CATÓLICA

UNIVERSIDADE CATÓLICA PORTUGUESA | PORTO

Escola Superior de Biotecnologia

MODULATION OF AROMA VOLATILES AND PHYTOCHEMICAL QUALITY OF FRESH-CUT MELON (*Cucumis melo* L.) BY OXYGEN LEVELS, 1-METHYLCYCLOPROPENE AND LYSOPHOSPHATIDYLETHANOLAMINE

Thesis submitted to the *Universidade Católica Portuguesa* to attain the degree of PhD in Biotechnology – with specialisation in Food Science and Engineering

By
Ana Luísa Leite Fernandes Amaro

Under the supervision of Prof. Dr. Domingos Paulo Ferreira de Almeida

June 2012

Aos meus pais, ao Paulo e ao meu filho,

Rodrigo.

ABSTRACT

Current fresh-cut technologies are effective in the effort of maintaining visual quality during the fresh-cut fruit supply chain. However, studies on extended shelf life based on appearance attributes or microbial stability have neglected the understanding of the effect of processing technologies and conservation techniques in the aromatic, nutritional, and functional properties of processed fruit. Three technologies were evaluated for their effects on quality of fresh-cut melon, with emphasis on aroma volatiles: oxygen levels, the ethylene action inhibitor 1-methylcyclopropene (1-MCP), and lysophosphatidylethanolamine (LPE), an inhibitor of phospholipase D (PLD). Modified atmosphere packaging (MAP) is widely used in fresh-cut fruit, with the objective of extending shelf-life. Nevertheless, MAP requirements and recommendations for fresh-cut fruit target mostly color and texture maintenance, neglecting the effects on aroma production. In this thesis, the quality attributes and aroma production of fresh-cut melon were monitored during MAP storage. Cantaloupe and honeydew melon cultivars were processed into cubes and stored at 5 ºC under high passive MAP or reduced controlled atmosphere (CA; 5 kPa O_2 + 10 kPa CO_2, balance N_2). Oxygen levels during storage had a greater impact on aroma than on other quality attributes during storage of fresh-cut climacteric and non-climacteric melon. Cantaloupe and honeydew melon flavor-important volatiles were differently affected by oxygen levels inside the packages. Also, storage time had a significant effect on volatile production of fresh-cut cantaloupe and honeydew melons.

1-MCP application to whole fruit, its effects on quality, ethylene production and perception have been the subject of many studies. In contrast, fewer studies have focused on 1-MCP application to fresh-cut fruit. For its known inhibitory effect on ethylene production, we anticipated 1-MCP to have beneficial effects on some quality attributes of fresh-cut melon, while altering aroma profile. The effect of 1-MCP application in quality and synthesis of flavor-important volatile compounds in melon

was evaluated during storage of fresh-cut cantaloupe. Cantaloupe melons were processed and treated with 1.0 µL L^{-1} of 1-MCP, for 24 h at 5 ºC, packaged in vented plastic clamshells and stored under normal atmosphere at 5 ºC for 9 days. Treatment with 1-MCP did not affect the main quality attributes of fresh-cut melon, but affected nine of the flavor-important volatiles, particularly those with isoleucine and phenylalanine as precursors. 1-MCP affected fresh-cut cantaloupe production of propyl acetate, 2-methylbutyl acetate, methyl butanoate, methyl 2-methyl butanoate, methyl hexanoate, 2-methylbutyl alcohol, phenethyl alcohol, benzyl alcohol and heptanal.

LPE is reported to inhibit PLD activity, reduce ethylene production, and extend shelf-life of many horticultural commodities. Therefore, LPE could provide a novel treatment to improve the quality of fresh-cut fruit, via maintenance of membrane integrity and reduction of the wound response. The effect of LPE on PLD activity, quality and volatile production of fresh-cut melon was investigated during storage. Charentais-type melons were processed into cubes and vacuum-infiltrated with 200 mg L^{-1} of LPE, packaged in plastic clamshells and stored at 5 ºC for 9 days. LPE was not effective in maintaining overall quality of fresh-cut melon but induced a reduction of aldehydes production in the first three days of storage. LPE inhibited PLD activity in the first day after processing, which may have induced the reduction of aldehydes observed at day three of storage of fresh-cut melon.

