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JOSÉ JADILSON NUNES DE MACEDO

INFLUÊNCIA DA LUZ PULSADA SOBRE A FISIOLOGIA E A QUALIDADE PÓS-COLHEITA DE ACEROLA (*Malpighia emarginata* DC)

FORTALEZA
2019

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Tese apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Ceará, como parte dos requisitos para obtenção do título de Doutor em Bioquímica. Área de concentração: Bioquímica Vegetal.

Orientadora: Prof.ª Dra. Maria Raquel Alcântara de Miranda.
Coorientador: Prof. Dr. Carlos Farley Herbster Moura.

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Aprovada em: 23/05/2019

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Aos meus pais, Jair Macedo e Bernadete Macedo.

À minha querida esposa, Elisabete Macedo,
aos meus filhos, Daniel, Raissa e Maria Clara Macedo e aos meus irmãos Jailson, Marlos e Débora Macedo.

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RESUMO

Acerola 'Okinawa', fisiologicamente madura, foi submetida a radiação de luz pulsada (LP) (0,6 $J.cm^{-2}$), armazenada a 10 ºC e avaliada nos dias 0, 1, 4, 10 e 16 para firmeza e variáveis associadas, marcador de estresse oxidativo e constituintes do metabolismo antioxidante, respiração e metabolismo de etileno, metabolismo de poliamina, metabolismo de cor e fenólico e conteúdo de vitamina C. Quando comparado ao controle de frutas não tratadas, o tratamento com LP reduziu o pico respiratório no climatério em 5% ($P < 0,05$) com 78,6 $mL.Kg^{-1}.h^{-1}$ e retardou o pico de etileno para o dia 3 com 2,65 $\mu L.Kg^{-1}.h^{-1}$ Acerola LP-tratada foi 12% significativamente mais firmes, no dia 10 e perdeu 13,4% menos peso, sem diferenças significativas nos atributos de cor, perda de peso e croma. O marcador de estresse oxidativo H_2O_2 foi significativamente menor em frutas tratadas com LP e a atividade das enzimas antioxidantes dismutase do superóxido (SOD) e catalase (CAT) foram estimuladas. O menor teor de espécies reativas de oxigênio (ROS) refletiu-se no menor grau de peroxidação lipídica da membrana, enquanto a atividade hidrolítica da parede celular também foi inibida, contribuindo para maior firmeza. A LP reduziu o conteúdo de Ácido 1-carboxílico-1-aminociclopropano (ACC), precursor de etileno, em 20% e inibiu a atividade da oxidase do ACC (ACO) em 16%. Os níveis de poliaminas putrescina e espermidina foram 21% e 12% maiores, respectivamente, que o controle devido ao estímulo de sua síntese (atividade da descarboxilase da arginina - ADC) e inibição de sua degradação (atividade da oxidase de diamina - DAO). O tratamento com LP resultou em níveis significativamente maiores ($P< 0,05$) de vitamina C e ácido ascórbico (AsA). O tratamento com LP resultou em maior conteúdo de polifenol devido à síntese aumentada pela fenilalanina amônia-liase (PAL). Portanto, a LP, sob as condições aqui aplicadas, influenciou a fisiologia da acerola não como agente estressor, mas como eliciadora do sistema de defesa antioxidante, prevenindo possíveis danos oxidativos devido ao desequilíbrio entre a produção de ROS e sua eliminação, promovendo a qualidade durante o armazenamento.

Palavras-chave: Acerola. Radiação. Climatério. Poliamina. Antioxidante. Ascorbato.

ABSTRACT

Physiologically mature acerola 'Okinawa' were submitted to Pulsed light (PL) radiation (0.6 $J.cm^{-2}$), stored at 10 ºC and evaluated at days 0, 1, 4, 10 and 16 for firmness and associated variables, oxidative stress marker and constituents of antioxidant metabolism, respiration and ethylene metabolism, polyamine metabolism, color and phenolic metabolism and vitamin C content. When compared to the control of untreated fruits, PL treatment reduced climacteric respiratory peak in 5% lower ($P< 0.05$) with 78.6 $mL.Kg^{-1}.h^{-1}$ and delayed ethylene peak to day 3 with 2.65 $\mu L.Kg^{-1}.h^{-1}$. PL-treated acerola were significantly firmer 12%, at day 10 and lost 13.4% less weight with no significant differences in color attributes lightness and chroma. Oxidative stress marker H_2O_2 was significantly lower in PL-treated fruit and antioxidant enzymes SOD and CAT activities were stimulated. The lower ROS content reflected in lower membrane lipid peroxidation degree, while cell wall hydrolytic activity was also inhibited, contributing to greater firmness. PL reduced ethylene precursor free ACC content in 20% and inhibited ACO activity in 16%. Polyamines putrescine and spermidine levels were 21% and 12% higher, respectively, than control due to stimulus of its synthesis (ADC activity) and inhibition of its degradation (DAO activity). PL-treatment resulted in significantly ($P< 0.05$) higher total vitamin C and AsA levels. LP treatment resulted higher polyphenol content due to withted the enhanced synthesis by PAL.Therefore, PL, under the conditions here applied, influenced acerola physiology not as a stress agent, but as an elicitor of the antioxidant defense system preventing possible oxidative damages due to unbalance between ROS production and scavenging mechanisms, promoting quality during storage.

Keywords: Acerola. Radiation. Climacteric. Polyamine. Antioxidant. Ascorbate.

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ACC -	Ácido 1-carboxílico-1-aminociclopropano
ACD -	Desaminase do ACC
ACO -	Oxidase do ACC
ACS -	Sintase do ACC
ADC -	Descarboxilase da arginina
APX -	Peroxidase do ascorbato
AsA -	Ácido ascórbico
ASC -	Ascorbato reduzido
CAT -	Catalase
DAO -	Oxidase de diamina
DHA -	Desidroascorbato
DHAR -	Redutase do desidroascorbato
DNS -	Ácido 3,5-dinitrosalicílico
FAD -	Flavina-adenina-dinucleotídeo
FDA -	Food and Drug Administration
MDA -	Malondialdeído
MET -	Metionina
ODC -	Descarboxilase da ornitina
PAL -	Fenilalanina amônia-liase
PAO -	Oxidase de poliamina
PG -	Poligalacturonase
PL -	Luz pulsada
PLP -	Piridoxal-5`- fosfato
PME -	Pectinametilesterase
PUT -	Putrescina
ROS -	Espécies reativas de oxigênio
SAM -	S-adenosilmetionina
SAMDC -	Descarboxilase do S-adenosilmetionina
SOD -	Dismutase do superóxido
SPD -	Espermidina
SPDS -	Sintase da espermidina

SPM - Espermina

SPMS - Sintase da espermina

TAA - Atividade Antioxidante Total

UV - Ultravioleta

UV-C - Ultravioleta Contínuo

SUMÁRIO

1 INTRODUÇÃO

A aceroleira (*Malpighia emarginata* DC.) é nativa da América Central, porém desde 2012, o Brasil é o maior produtor mundial de acerola com 50000 ton/ano e mais de 45 cultivares, dentre as quais, 'Okinawa' destaca-se por suas frutas mais firmes e resistentes à manipulação pós-colheita (MOURA *et al*., 2007). A acerola madura é rica em vitamina C e fenólicos como flavonóides e antocianinas (OLIVEIRA *et al*., 2011; SOUZA *et al*., 2014). Apesar de sua excelente composição nutricional, a vida pós-colheita da acerola é restrita de dois a quatro dias à temperatura ambiente (25 ºC), devido ao rápido amadurecimento e senescência. Portanto, a cadeia de comercialização da acerola é bastante dependente de tecnologias pós-colheita como processamento e refrigeração de polpas processadas e frutas inteiras, que podem ser armazenadas a -18 ºC por até 12 meses (OLIVEIRA *et al*., 2011).

A tecnologia da luz pulsada (LP) consiste na aplicação de intensos flashes de amplo espectro (200-1100 nm) com duração de 1 μs a 0,1 s e fluência até 12 $J.cm^{-2}$ que exerce efeito direto sobre microorganismos, além de induzir mudanças bioquímicas e fisiológicas nos tecidos de frutos quando aplicada em pós-colheita (URBAN *et al*., 2018). Entre as respostas biológicas induzidas por elicitores físicos como LP, estão aquelas associadas à defesa e resistência de plantas através da indução de espécies reativas de oxigênio (EROS) e metabólitos secundários como fitoalexinas (SHAMA e ALDERSON, 2005).

O tratamento pós-colheita com LP induziu diferentes respostas em polpa e casca de tecidos de manga 'Tommy Atkins'. Na celulose, um estresse oxidativo foi estabelecido devido a superprodução de EROs que teria desencadeado o sistema de defesa antioxidante, enquanto em casca, o sistema de defesa antioxidante foi estimulado sem mediação por EROS. Portanto, a LP penetrou na casca de manga atingindo a polpa e influenciando sua fisiologia, porém não afetou as variáveis estruturais de nenhum dos tecidos (LOPES *et al*. 2016). Uma vez que o tratamento com LP penetra na casca de manga com 1-1,5 mm de espessura, supõe-se que ela penetra facilmente na casca de acerola, que é mais fina com 0,5 mm influenciando sua fisiologia. Quanto à isso, não há informações publicadas sobre a influência do tratamento da LP sobre a acerola quanto ao padrão de amadurecimento do climatério, metabolismo da poliamina, estrutura, metabolismo da vitamina C. Assim, esse trabalho teve como objetivo estudar os efeitos da aplicação pós-colheita de LP sobre A qualidade e processos fisiológicos relacionados ao amadurecimento de acerola.

2 REVISÃO DE LITERATURA

2.1 Acerola

A aceroleira (*Malpighia emarginata* DC) é uma planta frutífera perene de porte pequeno, variando de 1,3 a 3,2 m, com tronco único e copa ramificada (MANICA *et al*., 2003). Teve origem na América Central e dispersou-se por todo o continente americano devido à sua boa adaptação à diferentes solos e climas. Em 1958, as sementes foram trazidas de Porto Rico para o Brasil e implantadas na Universidade Federal Rural de Pernambuco, onde foram multiplicadas e distribuídas para vários Estados do Nordeste e regiões do país. Porém, somente no final dos anos 80 e início dos anos 90 houve forte crescimento dos plantios comerciais no país (VENDRAMINI e TRUGO, 2000; CARRINGTON e KING, 2002; MONTIM, OLIVEIRA e VIEIRA, 2010).

Também conhecida por cereja das Antilhas, cereja de Barbados e cereja das Índias Ocidentais, a acerola é um fruto carnoso do tipo drupa que apresenta tamanho entre 1 e 4 cm de diâmetro, com formato ovalado e peso variando de 2 a 15 g. A sua maturação ocorre de 3 a 4 semanas após a floração (MANICA *et al*., 2003; MOURA *et al*., 2003; ERGUN *et al*., 2014). Suas flores são perfeitas, com coloração do rosa-claro ao vermelho, e ficam dispostas em cachos de 3 a 5 unidades nas regiões axiais dos ramos (SAZAN *et al*., 2014).

Atualmente, o Brasil é o maior produtor, exportador e consumidor mundial dessa fruta (DE ASSIS *et al*. 2008; PRAKASH e BASKARAN, 2018). Dados recentes mostram que a quantidade de acerola produzida no Brasil ultrapassa 140 mil toneladas (IBGE, 2017). O Nordeste brasileiro é uma das regiões com as melhores condições adaptativas para o desenvolvimento da planta, despertando o interesse na abertura de estabelecimentos agroindustriais para a cultura (DE PAIVA *et al*., 1999). Grande parte de sua produção é vendida na forma de polpa, e a massa de seus frutos verdes utilizada como matéria-prima para a confecção de cápsulas de vitamina C (MENEZES *et al*., 2009), que é aplicada pelas indústrias como aditivo para aumentar o valor nutricional de suplementos alimentícios e produtos farmacêuticos (DELVA e SCHNEIDER, 2013; PRAKASH e BASKARAN, 2018).

De acordo com os dados do Censo Agropecuário do IBGE de 2017, a produtividade de frutos de acerola da região Norte foi de 85.176,42 toneladas distribuídas em 1.209 estabelecimentos agropecuários. Em segundo lugar aparece a região Nordeste com uma produtividade de 49.184,61 toneladas em 4.475 estabelecimentos. O Ceará é o segundo maior produtor nordestino, com área plantada de 2.047 hectares, que renderam um total de 12.772

mil toneladas de frutos colhidos em maio de 2018, convertidos em pouco mais de 22 milhões reais para o estado (CIDRA.IBGE, 2018).

O epicarpo de seus frutos apresenta uma película fina com coloração que vai do verde, quando imaturo, a variações de amarelo, vermelho, vermelho-amarelado, vermelho-alaranjado à vermelho-púrpura, quando maduro. O mesocarpo (polpa) assume tonalidades de amarelo, laranja ou vermelho e representa de 70 a 80% da massa do fruto. O endocarpo é constituído por três sementes protegidas por invólucro que dão ao fruto o aspecto trilobado (ALMEIDA *et al*., 2002; FREITAS *et al*., 2006; LIMA *et al*., 2014).

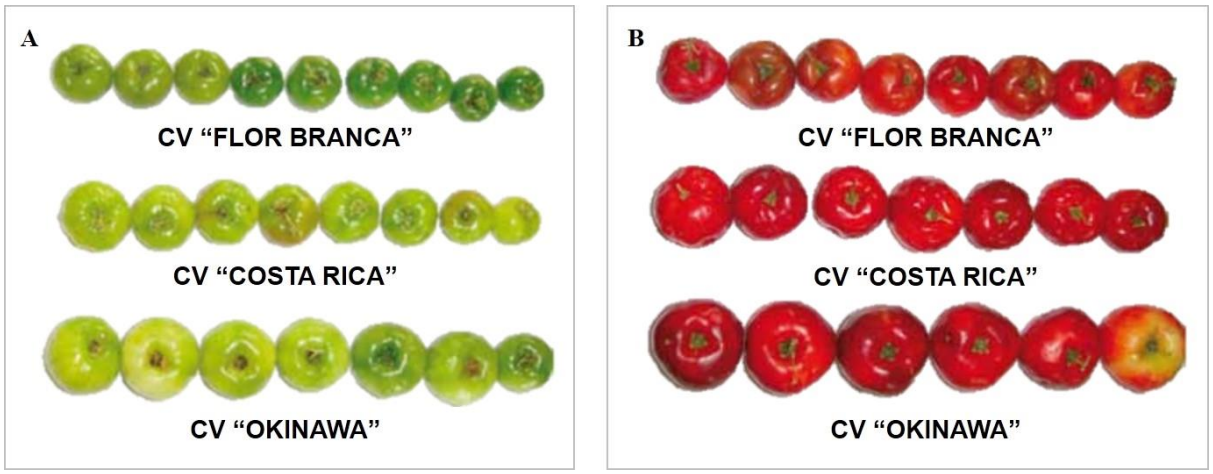
A acerola é uma das fontes naturais mais ricas em vitamina C e importantes para o ser humano (HANAMURA *et al*., 2008; DELVA e SCHNEIDER 2013), variando de 1000 a 4500 mg. $100g^{-1}$ de polpa, cerca de 50 a 100 vezes maior que o conteúdo encontrado no limão e na laranja, respectivamente (ALMEIDA *et al*., 2014). Tal atributo tem estimulado sua exportação para vários países, incluindo Estados Unidos, França, Japão, Coréia do Sul e Holanda (Panorama do Agronegócio Brasileiro – MAPA, 2017). Desta forma, o fruto da acerola está cada vez mais popular como um alimento que apresenta propriedades nutricionais e antioxidantes apreciada por consumidores que optam em obter essa vitamina de origens naturais, devido sua melhor absorção quando comparado àquela de fonte sintética (DE ASSIS *et al*., 2008; OLIVEIRA *et al*., 2012).

