three phenolic acids identified diminished. However, caffeoyltartaric acid, caffeoylquinic acid and caffeoylmalic acid showed a significant accumulation in lettuce after two weeks of UV-B treatment in comparison to those non-treated (Table 1). The caffeoylquinic acid (chlorogenic acid), a hydroxycinnamic acid derivative in lettuce, provide antioxidant properties (FIOL *et al.*, 2012). The absorption maxima of hydroxycinnamic acids range from 318 to 328 nm, thereby suggesting that they take an active response when exposed to UV-B radiation (NEUGART *et al.*, 2014).

In plants, UV-B-absorbing phenolic compounds accumulation is generally described as a key element in acclimation to UV-B exposure (BASSMAN, 2004). Flavonoids broadly have a role in many facets of plant physiology. They can act as antioxidants or ROS scavengers (ZIVCAK, 2017). According to Becker *et al.* (2015), the antioxidant activity of quercetin, luteolin and cyanidin glycosides as well as caffeic acid derivatives is equal to or even outperforms of ascorbic acid and α-tocopherol.

Anthocyanins were not detected in green lettuce and could shield the photosynthetic apparatus from excess radiation (STEYN *et al.*, 2002), turning green lettuce potentially more vulnerable to radiation than red cultivars (BECKER *et al.*, 2015).

Tsormpatsidis *et al.* (2010), studying lettuce plants in polyethylene clad tunnels exposed to either ambient or UV-free conditions, reported high yields and high phytochemical content if the plants are exposed to UV before the harvest.

3.1 Effects of UV-B on chlorophylls and carotenoids in green lettuce

Chlorophylls content showed significant difference after two weeks in green lettuce treated with UV-B (Table 2). In addition, photochemical efficiency values suggest that the UV-B treated lettuce did not suffer photoinhibitory damage, since they presented Fv/Fm (maximum quantum yield) higher than 0.75 (data not shown). This index reflects the status of the plant at that time, in relation to its own metabolism and with the environment in which it is found (SANTOS *et al.*, 2010).

Table 2. Chlorophylls and carotenoids determined after HPLC-UV-DAD analysis of green lettuce treated (UV-B) and non-treated (Control) for 1 and 2 weeks. Results expressed as $\mu g.cm^{-2}$. Data represent the mean of 6 replicates ± SD.

		Time of treatment	
		Week 1	Week 2
Chlorophyll *a*	Control	30.88±3.61aA	28.42±5.43aB
	UV-B	31.55±5.66aA	34.51±2.62aA
Chlorophyll *b*	Control	7.16±1.01aA	7.16±1.07aB
	UV-B	7.21±1.19aA	8.16±0.66aA
Total chlorophyll	Control	38.03±4.58aA	35.58±6.46aB
	UV-B	38.77±6.84aA	42.67±3.23aA
Chl *a*/chl *b* ratio	Control	4.33±0.22aA	3.95±0.24bB
	UV-B	4.37±0.14aA	4.23±0.15aA
Lutein	Control	1.45±0.23aA	1.17±0.22bB
	UV-B	1.22±0.22aB	1.40±0.12aA
Neoxanthin	Control	0.46±0.12aA	0.39±0.08aB
	UV-B	0.39±0.06aA	0.45±0.05aA
V+A+Z	Control	1.30±0.20aA	1.04±0.22bB
	UV-B	0.94±0.22aB	1.20±0.13aA
Total xanthophylls	Control	3.22±0.51aA	2.65±0.50bB
	UV-B	2.50±0.43aB	3.05±0.29aA
β-carotene	Control	1.80±1.85aB	3.79±0.93aB
	UV-B	3.21±0.88aA	4.04±0.27aA
Total carotenoids	Control	5.02±1.80aA	6.44±1.42aB
	UV-B	5.70±1.27aA	7.09±0.52aA

Significant differences between UV-B and control are indicated with different uppercase letters at the same column ($p \leq 0.05$) and significant differences between weeks are indicated with different lowercase letters at the same line ($p \leq 0.05$).

Regarding carotenoid content, lutein, neoxanthin and V+A+Z (where V means violaxanthin; A means antheraxanthin and Z means zeaxanthin) showed increased values after two weeks of UV-B treatment in comparison to the non-treated lettuces (Table 2). An increase in V+A+Z content demonstrates the ability to dissipate excess of luminous energy from the leaf in the xanthophyll cycle. This mechanism is also known to protect plant against photo-oxidation.

According to Becker *et al.* (2015), lutein, β-carotene, neoxanthin and violaxanthin are major xanthophyll pigments of the light harvesting complex of lettuce photosystem II and light harvesting antenna. Apart from being accessory pigments, they are involved in non-photochemical quenching and are also potent ROS scavengers (JAHNS e HOLZWARTH, 2012).

The absence of α-carotene in the green lettuce may be due to complete conversion of the compound to lutein, which is reported previously in other leafy vegetables, according to Saini *et al.* (2016).

Besides the importance for the plant metabolism, β-carotene and lutein are important for human eye health and are used as supplements to reduce the risk of glaucoma and age-related macular degeneration (SCHRÖTER *et al.*, 2017). Carotenoids also have become of interest because of their association with the prevention of atherosclerosis and the risk reduction of degenerative diseases (ROJAS-GARBANZO *et al.*, 2017). Some of the analyzed carotenoids also have provitamin A activity, like lutein, β-carotene and zeaxanthin (part of V +A+Z).

3.2 Effects of UV-B on flavonoids in red lettuce

Quercetins (Table 3), as well as caffeoyltartaric acid and caffeoylquinic acid of red lettuce showed a significant increase after UV-B treatment at the first and second weeks in comparison with control lettuces. However, caffeoylmalic acid in red lettuce extracts was not affected, but rather it decreased in both samples during the treatment.

Quercetin is one of the main dietary flavonoids present as glycoside in the plant while the main hydroxycinnamic acid is chlorogenic acid (ZIETZ *et al.*, 2010). According to Neugart and Schreiner (2018), quercetin and poly-hydroxylated flavonoids are enhanced, while kaempferol and mono-hydroxylated flavonoids remain unaffected by UV-B, which is suggested by the accumulation of quercetin glycoside in treated lettuces in relation to control.

Table 3. Flavonoids determined after HPLC-UV-DAD analysis of red lettuce treated (UV-B) and non-treated (Control) for 1 and 2 weeks. Results expressed as $mg.g^{-1}$. Data represent the mean of 3 replicates ± SD.

		Time of treatment	
		Week 1	Week 2
Caffeoyltartaric acid	Control	15.37±0.63aB	10.68±0.70bB
	UV-B	17.06±0.14aA	15.84±1.38bA
Chlororgenic/Caffeoylquinic acid	Control	5.25±0.01aB	4.51±0.32bB
	UV-B	7.31±0.23aA	6.45±0.27bA
Caffeoylmalic acid	Control	0.78±0.01aA	0.59±0.08bA
	UV-B	0.78±0.02aA	0.53±0.05bA
Quercetin-3-*O*-(6‘‘-O-malonyl)-glucoside isomer 1	Control	5.07±0.28aB	5.92±0.70aB
	UV-B	19.41±0.01aA	17.29±0.14bA
Quercetin-3-*O*-(6‘‘-O-malonyl)-glucoside isomer 2	Control	0.18±0.01aB	0.21±0.02aB
	UV-B	0.69±0.07aA	0.77±0.11aA
Quercetin-3-*O*-glucoside/Quercetin-3-*O*-glucuronide	Control	2.35±0.03aB	0.93±0.09bB
	UV-B	3.56±0.28aA	2.56±0.47bA
Luteolin-3-glucoside	Control	10.01±0.02aB	3.95±0.60bB
	UV-B	14.77±0.01aA	11.66±0.22bA
Cyanidin-3-*O*-(6‘‘-O-malonyl)-glucoside	Control	0.17±0.03aB	0.14±0.01aB
	UV-B	0.28±0.01bA	0.33±0.01aA

Significant differences between UV-B and control are indicated with different uppercase letters at the same column ($p \leq 0.05$) and significant differences between weeks are indicated with different lowercase letters at the same line ($p \leq 0.05$).

The results of phenolic compounds found in red lettuce are in agreement to those reported by Marin *et al.* (2015), in which the major phenolic compounds in red leaf lettuce are: quercetins, luteolin, cyanidin, caffeoyltartaric acid (chicoric acid), caffeoylquinic acid (chlorogenic acid) and caffeoylmalic acid.

According to Pérez-López *et al.* (2014), several authors have studied the bioactive compounds of different cultivars of lettuce demonstrating a higher antioxidant power in red versus green lettuce and indicating the importance of eating a particular variety of food

sources such as colored foods. In the present study, the green lettuces treated with UV-B after two weeks reached a total of 9.98 $mg.g^{-1}$ of flavonoids, while the red ones reached 55.1 $mg.g^{-1}$. Red lettuce varieties have been characterized by a higher content of hydroxycinnamic acids, flavones, flavonols and anthocyanins compared to the green varieties of lettuce plants (ZIVCAK, 2017). Ozgen and Sekerci (2011) studying the effect of leaf posistion on phytochemicals of green and red lettuce, also reported significant differences in phenolics content and related antioxidant activity between the samples, being that the red ones presented larger accumulations of bioactive compounds.

Among environmental factors, radiation and temperature are the two most influential climatic variables for the phenolic biosynthesis of red lettuce (MARIN *et al.*, 2015). Besides to light intensity, light quality also affects the biosynthesis of anthocyanins and especially UV light has been associated with regulation of anthocyanin biosynthesis in food (JAAKOLA, 2013). The content of cyanidin-3-maonylgluc in red lettuces after one and two weeks was positively affected by the UV-B treatment. Rodriguez *et al.* (2014) observed that UV-B exposed plants showed a stronger red colouration in the leaves and suggested that very low amounts of UV-B are enough to induce anthocyanin synthesis and improve the quality of red leaf lettuce. Color is one of the most important attributes affecting consumer perception of quality. As such, it plays a key role in food preference and acceptability and may even influence taste thresholds and sweetness perception (MARIN *et al.*, 2015).

These data are important once the flavonoids act as antioxidants and can offer protective effect against degenerative diseases when this type of food is part of the diet. According to Saha *et al.* (2016), the variability in the compositions and quantities of compounds in different foods indicate the importance of eating a variety of foods, especially the coloured ones.

3.1 Effects of UV-B on chlorophylls and carotenoids in red lettuce

Chlorophylls and carotenoids content did not present significant difference between the analyzed weeks for the red lettuces. There is the possibility that the biosynthesis of anthocyanins, which absorb in the photosynthetic part of the spectrum, reduces the photosynthetic capability of the leaves or that the increased production of secondary compounds acts in direct competition for assimilated carbon to the plants growth process (GARCÍA-MACÍAS *et al.*, 2007).

This same trend of results was observed by Caldwell *et al.* (2006) that reported that supplemental UV-B increased the carotenoid and chlorophyll concentration of green leaf lettuce, while can even reduce the levels of these compounds in red leaf lettuce.

The research of Mou *et al.* (2005) corroborates with the data of this work. Analysing the contents of carotenoids in 22 crisphead cultivars, between green and red ones, the researchers reported the combined level of β-carotene and lutein was the highest in green leaf followed by red leaf types. Based on studies reporting carotenoid contents, green lettuce is a valuable source of carotenoids, in particular β-carotene and lutein (KIM *et al.*, 2016).

Exposure of red lettuce to UV-B radiation during the second week of cultivation allowed the cyanidin glycoside concentration to increase by 101.35 %. The finding that cyanidin is the main anthocyanin is consistent with previous reports (GARCÍA-MACÍAS *et al.*, 2007), where differences induced by exposure to UV light were most clearly seen in the color of the leaves, due to the anthocyanin content.

Tabela 4. Chlorophylls and carotenoids determined after HPLC-UV-DAD analysis of red lettuce treated (UV-B) and non-treated (Control) for 1 and 2 weeks. Results expressed as $\mu g.cm^{-2}$. Data represent the mean of 6 replicates ± SD.