In conclusion, oxygen levels significantly affect the volatile profile of fresh-cut melon, and there may be opportunity to optimize oxygen recommendation for aroma preservation. 1-MCP and LPE altered the volatile profile of fresh-cut melon but had minimal or no effect on other quality attributes.

RESUMO

As atuais tecnologias de processamento mínimo são eficazes relativamente à manutenção da aparência da fruta minimamente processada, durante o período mínimo de 7 a 14 dias, necessários para o funcionamento da cadeia de abastecimento. No entanto, os estudos acerca da extensão da vida útil baseada em atributos visuais geralmente negligenciam o efeito das tecnologias de processamento e conservação nas propriedades aromáticas, nutricionais e funcionais da fruta minimamente processada. Neste estudo foram avaliados os efeitos de três tecnologias na qualidade de melão minimamente processado, enfatizando o efeito no aroma: disponibilidade de oxigénio durante o armazenamento, o inibidor da produção e percepção de etileno, 1-metilciclopropeno, e o inibidor da atividade da fosfolipase D, lisofosfatidiletanolamina. Uma das tecnologias mais utilizadas na extensão da vida útil da fruta minimamente processada é a atmosfera modificada. Contudo, os atuais requisitos e recomendações de atmosfera para fruta minimamente processada têm como objetivo a manutenção da cor e da textura e não a produção de aroma. Nesta tese foram monitorizados os atributos de qualidade e o aroma de melão minimamente processado, quando armazenado em atmosfera modificada. Frutos das variedades cantaloupe e honeydew foram processados em cubos e armazenados a 5 °C em atmosfera modificada passiva ou atmosfera controlada (5 kPa O_2 + 10 kPa CO_2, equilíbrio em N_2). Durante o armazenamento das variedades climatérica e não-climatérica, o nível de oxigénio teve um maior impacto no aroma do que nos restantes atributos de qualidade. Os níveis de oxigénio durante o armazenamento afetaram significativamente os voláteis importantes no aroma das variedades cantaloupe e honeydew. O tempo de armazenamento teve também um efeito significativo na produção de voláteis.

A aplicação de 1-metilciclopropeno a frutos inteiros, os seus efeitos na qualidade e na produção e perceção do etileno têm sido alvo de muitos estudos. Em

contrapartida, poucos estudos se têm focado na aplicação do 1-metilciclopropeno a frutos minimamente processados. Pelo seu conhecido efeito na inibição da produção de etileno, foram antecipados efeitos benéficos do 1-metilciclopropeno em alguns atributos de qualidade de melão minimamente processado, mas simultaneamente, uma alteração no perfil aromático. Neste estudo foi avaliado o efeito da aplicação do 1-metilciclopropeno na qualidade e síntese de compostos voláteis importantes no aroma de melão minimamente processado. Melões da variedade cantaloupe foram processados em cubos e tratados com 1.0 µL L^{-1} 1-metilciclopropeno, durante 24 h, a 5 °C, e armazenados durante 9 dias em embalagens de plástico, sob atmosfera normal. O tratamento com 1-metilciclopropeno não afetou os principais atributos de qualidade de melão minimamente processado mas afetou nove dos compostos voláteis importantes no aroma, em particular os derivados dos aminoácidos isoleucina e fenilalanina. O 1-metilciclopropeno afetou a produção dos esteres acetatos de propilo e de 2-metil butil, os butanoatos metil e metil 2-metil, o hexanoato metil, os álcoois 2-metil butil, fenetil e benzyl, e o aldeído heptanal.