Visando atender a essa demanda, pesquisas realizadas pela Embrapa Agroindústria Tropical, no Ceará em parceria com a Empresa de Frutas do Ceará S/A – FRUCESA e posteriormente com a multinacional Amway Nutrilite do Brasil resultaram no desenvolvimento de cultivares comerciais de alta produtividade, rendimento de polpa e teor de ácido ascórbico (DE PAIVA *et al*., 1999; EMBRAPA AGROINDÚSTRIA TROPICAL, 2012). Dentre os vários materiais genéticos de acerola cultivados no Brasil, estão as cultivares Apodi (BRS 235), BRS Cabocla, Cereja. (BRS 236), Flor Branca, Frutacor (BRS 238), Jaburu (BRS 366), Okinawa, Roxinha (BRS 237), BRS Rubra, Sertaneja (BRS 152), registrados no Ministério da Agricultura, Pecuária e Abastecimento (MAPA, 2017). A cultivar Okinawa é uma planta muito robusta e apresenta frutos com 5 g a 9 g, alto teor de vitamina C, coloração e resistência ao transporte apreciáveis, quando comparadas com as cultivares Flor Branca, que apresenta menor vigor e frutos menores (3 g a 5 g), e com a cultivar Costa Rica que apresenta vigor intermediário e também frutos menores (4 g a 6 g), figura 1. (EMBRAPA, 2012).

A constituição química da acerola é influenciada principalmente por condições ambientais como a variação da temperatura e a quantidade de água disponível (FARIAS *et al*., 2012), no entanto, a variabilidade genética e o estádio de maturação dos frutos também são

fatores determinantes (FREITAS *et al*., 2006; MACIEL *et al*., 2010; NASSER e ZONTA, 2014).

Figura 1 - Frutos verdes (**A**) e maduros (**B**) das CVS Flor Branca, Costa Rica e Okinawa.



Fonte: José Egídio Flori (EMBRAPA, 2012)

Um dos principais componentes nutricionais encontrado em acerolas é o ácido ascórbico (vitamina C), um importante antioxidante (GEST, GAUTIER e STEVENS, 2013). Tipicamente, o ácido ascórbico é acumulado nos frutos no estádio verde (3756,06 mg.100 g^{-1}), sofrendo intensa redução no seu conteúdo à medida que amadurece (862,86 a 1465,22 mg.100 g^{-1}) (BADEJO *et al*., 2009; OLIVEIRA *et al*., 2012; FIGUEIREDO NETO *et al*., 2014; SOUZA *et al*., 2014). Sendo essa redução associada ao tratamento e armazenamento dos produtos da acerola, de acordo com o método e instrumentos utilizados (MATSUURA *et al*., 2002).

Um dos principais componentes químicos da parede celular é a pectina; polissacarídeo ramificado capaz de proteger a célula contra a citotoxicidade gerada por peróxido de hidrogênio devido a redução dos níveis intracelulares de espécies reativas de oxigênio (EROs). Em acerolas os níveis desse composto têm variado de 94,93 a 246,71 mg.100 g^{-1} (BATISTA *et al*., 2015; KLOSTERHOFF *et al*., 2018).

Outro composto importante e abundante em acerolas são os polifenóis. Com atuação antioxidante oriundo do metabolismo secundário (OLIVEIRA *et al*., 2012), apresenta elevado potencial para a melhoria da saúde humana e para a ampliação do período de conservação pós-colheita do próprio fruto. Segundo Mariano-Nasser *et al*. (2017), acerola madura apresenta altas concentrações de polifenóis totais que variam entre 914,2 a 2428,3 mg de ácido gálico 100 g^{-1}. Dentre os compostos fenólicos mais abundantes em acerolas, estão as

antocianinas, catequinas, flavonoides, flavonóis e procianidinas (DELVA e GOODRICH, 2010; BETAGLION *et al*., 2015).

O amadurecimento representa o estádio terminal do desenvolvimento do fruto. Durante o amadurecimento, ocorrem alterações bioquímicas complexas nos metabolismos primário e secundário que culminam em mudanças específicas na cor, na firmeza e no sabor, tornando os frutos comestíveis e mais atraentes aos organismos dispersores das sementes (DE ASSIS *et al*., 2008; GIOVANNONI *et al*., 2017).

O fruto maduro da aceroleira é excessivamente perecível, de modo a apresentar vida de prateleira limitada de 2 a 4 dias à temperatura ambiente (VENDRAMINI e TRUGO, 2000; SCALON *et al*., 2004). Seu tempo de amadurecimento e senescência são acelerados, o que dificulta a manipulação e a estocagem após a colheita. O epicarpo muito fino da acerola a torna muito suscetível a danos físicos, a redução de umidade quando armazenados em altas temperaturas e baixa umidade relativa, ou quando acondicionados a baixas temperaturas (MOHAMMED, 2011). No entanto, o principal motivo dessa alta perecibilidade e fragilidade é o comportamento de maturação climatérica desse fruto. Estudos anteriores relataram uma taxa respiratória de 80 $mg.Kg^{-1}.h^{-1}$ de CO_2 (ALVES *et al*., 1995); e 900 $mL.Kg^{-1}.h^{-1}$, porém uma baixa produção do gás etileno 3.0 $\mu L.Kg^{-1}.h^{-1}$ (CARRINGTON e KING, 2002).

Em vista disso, o processo de senescência acelerado dos frutos é responsável por perdas econômicas notáveis no manejo pós-colheita, exigindo a aplicação de tecnologias de conservação adequadas. Neste sentido, a comercialização da acerola demanda transporte rápido e o uso de recipientes refrigerados adequados na tentativa de retardar a respiração (DE ASSIS *et al*., 2008). O armazenamento de acerolas a 10 °C possibilitou manter a qualidade dos frutos durante períodos de estocagem mais longos e prolongar o sabor por até 12 dias (ARAÚJO *et al*., 2009).

O uso de alguns mediadores de crescimento, como as poliaminas, têm sido pouco estudados no contexto do amadurecimento e manutenção da qualidade dos frutos de acerola. Deveras, a maioria dos estudos sobre o desenvolvimento e amadurecimento de frutas tem sido focado por exemplo, em tomate, morango, ameixa e etc. (MEHTA *et al*., 2002; PÉREZ-VICENTE *et al*, 2002; KHOSROSHAHI, ESNA-ASHARI, ERSHADI, 2007; MATTOO HANDA, 2008).

2.2 O metabolismo do etileno

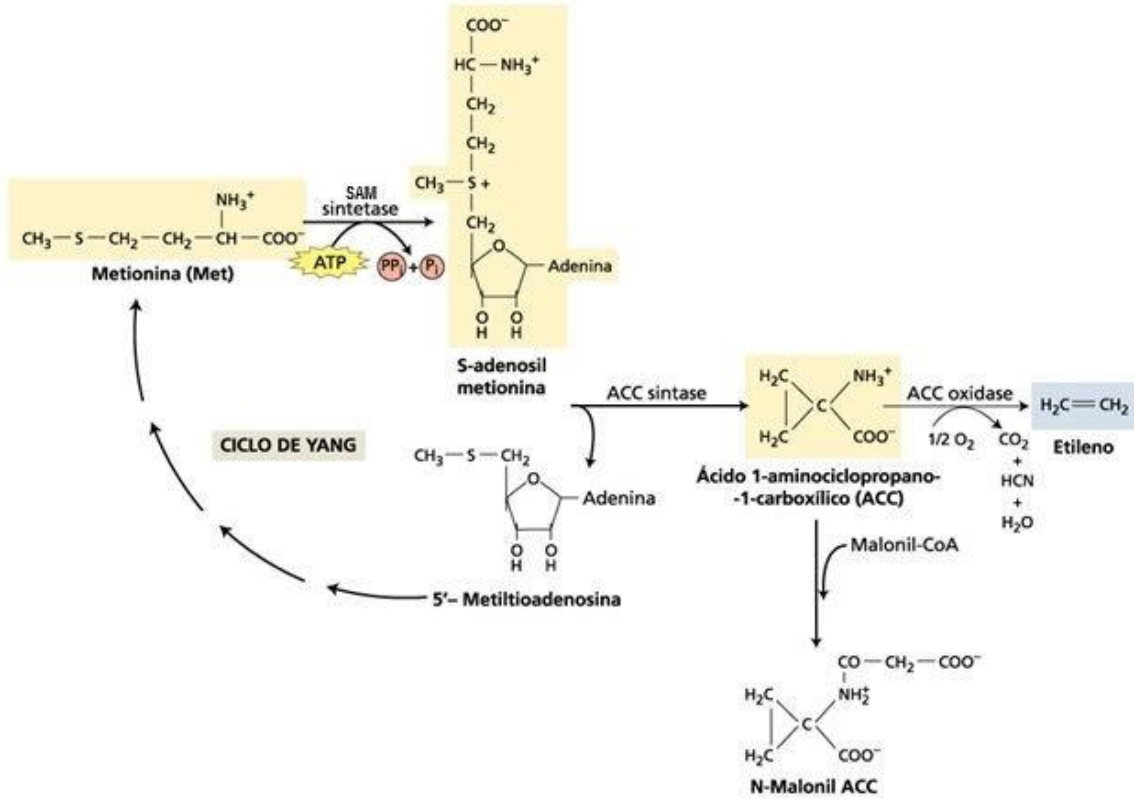
O etileno é um hormônio central para a iniciação e o controle do amadurecimento de frutos (GENARD, 2005; PAUL e PANDEY, 2014). Este hormônio atua na regulação de processos como: a firmeza, mudança de cor, produção e acúmulo de compostos orgânicos e de metabólitos secundários dos frutos (CHERIAN e FIGUEROA, 2014). Com base nas distinções dos perfis respiratórios e de produção de etileno durante o amadurecimento, os frutos são tipicamente classificados como climatéricos e não-climatéricos. Os frutos climatéricos (tomate, banana, maça, pera, manga, mamão) apresentam respiração e biossíntese de etileno elevados, destacando-se um pico climatérico característico, que ativa o amadurecimento, enquanto os não-climatéricos (morango, uva, limão e laranja) apresentam níveis basais ou diminuídos de respiração e de etileno ao longo do amadurecimento (CARRINGTON e KING, 2002; BAPAT *et al*., 2010; KARLOVA *et al*., 2014). A acerola, no entanto, é um fruto climatérico com pico respiratório muito alto e com baixa taxa de produção de etileno (CARRINGTON e KING, 2002).

A figura 2 apresenta esquematicamente a síntese biológica do etileno. Inicialmente, a metionina é convertida em S-adenosilmetionina (SAM) por ação da sintase do SAM, e logo após em ácido 1-carboxílico-1-aminociclopropano (ACC), pela atividade da sintase do ACC (ACS). Nesse processo bioquímico também é produzido a 5`-metiltioadenosina, que é utilizada para a renovação da metionina via ciclo de Yang (LIN; ZHONG; GRIERSON, 2009). Finalmente, o ACC é convertido pela oxidase do ACC (ACO) em etileno (ADAMS; YANG, 1979; CROZIER *et al*., 2000).

Ambas as enzimas, ACS e ACO sofrem regulação (pós-) transcricional e/ou traducional, por isso constituem etapas limitantes da produção de etileno (TAIZ; ZEIGER, 2013), que por sua vez, define dois sistemas distintos (sistemas I e II). O sistema I atua em condições de crescimento vegetativo e em frutos não-climatéricos, sendo caracterizado por níveis basais de etileno, que auto-inibe sua síntese por ser um regulador negativo de isoformas especificas da ACS. O sistema II, caracterizado por elevada concentração celular de etileno é ativado durante o amadurecimento de frutos climatéricos ou senescência pela indução autocatalítica de isoformas da ACS (não inibidas pelo etileno) (GRIERSON, 2013). A ACS, pertencente à classe das liases, é uma enzima citosólica caracterizada nos diversos seres vivos como um homodímero dependente de piridoxal-5´-fosfato (ADAMS; YANG, 1979; YAMAGAMI *et al*., 2003; ARGUESO; HANSEN; KIEBER, 2007). Já a ACO, pertence à classe das oxidorredutases, é uma enzima que requer o ferro (Fe^{+2}) para a sua atividade

(ZHANG *et al*., 2004; LIN; ZHONG; GRIERSON, 2009).

Figura 2. Biossíntese do etileno.



Fonte: TAIZ; ZEIGER, 2013.

De acordo com De Poel; Der Straeten (2014), a regulação da produção de etileno ocorre também nos níveis de ACC disponíveis. Em plantas, observou-se que o ACC pode ser conjugado em malonil-ACC (MACC), jasmonil-ACC e γ-glutamil-ACC, sendo o MACC, o conjugado mais abundante e o mais bem estudado (VAN DE POEL; VAN DER STRAETEN, 2014). Diversos estudos relataram que o ACC conjugado não pode ser oxidado a etileno, constituindo um importante mecanismo de controle da biossíntese de etileno (WANG; LI; ECKER, 2002; ZAPATA *et al*., 2007; LIN; ZHONG; GRIERSON, 2009; TAIZ; ZEIGER, 2013).