		Time of treatment	
		Week 1	Week 2
Chlorophyll *a*	Control	32.77±3.11aB	34.53±7.06aA
	UV-B	38.72±6.40aA	39.63±4.70aA
Chlorophyll *b*	Control	10.49±0.27aA	8.59±1.55bA
	UV-B	9.50±1.49aA	9.83±1.04aA
Total chlorophyll	Control	43.26±3.07aA	43.12±8.58aA
	UV-B	48.22±7.88aA	49.46±5.73aA
Chl *a*/chl *b* ratio	Control	3.13±0.32bB	4.00±0.20aA
	UV-B	4.07±0.09aA	4.03±0.08aA
Lutein	Control	1.77±0.04aA	1.43±0.26bA
	UV-B	1.53±0.27aB	1.57±0.16aA
Neoxanthin	Control	0.41±0.06aB	0.46±0.08aA
	UV-B	0.54±0.11aA	0.52±0.09aA
V+A+Z	Control	1.08±0.15aA	1.12±0.18aB
	UV-B	1.25±0.33aA	1.32±0.12aA
Total xanthophylls	Control	3.26±0.21aA	3.00±0.49aA
	UV-B	3.32±0.68aA	3.42±0.35aA
β-carotene	Control	3.66±0.44aB	3.77±0.88aA
	UV-B	4.55±0.97aA	4.34±0.60aA
Total carotenoids	Control	6.93±0.64aA	6.78±1.36aA
	UV-B	7.86±1.64aA	7.76±0.93aA

Significant differences between UV-B and control are indicated with different uppercase letters at the same column ($p \leq 0.05$) and significant differences between weeks are indicated with different lowercase letters at the same line ($p \leq 0.05$).

4 Conclusions

The content of flavonoids of extracts from red lettuce and the content of carotenoids from green lettuce were enhanced drastically by growing the crop under supplementary UV-B light, mainly after two weeks. This provides scope to consider lettuce as a potential source of bioactive compounds, essentially carotenoids, quercetin and cyanidin. Furthermore, UV-B radiation has proved to be a promising technology for increasing the healthy properties of the lettuce.

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References

ASSUMPÇÃO, C. F.; HERMES, V. S.; PAGNO, C.; CASTAGNA, A.; MANNUCCI, A.; SGHERRI, C.; PINZINO, C.; RANIERI, A.; FLÔRES, S. H.; RIOS, A. D. O. Phenolic enrichment in apple skin following post-harvest fruit UV-B treatment. **Postharvest Biology and Technology,** v. 138, p. 37-45, 2018.

BASSMAN, J. H. Ecosystem consequences of enhanced solar ultraviolet radiation: secondary plant metabolites as mediators of multiple trophic interactions in terrestrial plant communities. **Photochemistry and Photobiology,** v. 79, p. 382-398, 2004.

BECKER, C.; URLIĆ, B.; JUKIĆ ŠPIKA, M.; KLÄRING, H.-P.; KRUMBEIN, A.; BALDERMANN, S.; GORETA BAN, S.; PERICA, S.; SCHWARZ, D. Nitrogen limited red and green leaf lettuce accumulate flavonoid glycosides, caffeic acid derivatives, and sucrose while losing chlorophylls, B-carotene and xanthophylls. **PLoS ONE,** v. 10, n. 11, p. e0142867, 2015.

CALDWELL, C. R.; BRITZ, S. J. Effect of supplemental ultraviolet radiation on the carotenoid and chlorophyll composition of green house-grown leaf lettuce (*Lactuca sativa* L.) cultivars. **Journal of Food Composition and Analysis,** v. 19, p. 637-644, 2006.

CALVENZANI, V.; MARTINELLI, M.; LAZZERI, V.; GIUNTINI, D.; DALL'ASTA, C.; GALAVERNA, G. Response of wild-type and high pigment-1 tomato fruit to UV-B depletion: flavonoid profiling and gene expression. **Planta,** v. 231, p. 755-765, 2010.

CASTAGNA, A.; NALI, C.; CIOMPI, S.; LORENZINI, G.; SOLDATINI, G. F.; RANIERI, A. Ozone exposure affects photosynthesis of pumpkin (*Cucurbita pepo*) plants. **New Phytologist,** v. 152, p. 223-229, 2001.

CHEYNIER, V.; COMTE, G.; DAVIES, K. M.; LATTANZIO, V.; MARTENS, S. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. **Plant Physiology and Biochemistry,** v. 72, p. 1-20, 2013.

DUPONT, M. S.; MONDIN, Z.; WILLIAMSON, G.; PRICE, K. R. Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. **Journal of Agricultural and Food Chemistry,** v. 48, n. 9, p. 3957-3964, 2000.

FIOL, M.; ADERMANN, S.; NEUGART, S.; ROHN, S.; MÜGGE, C.; SCHREINER, M.; KRUMBEIN, A.; KROH, L. W. Highly glycosylated and acylated flavonols isolated from kale (*Brassica oleracea* var. sabellica) structure−antioxidant activity relationship. **Food Research International,** v. 47, p. 80−89, 2012.

GARCÍA-MACÍAS, P.; ORDIDGE, M.; VYSINI, E.; WAROONPHAN, S.; BATTEY, N. H.; GORDON, M. H.; HADLEY, P.; JOHN, P.; LOVEGROVE, J. A.; WAGSTAFFE, A. Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. **Journal of Agricultural and Food Chemistry,** v. 55, p. 10168-10172, 2007.

GOETZ, M.; ALBERT, A.; STICH, S.; HELLER, W.; SCHERB, H.; KRINS, A.; LANGEBARTELS, C.; SEIDLITZ, H. K.; ERNST, D. PAR modulation of the UV dependent levels of flavonoid metabolites in *Arabidopsis thaliana* (L.) Heynh. leaf rosettes: cumulative effects after a whole vegetative growth period. **Protoplasma,** v. 243, p. 95−103, 2010.

JAAKOLA, L. New insights into the regulation of anthocyanin biosynthesis in fruits. **Trends in Plant Science,** v. 18, n. 9, p. 477-483, 2013.

JAHNS, P.; HOLZWARTH, A. R. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. **Biochimica et Biophysica Acta - Bioenergetics,** v. 1817, p. 182-193, 2012.

JANSEN, M. A. K.; GABA, V.; GREENBERG, B. M. Higher plants and UV-B radiation: balancing damage, repair and acclimation. **Trends in Plant Science,** v. 3, p. 131-135, 1998.

KIM, M. J.; MOON, Y.; TOU, J. C.; MOU, B.; WATERLAND, N. L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). **Journal of Food Composition and Analysis,** v. 49, p. 19-34, 2016.

LI, Z.; ZHAO, X.; SANDHU, A. K.; GU, L. Effects of exogenous abscisic acid on yield, antioxidant capacities, and phytochemical contents of greenhouse grown lettuces. **Journal of Agriculture and Food Chemistry,** v. 58, p. 6503-6509, 2010.

MARIN, A.; FERRERES, F.; G., B. G.; GIL, M. I. Weather variability influences color and phenolic content of pigmented baby leaf lettuces throughout the season. **Journal of Agricultural and Food Chemistry,** v. 63, p. 1673−1681, 2015.

MOELLER, S. M.; JACQUES, P. F.; BLUMBERG, J. B. The potential role of dietary xanthophylls in cataract and age-related macular degeneration. **Journal of the American College of Nutrition,** v. 19, p. 522S-527S, 2000.

MOU, B. Genetic variation of beta-carotene and lutein contents in lettuce. **Journal of the American Society for Horticultural Science,** v. 130, n. 6, p. 870-876, 2005.

MULABAGAL, V.; NGOUAJIO, M.; NAIR, A.; ZHANG, Y.; GOTTUMUKKALA, A. L.; NAIR, M. G. In vitro evaluation of red and green lettuce (*Lactuca sativa*) for functional food properties. **Food Chem.,** v. 118 p. 300-306, 2010.

MURATA, N.; TAKAHASHI, S.; NISHIYAMA, Y.; ALLAKHVERDIEV, S. I. Photoinhibition of photosystem II under environmental stress. **Biochimica et Biophysica Acta,** v. 1767, p. 414-421, 2007.

NEUGART, S.; FIOL, M.; SCHREINE, M.; ROHN, S.; ZRENNER, R.; JROH, L. W.; KRUMBEIN, A. Interaction of moderate UV-B exposure and temperature on the formation of structurally different flavonol glycosides and hydroxycinnamic acid derivatives in kale (*Brassica oleracea* var. sabellica). **Journal of Agricultural and Food Chemistry,** v. 62, p. 4054−4062, 2014.

NEUGART, S.; SCHREINER, M. UVB and UVA as eustressors in horticultural and agricultural crops. **Scientia Horticulturae**, 2018.

NISHIYAMA, Y.; YAMAMOTO, H.; ALLAKHVERDIEV, S. I.; INABA, M.; YOKOTA, A.; MURATA, N. Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. **EMBO Journal,** v. 20, p. 5587-5594, 2001.

OZGEN, S.; SEKERCI, S. Effect of leaf position on the distribution of phytochemicals and antioxidant capacity among green and red lettuce cultivars. **Spanish Journal of Agricultural Research,** v. 9, p. 801-809, 2011.

PÉREZ-LÓPEZ, U.; PINZINO, C.; QUARTACCI, M. F.; RANIERI, A.; SGHERRI, C. Phenolic composition and related antioxidant properties in differently colored lettuces: a study by Electron Paramagnetic Resonance (EPR) Kinetics. **Journal of Agricultural and Food Chemistry,** v. 62, p. 12001−12007, 2014.

RODRIGUEZ, C.; TORRE, S.; SOLHAUG, K. A. Low levels of ultraviolet-B radiation from fluorescent tubes induce an efficient flavonoid synthesis in Lollo Rosso lettuce without negative impact on growth. **Acta Agriculturae Scandinavica, Section B - Soil & Plant Science**, p. 178-184 2014.

ROJAS-GARBANZO, C.; GLEICHENHAGEN, M.; HELLER, A.; ESQUIVEL, P.; SCHULZE-KAYSERS, N.; SCHIEBER, A. Carotenoid profile, antioxidant capacity, and chromoplasts of pink guava (*Psidium guajava* L. Cv. 'Criolla') during fruit ripening. **Journal of Agricultural and Food Chemistry,** v. 65, p. 3737−3747, 2017.

SAHA, S.; KALIA, P.; SUREJA, A. K.; SRITVASTAVA, A.; SARKAR, S. K. Genetic analysis of bioactive compounds and antioxidant properties in lettuce (*Lactuca sativa*). **Indian Journal of Agricultural Sciences,** v. 86, n. 11, p. 1471-1476, 2016.

SAINI, R. K.; SHANG, X. M.; KO, E. Y.; CHOI, J. H.; KIM, D.; KEUM, Y. Characterization of nutritionally important phytoconstituents in minimally processed ready-to-eat baby-leaf vegetables using HPLC-DAD and GC-MS. **Food Measure,** v. 10, p. 341-349, 2016.

SANKARI, M.; HRIDYA, H.; SNEHA, P.; GEORGE PRIYA DOSS, C.; RAMAMOORTHY, S. Effect of UV radiation and its implications on carotenoid pathway in Bixa orellana L. **Journal of Photochemistry and Photobiology B: Biology,** v. 176, p. 136-144, 2017.

SANTOS BUELGA, C.; MATEUS, N.; FEITAS, V. D. Anthocyanins. Plant pigments and beyond. **Journal of Agricultural and Food Chemistry,** v. 62, n. 29, p. 6879-6884, 2014.

SANTOS, C. M. D.; GONÇALVES, E. R.; ENDRE, L.; JADOSKI, C. J.; NASCIMENTO, L. A. D.; SANTOS, E. D. D. Atividade fotossintética em alface (*Lactuca sativa* L.) submetidas a diferentes compostagens de resíduos agroindustriais. **Pesquisa Aplicada & Agrotecnologia,** v. 3, n. 3, p. 1983-6325, 2010.

SCHMIDT, S.; ZIETZ, M.; SCHREINER, M.; ROHN, S.; KROH, L. W.; KRUMBEIN, A. Identification of complex, naturally occurring flavonoid glycosides in kale (*Brassica oleracea* var. sabellica) by high-performance liquid chromatography diode-array detection/electrospray ionization multi-stage mass spectrometry. **Rapid Communications in Mass Spectrometry,** v. 24, p. 2009−2022, 2010.

SCHREINER, M.; HUYSKENS-KEIL, S. Phytochemicals in Fruit and Vegetables: Health Promotion and Postharvest Elicitors. **CRC Crit Rev Plant Sci,** v. 25, n. 3, p. 267-278, 2006.