A lisofosfatidiletanolamina é descrita na literatura como capaz de inibir a atividade da fosfolipase D, de reduzir a produção de etileno e prolongar a vida útil de muitos produtos hortícolas. Assim sendo, a lisofosfatidiletanolamina poderia representar um tratamento inovador na melhoria da qualidade da fruta minimamente processada, através da manutenção da integridade da membrana celular e da redução da resposta ao ferimento. O efeito da aplicação da lisofosfatidiletanolamina na atividade da fosfolipase D, na qualidade e produção de compostos voláteis foi avaliado durante o armazenamento de melão minimamente processado. Melões tipo Charentais foram processados em cubos, infiltrados a vácuo com lisofosfatidiletanolamina (200 mg L^{-1}), colocados em embalagens de plástico e armazenados a 5 °C, durante 9 dias. A lisofosfatidiletanolamina não foi eficaz na manutenção da qualidade geral de melão minimamente processado mas induziu

a redução na produção de aldeídos nos primeiros 3 dias de armazenamento. A lisofosfatidiletanolamina inibiu a atividade da fosfolipase D no primeiro dia de armazenamento, o que pode ter induzido a redução de aldeídos observada.

Concluindo, os níveis de oxigénio afetam significativamente o perfil aromático de melão minimamente processado, o que representa uma oportunidade de otimizar as atuais recomendações para a preservação do aroma. O 1-metilciclopropeno e a lisofosfatidiletanolamina alteraram o perfil aromático de melão minimamente processado não afetando significativamente os restantes atributos de qualidade.

ACKNOWLEDGMENTS

This PhD program and dissertation were made possible due to the contribution of institutions and special persons, to whom I would like to express my gratitude:

To Fundação para a Ciência e a Tecnologia (Portugal) for my PhD grant (SFRH/BD/39143/2007);

To Escola Superior de Biotecnologia of Universidade Católica Portuguesa for the opportunity and excellent research conditions made available for me as a PhD student;

To my Supervisor, Dr. Domingos P. F. Almeida for believing in my capabilities and in this project, for his guidance, availability, motivation, help and support over the years. His knowledge and commitment to science were essential to my research, enriching it greatly;

To Dr. John Beaulieu and members of his group at the Southern Regional Research Center, New Orleans, USA, and Dr. Juan Pablo Fernández-Trujillo, and members of his group at Universidad Politécnica de Cartagena, Spain, for the opportunity to work at their labs, for the knowledge transfer, technical assistance, encouragement, suggestions and valuable discussions throughout the years;

To Dr. Ana Gomes and Dr. Manuela Pintado for their friendship, affection, support and guidance during my research work;

To my friends Joana Fundo, Manuela Amorim and Ana Raquel Madureira, for their strong friendship, for their help and company during the research experiments, for their affection, support and encouragement. Your friendship and support made the difference.

To my friends and research group colleagues Lígia Pimentel, Vincenza Ferraro, Maria Gião, Ana Catarina Guedes, Alejandra Cardelle, Ana

Oliveira, Helena Gomes, Ana Carvalho, Isabel Franco, Freni Tavaria, Raquel Branquinho, Susana Carvalho, Arménia Santos;

To my friends, Bruno Montenegro, Dina Coito, Fátima Macedo, Fátima Miller, Gabriela Sotto Mayor, Hugo Miller, Nelson Rocha and Pedro Monteiro for their friendship, affection and support. Thank you for sharing with me all the moments of happiness and for the support and company through the hard moments in this journey.

In particular, I would like to extend my deepest gratitude to my parents and brother, for all that they represent to me, for the encouragement to pursuit a dream and for being there through every day of the pathway. Thank you for showing me daily that with you in my life and with your love, strength and emotional support, I can have a dream and reach it…

Finally, I want to thank my husband Paulo and my son, Rodrigo. Paulo, thank you for holding my hand in every challenge I face in life, for your love, for the strength and courage that you give me at every obstacle, for always understanding my doubts, for believing in me and for always making me want to be better. Rodrigo, thank you for all the smiles that you gave in every return home from work, making my days so brighter and my achievements so meaningful. All the difficulties of life seem very small near the greatness of my feelings for you both…