2.3 Metabolismo das poliaminas

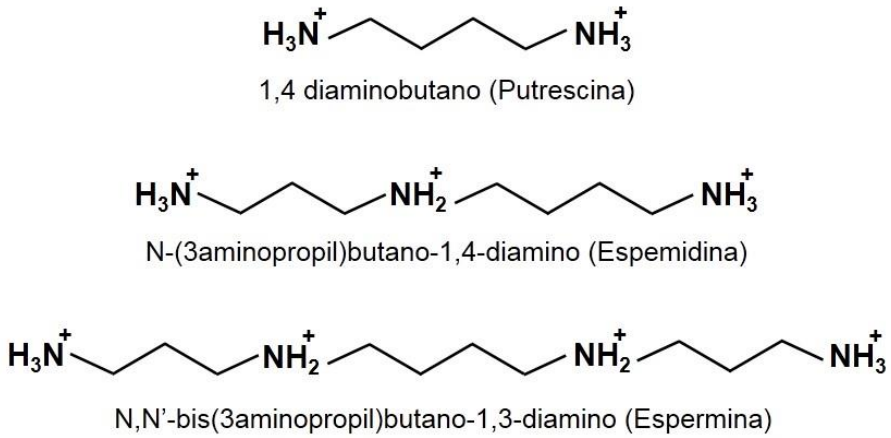
As poliaminas são componentes bioquímicos caracterizados como aminas policatiônicas alifáticas de baixo peso molecular. Em pH fisiológico apresentam-se carregadas

positivamente, uma propriedade que propicia sua ligação com macromoléculas como proteínas, fosfolipídios, componentes da parede celular, DNA e RNA (BARON e STASOLLA, 2008).

Essas aminas são encontradas em animais, vegetais, fungos e bactérias (WALLACE *et al*., 2003). Em plantas, estão presentes em todas as partes da célula, de modo que sua concentração total e individual varia de acordo com o estádio de desenvolvimento (KUZNETSOV *et al*., 2006). Além disso, há evidências da sua participação em vários processos básicos, tais como estabilização de membranas e modulação da atividade de enzimas, duplicação do DNA, transcrição e tradução (GUPTA; DEY; GUPTA, 2013).

A diamina putrescina (Put), a triamina espermidina (Spd) e a tetramina espermina (Spm) são as poliaminas encontradas em maior concentração nos seres vivos (Figura 3). Elas derivam da descarboxilação de aminoácidos (arginina, lisina e ornitina). Além disso, a metionina participa, especificamente, com o grupo aminopropil para a composição da espermidina e espermina (BAGNI; TASSONI, 2001).

Figura 3 - Estrutura condensada linear de cada uma das principais poliaminas e nomenclatura de acordo com a IUPAC.



Fonte: BAGNI; TASSONI, 2001.

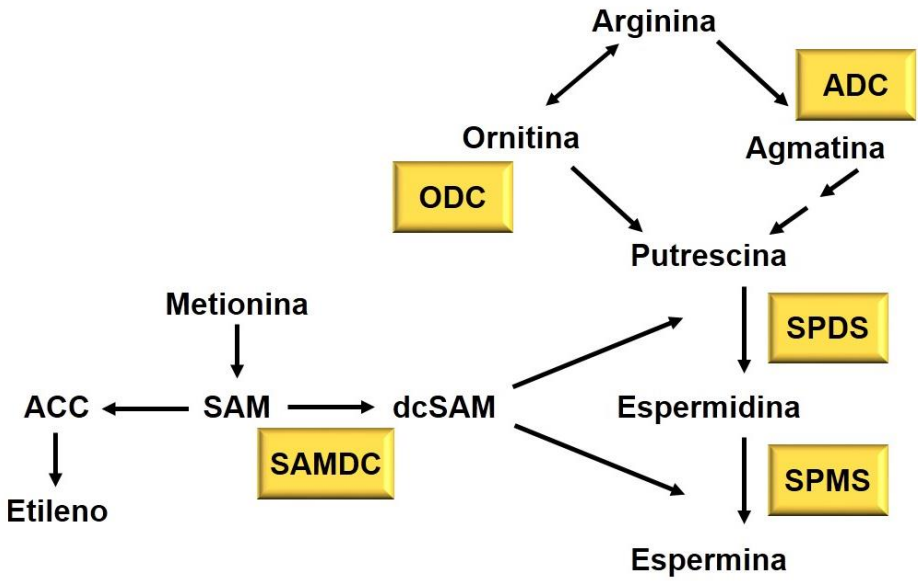
Em plantas, as três maiores categorias de poliaminas estão presentes nas formas livre, conjugada solúvel e conjugada insolúvel. Poliaminas conjugadas solúveis estão covalentemente ligadas a moléculas pequenas, como por exemplo o ácido cumárico, ácido caféico e ácido ferúlico, enquanto as poliaminas conjugadas insolúveis estão covalentemente ligadas a macromoléculas, como os ácidos nucleicos e proteínas. De maneira geral, cerca de

90% de seu conteúdo pode formar conjugados (BAIS *et al*., 2001; DUAN *et al*., 2008; BASSARD *et al*., 2010).

A literatura científica sugere que as poliaminas conjugadas solúveis, bem como os seus compostos de origem (Put, Spd, Spm), participam de vários processos do desenvolvimento vegetal, como: crescimento e desenvolvimento, divisão celular, floração, proteção da parede celular e nas respostas das plantas a estresse (GRIENENBERGER *et al*., 2009; LUO *et al*., 2009; BASSARD *et al*., 2010; MOSCHOU *et al*., 2012).

As concentrações das poliaminas são extremamente reguladas por reações anabólicas (Figura 4) e catabólicas (Figuras 5), bem como pela conjugação com ácidos orgânicos (ALCÁZAR *et al*., 2010).

Figura 4 - Via de biossíntese das poliaminas putrescina, espermidina e espermina.

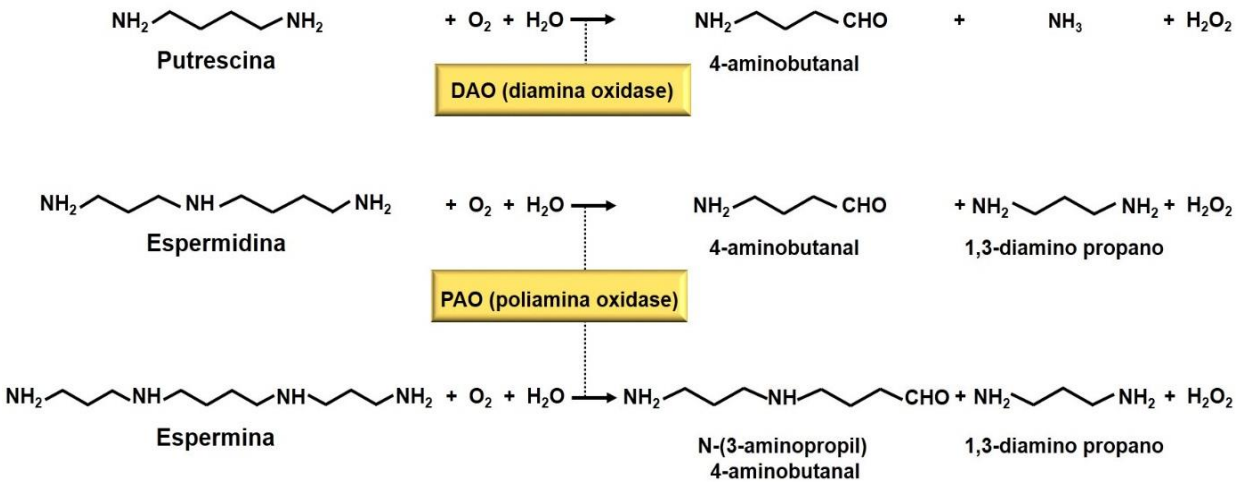


Fonte: LIU *et al*., 2007. (Com adaptações)

A putrescina (Put) é sintetizada de modo direto pela reação da descarboxilase da ornitina (ODC) ou de forma indireta pela descarboxilase da arginina (ADC) por meio da produção dos intermediários agmatina e N-carbamoilputrescina (Figura 4) (ALCÁZAR *et al*., 2006). Posteriormente, a Put pode ser transformada em espermidina (Spd) e esta, depois, em espermina (Spm) pelas ações consecutivas das enzimas, sintase da espermidina (SPDS) e sintase da espermina (SPMS), respectivamente (GUPTA; DEY; GUPTA, 2013). Outra importante rota de biossíntese das poliaminas tem como fonte a metionina, que é convertida em adenosilmetionina (SAM), e posteriormente, transformada em S-adenosilmetionina descarboxilado pela atividade da enzima descarboxilase do S-adenosilmetionina (SAMDC)

(Figura 4). SAMDC é considerada um ponto crucial de regulação da homeostase de poliaminas (TAKAHASHI; KAKEHI, 2010). Todas as SAMDCs são sintetizadas como proenzimas (BALE; EALICK, 2010) e em sua reação a SAM é descarboxilado produzindo a molécula de 5`-metiltioadenosina, em que é posteriormente reciclado para metionina (Met) através ciclo de Yang (ciclo da Metionina) (MIYAZAKI; YANG, 1987).

Figura 5. Via de degradação de poliaminas. PAO - oxidase de poliamina e a DAO - oxidase de diamina.



Fonte: LIU *et al*., 2007. (Com adaptações)

A enzima ODC pertence à classe das liases e nas plantas está presente como um homodímero dependente de piridoxal-5´-fosfato (PLP) (PANAGIOTIDIS; GEORGATSOS; KYRIAKIDIS, 1982; KOROMILAS; KYRIAKIDIS, 1988). Esta enzima geralmente exerce função relacionada com a regulação do crescimento por apresenta-se altamente ativa em tecidos de rápido crescimento (FLORES, 1991, citado por BASSARD *et al*., 2010), ao passo que a ADC é comumente considerada como a principal enzima implicada nas respostas aos estresses abióticos (URANO *et al*., 2004; JIMÉNEZ-BREMONT; RUIZ; RODRÍGUEZ-KESSLER, 2007; ALCÁZAR *et al*., 2010; GUPTA; DEY; GUPTA, 2013).

A ADC é uma enzima, também detentora do grupo prostético PLP, (NAM; LEE; LEE, 1997) que produz a Put de forma indireta, através da descarboxilação do aminoácido arginina, em duas etapas catalisadoras, pelas enzimas iminohidrolase da agmatina e amidohidrolase do N-carbamoilputrescina, nessa ordem (ALCÁZAR *et al*., 2006).

A oxidação das poliaminas é atribuída principalmente a duas oxidases: a PAO - oxidase de poliamina e a DAO - oxidase de diamina (LIU *et al*., 2007). A DAO é uma enzima homodimérica que possui o cofator (2,4,5-triidroxifenilalanina quinona) constituído por uma transformação autocatalítica pós-traducional de um resíduo de tirosina na região ativa da enzima e um íon Cu^{2+} em cada uma de suas subunidades (CONA *et al*., 2006). Esta enzima está amplamente posicionada na parede celular e é encarregada da oxidação do grupo amino primário da Put, produzindo os produtos NH_3, H_2O_2 e 4-aminobutanal, o qual pode ser metabolizado posteriormente ao ácido γ-aminobutírico através da formação do mediador Δ-pirrolina (BAGNI; TASSONI, 2001; CONA *et al*., 2006).

A PAO é uma enzima monomérica da família das oxidorredutases flavo-proteicas e, portanto, detém uma molécula de FAD como grupo prostético (ŠEBELA *et al*., 2001). A sua função no metabolismo das poliaminas é acelerar a oxidação do grupo amino secundário de poliaminas, como a Spd e Spm e, dessa forma, auxiliar para a regulação do acúmulo dessas poliaminas dentro das células (TAVLADORAKI *et al*., 2012). Estudos têm atestado que, do mesmo modo que a PAO de animais, essa enzima, em vegetais, também é capaz de agir no sentido inverso, convertendo Spm em Spd e, em seguida, em Put (TAKAHASHI; KAKEHI, 2010; LIU *et al*., 2014).

Os frutos têm sido caracterizados como ricos em Put, ao passo que outras partes vegetais exibem altos teores de Spd (KALAC *et al*., 2005; MORET *et al*., 2005). Tem sido observado que as poliaminas estão relacionadas à propriedades que retardam a senescência, contudo, na maioria das frutas, a fração de poliaminas frequentemente diminuem no decorrer do processo de envelhecimento. Tal diminuição peculiar nas quantidades de poliaminas afeta desfavoravelmente as características de textura e o período de utilização de frutas e vegetais (KUMAR *et al*., 1997).

As poliaminas são mais abundantes no desenvolvimento de frutos durante as etapas de divisão e a expansão celular (YAHOO *et al*., 2001, LIU *et al*., 2006) de modo a promover uma ação protetora contra muitos tipos de danos, uma vez que mantem/garantem a firmeza das membranas celulares (BAE *et al*., 2008, ZHANG *et al*., 2013). A aplicação endógena e exógena de poliaminas livres em frutos, refletiu em retardo da senescência, atuando principalmente no aumento da firmeza, alteração de cor, retardo e diminuição do pico de etileno e taxa respiratória, redução dos sintomas de frio e resistência mecânica (VALERO *et al*,. 1999).

Estudos seguindo esse raciocínio têm demonstrado que a aplicação exógena de poliaminas expande o prazo de consumo e retém a qualidade estrutural em várias frutas como

ameixa (PÉREZ-VICENTE *et al*., 2002), damasco (MARTÍNEZ-ROMERO *et al*., 2002), manga (MALIK e SINGH, 2005) e morango (KHOSROSHAHI, ESNA-ASHARI e ERSHADI, 2007).

Kramer, Wang e Conway, 1989, descreveram que o acúmulo de poliaminas, sobretudo de Put, favoreceu a redução do dano causado por estresse a frio. Muitos trabalhos relatam que a aclimatação do fruto à baixa temperatura foi relacionada a um aumento constante da Put e à atenuação de Spd e Spm em abobrinha (WANG e JI, 1989), citros (MCDONALD e KUSHAD, 1986) e cherimoya (ESCRIBANO e MERODIO, 1994).

Na uva, fruto não-climatérico, o conteúdo de Put e Spd decresceu vigorosamente ao longo do amadurecimento, ao passo que a quantidade de Spm não alterou significativamente durante esse período (SHIOZAKI *et al*., 2000). No tomate, fruto climatérico, os níveis de Put aumentaram gradativamente ao longo da maturação dos frutos, alcançando o seu maior valor no estágio vermelho maduro, enquanto os valores de Spd e Spm reduziram neste estágio (TSANIKLIDIS *et al*., 2016). Portanto, o conteúdo de poliaminas e seu papel funcional parecem distinguir a depender do tipo de fruto e do estágio de maturação. Estas distinções podem estar amplamente correlacionadas com as enzimas implicadas no metabolismo de poliaminas.