SCHRÖTER, D.; BALDERMANN, S.; SCHREINER, M.; WITZEL, K.; MAUL, R.; ROHN, S.; NEUGART, S. Natural diversity of hydroxycinnamic acid derivatives, flavonoid glycosides, carotenoids and chlorophylls in leaves of six different amaranth species. **Food Chem.**, 2017.

SHENG, K.; ZHENG, H.; S., S.; YAN, L.; LIU, C.; ZHENG, L. Comparison of postharvest UV-B and UV-C treatments on table grape: Changes in phenolic compounds and their transcription of biosynthetic genes during storage. **Postharvest Biology and Technology,** v. 138 p. 74-81, 2018.

STEYN, W. J.; WAND, S. J. E.; HOLCROFT, D. M.; JACOBS, G. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. **New Phytologist,** v. 155, p. 349-361, 2002.

TSORMPATSIDIS, E.; HENBEST, R. G. C.; BATTEY, N. H.; HADLEY, P. The influence of ultraviolet radiation on growth, photosynthesis and phenolic levels of green and red lettuce: potential for exploiting effects of ultraviolet radiation in a production system. **Annals of Applied Biology,** v. 156 p. 357-366, 2010.

YOUSUF, B.; GUL, K.; WANI, A. A.; SINGH, P. Health benefits of anthocyanins and their encapsulation for potential use in food systems: a review. **Critical Reviews in Food Science and Nutrition,** v. 56, n. 13, p. 2223-2230, 2016

ZHANG, W. J.; BJÖRN, L. O. The effect of ultraviolet radiation on the accumulation of medicinal compounds in plants. **Fitoterapia,** v. 80, p. 207-218, 2009.

ZIETZ, M.; WECKMULLER, A.; SCHMIDT, S.; ROHN, S.; SCHREINER, M.; KRUMBEIN, A.; KROH, L. W. Genotypic and climatic influence on the antioxidant activity of flavonoids in kale (*Brassica oleracea* var. sabellica). **Journal of Agricultural and Food Chemistry,** v. 58, p. 2123-2130, 2010.

ZIVCAK, M. B., K.; SYTAR, O.; BRESTIE, M.; OLSOVSKA, K.; ALLAKHVERDIEV, S. I. Lettuce flavonoids screening and phenotyping by chlorophyll fluorescence excitation ratio. **Planta,** v. 245, n. 6, p. 1215-1229, 2017.

CAPÍTULO 7

DISCUSSÃO GERAL

Este trabalho foi desenvolvido com o objetivo de avaliar os efeitos da radiação UV-B suplementar no conteúdo de compostos bioativos de frutas como caqui, goiaba e maçã na pós-colheita e de alfaces verdes e roxas durante o cultivo.

No Capítulo 4 está apresentado o estudo em que foi investigada a efetividade da radiação UV-B suplementar em aumentar o conteúdo de metabólitos secundários em frutas climatéricas como o caqui e a goiaba. Essas frutas foram selecionadas por representarem uma grande porção do consumo nos estados do sul do Brasil. Além disso, ambas são frutas climatéricas, o que leva a crer que seu comportamento é sensível à radiação UV-B suplementar.

Os dados obtidos revelaram que a radiação UV-B é uma técnica por meio da qual obtém-se caquis e goiabas com maiores teores de carotenoides, apesar de este acúmulo ocorrer em momentos distintos. Nos frutos de caquis, sugere-se que a via de síntese dos carotenoides foi influenciada após 30 horas de tratamento, enquanto que para os frutos de goiaba, essa influência deu-se até as primeiras 30 horas de tratamento. Apesar de possuírem perfis diferentes de carotenoides, os frutos de caquis e goiabas apresentaram acúmulos semelhantes ao fim do tratamento com radiação UV-B suplementar: 8 % e 9,4 % a mais de carotenoides totais nos frutos tratados em relação aos frutos controle. Estes valores foram inferiores aos observados para as alfaces verdes e roxas estudadas no Capítulo 6. Apesar de não terem sido observadas diferenças significativas para os carotenoides e clorofilas entre as alfaces roxas tratadas ou não com radiação UV-B suplementar, as alfaces verdes tratadas tiveram mais de 14 % de aumento no teor de carotenoides em relação às alfaces controle.

Esta amplitude de resultados pode ser explicada pelas diferenças entre os tratamentos oferecidos. Uma vez que foram obtidos dados positivos no estudo da suplementação de radiação UV-B na pós-colheita para os frutos de caquis e goiabas, o Capítulo 6 dedicou-se a investigar os efeitos desta tecnologia durante o cultivo de alfaces verdes e roxas. Este experimento foi realizado em parceria com o Leibniz Institute of Vegetable and Ornamental Crops, em Groβbeeren na Alemanha.

As plantas foram cultivadas por até 2 semanas recebendo 1 hora de radiação suplementar por dia. Os resultados positivos para os conteúdos de clorofila tornaram possível

saber que as plantas estavam protegidas contra os possíveis danos oxidativos da radiação suplementar. A dose de UV-B aplicada aumentou significativamente o conteúdo de flavonoides nas alfaces roxas e o conteúdo de carotenoides nas alfaces verdes ao fim do experimento.

Após duas semanas, as alfaces roxas tratadas com UV-B apresentaram quase 3 vezes mais cianidina em relação às alfaces controle. Este valor é bastante próximo àquele observado nas cascas de maçãs tratadas com radiação UV-B suplementar por 36 horas, conforme Capítulo 5. Porém, ao acontrário do observado nas maçãs, as quercetinas presentes nas alfaces roxas foram positivamente afetadas pelo tratamento.

Os alimentos estudados nos Capítulos 5 e 6 apresentaram maiores teores de ácidos fenólicos, com destaque para o ácido clorogênico, após os tratamentos aplicados. Este resultado é de suma importância no que diz respeito ao potencial antioxidante destes alimentos quando tratados com radiação UV-B suplementar.

O Capítulo 5 foi desenvolvido durante o estágio sanduíche realizado na Università di Pisa, na Itália, em que foi avaliada a influência da radiação UV-B suplementar no aumento de compostos bioativos de cascas de maçãs Red Delicious, sem que sua qualidade físico-química durante o armazenamento fosse prejudicada. As maçãs cultivadas na Itália são conhecidas mundialmente pela sua qualidade. Assim como no Capítulo 4, optou-se por analisar somente as cascas por serem a fatia que recebe a radiação UV-B mais intensa. A partir da polpa da fruta, os efeitos da radiação já são menos percebidos.

Os resultados obtidos nestes três artigos sugerem que a radiação UV-B suplementar representa uma tecnologia válida para a modulação de compostos bioativos tanto na pós-colheita de frutas quanto durante o cultivo de hortaliças.

Neste sentido, foi delineado e executado um estudo piloto em parceria com uma indústria gaúche de câmaras de cultivo de alimentos *indoor*, a Plantário. O estudo dedicou-se a investigar uma possibilidade de aplicação da tecnologia de radiação UV-B suplementar utilizando tecnologia desenvolvida no Brasil (Apêndice A).

O estudo prévio com lâmpadas de LED brancas e infravermelho forneceu dados importantes sobre o cultivo *indoor* de alfaces, como a quantidade de plantas utilizadas, o encurtamento do ciclo produtivo e a influência da quantidade de luz fornecida sobre o conteúdo de compostos bioativos, principalmente antocianinas. O perfil de compostos bioativos obtido neste estudo piloto foi bastante semelhante ao obtido nas análises em laboratório na Alemanha, conforme Capítulo 6. Não por acaso, o uso de cultivares de alface é comumente indicado por se tratarem de plantas mundialmente consumidas que possuem um

ciclo produtivo médio de 30 dias, além de representarem um bom modelo de estudo para efeitos da luz e serem consideradas fontes de compostos bioativos.

Neste contexto, a radiação UV-B está inserida no conceito de consumo local e agricultura urbana, representando uma tecnologia *eco-friendly* capaz de modular a disponibilidade de compostos bioativos nos alimentos. A partir deste estudo piloto sugere-se que novos estudos, incluindo a radiação UV-B suplementar, sejam aplicados futuramente em câmaras de cultivo *indoor* com perspectivas de ótimos resultados.

As diferenças de resposta do alimento em relação à radiação UV-B podem ser observadas em diferentes cultivares de um mesmo alimento. Portanto, as pesquisas futuras são importantes também para indicar a intensidade adequada de radiação nesse sentido e para adequar lâmpadas ao espaço de cultivo.

REFERÊNCIAS

A-H-MACKERNESS, S.; JOHN, C. F.; JORDAN, B.; THOMAS, B. Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. **FEBS Letters,** v. 489, p. 237-242, 2001.

AMODIO, M. L.; DEROSSI, A.; COLELLI, G. Modeling phenolic content during storage of cut fruit and vegetables: A consecutive reaction mechanism. **Journal of Food Engineering,** v. 140, p. 1-8, 2014.

ANDERSEN, M.; JORDHEIM, M. The anthocyanins. In: ANDERSEN, O. M.;MARKHAM, K. R. (Ed.). **Flavonoids: Chemistry, Biochemistry and Applications**. 2nd. Boca Raton, FL: CRC Press, 2006. p.452-471.

BAE, J.-M.; LEE, E. J.; GUYATT, G. Citrus fruit intake and stomach cancer risk: a quantitative systematic review. **Gastric Cancer,** v. 11, n. 1, p. 23-32, 2008.

BAKHSHI, D.; ARAKAWA, O. Effects of UV-B irradiation on phenolic compound accumulation and antioxidant activity in 'Jonathan' apple influenced by bagging, temperature and maturation. **Journal of Food, Agriculture & Environment,** v. 4, n. 1, p. 75-79, 2006.

BARRETT, D. M.; LLOYD, B. Advanced preservation methods and nutrient retention in fruits and vegetables. **Journal of the Science of Food and Agriculture,** v. 92, n. 1, p. 7-22, 2012.

BASLAM, M.; MORALES, F.; GARMENDIA, I.; GOICOECHA, N. Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. **Scientia Horticulturae,** v. 151, p. 103-111, 2013.

BAUCHER, M.; MONTIES, B.; VAN MONTAGU, M.; BOERJAN, W. Biosynthesis and genetic engineering of lignin **Critical Review in Plant Sciences,** v. 17, p. 125-197, 1998.

BECATTI, E.; CHKAIBAN, L.; TONUTTI, P.; FORCATO, C.; BONGHI, C.; RANIERI, A. M. Short-Term Postharvest Carbon Dioxide Treatments Induce Selective Molecular and Metabolic Changes in Grape Berries. **Journal of Agricultural and Food Chemistry,** v. 58, n. 13, p. 8012-8020, 2010/07/14 2010.

BECKER, C.; URLIĆ, B.; JUKIĆ ŠPIKA, M.; KLÄRING, H.-P.; KRUMBEIN, A.; BALDERMANN, S.; GORETA BAN, S.; PERICA, S.; SCHWARZ, D. Nitrogen Limited Red and Green Leaf Lettuce Accumulate Flavonoid Glycosides, Caffeic Acid Derivatives, and Sucrose while Losing Chlorophylls, B-Carotene and Xanthophylls. **PLoS ONE,** v. 10, n. 11, p. e0142867, 2015.

BECKMAN, C. H. Phenolics-storing cells: Keys to programmed cell dead and periderm formation in disease resistance and in general defense responses in plants? **Physiological and Molecular Plant Pathology,** v. 57, p. 101-110, 2000.

BOOKER, F. L.; BURKEY, K. O.; JONES, A. M. Re-evaluating the role of ascorbic acid and phenolic glycosides in ozone scavenging in the leaf apoplast of *Arabidopsis thaliana* L. **Plant, cell & environment,** v. 35, n. 8, p. 1456-1466, 2012.

BOVY, A.; VAN DEN BERG, C.; DE VRIEZE, G.; THOMPSON, W. F.; WEISBEEK, P.; SMEEKENS, S. Light-regulated expression of the Arabidopsis thaliana ferredoxin gene requires sequences upstream and downstream of the transcription initiation site. **Plant Molecular Biology,** v. 27, p. 27-39, 1995.

BROSCHÉ, M.; STRID, Å. Molecular events following perception of ultraviolet-B radiation by plants. **Physiology Plant,** v. 117, p. 1-10, 2003.