LIST OF SYMBOLS AND ABBREVIATIONS

ABA……..….Abcisic acid

ACC…….……1- Aminocyclopropane-1-carboxylic acid

AcInv…….Acid invertase

ACO……..…1- Aminocyclopropane-1-carboxylic acid oxidase

ACS……1- Aminocyclopropane-1-carboxylic acid synthase

ADH…….…….Alcohol dehydrogenase

ATT……..... Alcohol acetyltransferase

β-Gal……..β-galactosidase

β-Glc……...….β-glucanase

C*……..……...Chroma

CA…….……Controlled atmosphere

Chlase……Chlorophyllase

DAG……..Diacylglycerol

EPA…….Environmental Protection Agency

EPT……Ethanolaminephosphotransferase

FFA…….Free fatty acids

GGDP……..….... Geranylgeranyldiphosphate

h°……...……...Hue angle

HPD…….……Hydroperoxide dehydratase

HPL……..….Hydroperoxide lyase

HPLC-DAD……High-Performance Liquid Chromatography with Diode-Array Detection

L*……..…..……Lightness

LPE……Lysophosphatidylethanolamine

LOX……..Lipoxygenase

MAP……Modified atmosphere packaging

1-MCP……..1-Methylcyclopropene

PA……..…….Phosphatidic acid

PAL……...Phenylalanine ammonia lyase

PG……..……Polygalacturonase

POD……...Peroxidase

PLA……..……Phospholipase A

PLC…….Phospholipase C

PLD……..….Phospholipase D

PME…….…...Pectin methylesterase

PE……....…….Phosphatidylethanolamine

PP…….Phosphatidate phosphatase

PPO…….Polyphenol oxidase

PS…….Phytoene synthase

ROS……..…….Reactive oxygen species

SAM…….…...S-adenosyl-L-methionine

SBSE……..Stir bar sorptive extraction

SPME……Solid phase microextraction

SSC……...……Soluble solids content

KEYWORDS

Antioxidant activity

Aroma

β-Carotene

Controlled atmosphere

Cucumis melo

Ethylene

Lipoxygenase

Lysophosphatidylethanolamine

1-Methylcyclopropene

Minimal processing

Modified atmosphere packaging

Oxygen

Phenolics

Phospholipase D

Volatile compounds

SCOPE AND OUTLINE

This study aimed at understanding the effect of three, current and novel, technologies in the overall quality of minimally processed melon fruit, with emphasis on aroma volatiles. Oxygen levels in the atmosphere, the inhibition of ethylene action by 1-MCP and the PLD inhibitor LPE were evaluated for their effects on general quality, key-nutrients, and aroma volatiles of fresh-cut melon. These compounds are anticipated to have beneficial effects on some quality attributes of fresh-cut melon and to alter the profile of flavor volatiles. These studies contribute to the understanding of the effects of oxygen, ethylene and lipid catabolism to the synthesis of volatiles in fresh-cut melon and provide useful information for developing processes aimed at the modulation of aroma of minimally processed melon.

This thesis is organized in 6 chapters. Chapter 1 presents the state of the art. In this chapter, minimally processed fruits are defined, current fresh-cut technologies are briefly reviewed, and wound physiological responses and effects in fresh-cut fruit are described. Focus is placed on MAP, not only for its importance in the fresh-cut industry, recognized impact in quality but specifically for our interest in evaluating the effects of MAP on aroma production during storage. 1-MCP mode of action is presented and its use as a complementary technology for fresh-cut produce is described.