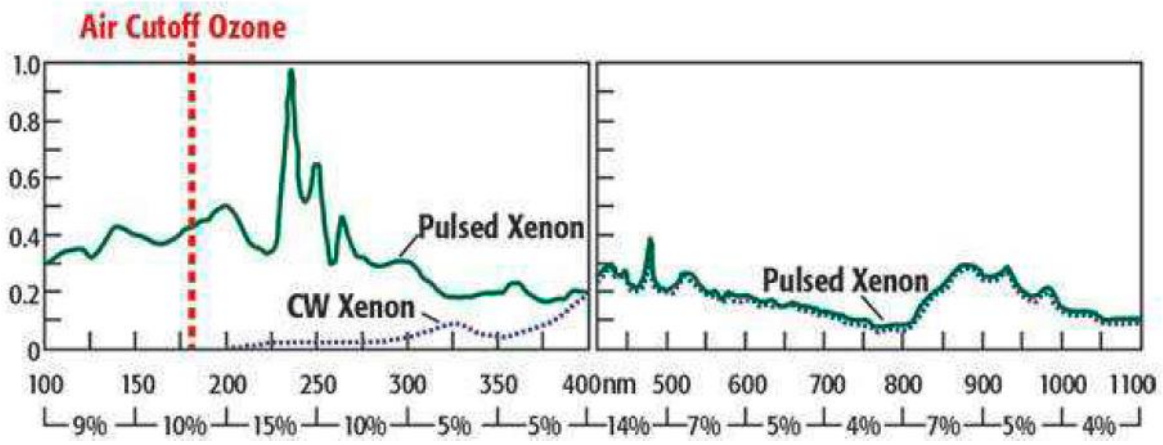
Embora, o papel das poliaminas no processo de amadurecimento de frutos ainda não seja completamente entendido (AGUDELO-ROMERO *et al*., 2013), sabe-se que mesmo em baixas concentrações elas atuam no amadurecimento de frutos climatéricos, regulando particularmente a produção dos níveis de etileno por competir pelo substrato S-adenosilmetionina (SAM) (BREGOLI *et al*., 2002, LIU *et al*., 2006). Desta forma, a interligação do metabolismo de poliaminas e do etileno, através do S-adenosilmetionina (SAM) (Figura 3), já está bem definido (SRIVASTAVA *et al*., 2007, AGUDELO-ROMERO *et al*., 2013).

2.4 Tecnologia pós-colheita da luz pulsada

Diversas tecnologias pós-colheita, dentre elas refrigeração, uso de inibidores da ação do etileno (SINGH *et al*., 2008), a atmosfera de armazenamento modificada (VALERO *et al*., 2013; SAHOO *et al*., 2015; TEIXEIRA *et al*., 2016) e a irradiação (PATARO *et al*., 2015) já foram utilizadas com sucesso para o retardo do amadurecimento pós-colheita de muitos frutos. A tecnologia de luz pulsada utiliza pulsos de luz, não térmicos, de elevada intensidade e rápida duração (1 μs a 0,1 s) com um variado espectro de radiação

eletromagnético (200-1100 nm), que inclui um amplo intervalo de radiação ultravioleta (100-400 nm), luz visível (400-700 nm) e infravermelho (700-1100 nm) (Figura 6) (BARBOSA-CANOVAS *et al*., 1999; PERL-TREVES, 2002; BHAVYA e HEBBAR, 2017).

Figura 6. Espectro de radiação eletromagnético da luz pulsada.

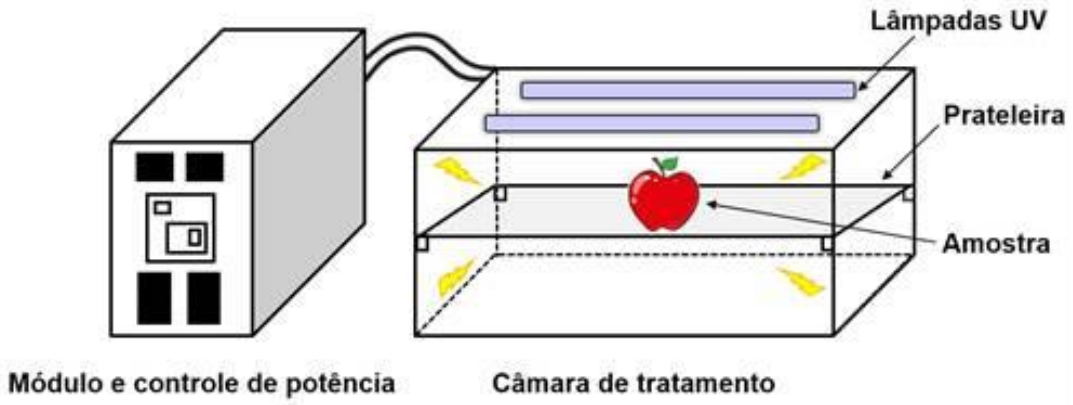


Comprimento de onda em nanômetros e distribuição de saída de luz em %

Fonte: Xenon Co (PERL-TREVES, 2002)

Os aparelhos de luz pulsada possuem um sistema de funcionamento que apresenta componentes parecidos, estruturados fundamentalmente por um módulo capacitor de energia e controle de potência e uma câmara para a realização do tratamento (Figura 7) (ELMNASSER *et al*., 2007; MAGED *et al*., 2012).

Figura 7: Esquema do sistema de luz pulsada de bancada.



Fonte: ELMNASSER *et al*., 2007. (Com adaptações)

Na câmara de tratamento do sistema e luz pulsada, a lâmpada de flash é a responsável pela transformação de até 50% da energia elétrica de entrada em energia radiante pulsada (XENON CORPORATION, 2005; ABIDA, RAYEES e MASSODI, 2014). Essa lâmpada contém, preferencialmente, o gás xenônio por promover maior eficiência de resolução e também por ser o gás mais adotado na maioria das aplicações de inativação microbiana (ELMNASSER *et al.,* 2007; ABIDA, RAYEES e MASSODI, 2014).

Nesse tipo de recurso, a energia eletromagnética é armazenada em um capacitor durante poucos segundos e, logo após, emitida na forma de luz resultando em ampliação da potência com utilização mínima de energia (DUNN *et al*., 1995). Diante disso, o uso da luz pulsada tem sido considerada como uma tecnologia mais moderna do uso de luz UV (PATARO *et al*., 2015).

A luz pulsada foi preliminarmente utilizada na pós-colheita para eliminar a contaminação superficial provocada por microrganismos em diversos produtos vegetais, principalmente em frutos (DUNN *et al*., 1995; BHAVYA e HEBBAR, 2017). Neste contexto, o tratamento com LP usa a faixa no espectro UV-C (200-280 nm) que promove melhor impacto sobre organismos vivos (ELMNASSER *et al*., 2007; PALGAN *et al*., 2011; GÓMEZ *et al*., 2012) e o pequeno tempo de exposição (segundos para LP e minutos ou horas para luz UV contínua) necessário para que o tratamento cause os efeitos desejados da tecnologia de LP em escala industrial (RODOV *et al*., 2012).

O uso da luz pulsada tem interessado muitos pesquisadores como um tratamento de conservação pós-colheita de frutos e hortaliças, por meio da incitação à produção de compostos bioativos instigados pelo estresse hermético (MANZOCCO, DA PIEVE e MAIFRENI, 2011; KOH *et al*., 2016; XU e WU, 2016; MOREIRA *et al*., 2017). Contudo, para a utilização da radiação pulsada nesses produtos é necessário a investigação de uma determinada dose que promova os benefícios esperados, como os já evidenciados em trabalhos realizados por Costa *et al*., 2006; Pongprasert *et al*., 2011; Kasim e Kasim, 2012; Khademi *et al*., 2013 e Lopes *et al*., 2016 em diversos tipos de frutos climatéricos e não climatéricos, hortaliças folhosas e tubérculos.

Resultados da aplicação de doses diferentes de luz pulsada em diversos frutos tem visado à melhoria de suas qualidades nutricionais no decorrer do armazenamento pós-colheita. O uso da tecnologia de LP revelou que a irradiação com UV-C promoveu o acúmulo de antioxidantes como a vitamina C e de poliaminas em frutos de manga (GONZÁLEZ-AGUILAR *et al*., 2007), banana, goiaba (ALOTHMAN; BHAT; KARIM, 2009a; 2009b),

mamão (RIVERA-PASTRANA *et al*., 2013) e morango (ERKAN; WANG; WANG, 2008) e o aumento relevante da qualidade e tempo de prateira devido a maior eficiência antioxidante.

Outros estudos mostraram que a aplicação da variação de doses de LP de 2 a 8 $J.cm^{-2}$ estimulou o acréscimo no conteúdo de licopeno, carotenoides, compostos fenólicos e a atividade antioxidante em tomates tratados (PATARO *et al*., 2015). O tratamento de framboesas, armazenadas por 10 dias, à uma taxa de fluência de 5,0 $J.cm^{-2}$, aumentou o conteúdo de antocianinas totais (XU e WU, 2016). Em manga processada e tratada com uma dose de 8 $J.cm^{-2}$ verificou-se que a coloração, a firmeza e o conteúdo de carotenoides mantiveram-se praticamente estável a 6 °C durante 7 dias (CHARLES *et al*., 2013). A aplicação de LP (2,80 $J.cm^{-2}$) foi considerada a dose ideal para conservar a qualidade de mangas "Tommy Atkins" minimamente processadas por conferir a redução na perda de massa fresca, a manutenção da aparência visual geral, além de manter alto o conteúdo de carotenoides e a atividade antioxidante durante sete dias de armazenamento a 6 °C (LOPES *et al*., 2017). De maneira diferente, Moreira *et al*. (2017) observaram que a LP não exerceu qualquer efeito sobre a qualidade e a atividade antioxidante de maçãs não processadas. Enquanto, em abacates, o tratamento com LP favoreceu um aumento na atividade respiratória (RAMOS-VILLARROEL *et al*., 2011).

3 PULSED LIGHT INFLUENCE ON ACEROLA POSTHARVEST PHYSIOLOGY AND QUALITY

Artigo científico referente à tese submetido à revista ¨Food Chemistry", a qual apresenta fator de impacto médio de 4.946 (2017) e classificação A2 de acordo com a avaliação trienal (2013-2016) realizada pela Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

Abstract

Physiologically mature acerola 'Okinawa' were submitted to pulsed light (PL) radiation (0.6 J cm^{-2}), stored at 10 ºC and evaluated at days 0, 1, 4, 10 and 16 for firmness and associated variables, oxidative stress marker and constituents of antioxidant metabolism, respiration and ethylene metabolism, polyamine metabolism, color, phenolic and vitamin C metabolism. When compared to control untreated fruit, PL treatment reduced climacteric respiratory peak in 5% lower ($P< 0.05$) with 78.6 $mL.Kg^{-1}.h^{-1}$ and delayed ethylene peak to day 3 with 2.65 $\mu L.Kg^{-1}.h^{-1}$. PL-treated acerola was significantly firmer 12%, at day 10 and lost 13.4% less weight with no significant differences in color attributes lightness and chroma. Oxidative stress marker H_2O_2 was significantly lower in PL-treated fruit and antioxidant enzymes SOD and CAT activities were stimulated. The lower ROS content reflected in lower membrane lipid peroxidation degree, while cell wall hydrolytic activity was also inhibited, contributing to greater firmness. PL reduced ethylene precursor free ACC content in 20% and inhibited ACO activity in 16%. Polyamines putrescine and spermidine levels were 21% and 12% higher, respectively, than control due to stimulus of its synthesis (ADC activity) and inhibition of its degradation (DAO activity). PL-treatment resulted in significantly ($P< 0.05$) higher total vitamin C and AsA levels, while DHA levels were lower. PL treatment resulted higher polyphenol content due to enhanced synthesis by PAL. Therefore, PL, under the conditions here applied, influenced acerola physiology not as a abiotic stress agent, but as an elicitor of the antioxidant defense system preventing possible oxidative damages due to imbalance between ROS production and scavenging mechanisms, promoting quality during storage.

Keywords: acerola, radiation, climacteric, polyamine, antioxidant, ascorbate.

3.1 Introduction

Acerola (*Malpighia emarginata* DC.) tree is native of Central America, but it has adapted well to Northeastearn region of Brazil. Based on that, since 2012, Brazil is the largest global acerola producer with 50000 ton/year and over 45 cultivars. Among, the cultivar 'Okinawa' stands out for its firmer fruit more resistant to postharvest handling (MOURA *et al*., 2007). Ripe acerola, also known as Caribbean cherries, are drupes with thin skin colored from orange to purplish-red, while fleshy pulp color ranges from yellow to red and represents up to 80% of total fruit mass, surrounding three seeds (LIMA *et al*., 2014).

Ripe acerola has very high vitamin C content reaching 2000 mg 100 g^{-1}, and is a rich source of phenolics reaching 2631 mg EAG 100 g^{-1} including flavonoids and anthocyanins (OLIVEIRA *et al*., 2011; SOUZA *et al*., 2014). Despite its outstanding nutritional composition, acerola postharvest life is restricted to two to four days at ambient (25 ^{o}C) due to fast ripening and senescence, as consequence of its climacteric respiratory pattern with 900 mL de $CO_2.Kg^{-1}.h^{-1}$ and ethylene production, 3 μL de $C_2H_4.Kg^{-1}.h^{-1}$ (CARRINGTON and KING, 2002). Therefore, acerola marketing chain is greatly dependent of postharvest technologies as processing and refrigeration of both processed pulp and whole fruit, which may be stored at -18 ^{o}C for up to 12 months (OLIVEIRA *et al*., 2011).

Pulsed light (PL) radiation is a non-thermal technology initially used for microbial decontamination of fruit surface due to intense broad-spectrum (200-1100 nm) light flashes with duration of 1 μs to 0.1 s and fluence up to 12 $J.cm^{-2}$, as approved by FDA in 1996. Besides the direct effects on microorganisms, PL may exert effects on fruit tissue inducing biochemical and physiological changes therefore acting as a physical elicitor (URBAN *et al*., 2018). Among the biological responses induced by physical elicitors as PL, are those associated with plant defense and resistance through induction of reactive oxygen species (ROS) and secondary metabolites as phytolalexins (SHAMA e ALDERSON, 2005).