BROWN, B. A.; CLOIX, C.; JIANG, G. H.; KAISERLI, E.; HERZYK, P.; KLIEBENSTEIN, D. J.; JENKINS, G. I. A UV-B-specific signaling component orchestrates plant UV protection. **Proceedings of the National Academy of Sciences,** v. 102, p. 18225-18230, 2005.

CABRITA, L.; FOSSEN, T.; ANDERSEN, M. Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. **Food Chem.,** v. 68, n. 1, p. 101-107, 2000.

CALVENZANI, V.; MARTINELLI, M.; LAZZERI, V.; GIUNTINI, D.; DALL'ASTA, C.; GALAVERNA, G. Response of wild-type and high pigment-1 tomato fruit to UV-B depletion: flavonoid profiling and gene expression. **Planta,** v. 231, p. 755-765, 2010.

CASTAGNA, A.; NALI, C.; CIOMPI, S.; LORENZINI, G.; SOLDATINI, G. F.; RANIERI, A. Ozone exposure affects photosynthesis of pumpkin (*Cucurbita pepo*) plants. **New Phytologist,** v. 152, p. 223-229, 2001.

CASTAGNA, A.; CHIAVARO, E.; DALL'ASTA, C.; RINALDI, M.; GALAVERNA, G.; RANIERI, A. Effect of postharvest UV-B irradiation on nutraceutical quality and physical properties of tomato fruits. **Food Chem.,** v. 137, n. 1, p. 151-158, 2013.

CASTAGNA, A.; DALL'ASTA, C.; CHIAVARO, E.; GALAVERNA, G.; RANIERI, A. Effect of post-harvest UV-B irradiation on polyphenol profile and antioxidante activity in flesh and peel of tomato fruits. . **Food Bioproc. Tech.,** v. 7, n. 8, p. 2241-2250, 2014.

CASTAÑEDA-OVANDO, A.; PACHECO-HERNÁNDEZ, M. D. L.; PÁEZ-HERNÁNDEZ, M. E.; RODRÍGUEZ, J. A.; GALÁN-VIDAL, C. A. Chemical studies of anthocyanins: A review. **Food Chem.,** v. 113, p. 859-871, 2009.

CHARLES, M. T.; GOULET, A.; ARUL, J. Physiological basis of UV-C induced resistance to *Botrytis cinerea* in tomato fruit: IV. Biochemical modification of structural barriers. **Postharvest Biology and Technology,** v. 47, n. 1, p. 41-53, 2008.

CHITARRA, M. I. F.; CHITARRA, A. B., 2005. P**ós colheita de frutos e hortaliças: fisiologia e manuseio**, 2nd ed. FAEPE, Lavras.

CONTRERAS-CALDERÓN, J.; CALDERÓN-JAIMES, L.; GUERRA-HERNÁNDEZ, E.; GARCÍA-VILLANOVA, B. Antioxidant capacity, phenolic content and vitamin C in pulp,

peel and seed from 24 exotic fruits from Colombia. **Food Research International,** v. 44, p. 2047-2053, 2011.

CRAKER, L. E.; SEIBERT, M. Light and the development of Grand Rapids lettuce. **Canadian Journal of Plant Science,** v. 63, p. 277-281, 1983.

DAMBECK, M.; SANDMANN, G. Antioxidative activities of algal keto carotenoids acting as antioxidative protectants in the chloroplast. **Photochemistry and Photobiology,** v. 90, p. 814-819, 2014.

DAOOD, H. G.; BIACS, P.; CZINKOTAI, B.; HOSCHKA, A. Chromatographic investigation of carotenoids, sugars and organic acids from Dyospiros kaki fruits. **Food Ckemistry,** v. 45, p. 151-155, 1992.

DENG, X. W.; QUAIL, P. H. Signalling in light-controlled development. **Cell & Developmental Biology,** v. 10, p. 121-129, 1999.

DIXON, R. A.; PAIVA, N. L. Stress-induced phenylpropanoid metabolism. **The Plant Cell,** v. 7, n. 7, p. 1085-1097, 1995.

DULING, D. R. Simulation of multiple isotropic spin trap EPR spectra. **Journal of Magnetic Resonance,** v. 104, p. 105-110, 1994.

DUTHIE, G. G.; GARDNER, P. T.; KYLE, J. A. M. Plant polyphenols: are they the new magic bullet? **Proceedings of the Nutrition Society,** v. 62, n. 3, p. 599-603, 2003.

EICHHOLZ, I.; HUYSKENS-KEIL, S.; KELLER, A.; ULRICH, D.; KHOH, L. W.; ROHN, S. UV-B-induced changes of volatile metabolites and phenolic compounds in blueberries (*Vaccinium corymbosum* L.). **Food Chem.,** v. 126, p. 60-64, 2011.

EICHHOLZ, I.; ROHN, S.; GAMM, A.; BEESK, N.; HERPPICH, W. B.; JROH, L. W.; ULRICHS, C.; HUYSKENS-KEIL, S. UV-B-mediated flavonoid synthesis in white asparagus (*Asparagus officinalis* L.). **Food Research International,** v. 48, p. 196-201, 2012.

FAOSTAT. FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS STATISTICS. 2017. Disponível em: < http://www.fao.org/faostat/en/#data/QC >. Acesso em: 26 de dezembro de 2017.

FAVORY, J. J.; STEC, A.; GRUBER, H.; RIZZINI, L.; ORAVECZ, A.; FUNK, M. Interaction of COP1 and UVR8 regulates UV-B-induced photomorphogenesis and stress acclimation in Arabidopsis. **European Molecular Biology Organization Journal,** v. 28, p. 591-601, 2009.

FERNÁNDEZ-GARCÍA, E. Skin protection against UV light by dietary antioxidants. **Food Function,** v. 5, p. 1994-2003, 2014.

FLESCHHUT, J.; KRATZER, F.; RECHKEMMER, G.; KULLING, S. E. Stability and biotransformation of various dietary anthocyanins in vitro. **European Journal of Nutrition,** v. 45, n. 1, p. 7-18, 2006.

FRASER, P.; BRAMLEY, P. The biosynthesis and nutritional uses of carotenoids. **Progress in Lipid Research,** v. 43, p. 228-265, 2004.

FROHNMEYER, H.; STAIGER, D. Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection. **Plant Physiology,** v. 133, n. 4, p. 1420-1428, 2003.

GALVÃO, V. C.; FRANKHAUSER, C. Sensing the light environment in plants: photoreceptors and early signaling steps. **Current Opinion in Neurobiology,** v. 34, p. 46-53, 2015.

GARCIA-FUENTES, M.; TORRES, D.; ALONSO, M. J. Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. **Colloids and Surfaces B - Biointerfaces,** v. 27, p. 159-168, 2003.

GIUNTINI, D.; GRAZIANI, G.; LERCARI, B.; FOGLIANO, V.; SOLDATINI, G. F.; RANIERI, A. Changes in carotenoid and ascorbic acid contents in fruits of different tomato genotypes related to the depletion of UVB radiation. **Journal of Agricultural and Food Chemistry,** v. 53, p. 3174-3181, 2005.

GIUNTINI, D.; LAZZERI, V.; CALVENZANI, V.; DALL'ASTA, C.; GALAVERNA, G.; TONELLI, C.; PETRONI, K.; RANIEI, A. Flavonoid profiling and biosynthetic gene expression in flesh and peel of two tomato genotypes grown under UV-B-depleted conditions during ripening. **Journal of Agricultural of Food Chemistry,** v. 56, p. 5905- 5915, 2008.

GIUSTI, M. M.; WROLSTAD, R. E. Acylated anthocyanins from edible sources and their applications in food systems. **Biochemical Engineering Journal,** v. 14, n. 3, p. 217- 225, 2003.

GONZÁLEZ, I. A.; OSORIO, C.; MELÉNDEZ-MARTÍNEZ, A. J.; GONZÁLEZ-MIRET, M. L.; HEREDIA, F. J. Application of tristimulus colorimetry to evaluate colour changes during the ripening of Colombian guava (*Psidium guajava* L.) varieties with different carotenoid pattern. **Food Sci and Technol,** v. 46, n. 4, p. 840-848, 2011.

GORALCZYK, R.; WERTZ, K. Skin photoprotection by carotenoids. In: BRITTON, G.;PFANDER, H., *et al* (Ed.). **Carotenoids**. Basileia, Switzerland: Birkhäuser Basel, 2009. p.335-362.

GÜLÇIN, I. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). **Toxicology,** v. 217, p. 213-220, 2006.

GWYNN-JONES, D. Short term impacts of enhanced UV-B radiation on photoassimilate allocation and metabolism: a possible interpretation for time dependent inhibition of growth. **Plant Ecology,** v. 154, p. 67-73, 2001.

HAGEN, S. F.; BORGE, G. I. A.; BENGTSSON, G. B.; BILGER, W.; BERGE, A.; HAFFNER, K.; SOLHAUG, K. A. Phenolic contents and other health and sensory related properties of apple fruit (*Malus domestica* Borkh, cv. Aroma): Effect of postharvest UV-B irradiation. **Postharvest Biology and Technology,** v. 45, p. 1-10, 2007.

HASAN, M. M.; BASHIR, T.; GHOSH, H.; LEE, S. K.; BAE, H. An overview of LEDs' effects on the production of bioactive compounds and crop quality. **Molecules,** v. 22, p. 1420-1432, 2017.

HE, F. J.; NOWSON, C. A.; LUCAS, M.; MACGREGOR, G. A. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. **Journal Of Human Hypertension,** v. 21, p. 717, 2007.

HEATH, R. L.; LEFOHN, A. S.; MUSSELMAN, R. C. Temporal processes that contribute to nonlinearity in vegetation responses to ozone exposure and dose. **Atmospheric Environment,** v. 43, p. 2919-2928, 2009.

HEO, J. W.; KANG, D. H.; BANG, H. S.; HONG, S. G.; CHUN, C.; KANG, K. H. Early growth, pigmentation, protein content, and phenylalanine ammonia-lyase activity of red curled lettuces grown under different lighting conditions. **Korean Journal of Horticulture Science and Technology,** v. 30, p. 6-12, 2012.

ILIĆ, S. Z.; MILENKOVIĆ, L.; DIMITRIJEVIĆ, A.; STANOJEVIĆ, L.; CVETKOVIĆ, D.; KEVREŠAN, Ž.; FALLIK, E.; MASTILOVIĆ, J. Light modification by color nets improve quality of lettuce from summer production. **Scientia Horticulturae,** v. 226, p. 389-397, 2017.

INTERDONATO, R.; ROSA, M.; NIEVA, C. B.; GONZÁLEZ, J. A.; HILAL, M.; PRADO, F. E. Effects of low UV-B doses on the accumulation of UV-B absorbing compounds and total phenolics and carbohydrate metabolism in the peel of harvested lemons. **Environmental and Experimental Botany,** v. 70, p. 204-211, 2011.

JANSEN, M. A. K.; VAN DEN NOORT, R. E.; TAN, M. Y.; PRINSEN, E.; LAGRIMINI, L. M.; THORNELEY, R. N. F. Phenol-oxidizing peroxidases contribute to the protection of plants from ultraviolet radiation stress. **Plant Physiology,** v. 126, n. 3, p. 1012-1023, 2001.

JANSEN, M. A. K.; HECTORS, K.; O'BRIEN, N. M.; GUISEZ, Y.; POTTERS, G. Plant stress and human health: Do human consumers benefit from UV-B acclimated crops? **Plant Science,** v. 175, p. 449-458, 2008.

JIANG, M.; LIU, Y.; REN, L.; LIAN, H.; CHEN, H. Molecular cloning and characterization of anthocyanin biosynthesis genes in eggplant (*Solanum melongena* L.). **Acta Physiologiae Plantarum,** v. 38, p. 1-13, 2016.

KASIGE, A.; TAKASHI, A. Carbon and nitrogen partitioning in the freshwater submerged macrophyte *Vallisneria gigantea* in response to ultraviolet-B irradiance. **Aquatic Ecology,** v. 43, p. 313-322, 2009.

KAWKA, B.; KWIECIEŃ, I.; EKIERT, H. Influence of culture medium composition and light conditions on the accumulation of bioactive compounds in shoot cultures of *Scutellaria lateriflora* L. (American Skullcap) grown in vitro. **Applied Biochemistry and Biotechnology,** v. 183, n. 4, p. 1414-1425, December 01 2017.