Recently, researchers started to evaluate how PL activates plant secondary metabolism and antioxidant system (WANG and FREI, 2011). In a study evaluating different mode of PL applications on fresh-cut 'Tommy Atkins' mangoes (LOPES *et al*., 2017), authors reported that four sequential pulses (2.80 $J.cm^{-2}$) preveny mass loss and improved carotenoid and antioxidant content during storage at 6 °C, when compared to one-pulse daily treatment repeated for four days. Fresh-cut carrots treated with PL (2.26 $J.cm^{-2}$) and stored at 8 °C for 14 days presented higher levels of β-carotene, β-glucose as well as polyacetylene as falcarindiol, falcarinol and falcarindiol-3-acetate, which have antifungal and health-promoting

activity (AGUILÓ-AGUAYO *et al*. 2017). PL treatment with fluences from 11 to 47 J.cm^{-2} also improved firmness of strawberry due to greater integrity of cell wall, when compared to plasmolysed and collapsed cells of control fruit (DUARTE-MOLINA *et al*. 2016). PL postharvest treatment induced different responses in 'Tommy Atkins' mango tissues pulp and peel. In pulp, an oxidative stress was established due to overproduction of ROS that would have triggered the antioxidant defense system, while in peel; the antioxidant defense system was stimulated without ROS mediation. Therefore, PL penetrated mango peel reaching the pulp and influencing its physiology, however, did not affect structural variables of neither tissue (LOPES *et al*. 2016).

Once, PL treatment penetrated mango peel that is 1-1.5 mm thick, it would easily penetrate acerola peel, which is thinner with 0.5 mm and possibly influence pulp physiology. Light penetration depths is one of the mains constraints of pulsed light technology, which has ultraviolet range as the component with most biological effects. Moreover, there is no published information regarding the influence of PL treatment on acerola regarding its climacteric ripening pattern, polyamine metabolism, structure, phenolic metabolism and vitamin C. Thereby, the work proposed to approach an amplitude of physiological processes related with ripening of acerola treated at postharvest with PL.

3.2 Material and methods

3.2.1 Plant material

Acerola (*Malpighia emarginata* DC, cv. Okinawa) were obtained from commercial grower located at Pacatuba-CE, Brazil (03º 59' 03" S and 38º 37' 13" W), with a tropical climate with average annual temperature of 25 ºC, irregular rainfall in average of 1305 mm and 70% average relative humidity (RH). Fruit were harvest at physiological maturity with peel turning red and afterwards, were selected for uniformity in size and absence of injuries.

3.2.2 Chemicals

Arginine, Agmatine, Spermine (spm), Spermidine (spd), Putrescine (put), Benzoyl chloride, Trans-cinnamic acid, 2,6-Dichloro-indophenol (DFI), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Ethylenediaminetetracetic acid (EDTA), Nitroblue tetrazolium chloride (NBT), Hydrogen peroxide (H_2O_2), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Ascorbic acid, Phenylalanine and Polygalacturonic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid was purchased from Acrós Organics (Belgium). All other chemicals were of analytical grade.

3.2.3 Pulsed light treatment and storage

At harvest day or day zero (D0), whole acerola were separated into two equal lots, one was not treated (control) and the other submitted to PL radiation treatment. Radiation was administered using a Xe-MaticA-2LXL system (SteriBeam® GmbH, Germany) from a distance of 10 cm applying two pulses (0.3 $J.cm^{-2}$ each) with a total incident fluence of 0.6 $J.cm^{-2}$. The chamber was equipped with two xenon flash lamps and Teflon® transparent support that allowed samples to be exposed 360º by both lamps, moreover to ensure uniformity, fruit position changed between dose applications. Lamps were positioned vertically, one on the left and another on the right side of fruit, and produced short-time pulses of 0.3 ms, delivering broad spectrum white light (200-1100 nm) with approximately 25% of all emitted energy in the UVC (220-280 nm).

At the same day (D0), control and treated samples (200 g) were placed on polystyrene trays covered with PVC film (14 microns) and stored at 10 ± 2 ºC and 80% RH. Fruit were evaluated at days 0, 1, 4, 10 and 16, and after physical analysis, whole fruit were homogenized for 2 min at 15000 rpm with an omnimixer (Ultra-turrax IKA® T25**,** Germany) and thereafter, all samples were stored at -80 ºC.

3.2.4 Firmness and associated variables

Whole fruit firmness was evaluated twice on opposite sides of each fruit with a texturometer (Brookfield® 25K CT3, USA) to measure the maximum force required to penetrate sample tissue to a depth of 10 mm using a 2-mm diameter cylindrical flat-tipped steel plunger at a shearing speed of 1 $mm.s^{-1}$. Results were expressed in Newton (N).

Biological membrane integrity estimated by lipid peroxidation degree was determined by malondialdehyde (MDA) content as described by Zhu *et al*. (2008). Pulp (1 g) was homogenized in 5 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 3300 x *g* for 20 min, at 4 °C. Supernatant (250 µL) was collected and added to 1 mL 0.5% thiobarbituric acid (TBA) in 20% TCA (v/v), thus incubated at 95 °C for 30 min. Following incubation, samples were, immediately cooled in ice bath and centrifuged (Sigma® 2-16 KL, Germany) at 3000 x *g* for 10 min. Supernatant absorbance was measured at 532 nm on microplate UV/Vis spectrophotometer (Biotek Sinergy® MX, USA), corrected for unspecific turbidity by subtracting from absorbance at 600 nm. Thus, thiobarbituric acid reactive substances (TBARS), as MDA, content was calculated using an extinction coefficient ε = 155 $mmol^{-1}.cm^{-1}$ (HEATH and PACKER 1968) and expressed as mol $MDA.Kg^{-1}$ fresh matter (FM)

Cell wall hydrolases were assayed and all procedures were carried out at 4 ºC. For pectinmethylesterase (PME, EC 3.1.1.11) assay, extraction started with pulp (5 g) homogenized in 20 mL of cold NaCl (0.2 M), then filtered through Whatman n.1 filter paper, filtrate was used as enzyme extract (JEN and ROBINSON, 1984). Enzyme activity was measured as 5 mL of extract plus 30 mL of citrus pectin substrate (1%) in NaCl (0.2 M, pH 7.0) were titrated with NaOH (0.01 N). One unit of enzyme activity (UEA) was defined as the amount enzyme capable to remove a methyl group from pectin and results were expressed as $UEA.Kg^{-1}$ $FM.s^{-1}$.

For polygalacturonase (PG, EC 3.2.1.15) assay, extract was prepared according to Pressey and Avants (1982), thus pulp (12.5 g) was homogenized with 25 mL of distilled water and then, centrifuged at 9000 x *g* for 10 min, at 4 °C. PG activity as described by Buescher

and Furmanski (1978) was expressed based on the difference of reducing sugar determined by 3,5-dinitrosalicylic acid (DNS). One unit of enzyme activity was defined as the amount of enzyme able to catalyze the formation of 1 nmol of reducing sugar $mg^{-1}.min^{-1}$ and results were expressed as $UEA.Kg^{-1}$ FM.

3.2.5 Oxidative stress and constituents of antioxidant metabolism

Hydrogen peroxide (H_2O_2) levels were assayed as oxidative stress marker, as described by Sergiev *et al*. (2001). Pulp tissue (0.5 g) was homogenized in ice bath with 5 mL 5% TCA (w/v) and then, centrifuged at 12000 x *g* for 15 min at 4 °C. The supernatant (50 µL) was added to 50 µL of 10 mM potassium phosphate buffer (pH 7.0) and 100 µL of 1 M KI. Absorbance was monitored at 390 nm, based on standard curve of H_2O_2, and results were expressed as $mmol.Kg^{-1}$ FM.

Total antioxidant activity (TAA) was determined using 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid radical cation ($ABTS^{*+}$) method as described by Re *et al*. (1999) and adapted by Rufino *et al*. (2010). Before the colorimetric assay, samples were subjected to extraction in 50% methanol and 70% acetone (Larrauri *et al*., 1997). Once the radical was formed, reaction was started by adding 3 µL of extract in 300 µL of radical solution, absorbance (734 nm) was measured after 6 min and decrement in absorption was used to calculate TAA. A calibration curve was prepared with different concentrations of trolox antioxidant (100 to 2000 µM) that were also evaluated against the radical. Antioxidant activity was expressed as trolox equivalent antioxidant capacity (TEAC) $mol.Kg^{-1}$ FM.

Antioxidant enzymes activity were determined using an enzymatic extract prepared with pulp (1 g) macerated for 5 min with ice-cold buffer solution (pH 7.0) containing 0.1 M potassium phosphate and 0.1 mM ethylenediaminetetracetic acid (EDTA) (YANG *et al*., 2009). Then, centrifuged at 12000 x g for 15 min at 4 °C and supernatant was used as crude enzyme extract. Here on, all enzymatic assay procedures were performed at 4 ºC and results were calculated as specific enzyme activity considering the total protein content of each sample as determined by Bradford (1976), using bovine serum albumin (BSA) as standard

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined based on inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT) (BEAUCHAMP and FRIDOVICH, 1971). Absorbance was measured at 560 nm and one unit enzyme activity was defined as the amount of enzyme required to cause a 50% inhibition in

the NBT photoreduction rate, and results were expressed as UAE.mg^{-1} protein (P). Catalase (CAT, EC 1.11.1.6) activity was assayed according to Beers and Sizer (1952) and decrement of H_2O_2 content was monitored by absorbance at 240 nm and quantified using its molar extinction coefficient (36 M^{-1}.cm^{-1}), results were expressed in µmol H_2O_2.min^{-1}.mg^{-1} P. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to Nakano and Asada (1981). In a water bath at 30 °C, 210 µL of potassium phosphate buffer 50 mM (pH 6.0) containing EDTA 0.05 mM was added to 20 µL of enzyme extract, and to substrates 10 µL of 0.03 M hydrogen peroxide (H_2O_2) and 10 µL of 15 mM AsA. The oxidation rate of ascorbate by H_2O_2 was monitored by the decrease in absorbance at 290 nm for 10 min, quantified using the extinction coefficient of H_2O_2 (Ɛ =2.8 mM^{-1}.cm^{-1}) and results were expressed in µmol H_2O_2. min^{-1}.mg^{-1} P.

3.2.6 Respiration and ethylene metabolism

Postharvest ethylene (C_2H_2) production and respiratory rate (CO_2 evolution) were determined on gas chromatograph mass spectrometer (Shimadzu® GCMS-QP2010 Plus, Japan), respectively, as described by Freitas *et al*. (2018). Acerola (50 g) were placed in hermetically closed vials for 1 h, thereafter 200 µL of headspace gas was injected in split mode (1:500), using helium as a carrier gas at a constant flow of 0.86 mL.min^{-1} on a RTX-5MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Restec, Bellefonte, USA). The oven temperature was set to 60 °C for 3 min, and injector, interface and ion source temperatures were set to 150, 200 and 200 ºC, respectively. Each sample ran thrice by the GC/MS and results were expressed as µL of ethylene.Kg^{-1}.h^{-1} and mL of CO_2.Kg^{-1}.h^{-1}, with reference to a standard curve.

Ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), content was determined according to Bulens *et al*. (2011). Total and free ACC were extracted and quantified with Shimadzu® GCMS-QP2010 Plus (Japan), while conjugated ACC was calculated by subtracting the amount of free ACC from that of total ACC. Samples were evaluated in triplicates and results were expressed as mol ACC.g^{-1} FM.

Activity of enzymes responsible for ethylene synthesis, ACC synthase (ACS, EC 4.4.1.14) and ACC oxidase (ACO, EC 1.4.3) was determined as described by Bulens *et al*. (2011) and specific activities for both enzymes were expressed as mol C_2H_4.h^{-1}.mg^{-1} P.

ACS activity assay started with pulp (1g) homogenized with 2 mL 100 mM Hepes buffer, pH 8.5 with 5 µM pyridoxal phosphate (PLP), 5 mM dithiothreitol (DTT), 0.5 mM

phenylmethylsulfonyl fluoride (PMSF) and 2% polyvinylpolipirrolidone (PVPP) (w/v) for 3 min with pistil and mortar, at 4 °C. Afterwards, the mixture was centrifuged at 12000 x *g* for 30 min, a 4 ºC and supernatant used as enzyme extract. ACS activity was determined with 100 µL of enzyme extract in 200 µL of 80 mM Hepes buffer, pH 8.5 with 20 µM PLP and its substrate, 100 µM S-adenosylmethionine (SAM) incubated for 2 h at 30 ºC. Then, reaction was terminated with 100 µL of cold 25 mM mercury chloride ($HgCl_2$) and ACC produced converted into ethylene, which was determined through GC/MS.

ACO activity assay started with pulp (2 g) homogenized with 2 mL 400 mM MOPS buffer, pH 7.2 with 10% glycerol (w/v), 30 mM ascorbic acid and 5% PVPP (w/v) for 2 min with pestle and mortar, at 4 °C. Afterwards, the mixture was kept in ice-bath under agitation for 10 min, then centrifuged at 12000 x *g* for 1 h, a 4 ºC and supernatant used as enzyme extract. ACS activity was determined with 40 µL of enzyme extract in 360 µL of 50 mM MOPS buffer, pH 7.2 with 10% glycerol (w/v), 5 mM ascorbic acid, 20 mM sodium bicarbonate ($NaHCO_3$), 0.02 mM ferrous sulfate ($FeSO_4$), 1 mM DTT and its substrate, 1 mM ACC incubated for 1 h at 30 ºC, in hermetically closed vials. Then, the ethylene produced from ACC was determined through GC/MS.

3.2.7 Polyamine metabolism

Free, conjugated and bound polyamines were determined according to Xu *et al*. (2011), with modifications. Pulp (3 g) was homogenized in 4 mL of 5% perchloric acid (PCA) (v/v) with pestle and mortar for 5 min at 4 ºC, kept in ice-bath for 1 h under agitation, then centrifuged at 27000 x *g* for 30 min, at 4 ºC. Supernatant (free) and precipitate were both used for acid hydrolysis with 1 mL of 12 M HCl for 18 h, at 110 ºC, in flame-sealed glass ampules to liberate soluble (conjugated) and insoluble bound polyamines, respectively. After hydrolysis, samples were filtered through microfilters (PVDF 22 mm, 0.22 µm), HCl evaporated by heating at 80 ºC for 48 h and the precipitate resuspended in 1 mL of 5% PCA. Polyamines recovered from the non-hydrolyzed supernatant (free), the hydrolyzed supernatant (free + soluble conjugated) and hydrolyzed precipitate (bound) were submitted to derivatization using benzoyl chloride according to Duan *et al*. (2008).