KIM, D.-O.; CHUN, O. K.; KIM, Y. J.; MOON, H.-Y.; LEE, C. Y. Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums. **Journal of Agricultural and Food Chemistry,** v. 51, n. 22, p. 6509-6515, 2003.

KIM, H. H.; GOINS, G. D.; WHEELER, R. M.; SAGER, J. C. Green-light supplementation for enhanced lettuce growth under red- and blue-light-emitting diodes. **Horticultural Science,** v. 39, p. 1617-1622, 2004.

KIM, M. J.; MOON, Y.; TOU, J. C.; MOU, B.; WATERLAND, N. L. Nutritional value, bioactive compounds and health benefits of lettuce (*Lactuca sativa* L.). **Journal of Food Composition and Analysis,** v. 49, p. 19-34, 2016.

KONCZAK, I.; ZHANG, W. Anthocyanins-more than nature's colours. **Journal of Biomedicine and Biotechnology,** v. 5, p. 239-240, 2004.

KONG, J. M.; CHIA, L. S.; GOH, N. K.; CHIA, T. F.; BROUILLARD, R. Analysis and biological activities of anthocyanins. **Phytochemistry,** v. 64, n. 5, p. 923-933, 2003.

KOSMA, C.; TRIANTAFYLLIDIS, V.; PAPASAVVAS, A.; SALAHAS, G.; PATAKAS, A. Yield and nutritional quality of greenhouse lettuce as affected by shading and cultivation season. **Emirates Journal of Food and Agriculture,** v. 25, p. 974-979, 2013.

LADANIYA, M. S. Fruit morphology, anatomy, and physiology. **Citrus Fruit - Biology, Technology and Evaluation**, v. 5, p. 103-124, 2008/.

LANCASTER, J. E.; DOUGALL, D. K. Regulation of skin color in apples. **CRC Critical Reviews in Plant Science,** v. 10, n. 6, p. 487-502, 1992/01/01 1992.

LAZZERI, V.; CALVENZANI, V.; PETRONI, K.; TONELLI, C.; CASTAGNA, A.; RANIERI, A. Carotenoid profiling and biosynthetic gene expression in flesh and peel of wild-type and hp-1 tomato fruit under UV-B depletion. **Journal of Agriculture and Food Chemistry,** v. 60, p. 4960−4969, 2012.

LEE, J. UV-B Signal transduction pathway in Arabidopsis. **Journal of Plant Biology,** v. 59, p. 223-230, 2016.

LEPINIEC, L.; DEBEAUJON, I.; ROUTABOUL, J. M.; BAUDRY, A.; POURCEL, L.; NESI, N.; CABOCHE, M. Genetics and biochemistry of seed flavonoids. **Annual Review of Plant Biology**, v. 57, p. 404-430, 2006.

LI, P.; DU, G.; MA, F. Phenolics concentration and antioxidant capacity of different fruit tissues of astringent versus non-astringent persimmons. **Scientia Horticulturae,** v. 129, p. 710-714, 2011.

LI, Z.; ZHAO, X.; SANDHU, A. K.; GU, L. Effects of exogenous abscisic acid on yield, antioxidant capacities, and phytochemical contents of greenhouse grown lettuces. **Journal of Agriculture and Food Chemistry,** v. 58, p. 6503-6509, 2010.

LIU, C.; HAN, X.; CAI, L.; LU, X.; YING, T.; JIANG, Z. Postharvest UV-B irradiation maintains sensory qualities and enhances antioxidant capacity in tomato fruit during storage. **Postharvest Biology and Technology,** v. 59, p. 232–237, 2011.

LIU, C.; CAI, L.; LU, X.; HAN, X.; YING, T. Effect of postharvest UV-C irradiation on phenolic compound content and antioxidante activity of tomato fruit during storage. **Journal of Integrative Agriculture,** v. 11, n. 1, p. 159-165, 2012.

LONG, S. P.; HUMPHRIES, S.; FALKOWSKI, P. G. Photoinhibition of photosynthesis in nature. **Annual Review of Plant Physiology and Plant Molecular Biology,** v. 45, p. 633-662, 1994.

LÓPEZ-RUBIO, A.; LAGARON, J. M. Improved incorporation and stabilisation of δ-carotene in hydrocolloids using glycerol. **Food Chemistry,** v. 125, p. 997-1004, 2011.

LULE, S. U.; XIA, W. Food phenolics, pros and cons: A review. **Food Reviews International,** v. 21, n. 4, p. 367-388, 2005.

MADHUJITH, T.; SHAHIDI, F. Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. **Food Chemistry,** v. 117, p. 615- 620, 2009.

MARTIN, C.; BUTELLI, E.; PETRONI, K.; TONELI, C. How can research contribute to promoting human health? . **Plant Cell,** v. 23, p. 1685-1699, 2011.

MARTINEZ-VALVERDE, I.; PERIAGO, M. J.; ROS, G. Significado nutricional de los compuestos fenólicos de la dieta. **Archivos Latinoamericanos de Nutrición,** v. 50, n. 1, p. 5-18, 2000.

MELO, E. A. M.; MACIEL, M. I. S.; LIMA, V. L. A. G.; NASCIMENTO, R. J. Capacidade antioxidante de frutas. **Brazilian Journal of Pharmaceutical Sciences,** v. 44, n. 2, p. 193-201, 2008.

MERCADANTE, A.; STECK, A.; PFANDER, H. Carotenoids from guava (*Psidium guajava* L.): isolation and structure elucidation. **Journal of Agricultural and Food Chemistry,** v. 47, p. 145−151, 1999.

MERCADANTE, A. Z.; RODRIGUEZ-AMAYA, D. B. Effects of Ripening, Cultivar Differences, and Processing on the Carotenoid Composition of Mango. **Journal of Agricultural and Food Chemistry,** v. 46, n. 1, p. 128-130, 1998.

MOON, J. Y.; LEE, S.; JEONG, S.; KI, J.; AHN, K. S.; MOSADDIK, A.; CHO, S. K. Free radical-scavenging activities and cytoprotective effect of polyphenol-rich ethyl acetate fraction of guava (*Psidium cattleianum*) leaves on H_2O_2-treated HEPG2 cell. **Journal of the Korean Society Applied Biological Chemistry,** v. 56, p. 687−694, 2013.

MURTHY, H. N.; LEE, E.; PAEK, K. Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. **Plant Cell, Tissue and Organ Culture,** v. 118, p. 1-16, 2014.

MUSA, K. H.; ABDULLAH, A.; SUBRAMANIAM, V. Flavonoid profile and antioxidant activity of pink guava. **Science Asia,** v. 41, p. 149-154, 2015.

NACZK, M.; SHAHIDI, F. Extraction and analysis of phenolics in food. **Journal of Chromatography A,** v. 1054, p. 95-111, 2004.

NEUGART, S.; FIOL, M.; SCHREINE, M.; ROHN, S.; ZRENNER, R.; JROH, L. W.; KRUMBEIN, A. Interaction of moderate UV-B exposure and temperature on the formation of structurally different flavonol glycosides and hydroxycinnamic acid derivatives in kale (*Brassica oleracea* var. sabellica). **Journal of Agricultural and Food Chemistry,** v. 62, p. 4054−4062, 2014.

NICHENAMETLA, S. N.; TARUSCIO, T. G.; BARNEY, D. L.; EXON, J. H. A. A review of the effects and mechanisms of polyphenolics in cancer. **Critical Reviews in Food Science and Nutrition,** v. 46, n. 2, p. 161-183, 2006.

NTSOANE, L. M.; SOUNDY, P.; JIFON, J.; SIVAKUMAR, D. Variety-specific responses of lettuce grown under the different-coloured shade nets on phytochemical quality after postharvest storage. **Journal of Horticultural Science and Biotechnology,** v. 91, p. 520-528, 2016.

ORAVECZ, A.; BAUMANN, A.; MATE, Z.; BRZEZINSKA, A.; MOLINIER, J.; OAKELEY, E. J.; ADAM, E.; SCHAFER, E.; NAGY, F.; ULM, R. Constitutively photomorphogenic1 is required for the UV-B response in Arabidopsis. **Plant Cell,** v. 18, p. 1975-1990, 2006.

PADULA, M.; RODRÍGUEZ-AMAYA, D. B. Characterization of the carotenoids and assessment of the vitamin A value of Brasilian guavas (*Psidium guajava* L.). **Food Chemistry,** v. 20, p. 11−19, 1986.

PARK, S. U.; AHN, D.-J.; JEON, H.-J.; KWON, T. R.; LIM, H.-S.; CHOI, B.-S.; BAEK, K.-H.; BAE, H. Increase in the contents of ginsenosides in raw ginseng roots in response to exposure to 450 and 470 nm light from light-emitting diodes. **Journal of Ginseng Research,** v. 36, n. 2, p. 198-204, 2012.

PELLEGRINI, N.; RE, R.; YANG, M.; RICE-EVANS, C. Screening of dietary carotenoids and carotenoid-rich fruit extracts for antioxidant activities applying 2,2'-azinobis (3-ethylenebenzothiazoline-6-sulfonic acid) radical cation decolorization assay. **Methods in Enzymology,** v. 299, p. 379–389, 1999.

PETRONI, K.; TONELLI, C. Recent advances on the regulation of anthocyanin synthesis in reproductive organs. **Plant Science,** v. 181, p. 219-229, 2011.

POLLASTRI, S.; TATTINI, M. Flavonoids: old compounds for old roles. **Annais of Botany,** v. 108, p. 1225-1233, 2011.

REIN, M. Copigmentation reactions and color stability of berry anthocyanins. 2005. (Dissertation). Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki

RICE-EVANS, C. A.; MILLER, N. J.; PAGANGA, G. Antioxidant properties of phenolic compounds. **Trends in Plant Science,** v. 4, p. 304-309, 2002.

RIJKE, E. D.; OUT, P.; NIESSEN, W. M. A.; ARIESE, F.; GOOIJER, C.; BIRNKMAN, U. A. T. Analytical separation and detection methods for flavonoids. **Journal of Chromatography A,** v. 1112, p. 31-63, 2006.

RIZZINI, L.; FAVORY, J. J.; CLOIX, C.; FAGGIONATO, D.; O'HARA, A.; KAISERLI, E. Perception of UV-B by the Arabidopsis UVR8 protein. **Science,** v. 332, p. 103-106, 2011.

ROJAS-GARBANZO, C.; GLEICHENHAGEN, M.; HELLER, A.; ESQUIVEL, P.; SCHULZE-KAYSERS, N.; SCHIEBER, A. Carotenoid profile, antioxidant capacity, and chromoplasts of pink guava (*Psidium guajava* L. Cv. 'Criolla') during fruit ripening. **Journal of Agricultural and Food Chemistry,** v. 65, p. 3737−3747, 2017.

SANDRE, A. A.; PINA, J. M.; MORAES, R. M.; FURLAN, C. M. Anthocyanins and tannins: is the urban air pollution an elicitor factor? . **Brazilian Journal of Botany,** v. 37, n. 1, p. 9-18, 2014.

SAURA-CALIXTO, F.; GOÑI, I. Antioxidant capacity of the Spanish Mediterranean diet. **Food Chem.,** v. 94, p. 442−447, 2006.

SCALBERT, A.; MANACH, C.; MORAND, C.; RÉMÉSY, C.; JIMÉNEZ, L. Dietary polyphenols and the prevention of diseases. **Critical Reviews in Food Science and Nutrition,** v. 45, n. 4, p. 287-306, 2005.

SCHMIDT, S.; ZIETZ, M.; SCHREINER, M.; ROHN, S.; KROH, L. W.; KRUMBEIN, A. Identification of complex, naturally occurring flavonoid glycosides in kale (*Brassica oleracea* var. sabellica) by high-performance liquid chromatography diode-array detection/electrospray ionization multi-stage mass spectrometry. **Rapid Communications in Mass Spectrometry,** v. 24, p. 2009−2022, 2010.

SCHREINER, M.; HUYSKENS-KEIL, S. Phytochemicals in Fruit and Vegetables: Health Promotion and Postharvest Elicitors. **CRC Critical Reviews in Plant Science,** v. 25, n. 3, p. 267-278, 2006/07/01 2006.