For derivatization, each polyamine fraction was mixed with 2 mL of 2 M NaOH and 15 µL of benzoyl chloride, vortexed for 10 s, and kept for 20 min at 37 ºC. Afterwards, derivatization was interrupted by 4 mL of NaCl saturated solution, then 2.5 mL of cold diethyl ether was added under agitation for 2 min, followed by centrifugation at 3000 x *g* for 10 min,

at 4 ºC. Supernatant was collected, dried under gaseous nitrogen flux, and resuspended in 500 µL of methanol. Benzoyl-derivatives of polyamines were separated by reverse-phase HPLC (Shimadzu®, Japan) equipped with a C18 column (Shim-pack®, CLC-ODS, 150 mm × 4.6 mm) with temperature set to 30 ºC. Samples (20 µL) were injected and eluted from the column with 64% methanol (in water, v/v) at flow rate of 1.0 mL.min^{-1}. Spermine (spm), spermidine (spd), putrescine (put) peaks were monitored at 240 nm in a SPD-20A detector (Shimadzu®, Japan) and determined as benzoylated polyamine standards were also separated by HPLC and used to generate standard regression curves. Conjugated polyamines contents were calculated by subtracting soluble from free polyamines and results expressed as mol. g^{-1} FM.

The activities of enzymes associated with polyamines metabolism were evaluated. Arginine decarboxylase (ADC, EC 4.1.1.19) activity was measured according to Duan *et al*. (2008), with some modifications made by Freitas *et al*. (2018). Pulp (2 g) were homogenized, for 3 min, in 100 mM potassium phosphate buffer (pH 7.5) containing 5 mM PMSF, 0.1 mM PLP, 5 mM DTT, 5 mM EDTA, 5 mM ascorbic acid and 0.1% polyvinylpyrrolidone (PVP), at 4 ºC. Then, centrifuged at 12000 x *g* for 40 min, at 4 ºC and the supernatant separated as enzyme extract. Enzyme activity was assayed with 150 µL of extract and 850 µL of 100 mM Tris-HCl buffer (pH 7.5), 40 µM PLP, 5 mM DTT, 5 mM EDTA and substrate 40 mM L-arginine for 1 h, at 37 ºC. Then, was stopped with 0.5 mL of 20% TCA. ADC activity was assayed by measuring agmatine (Agm) production from arginine decarboxylation. Agm was derivatized by silylation with N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS) and determined by GC/MS. Silyl-derivatized samples (1 µL) was injected in split mode (1:25) using helium as a carrier gas at constant flow of 1.48 mL/min on a RTX-5MS column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restec®, USA). The oven temperature was set to 150 °C for 2 min, raised 4 °C.min^{-1} to 165 °C, raised 2 °C.min^{-1} to 220 °C and finally raised 4 °C.min^{-1} to 280 °C and then, constant for 7 min. Injector, interface and ion source temperatures were set to 150, 200 and 200 ºC, respectively. Each sample ran thrice by the GC/MS and results for specific activity were expressed as mmol Agm.mg^{-1} P, with reference to a standard curve of Agm.

Diamine oxidase (DAO, EC 1.4.3.6) and polyamine oxidase (PAO, EC 1.5.3.11) activities were estimated based on the colorimetric assay of Δ-pyrroline (Holmstedt *et al*. 1961). Pulp (2 g) were homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 5 mM DTT, and then, centrifuged at 16000 x *g* for 20 min at 4 °C. The supernatant was used as enzyme extract. DAO and PAO activities were assayed with 225 µL

of extract and 125 µL of 50 mM potassium phosphate buffer (pH 7.5 for DAO and 6.0 for PAO) containing 20 mM EDTA, 50 units of catalase, 0.1% 2-aminobenzaldehyde and substrates 10 mM putrescine and 5 mM spermidine + 5 mM spermine, respectively. Each reaction was carried out for 3 h at 36 °C, stopped with 0.5 mL of 10% PCA, then centrifuged at 5000 x *g* for 15 min. Δ-pyrroline formation was monitored at 430 nm using a microplate UV/VIS spectrophotometer (Biotek® Synergy MX, USA), and calculated using its extinction coefficient (1.86 $mM^{-1}.cm^{-1}$), thus enzymes specific activities expressed as ηmol Δ-pyrroline.mg^{-1} P.

3.2.8 Color and phenolic metabolism

Color was determined using a chromameter (Konica Minolta® CR 400, Japan) with cylindrical coordinate system and color space for lightness (L*), chroma (C) and Hue angle (h). Peel color was evaluated twice on opposite sides of each fruit.

Total phenol content of acerola was measured by a colorimetric assay using Folin-Ciocalteau reagent as described by Obanda and Owuor (1997). Pulp (1 g) were extracted in 50% methanol followed by extraction with 70% acetone for 1 h, and after extraction period, samples were centrifuged at 16000 x *g* for 15 min, at 20 °C as described by Larrauri *et al*. (1997). The assay mixture consisted of 100 µL of extract (supernatant), 250 µL of Folin-Ciocalteau reagent (1 N), 500 µL of 20% Na_2CO_3 and 650 µL of distilled water that was homogenized and absorbance monitored at 700 nm. Results calculated with reference to a standard curve of gallic acid and expressed as gallic acid equivalents (GAE) g.Kg^{-1} FM.

Phenylalanine ammonia lyase (PAL, EC 4.3.1.24) catalyzes the initial reaction in polyphenol biosynthetic pathway and its activity was determined as described by El-Shora (2002), with modifications. Pulp (1 g) was homogenized for 3 min with 4 mL of 100 mM Tris-HCl buffer (pH 8.4) containing EDTA and PVP, at 4 ºC and then, centrifuged at 10000 x *g* for 10 min at 4 ºC and supernatant separated as enzyme extract. For activity assay, 25 µL of extract was homogenized with 5 µL of ß-mercaptoethanol, 580 µL of 100 mM Tris-HCl buffer (pH 8.4) and 50 µL of substrate 40 mM L-phenylalanine. The reaction was stopped with 25 µL of 6 M HCl and absorbance was monitored at 290 nm. PAL specific activity calculated with reference to a standard curve of trans-cinnamic acid and expressed as µmol trans-cinnamic acid.$h^{-1}.mg^{-1}$ P.

3.2.9 Vitamin C metabolism

Quantification of total vitamin C, reduced ascorbic acid (AsA) and oxidized dehydroascorbic acid (DHA) was performed according to Chen and Wang (2002). Pulp (1 g) was homogenized with 25 mL of 5% (w/v) TCA, then centrifuged at 15000 x *g* for 12 min at 4 °C and supernatant was used for the vitamin measurements. Total vitamin C content was measured by homogenizing 50 µL of supernatant, 252 µL of 100 mM phosphate buffer (pH 7.7) and 12.5 µL of 0.01 mM DTT, at room temperature (25 °C) for 10 min. Afterwards, the mixture was added to 175 µL of solution containing 10% TCA, 8.8% phosphoric acid, 0.8% 2,2-bipyridyl and 0.3% ferric trichloride, following incubation at 37 °C for 60 min, then absorbance was monitored at 525 nm. Results were calculated with reference to a standard curve of ascorbic acid and expressed as $mg.Kg^{-1}$ FM. For AsA quantification, a reaction mixture containing 50 µL supernatant, 12.5 µL of 100 mM phosphate buffer (pH 7.7) plus 175 µL of solution containing 10% TCA, 8.8% phosphoric acid, 0.8% 2,2-bipyridyl and 0.3% ferric trichloride was incubated at 37 °C for 60 min, then absorbance was monitored at 525 nm. Results were calculated with reference to a standard curve of ascorbic acid and expressed as AsA $mg.Kg^{-1}$ FM. DHA content was calculated from the difference between total vitamin C and AsA and expressed as DHA $mg.Kg^{-1}$ FM.

3.2.10 Statistical analysis

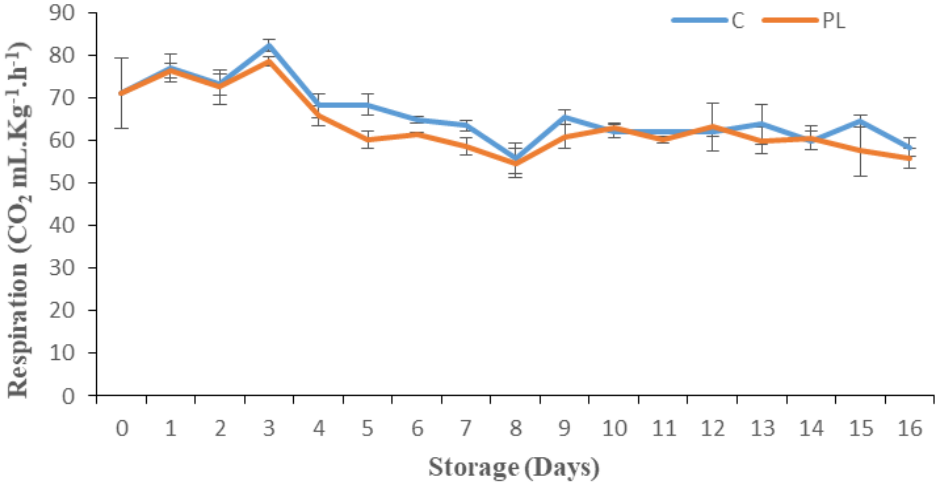
The experimental design was completely randomized (2 x 5, treatment and storage periods, respectively) with three replications with 200 g of acerola each and all analyzes were performed as triplicate. Data was subjected to analysis of variance (ANOVA) using SISVAR® 4.3 Statistical Assistance Software and means were compared by Tukey's test at 5% probability.

3.3 Results and discussion

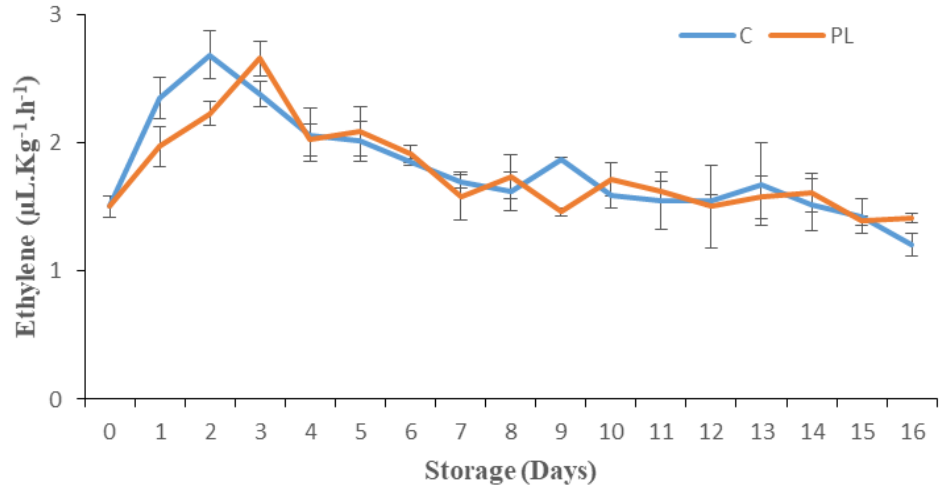
3.3.1 Postharvest quality attributes

Pulsed light (PL) treatment influenced acerola 'Okinawa' climacteric respiratory rate and ethylene production (Figures 8A and 8B). Respiratory peak occurred at day 3 of storage at 10 ºC for control reaching 82.3 mL.Kg^{-1}.h^{-1}, although it was 5% lower (P< 0.05) with 78.6 mL.Kg^{-1}.h^{-1} in PL-treated acerola (Figure 8A). Ethylene peaked at day 2 for control fruit with 2.68 µL.Kg^{-1}.h^{-1}, while in PL-treated acerola, it was delayed to day 3 with 2.65 µL.Kg^{-1}.h^{-1} (Figure 8B).

Figure 8: Respiration (A) and ethylene production (B) of 'Okinawa' acerola treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days.



A

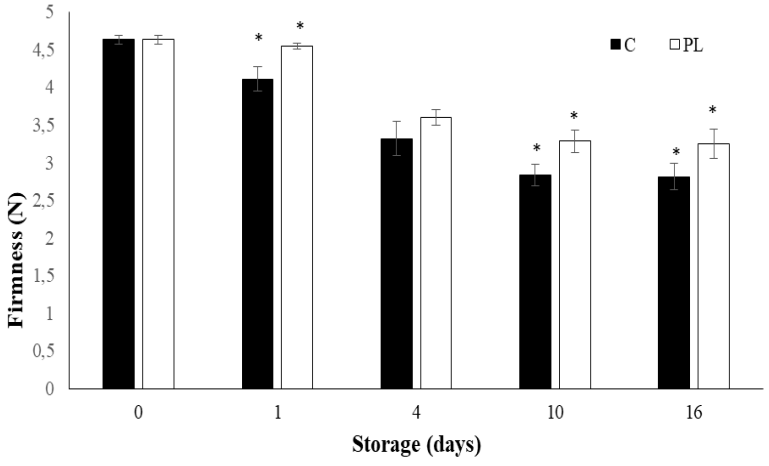


B

As PL treatment slowed down ‘Okinawa’ acerola physiology with inhibition or delay of climacteric, it affected positively quality attributes. Firmness decreased during storage, although PL-treated acerola were significantly firmer with 3.2 N, after day 10 of storage, a 12% greater firmness than control (Figure 9A). Color attributes were evaluated and lightness and chroma values did not differ ($P< 0.05$) among treatments during storage (data not shown). However, Hue angle (h) decreased even though it was statistically higher (36.5) in treated acerola than in control (31.9) at day 1 (Figure 9B). During 16 days of cold storage, acerola lost weight, although control lost significantly more (13.4%) than treated fruit with 12.2% of weight loss (Figure 9C).

Peaches fruits treated with ultraviolet-C (UV-C, 0.3 J.cm^{-2}), which is the main component of PL regarding biological effects, and stored at 20 ºC had greater firmness due to inhibition of respiration rate (YANG *et al*., 2014). The authors explained respiration was lower due to inhibition of succinic dehydrogenase and cytochrome C oxidase activities, besides a higher level of mitochondrial membrane fluidity. PL treatment (2.8 J.cm^{-1}) also improved fresh-cut ‘Tommy Atkins’ mango quality during storage at 6 ºC, through a reduction in respiratory rate that resulted in maintenance of color and lower mass loss (LOPES *et al*., 2017). Meanwhile, Ignat *et al*. (2014) treated fresh-cut ‘Golden Delicious’ apples with PL (0 to 15.7 J.cm^{-1}) and reported that fluences greater than 10.5 J.cm^{-1} increased weight loss, browning and cooked off-flavor of samples.