SGHERRI, C.; PINZINO, C.; QUARTACCI, M. F. Reactive oxygen species and photosynthetic functioning: past and present. In: SINGH, V. P.;SINGH, S., *et al* (Ed.). **Reactive oxygen species in plants: boon or bane? Revisiting the Role of ROS**. New York (USA): J. Wiley & Sons, 2017. p.137-155.

SHAHIDI, F.; NACZK, M. **Phenolics in food and nutraceuticals: Sources, applications and health effects**. Boca Raton , FL: CRC Press, 2004.

SHINKLE, J. R.; ATKINS, A. K.; HUMPHREY, E. E.; RODGERS, C. W.; WHEELER, S. L.; BARNES, P. W. Growth and morphological responses to different UV wavebands in cucumber (*Cucumis sativum*) and other dicotyledonous seedlings. **Physiologia Plantarum,** v. 120, p. 240-248, 2004.

SINGH, S.; AGRAWAL, S. B.; AGRAWAL, M. UVR8 mediated plant protective responses under low UV-B radiation leading to photosynthetic acclimation. **Journal of Photochemistry and Photobiology B: Biology,** v. 137, p. 67-76, 2014.

SMILLIE, R. M.; HETHERINGTON, S. E. Photoabatement by anthocyanin shields photosynthetic systems from light stress. **Photosynthetica,** v. 36, p. 451-463, 1999.

STINTZING, F. C.; CARLE, R. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. **Trends in Food Science and Technology,** v. 15, n. 1, p. 19-38, 2004.

STRATI, I. F.; OREOPOULOU, V. Process optimisation for recovery of carotenoids from tomato waste. **Food Chemistry,** v. 129, n. 3, p. 747-752, 2011.

STRATMANN, J. Ultraviolet-B radiation co-opts defense signaling pathways. **Trends in Plant Science,** v. 8, p. 526-533, 2003.

STRZAŁKA, K. Procesy anaboliczne. Związki fenolowe. In: (Ed.). **Fizjologia roślin pod redakcją Jana Kopcewicza i Stanisława Lewaka**. Warszawa: Wydawnictwo Naukowe PWN, 2002. p.376-378.

SUESSLIN, C.; FROHNMEYER, H. An Arabidopsis mutant defective in UV-B light-mediated responses. **Plant Journal,** v. 33, n. 3, p. 591-601, 2003.

TAN, X.; ZHAO, C.; PAN, J.; SHI, Y.; LIU, G.; ZHOU, B.; ZHENG, R. In vivo non-enzymatic repair of DNA oxidative damage by polyphenols. **Cell Biology International,** v. 33, n. 6, p. 690-696, 2009.

TANG, K.; ZHAN, J.; YANG, H.; HUANG, W. Changes of resveratrol and antioxidant enzymes during UV-induced plant defense response in peanut seedlings. **Journal of Plant Physiology,** v. 167, p. 95-102, 2010.

TEGELBERG, R.; JULKUNEN-TIITTO, R.; APHALO, P. J. The effects of long-term elevated UV-B on the growth and phenolics of field-grown silver birch (*Betula pendula*). **Global Change Biology,** v. 7, p. 839-848, 2001.

TERAMURA, A. H. Effects of ultraviolet-B radiation on the growth and yield of crop plants. **Physiologia Plantarum,** v. 58, n. 3, p. 415–427, 2006.

TERRY, L. A.; JOYCE, D. C. Elicitors of induced disease resistance in postharvest horticultural crops: A brief review. **Postharvest Biology and Technology,** v. 32, n. 1, p. 1-13, 2004.

TESSMER, M. A.; KLUGE, R. A.; APPEZZATO-DA-GLÓRIA, B. The accumulation of tannins during the development of 'Giombo' and 'Fuyu' persimmon fruits. **Scientia Horticulturae,** v. 172, p. 292-299, 2014.

TREUTTER, D. Managing phenol contents in crop plants by phytochemical farming and breeding - Visions and constraints. **International Journal of Molecular Sciences,** v. 11, n. 3, p. 807-857, 2010.

TRIVELLINI, A.; LUCCHESINI, M.; MAGGINI, R.; MOSADEGH, H.; VILLAMARIN, T. S. S.; VERNIERI, P.; MENSUALI-SODI, A.; PARDOSSI, A. Lamiaceae phenols as multifaceted compounds: bioactivity, industrialprospects and role of "positive-stress". **Industrial Crops and Products,** v. 83, p. 241-254, 2016.

TROJAK, M.; SKOWRON, E. Role of anthocyanins in high-light stress response. **World Scientific News,** v. 81, p. 150-168, 2017.

VAN BENNEKUM, A.; WERDER, M.; THUAHNAI, S. T.; HAN, C. H.; DUONG, P.; WILLIAMS, D. L.; WETSTEIN, P.; SCHULTHESS, G.; PHILLIPS, M. C.; HAUSER, H. Class B scavenger receptor mediated intestinal absorption of dietary beta-carotene and cholesterol. **Biochemistry,** v. 44, p. 4517-4525, 2005.

VASCONCELOS, A. G.; AMORIM, A. D. G. N.; DOS SANTOS, R. C.; SOUZA, J. M. T.; DE SOUZA, L. K. M.; ARAÚJO, T. D. S. L.; NICOLAU, L. A. D.; DE LIMA CARVALHO, L.; DE AQUINO, P. E. A.; DA SILVA MARTINS, C.; ROPKE, C. D.; SOARES, P. M. G.; KUCKELHAUS, S. A. S.; MEDEIROS, J.-V. R.; LEITE, J. R. D. S. A. Lycopene rich extract from red guava (*Psidium guajava* L.) displays anti-inflammatory and antioxidant profile by reducing suggestive hallmarks of acute inflammatory response in mice. **Food Research International,** v. 99, p. 959-968, 2017/09/01/ 2017.

VAUZOUR, D.; RODRIGUEZ-MATEOS, A.; CORONA, G.; ORUNA-CONCHA, M. J.; SPENCER, J. P. E. Polyphenols and human health: prevention of disease and mechanisms of action. **Nutrients,** v. 2, p. 1106-1131, 2010.

VÁZQUEZ-GUTIERREZ, J. L.; QUILES, A.; HERNANDO, I.; PÉREZ-MUNUERA, I. Changes in the microstructure and location of some bioactive compounds in persimmons treated by high hydrostatic pressure. **Postharvest Biology and Technology,** v. 61, p. 137-144, 2011.

VEBERIC, R.; JURHAR, J.; MIKULIC-PETKOVSEK, M.; STAMPAR, F.; SCHMITZER, V. Comparative study of primary and secondary metabolites in 11 cultivars of persimmon fruit (*Diospyros kaki* L.). **Food Chem.,** v. 119, p. 477-483, 2010.

WEGER, H. G.; SILIM, S. N.; GUY, R. D. Photosynthetic acclimation to low temperature by western red cedar seedlings. **Plant, Cell and Environment,** v. 16, p. 711-717, 1993.

WHO. **Diet, nutrition and the prevention of chronic diseases**. WHO, Technical Report Series 916. Geneva. 2003

WINKEL-SHIRLEY, B. Biosynthesis of flavonoids and effect on stress. **Current Opinion in Plant Biology,** v. 5, p. 218-223, 2000.

WINKEL-SHIRLEY, B. It takes a garden: how work on diverse plant species has contributed to an understanding of flavonoid metabolism. **Plant Physiology,** v. 127, p. 1399-1404, 2001.

WOOTTON-BEARD, P. C.; RYAN, L. Improving public health? The role of antioxidant-rich fruit and vegetable beverages. **Food Research International,** v. 44, p. 3135-3148, 2011.

WRIGHT, M. E.; PARK, Y.; SUBAR, A. F.; FREEDMAN, N. D.; ALBANES, D.; HOLLENBECK, A.; LEITZMANN, M. F.; SCHATZKIN, A. Intakes of fruit, vegetables, and specific botanical groups in relation to lung cancer risk in the NIH-AARP diet and health study. **American Journal of Epidemiology,** v. 168, n. 9, p. 1024-1034, 2008.

ZHANG, Y. Z.; XU, S. Z.; CHENG, Y. W.; YA, H. Y.; HAN, J. M. Transcriptome analysis and anthocyanin-related genes in red leaf lettuce. **Genetics and Molecular Research,** v. 15, p. 1-11, 2016.

APÊNDICE

AVALIAÇÃO DO CRESCIMENTO, DESENVOLVIMENTO E PERFIL DE COMPOSTOS FENÓLICOS DE ALFACES ROXAS PRODUZIDAS EM CULTIVO *INDOOR*

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Resumo

Indicadores de crescimento e desenvolvimento e perfil de compostos bioativos da cultivar de alface roxa Mimosa foram analisados por meio de um experimento em câmara de cultivo *indoor* comparado a um tratamento simulando o cultivo doméstico. As mudas foram adquiridas em comércio local de Porto Alegre e foram cultivadas por um período de 28 dias. As plantas cultivadas na câmara apresentaram aumento no número de folhas, diâmetro do caule, área foliar, taxa de crescimento absoluto e produtividade. As folhas cultivadas no tratamento controle sofreram o fenômeno do estiolamento, o que prejudicou seu crescimento e desenvolvimento total, sugerindo vantagens significativas no uso da câmara de cultivo.

Palavras-chave: área foliar, assimilação, HPLC-DAD-MS, *Lactuca sativa*, crescimento, desenvolvimento.

1 Introdução

O advento dos sistemas alimentares locais tem se delineado como um movimento multifacetado que defende causas diversas como saúde, nutrição e estilo de vida, justiça social, segurança alimentar, conservação ambiental, além de desenvolvimento comunitário e econômico (KREMER e DELIBERTY, 2011). Segundo Pölling *et al.* (2016), a agricultura urbana é um tópico bastante pertinente nas sociedades ao redor do mundo e pode ser analisada cientificamente dentro de diversas áreas. Além disso, o alimento produzido dentro do conceito

de *local food* permite ao consumidor saber sobre como e onde o seu alimento foi cultivado, o que influencia na sua tomada de decisão.

Dentro deste contexto, na última década, com o advento das lâmpadas de LED (*light emitting diode*), o cultivo de alimentos *indoor* evoluiu rapidamente para uma abordagem comercialmente viável e em larga escala. A indústria do setor tem voltado suas atenções à produção de alimentos dentro de centros urbanos. Hoje em dia é possível produzir alimentos dentro do próprio lar sem a necessidade de dispor de espaços ensolarados.

Como um bom modelo de estudo para os efeitos da luz, a alface é destaque de produção e consumo em termos mundiais. No Estado do Rio Grande do Sul, é produzida durante todo o ano, com a existência de dois períodos com condições climáticas pouco favoráveis: o verão, quando ocorrem elevadas temperatura do ar e radiação solar, o que favorece o pendoamento precoce das plantas; e o inverno com ocorrência de baixas temperaturas e precipitações prolongadas que podem retardar o crescimento e danificar as plantas (SEGOVIA *et al.*, 1997).

Diante do exposto, o presente trabalho teve como objetivo comparar o crescimento, o desenvolvimento e o perfil de compostos bioativos de alfaces roxas (cv. Mimosa) produzidas em cultivo *indoor*, utilizando uma câmara de cultivo da empresa Plantário®.

2 Material e Métodos

O experimento foi conduzido no Laboratório de Pós-colheita do Departamento de Horticultura e Silvicultura/Faculdade de Agronomia da Universidade Federal do Rio Grande do Sul (Porto Alegre, RS-Brasil), localizado em 30° 04’ de latitude sul e 51° 08’ de longitude, durante o período de julho a setembro de 2017. Em 26 de julho, mudas de alface Mimosa Roxa, adquiridas no comércio local, foram transplantas em vasos plásticos com capacidade de 1,7 L cada, que foram previamente lavados e preenchidos com perlita (substrato) antes do transplantio.

As plantas foram cultivadas *indoor* em diferentes condições, uma em câmara de cultivo sob luz de LED brancas e infravermelho, conforme protótipo disponibilizado pela empresa Plantário® (Figura 1). E uma segunda condição, para simular o cultivo doméstico, foi conduzido um tratamento controle de lâmpadas brancas. Os sistemas de cultivo *indoor* testados foram mantidos em uma sala a temperatura constantes de 18 °C enquanto os

fotoperíodos da câmara de cultivo e do controle foram de 18:6 e 10:14 horas de luz e escuro, respectivamente.

Figura 1. Protótipo disponibilizado pela empresa Plantário®.