Figure 9: Firmness (A), color parameter Hue^{o} (B) and weight loss (C) of ‘Okinawa’ acerola treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days. *Represents significant differences at $P< 0.05$ between treatments at each storage period.



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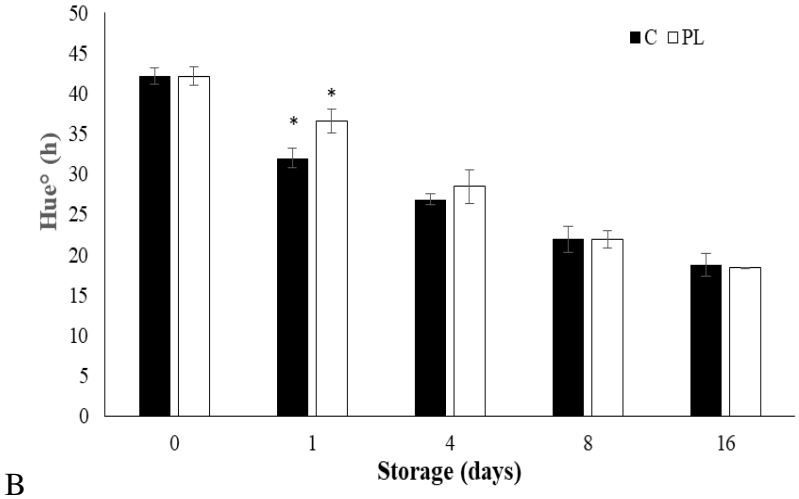

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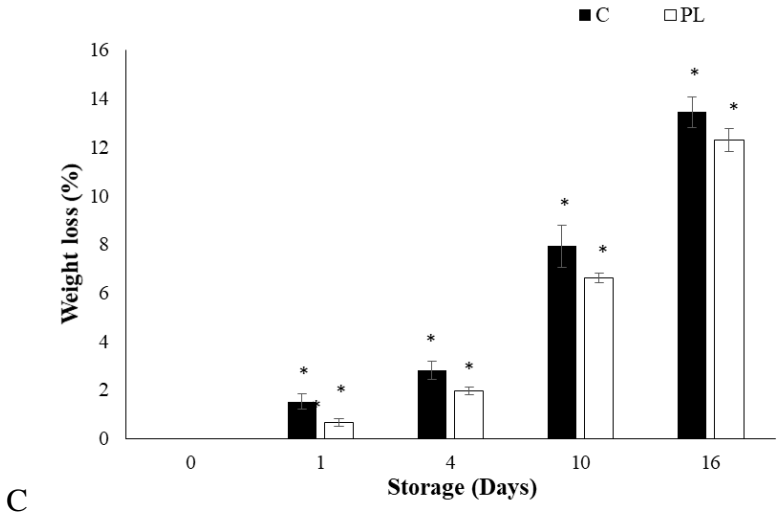

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3.3.2 Oxidative and antioxidant metabolism

ROS are natural products of respiratory process and are used as oxidative stress markers. As acerola progressed into ripening, the hydrogen peroxide (H_2O_2) content increased (Table 1). Although, this increase was significantly slower in PL-treated fruit (58.2 mmol. Kg^{-1}) when compared to control (77.2 mmol. Kg^{-1}), at day 10. Thus, PL treatment inhibited respiration of acerola (Figure 8A) justifying the lower H_2O_2 content.

The antioxidant defense metabolism consists of enzymatic and non-enzymatic compounds that contribute to the total antioxidant capacity of a tissue. Thus, activity of antioxidant enzymes was evaluated in acerola treated with pulsed light and results show that besides the lower respiratory rate, PL stimulated the antioxidant defense mechanism (Table 1). SOD activity varied little during storage, but was statistically higher in PL-treated acerola with 116.2 UAE.mg^{-1} P than in control with 94.6 UAE.mg^{-1} P, at day 16. The reaction

catalyzed by SOD produces H_2O_2 that may be neutralized by CAT activity, which was higher ($P< 0.05$) in PL-treated acerola with 435.5 µmol $H_2O_2.min^{-1}.mg^{-1}$ P than control with 342.0 µmol $H_2O_2.min^{-1}.mg^{-1}$ P, at day 1, and then decreased to the end of storage. In the beginning of storage, increases in CAT activity stimulated by PL explains the lower H_2O_2 content, observed at day 10. Acerola total antioxidant activity (TAA) determined by ABTS method incresed after day 1 of storage, when PL-treated fruit had significantly higher activity of 169.54 mmol TEAC.Kg^{-1} FM while control had 160.15 mmol TEAC.Kg^{-1} FM.

Yang *et al*. (2014) observed that UV-C treatment (0.3 $J.cm^{-2}$) in peaches enhanced the activities of SOD, CAT and ascorbate peroxidase (APX) resulting in lower levels of superoxide radicals (O_2^-) and H_2O_2, which inhibited opening of mitochondrial permeability transition pore contributing to maintenance of mitochondrial membrane integrity and slowing down fruit senescence. Therefore, results presented here corroborate to the idea that PL, under the conditions here applied, influenced acerola physiology not as a stress agent, but as an elicitor of the antioxidant defense system preventing possible oxidative damages due to imbalance between ROS production and scavenging mechanisms.

APX activity increased to day 10 of storage, and thereafter declined to 0.58 µmol H_2O_2. $min^{-1}.mg^{-1}$P in control fruit, at day 16, without significant differences between treatments (Table 1). Only at day 4, PL treatment induced an 18% higher APX activity when compared to control with 0.82 µmol H_2O_2. $min^{-1}.mg^{-1}$P. The increase in APX activity may reflect the need to remove H_2O_2 (Table 1) produced as a by-product of acerola respiration (Figure 8A), besides resulting in decreased AsA (Figure 10B) and increased oxidized DHA content (Figure 10C). Moreover, in PL treated fruit, the higher APX activity explains the lower H_2O_2 content.

3.3.3 Variables associated with firmness

Fruit tissue firmness (Figure 9A) is associated to cell wall and membrane structural integrity; therefore, these variables were evaluated in 'Okinawa' acerola in response to PL treatment. Lipid peroxidation degree reflects biological membrane structure and results show an increase during storage from 61.04 to 136.86 µmol MDA. Kg^{-1} FM, in control fruit, however PL-treated acerola showed significantly 21% lower peroxidation degree, only day 10 (Table 2). Cell wall hydrolytic activity differed significantly ($P< 0.05$) between treatments (Table 2). Pectin methyl esterase (PME) activity increased at day 1 to 45.81 $x10^3$ UEA.$Kg^{-1}.s^{-1}$ in control, and decreased thereafter with significantly lower levels in treated fruit up to day

10. Meanwhile, polygalacturonase (PG) activity peaked at day 10 with 26.62 x10^3 UEA.kg^{-1} FM in control samples, although PL-treated samples had 8% lower activity. At the beginning of storage period, the lower PME cell wall hydrolytic activity of PL-treated fruit possibly contributed to the significantly greater firmness (Figure 9A), while at the end of storage, the inhibition of membrane lipid peroxidation and PG activity were the main responsible.

ROS may inflict oxidative damage to different cell structures, but its action on biological membranes is obviously perceived as it affects tissue firmness. As observed by results here presented, increase in lipid peroxidation degree is part of softening process during fleshy acerola ripening, however the lower ROS content, in PL-treated fruit, reflected in lower peroxidation degree and greater firmness. Huan *et al*. (2016) stated that, in 'Xiahui 5' peaches, ROS were mainly generated from respiration and acted as potential signaling molecules during fruit development and as toxic molecules stimulating lipid peroxidation during ripening.

The dissolution of cell wall structure results mainly from activity of cell wall hidrolases as PME and PG (Table 2), which presented lower activity levels in PL-treated acerola. 'Tommy Atkins' mangoes treated with PL (0.6 J.cm^{-2}) did not show obvious difference on skin nor pulp microstructure when compared to control, during 7 days of storage at 20 ºC (LOPES *et al*., 2016). Another histological analysis performed on minimally processed 'Cantaloupe' melons treated with PL (0.9 J.cm^{-2}) application every 48 h resulted in samples with better delimited cell walls and more turgid cells when compared to collapsed and flattened control cells, at the end of 28 days storage at 4 °C (KOH *et al*., 2016).

Table 1. Variables associated with oxidative stress and antioxidant metabolism of 'Okinawa' acerolas treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days.

STORAGE (Days)	H_2O_2 (mmol. Kg^{-1} FM)		SOD (UAE. mg^{-1} P)		CAT (µmol H_2O_2.min^{-1}.mg^{-1} P)		APX (µmol H_2O_2.min^{-1}.mg^{-1} P)		TAA (mmol TEAC.Kg^{-1} FM)	
	C	**PL**	**C**	**PL**	**C**	**PL**	**C**	**PL**	**C**	**PL**
0	25.86	25.86	104.05	104.05	139.22	139.22	0.66	0.66	134.41	134.11
1	31.01	40.61	108.13	101.10	342.05*	435.59*	0.83	0.90	160.15*	169.45*
4	34.78	39.76	107.47	118.33	251.07*	337.34*	0.82 *	1.01 *	110.88	111.69
10	77.27*	58.28*	117.66	105.13	242.46	267.79	1.07	1.16	108.78	110.68
16	86.04	82.94	94.69*	116.25*	110.21	113.18	0.58	0.51	59.69	64.91

*Represents significant differences at $P < 0.05$ between treatments at each storage period. Legend: Hydrogen peroxide (H_2O_2), Superoxide dismutase (SOD), Catalase (CAT), Ascorbate peroxidase (APX) and Total antioxidant activity (TAA).

Table 2. Variables associated with firmness of 'Okinawa' acerolas treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days, as activity of cell wall enzymes pectin methyl esterase (PME) and polygalacturonase (PG).

STORAGE (Days)	Lipid Peroxidation Degree (µmol MDA. Kg^{-1} FM)		PME (x10^3 UEA.Kg^{-1} FM.s^{-1})		PG (x10^3 UEA.Kg^{-1} FM)	
	C	PL	C	PL	C	PL
0	61.04	61.04	31.67	31.67	19.90	19.90
1	61.38	69.37	45.81*	42.89*	15.04*	17.96*
4	61.93	69.59	29.41*	28.16*	15.44*	16.34*
10	118.03*	93.43*	24.26*	21.22*	26.62*	24.63*
16	136.86	139.02	16.94*	19.87*	17.62*	13.65*

*Represents significant differences at $P < 0.05$ between treatments at each storage period.

3.3.4 Ethylene and polyamine metabolism

At day 1, ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) total content was highest. The PL treatment induced an ACC content 18% lower ($P < 0.05$) than control with 2679 µmol.Kg^{-1} MF (Table 3). Besides reducing total ACC content, PL enhanced ACC conjugation over 30% a response that was accompanied by reduced of 20% in free ACC when compared to control, at day 4 (Table 3). Thus, PL treatment of acerola delayed ethylene climacteric peak (Figure 8B) due to lower total ACC and higher conjugated ACC levels, which resulted in lower free ACC content.

Ethylene synthesis relies on ACC synthase (ACS) to produce ACC from precursor S-adenosyl-L-methionine (SAM). Here, ACS activity decreased during storage of 'Okinawa' acerola (Table 3). However, at day 1, PL enhanced such activity to 7.87 µmol C_2H_4 h^{-1}.mg^{-1} P, 11% higher than control. Additionally, enzyme ACC oxidase (ACO) catalyzes ACC oxidation into volatile ethylene and its activity also peaked at day 1, although it was 16% lower in PL-treated acerola, 59.63 µmol C_2H_4 h^{-1}.mg^{-1}P, when compared to control (Table 3). Tiecher *et al.* (2013) submitted 'Flavortop' tomatoes to UV-C treatment (0.37 J.cm^{-2}) and observed that *ACS* and *ACO* transcripts were upregulated one day after treatment, and although the authors did not determine these enzymes activities, their result could explain the higher ACS and ACO activities observed for 'Okinawa' acerola, at day 1, independent of PL treatment.

PL treatment influenced ACC synthesis in an opposite manner as compared to its oxidation by ACO and although ACS activity was stimulated by PL, it did not reflect on

higher total ACC levels. The higher ACS activity could be a direct effect of light on its stability/turnover rate as reported by Booker and DeLong (2015). Our findings suggest that ACO activity not ACS is a rate limiting for ethylene production during fruit ripening. Therefore, results here presented corroborate with ACO activity, inhibited by PL, being the rate limiting reaction for ethylene synthesis during acerola ripening.

Ethylene and polyamines may act as regulators of plant development, as well as elicitors of resistance against environmental stresses (GUPTA; DEY; GUPTA, 2013). These two regulators are biochemically related once they share SAM as common precursor in their biosynthetic routes, indicating a competition between ethylene and polyamine biosynthesis (GRZESIAK *et al*., 2013). Polyamines are low-molecular weight aliphatic amines whose intracellular levels are highly regulated by anabolic and catabolic processes, in plants. Putrescine (Put), a diamine and smaller polyamine, may be synthetized from arginine by arginine decarboxilase (ADC) with agmatine (Agm) as an intermediary, representing the main pathway involved in responses to abiotic stresses (ALCÁZAR *et al*., 2010; GUPTA; DEY; GUPTA, 2013). The tri and tetra-amines Spermindine (Spd) and Spermine (Spm) formation by addition of aminopropyl groups derived from decarboxylation of SAM to putrescine represent a critical point for regulation of polyamine homeostasis.

Free putrescine levels were increased during storage of 'Okinawa' acerola, however at day 1, PL-treated fruit had 21% statistically higher levels with 4.98 $\mu mol.g^{-1}$ FM, when compared to control (Table 4). Free spermidine and spermine levels did not change with storage period, however, only free spermidine was influenced by PL-treatment with levels 12% higher than control (Table 4). Soluble and insoluble conjugated polyamine levels were not influenced by storage nor PL-treatment (data not shown). UV-C treatment enhanced Put and Spd levels in peaches and tomatoes, but not Spm content (GONZÁLEZ-AGUILAR *et al*. 2004, TIECHER *et al*., 2013, URBAN *et al*., 2016). PL increased ADC activity by a 13% with 45,520 mmol $Agm.mg^{-1}$ P, when compared to control at day 1. Meanwhile, activity of diamine oxidase (DAO), enzyme associated with putrescine degradation, was inhibited over 30% by PL treatment to 51.21 ηmol $pyrroline.mg^{-1}$ P, when compared to control at day 1 (Table 4). In contrast, the activity of polyamine oxidase (PAO), responsible for degradation of Spm and Spd, was enhanced 18% by PL-treatment to 94.56 ηmol $pyrroline.mg^{-1}$P, when compared to control at day 1.