A solução nutritiva, o substrato e a irrigação foram os mesmos para ambos os tratamentos. A solução nutritiva fornecida às plantas possúia composição química de macronutrientes: $Ca(NO_3)_2$ (42,32 %), KNO_3 (26,45 %) $NH_4H_2PO_4$ (7,93 %), $MgSO_4$ (21,16 %), KH_2PO_4 (0,53 %); e de micronutrientes: Fe e Fe-EDTA (1,58 %). O pH e a condutividade elétrica inicial da solução nutritiva utilizada foram de 5,45 e 2,016 mS, respectivamente. O nível da solução nutritiva de cada unidade experimental foi mantido pela reposição do volume consumido com água, sendo realizada quando houve redução de 20 % do volume de cada vaso, que correspondeu à descida de 2,5 cm no nível da solução no reservatório.

A irrigação foi automatizada por meio de um timer que acionava a bomba 3 vezes ao dia, durante 15 minutos (às 6 h, às 12 h e às 18 h do dia). Não foi aplicada nenhum defensivo durante o cultivo das plantas. A solução nutritiva, o substrato e a irrigação foram os mesmos para ambos os tratamentos.

O experimento foi conduzido em dois blocos inteiramente casualizados. As alfaces foram colhidas e analisadas de forma destrutiva para obtenção dos parâmetros de produção,

como crescimento e desenvolvimento no dia da instalação do experimento (0 DAT), aos 20 dias após o transplantio (20 DAT) e aos 28 dias após o transplantio (28 DAT). Cada coleta para cada tempo e tratamento consistiu em 3 plantas inteiras (randomicamente uma de cada vaso pré-selecionado).

Durante as avaliações, a umidade relativa do ar foi de ≈ 80 ± 5 %, a temperatura do ambiente variou de 18 a 20 °C, a irradiância de saturação de 400 µmol de fótons m^{-2} s^{-1} dentro da câmara de cultivo (Plantário®) e de 200 µmol de fótons m^{-2} s^{-1} no tratamento Controle.

Os resultados obtidos foram submetidos à análise de variância utilizando-se o programa estatístico Statistica, sendo os tratamentos comparados pelo teste de Tukey a 5% de significância.

2.1 Determinação dos parâmetros de desenvolvimento de alfaces

Nas três colheitas (0DAT, 20DAT e 28DAT), contabilizou-se o número de folhas por planta, considerando apenas as folhas comerciais. Determinou-se a área foliar, utilizando-se um integrador de área, modelo LI3100 da Licor, sendo utilizadas todas as folhas de cada planta. A altura da parte aérea foi determinada logo após a separação do sistema radicular com o auxílio de uma régua graduada. O diâmetro dos caules foi determinado por meio de paquímetro e expresso em milímetro (mm). O peso da massa fresca da parte aérea das plantas foi determinado logo após a colheita, com balança digital de precisão (0,01 g). Após as plantas foram acondicionadas em sacos de papel previamente identificados e postas para secagem em estufa com circulação forçada de ar, na temperatura de 65±1 ºC, onde permaneceram até atingirem peso constante; em sequência, as plantas foram pesadas em balança digital de precisão (0,01 g), e determinada a massa seca da parte aérea. A taxa de crescimento absoluto (TAC) foi calculada pela seguinte expressão: TCA = (P2-P1)/(T2-T1), em que P2 - P1 é a diferença de massa de matéria seca (g), em determinada área, e T2 - T1 é o intervalo de tempo (dias) entre as duas amostragens. Os valores estão expressos em $g.dia^{-1}$. A produtividade foi calculada pela divisão da massa seca total pela área de cultivo. Os valores estão expressos em $g.cm^{-2}$.

2.2 Extração e determinação do perfil de compostos fenólicos por HPLC-DAD-MS

Amostras congeladas e liofilizadas (0.5 g) foram trituradas com nitrogênio líquido para obtenção de um pó fino. O material foi extraído em triplicada utilizando o método descrito por Becatti *et al.* (2010), seguindo extrações em ultrassom com metanol 80% e

centrifugação com recuperação do sobrenadante. Os compostos fenólicos foram determinados de acordo com o protocolo descrito por Rodrigues *et al.* (2013). As análises cromatográficas foram realizadas usando um cromatógrafo Shimadzu (Kyoto, Japão) equipado com duas bombas, desgaseificador, forno da coluna, ligado em série a um detector de arranjo de diodo e um espectrômetro de massas com analisador Q-TOF e fonte de ionização por electrospray (ESI) (Bruker Daltonics, modelo micrOTOF-QII, Bremen, Alemanha). Os extratos preparados foram filtrados com membranas de 0,22 μm de acetato de celulose (Millipore®, Massachusetts, EUA) e injetados no volume de 20 μL ao sistema cromatográfico. O compostos fenólicos foram separados em coluna Atlantis C18 RP-T3 (5 μm, 250 × 4,6 mm) e coluna Synergi Hydro-RP C18 (4 μm, 250 × 4,6 mm, Phenomenex), a um fluxo de 0,7 $mL.min^{-1}$ e temperatura da coluna de 29 ºC, usando duas soluções de fase móvel: (A) solução de água ultrapura (Milli-Q®, Millipore®) acidificada com ácido fórmico (99,5:0,5 v/v); (B) solução de acetonitrila acidificada com ácido fórmico (99,5:0,5 v/v). Um gradiente linear para as soluções de fase móvel foi utilizado, iniciando com a proporção de 99:1 para A/B, aumentando linearmente a proporção da fase móvel B até alcançar 50:50 (A/B) em 50 min. Na sequência, foi aumentado novamente a proporção de B até 1:99 (A/B) em 5 min. Essa relação anterior (1:99) foi mantida por mais 5 minutos e após esse tempo o sistema voltou a condição inicial de 99:1 para A/B. Os espectros de UV-vis foram obtidos entre 200 e 800 nm e os cromatogramas foram processados a 320, 360, e 520 nm. O eluato da coluna foi dividido para permitir a entrada de apenas 0,35 $mL.min^{-1}$ na interface de ESI do espectrômetro de massas. Os espectros de massas foram adquiridos com uma faixa de varredura de 100 a 700 m/z. Os parâmetros de MS foram os seguintes: fonte ESI nos modos de ionização positivo e negativo; voltagem do capilar: 2000 V (positivo) ou -3000 V (negativo); end plate offset: -500 V; gás de secagem: N_2; temperatura: 310 ºC; vazão de nitrogênio: 8 $L.min^{-1}$; gás nebulizador: 2 bar. A fragmentação (MS_2) foi obtida no modo automático, aplicando uma energia de fragmentação de 34 eV. Os compostos fenólicos foram identificados com base nas seguintes informações: ordem de eluição e tempo de retenção na coluna de fase reversa, UV-vis e características dos espectros de massas (MS e MS2) comparados com padrões analisados nas mesmas condições e com dados disponíveis na literatura.

3 Resultados e Discussão

Conforme resultados da Figura 2A, pode ser observado que o número de folhas comerciais por planta tende a um aumento mais rápido no interior da câmara (Plantário®) em

relação ao controle. Por outro lado, quanto à altura das plantas (Figura 2C), com a ocorrência do fenômeno de estiolamento nas plantas cultivadas no tratamento Controle, não foi possível observar diferença entre os tratamentos. No entanto, o consumidor busca uma alface com um maior número de folhas/planta, sendo esta a variável que confere valor comercial a este tipo de alimento.

O índice de área foliar (Figura 2D) mostra uma tendência semelhante aos valores de número de folhas comerciais/planta e de diâmetro do caule (Figura 2B), porém a amplitude da diferença é maior. Os estudos de Segovia *et al.* (1997) sugerem resultados semelhantes quando comparados o crescimento e o desenvolvimento de três cultivares de alface no interior de uma estufa de polietileno.

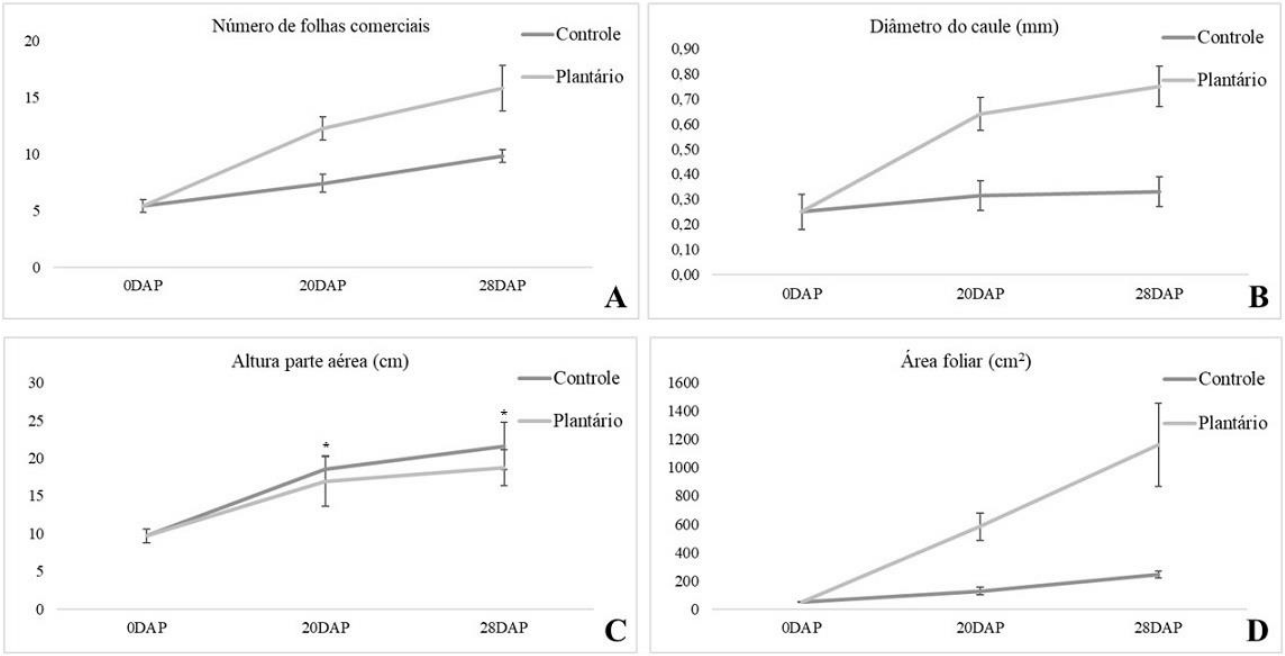

Figura 2. Número de folhas comerciais (intactas)/planta (A), diâmetro do caule (mm) (B), altura da parte aérea (cm) (C) e área foliar (cm^2) (D) de alfaces roxas (cv. Mimosa) cultivadas em câmara de cultivo indoor (Plantário®) e simulando cultivo doméstico (Controle). 0DAP, 20DAP e 28 DAP representam a quantidade de dias após o transplantio das mudas. * significa que não houve diferença significativa ($p < 0,05$).

Segundo Radin *et al.* (2004), os parâmetros da massa foliar e do índice de área foliar indicam que a massa específica das folhas tende a ser menor no interior das estufas, sugerindo que no interior de estufas as folhas se expandem mais rapidamente devido aos teores mais elevados da umidade relativa do ar. Essa característica favorece atributos como a aparência do alimento, mas é negativa do ponto de vista da resistência ao transporte e da conservação pós-

colheita. Porém, o alimento estudado neste trabalho é desenvolvido para o cultivo no próprio local de consumo, ou seja, estes alimentos não necessitam ser transportados.

Segundo Despommier (2013), o cultivo *indoor* atrelado a este tipo de sistema local oferece muitas vantagens em relação à agricultura tradicional baseada no solo; o mais importante é o controle total das condições necessárias para alcançar uma sobrevivência, crescimento e maturação ótimos de qualquer cultura, o que pode garantir assim o rendimento máximo da produtividade. Segundo os autores, este sistema representa uma das poucas oportunidades de exploração nas próximas décadas.

Os resultados mostraram que as plantas de alface roxa cultivadas no interior da câmara Plantário® apresentaram uma taxa de crescimento maior, conforme Figura 3A, demonstrando que a câmara proporciona uma maior eficiência no acúmulo de massa por dia em relação ao controle. Além disso, tem maior eficiência também no acúmulo de massa por área (Figura 3B) e em produtividade, parâmetro no qual o Plantário® apresentou uma diferença de 300 $g.cm^{-2}$ a mais que o Controle.

Contudo, verifica-se que o Plantário® proporcionou precocidade de colheita, maior produção folhas por planta e por área que aquela obtida com o cultivo doméstico simulado pelo Controle.

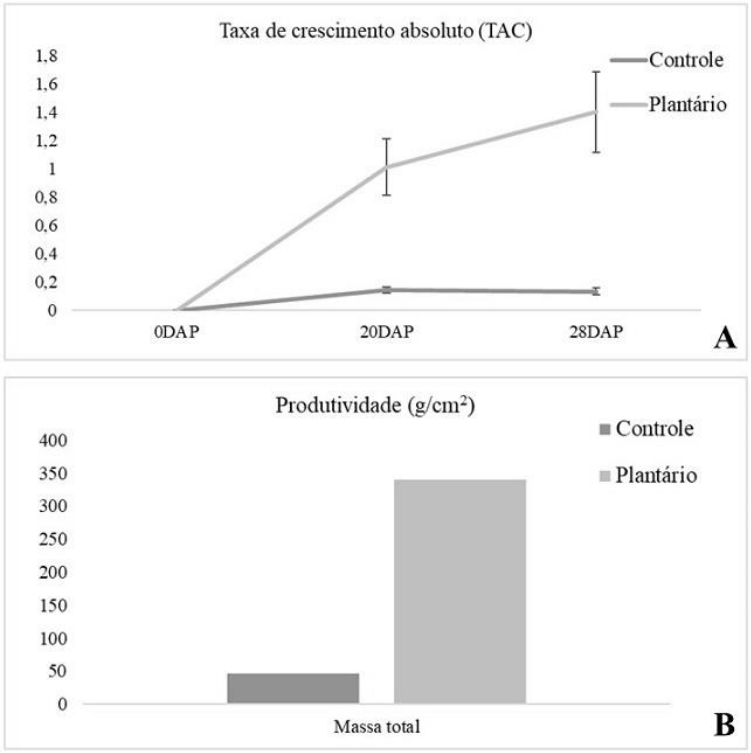

Figura 3. Taxa de crescimento absoluto (TAC) (A) e produtividade (B) de alfaces roxas (cv. Mimosa) cultivadas em câmara de cultivo *indoor* (Plantário®) e simulando cultivo doméstico (Controle). 0DAP, 20DAP e 28 DAP representam a quantidade de dias após o plantio das mudas.

Sete compostos fenólicos foram identificados por HPLC-DAD-MS baseados na combinação de informações obtidas pela eluição cromatográfica na coluna C18, UV-vis e espectro de massas (Figura 3 e Tabela 1). Os fragmentos característicos de cada estrutura química permitiram a confirmação dos grupos.

Tabela 1. Compostos fenólicos identificados em alface roxa (cv. Mimosa) por HPLC-DAD-MS. Os detalhes das condições cromatográficas estão descritos na Seção 2.3.

Número do pico	**Composto fenólico**	**Tempo de retenção (min)**	**λ de análise (nm)**	**Aduto**
1	Ácido clorogênico	23,3	320	$[M-H]^-$
2	Ácido cafeico (3,4-ácido dihidroxicinâmico)	24,7	320	$[M-H]^-$
3	Ácido chicórico	35,2	320	$[M-H]^-$
4	Luteolina-7-O-glucoronídeo	31,3	360	$[M-H]^-$
5	Quercetina-3-malonilglucosídeo	31,7	360	$[M-H]^-$
6	Quercetina acetil hexosídeo	33,3	360	$[M-H]^-$
7	Cianidina 3-O-malonilglucosídeo	25,8	520	$[M+H]^+$

Ambos os tratamentos (Plantário® e Controle) mostraram perfis de compostos fenólicos semelhantes (Figura 4). A identificação de luteolina, cianidina, ácido chicórico, ácido clorogênico e isômeros de quercetina é corroborada pelos resultados de Becker *et al.* (2014) no estudo de alfaces roxas.

O perfil dos compostos bioativos de diferentes cultivares de alface tem sido estudado por diversos autores (PÉREZ-LÓPEZ *et al.*, 2014; MARIN *et al.*, 2015). A superioridade da capacidade antioxidante das alfaces roxas em relação às de folhas verdes indica a importância de uma alimentação diversificada. Os flavonoides que conferem coloração roxa às alfaces em estudo são as antocianinas, aqui representadas pela cianidina.

O potencial de flavonoides na redução do risco de diversas doenças cardiovasculares, cânceres e aterosclerose já é bastante conhecido. Segundo Pérez-López *et al.* (2014), a capacidade antioxidante de alfaces roxas é majoritariamente atribuída às quercetinas e

antocianinas, sugerindo que o consumo desse tipo de alimento é potencialmente benéfico à saúde humana, sendo que produção *indoor* pode contribuir para aproximar o consumidor de uma alimentação mais saudável, com produtos livres de agrotóxicos e contaminantes ambientais.

A formação de metabólitos secundários em alimentos como os identificados neste trabalho depende de diversos fatores, entre eles a disponibilidade de luz (GARCÍA-MACÍAS *et al.*, 2007). Neste sentido, o Plantário® fornece condições suficientes para a formação do perfil de compostos bioativos normalmente encontrados neste tipo de cultivo.

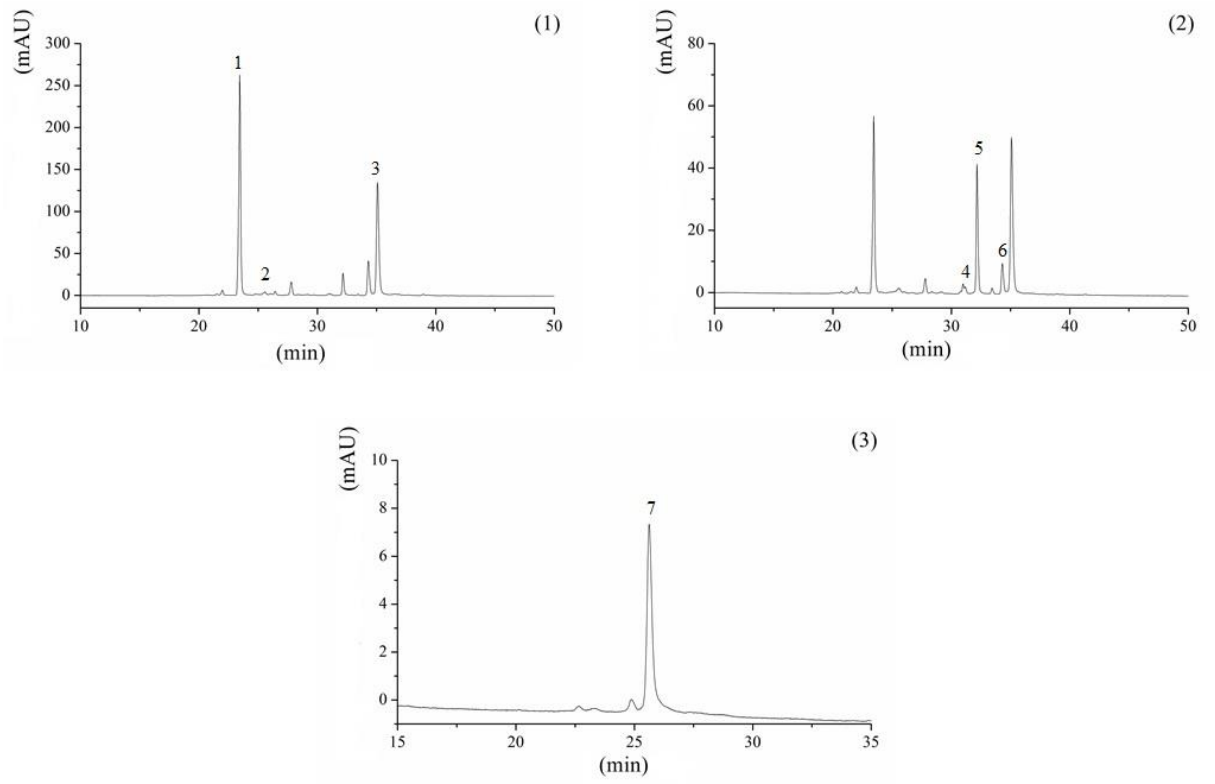

Figura 4. Cromatogramas da identificação de compostos fenólicos em diferentes comprimentos de onda (1: 320 nm; 2: 360 nm; 3: 520 nm) em alfaces roxas (cv. Mimosa) cultivadas em câmara de cultivo indoor (Plantário®) e simulando cultivo doméstico (Controle).

4 Conclusão

O cultivo *indoor* utilizando a câmara Plantário® proporcionou maior crescimento e desenvolvimento das plantas de alface roxa (cv. Mimosa) em relação ao controle. Os resultados indicam que a câmara de cultivo *indoor* é uma alternativa eficiente para obter uma maior produtividade e qualidade geral das alfaces roxas, além de ser uma opção viável que proporciona ao consumidor uma alimentação mais natural, saudável e local.

Referências

BECATTI, E.; CHKAIBAN, L.; TONUTTI, P.; FORCATO, C.; BONGHI, C.; RANIERI, A. M. Short-Term Postharvest Carbon Dioxide Treatments Induce Selective Molecular and Metabolic Changes in Grape Berries. **Journal of Agricultural and Food Chemistry,** v. 58, n. 13, p. 8012-8020, 2010/07/14 2010.

BECKER, C.; KLAERING, H.; KROH, L. W.; KRUMBEIN, A. Cool-cultivated red leaf lettuce accumulates cyaniding -3-O-(6''-O-malonyl)-glucoside and caffeoylmalic acid. **Food Chem.,** v. 146, p. 404-411, 2014.

DESPOMMIER, D. Farming up the city: the rise of urban vertical farms. **Trends in Biotechnology,** v. 31, n. 7, p. 388-389, 2013.

GARCÍA-MACÍAS, P.; ORDIDGE, M.; VYSINI, E.; WAROONPHAN, S.; BATTEY, N. H.; GORDON, M. H.; HADLEY, P.; JOHN, P.; LOVEGROVE, J. A.; WAGSTAFFE, A. Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. **Journal of Agricultural and Food Chemistry,** v. 55, p. 10168-10172, 2007.

KREMER, P.; DELIBERTY, T. L. Local food practices and growing potential: Mapping the case of Philadelphia. **Applied Geography,** v. 31, p. 1252-1261, 2011.

MARIN, A.; FERRERES, F.; G., B. G.; GIL, M. I. Weather variability influences color and phenolic content of pigmented baby leaf lettuces throughout the season. **Journal of Agricultural and Food Chemistry,** v. 63, p. 1673−1681, 2015.

PÉREZ-LÓPEZ, U.; PINZINO, C.; QUARTACCI, M. F.; RANIERI, A.; SGHERRI, C. Phenolic composition and related antioxidant properties in differently colored lettuces: a study by Electron Paramagnetic Resonance (EPR) Kinetics. **Journal of Agricultural and Food Chemistry,** v. 62, p. 12001−12007, 2014.

PÖLLING, B.; MERGENTHALER, M.; LORLEBER, W. Professional urban agriculture and its characteristic business models
in Metropolis Ruhr. **Germany Land Use Policy,** v. 58, p. 366-379, 2016.

RADIN, B.; REISSER JÚNIOR, C.; MATZENAUER, R.; BERGAMASCHI, H. Crescimento de cultivares de alface conduzidas em estufa e a campo. **Horticultura Brasileira,** v. 22, n. 2, p. 178-181, 2004.

RODRIGUES, E.; MARIUTTI, L. R. B.; MERCADANTE, A. Z. Carotenoids and phenolic compounds from Solanum sessiliflorum, an unexploited Amazonian fruit, and their scavenging capacities against reactive oxygen and nitrogen species. **Journal of Agricultural and Food Chemistry,** v. 61, n. 12, p. 3022-3029, 2013.

SEGOVIA, J. F. O.; ANDRIOLO, J. L.; BURIOL, G. A.; SCHNEIDER, F. M. Comparação do crescimento e desenvolvimento da alface (*Lactuca sativa* L.) No interior e no exterior de uma estufa de polietileno em Santa Maria, RS. **Ciência Rural,** v. 27, n. 1, p. 37-41, 1997.