The greater Put levels reflects the stimulus of its synthesis (ADC activity) and inhibition of its degradation (DAO activity) by PL. Moreover, the higher Spd and Put levels observed in PL-treated acerola must also have contributed to the lower ethylene production

(Figure 8B), once Spd formation depends on SAM, the same precursor as ethylene synthesis. These results corroborate with the idea that PL delayed 'Okinawa' acerola ripening and senescence through competition between ethylene and polyamines, the latter may also be associated with fruit firmness maintenance (Figure 9A) through due to reduced membrane-lipid peroxidation degree and PG cell wall hydrolytic activity (Table 2). It has been reported that UV-C treatment contributed to fruit firmness through stabilization of cell membranes due to association with negatively charges phospholipids or to formation of cross-links with cell wall polysaccharides impeding cell wall hydrolytic activity (GONZÁLEZ-AGUILAR *et al*. 2004). Morevoer, Freitas *et al*. (2018) showed that ethylene contributes to H_2O_2 generation, thus fine-tuning the signaling events involving the regulation of polyamine catabolic enzymes, under oxidative stress condition.

Table 3. Variables associated with ethylene synthesis of 'Okinawa' acerolas treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days.

STORAGE (Days)	Total ACC (µmol.Kg^{-1} MF)		Free ACC (µmol.Kg^{-1} MF)		Conjugated ACC (µmol.Kg^{-1} MF)		ACS (µmol C_2H_4.h^{-1}.mg^{-1} P)		ACO (µmol C_2H_4 h^{-1}.mg^{-1} P)	
	C	**PL**	**C**	**PL**	**C**	**PL**	**C**	**PL**	**C**	**PL**
0	1687	1687	371	371	1317	1317	8.65	8.65	48.28	48.28
1	2679*	2219*	1627*	1125*	1053	1094	7.02*	7.87*	70.31*	59.63*
4	1923	1998	1332*	1071*	591*	927*	5.47	5.95	54.78*	33.28*
10	1911	1865	892	845	1019	1021	4.73	5.27	25.09	24.38
16	1575	1615	991*	821*	585	794	5.18	5.17	24.28*	17.81*

*Represents significant differences at $P < 0.05$ between treatments at each storage period. Legend: 1-aminocyclopropane-1-carboxylic acid (ACC), 1-aminocyclo-propane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO).

Table 4. Variables associated with polyamine metabolism of 'Okinawa' acerolas treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days. Enzymes evaluated Arginine decarboxylase (ADC), Diamine oxidase (DAO) and Polyamine oxidase (PAO).

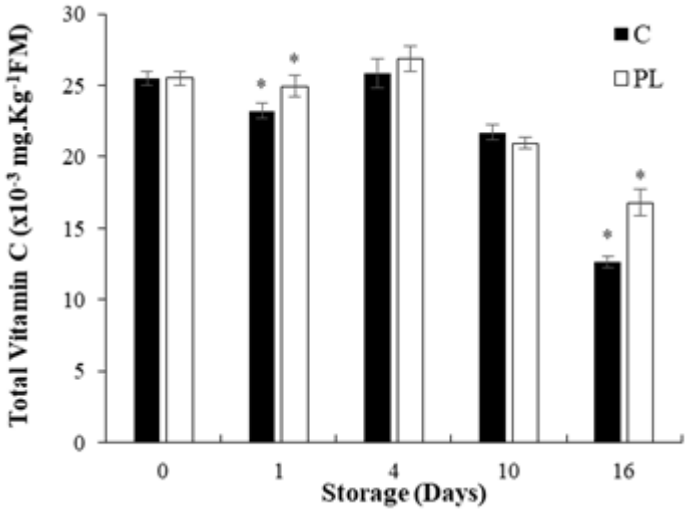
STORAGE (Days)	Free Putrescine (µmol.g^{-1} FM)		Free Spermidine (µmol.g^{-1} FM)		Free Spermine (µmol.g^{-1} FM)		ADC (mmol Agm.mg^{-1} P)		DAO (ηmol pyrroline.mg^{-1} P)		PAO (ηmol pyrroline.mg^{-1} P)	
	C	PL	C	PL	C	PL	C	PL	C	PL	C	PL
0	4.45	4.45	2.79	2.79	1,51	1,50	32830	32830	55.08	55.08	73.05	73.05
1	3.95*	4.98*	2.57*	2.91*	1,46	1,47	39630*	45520*	76.41*	51.21*	77.84*	94.56*
4	7.44	7.36	2.81	2.78	1,48	1,49	34540	37390	58.94	54.09	66.55	61.14
10	10.83	11.41	2.79	2.74	1,48	1,49	25140	28510	32.93	31.68	62.71	57.72
16	14.42	14.6	2.67*	2.94*	1,48	1,51	18970	17770	26.78	26.29	20.43	19.49

*Represents significant differences at $P < 0.05$ between treatments at each storage period.

3.3.5 Vitamin C metabolism

Total vitamin C consists of reduced ascorbic acid (AsA) and oxidized dehydroascorbic acid (DHA) forms. In 'Okinawa' acerola (Figure 10A), total vitamin C content was initially 25,515.38 mg.Kg^{-1} FM and declined after four days of storage. But at day 16, PL-treatment resulted in significantly ($P < 0.05$) higher levels with 16,790.12 mg.Kg^{-1} FM, while control had 12,624.69 mg.Kg^{-1} FM. A similar behavior was observed for AsA content at 16 days of storage (Figure 10B), PL-treated acerola presented significantly higher content than control with 15,081.48 and 10,548.15 mg.Kg^{-1} FM, respectively. On the contrary, oxidized DHA increased during storage with significantly higher levels for control samples (2,743.21 mg.Kg^{-1} FM), at day 10 of storage and thereafter, decreased (Figure 10C). However, at day 16, PL-treated fruits had ($P < 0.05$) higher contents with 1,365.24 mg. Kg^{-1} FM than control, 1,070.03 mg.Kg^{-1} FM. At the beginning of acerola storage, the high AsA and low DHA concentration indicate that AsA recycling and synthesis mechanisms were active, but as fruit progressed into ripening and senescence, the ability to synthesize and recycle AsA declined, possibly allied to increases in degradation rate. Lopes *et al*. (2016) also reported an enhancement of total vitamin C contents by PL (0.6 J/cm^{-2}) in 'Tommy Atkins' pulp and peel tissues.

Figure 10. Total vitamin C (A), ascorbic acid (AsA, B) and dehydroascorbic acid (DHA, C) content of 'Okinawa' acerola treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days. *Represents significant differences at $P < 0.05$ between treatments at each storage period.



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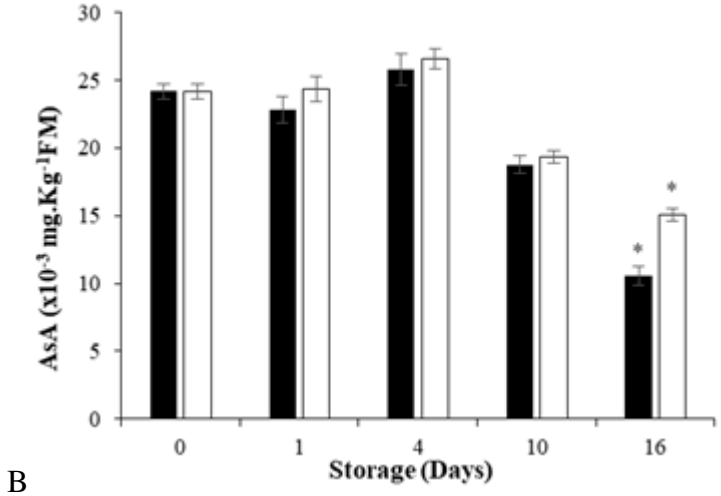

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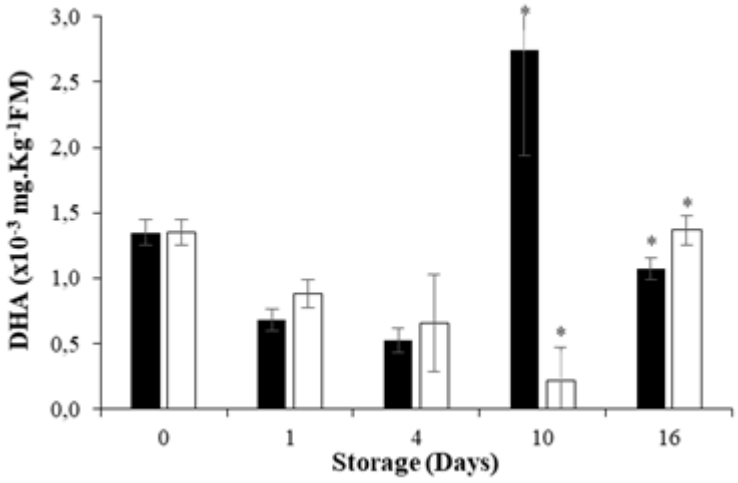

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3.3.6 Phenolic metabolism

In 'Okinawa' acerola, the concentration of total extractable polyphenols decreased with storage time (Table 5), however, the LP-treated acerola presented significantly higher content with 21.42 g GAE.Kg^{-1} FM than control with 19.35 g GAE.Kg^{-1} FM, at day 16.

Phenylalanine ammonia lyase (PAL) is the primary enzyme of phenolic biosynthetic pathway and its activity decreased during storage of acerola (Table 5). Although, PL treatment slowed down significantly PAL decline, thus at day 16, PAL activity of treated acerola was 108.27 µmol trans-cinnamic acid.h^{-1}.mg^{-1} P, 13% higher than control with 94.49 µmol trans-cinnamic acid.h^{-1}.mg^{-1} P, justifying the higher polyphenol content of treated acerola indicating LP stimulated PAL activity and phenolic synthesis. However, Tiecher *et al.* (2013) reported that, during ripening of 'Flavortop' tomatoes, *PAL* transcription was

upregulated with increased phenolic levels, although without significant differences between control and UV-C treatment. Therefore, as proposed by Urban *et al*. (2016), PAL activity is likely induced by PL at the post-transcriptional level.

Table 5. Variables associated with phenolic metabolism of 'Okinawa' acerolas treated with pulsed light (PL, 0.6 J.cm^{-2}) and stored under refrigeration (10 °C) for 16 days. Enzyme evaluated Phenylalanine ammonia lyase (PAL). *Represents significant differences at $P < 0.05$ between treatments at each storage period.

STORAGE	**Total Polyphenol**		**PAL**	
(Days)	**(g GAE.Kg^{-1} FM)**		**(µmol cinnamic acid.h^{-1}.mg^{-1} P)**	
	C	**PL**	**C**	**PL**
0	38.94	38.94	145.93	145.93
1	37.60	37.03	138.16*	124.14*
4	32.97	33.73	132.89	136.70
10	27.78*	25.87*	119.03	124.98
16	19.35*	21.42*	94.49*	108.27*

3.3 Conclusion

Summarizing, we may to explain that pulsed light (0.6 $J.cm^{-2}$) treatment slowed down 'Okinawa' acerola climacteric respiration and ethylene production preventing fruit softening and weight loss. PL reduced H_2O_2 content and stimulated the antioxidant enzymatic defense mechanism, thus inhibiting membrane lipid peroxidation, which together with the lower cell wall hydrolytic activity contributed to the greater firmness. The delayed ethylene climacteric peak resulted from inhibition of enzyme ACC oxidase and although, ACS activity was stimulated by PL, it did not reflect on total ACC levels that were lower total while conjugated ACC levels were higher, leading in lower free ACC for ethylene synthesis. Polyamines putrescine and spermidine levels were stimulated by PL as reflection of stimulus of its synthesis by ADC and inhibition of its degradation by DAO. Moreover, the higher polyamines level contributed to the lower ethylene and to firmer fruit. There is no previous report on the influence of ascorbate metabolism by PL in fruits and results here presented show PL-treatment induced a higher total vitamin C and enhanced the synthesis PAL, leading to statistically higher polyphenol content. In conclusion, the results presented here corroborate to the idea that PL, under the conditions here applied, influenced acerola physiology not as a stress agent, but as an elicitor of the antioxidant defense system preventing possible oxidative damages due to imbalance between ROS production and scavenging mechanisms, promoting quality during storage.

4 CONCLUSÃO

Resumindo, podemos explicar que o tratamento com luz pulsada (LP) (0,6 J.cm-2) reduziu o pico respiratório no climatério e retardou o pico de etileno em acerola 'Okinawa', impedindo o amolecimento dos frutos e a perda de peso. A LP também reduziu o conteúdo de H_2O_2 e estimulou o mecanismo de defesa enzimática antioxidante, inibindo assim a peroxidação lipídica da membrana, que juntamente com a menor atividade hidrolítica da parede celular contribuiu para a maior firmeza. Já o retardo do pico climatério de etileno resultou da inibição da enzima ACC oxidase e, embora a atividade da ACS tenha sido estimulada pela LP, não refletiu sobre os níveis totais de ACC que estavam mais baixos, enquanto os níveis de ACC conjugados estavam mais elevados, levando uma menor quantidade de ACC livre para síntese de etileno. Os níveis das poliaminas putrescina e espermidina foram estimulados pela LP como reflexo do estímulo de sua síntese pela ADC e inibição de sua degradação pela DAO. Além disso, o maior nível de poliaminas contribuiu para a menor concentração de etileno e, consequentemente, para frutas mais firmes. Não há registros prévios sobre a influência do metabolismo do ascorbato por LP em frutas e os resultados aqui apresentados mostram que o tratamento com LP resultou em níveis mais altos de vitamina C total e na síntese aumentada da PAL, levando a um aumento estatístico no teor de polifenóis. Em conclusão, os resultados apresentados acima ou dessa pesquisa corroboram a ideia de que a LP, nas condições aqui aplicadas, influenciou a fisiologia da acerola não como agente estressor, mas como eliciadora do sistema de defesa antioxidante, prevenindo possíveis danos oxidativos devido ao desequilíbrio entre a produção de ROS e sua eliminação, promovendo, assim, a qualidade durante o armazenamento.

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