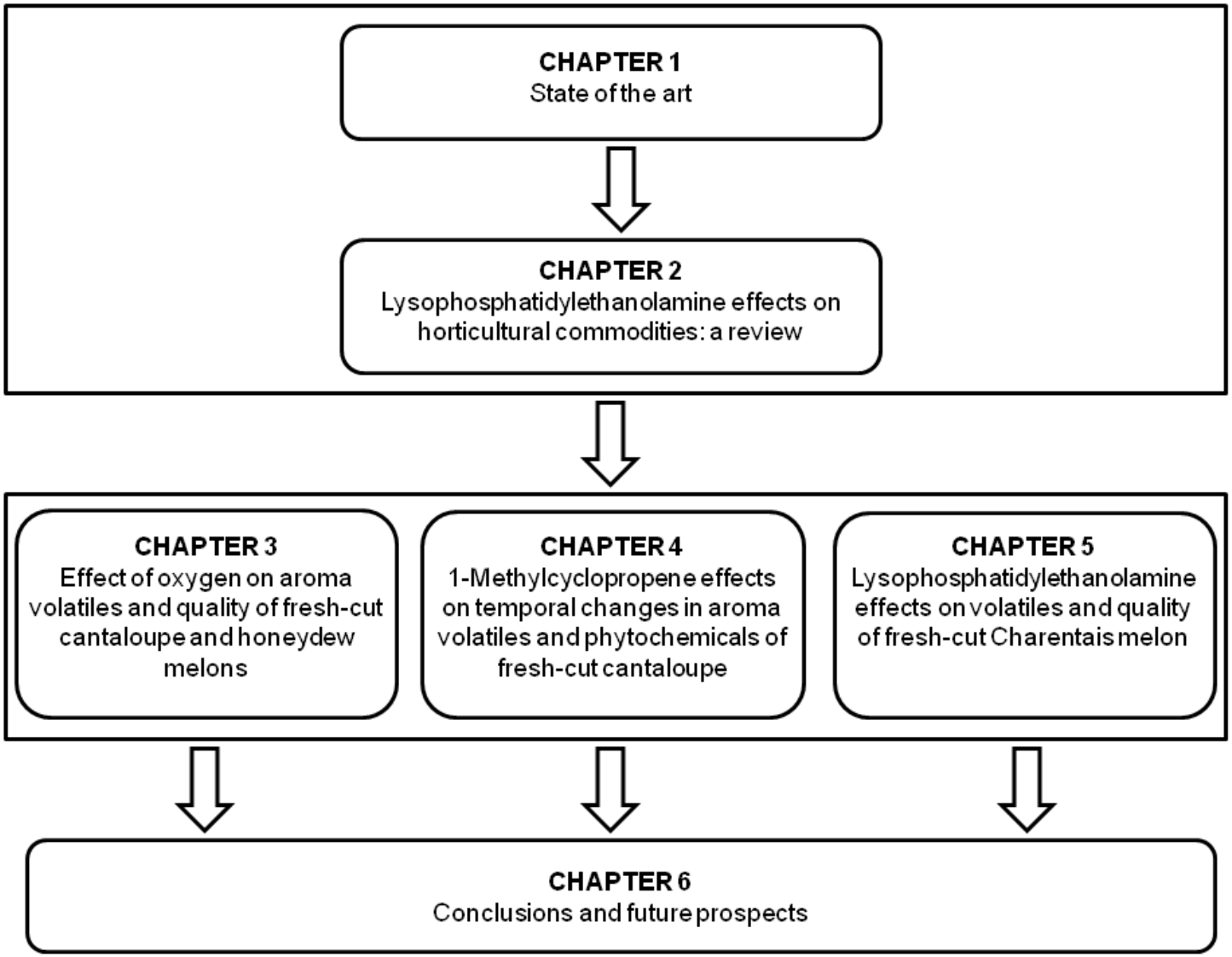
Chapter 2 consists on a comprehensive and critical review of LPE effects on horticultural commodities. Physiological and biochemical LPE effects are listed and the existing knowledge gaps regarding the regulatory role of exogenously applied LPE are highlighted. In the pursue of understanding the regulatory role of LPE in plant tissues, based on literature described effects, possible modes of action were analyzed and a tentative model to explain the diversity of effects is proposed.

To establish baseline information on aroma volatiles and other quality changes during storage of fresh-cut melon, two cultivars, with different climacteric behaviors

were analyzed during storage at 5 ºC under MAP and CA (5 kPa O_2+10 kPa CO_2). These results and the particular effects of oxygen levels on aroma volatiles are reported in Chapter 3.

1-MCP and LPE effects on fresh-cut cantaloupe quality during storage with emphasis in aromatic profile, and the evolution of the content in main phytochemicals responsible for the functional properties of fresh-cut melon, were evaluated and are presented in Chapters 4 and 5, respectively.

The results from these studies allow the integration of physiological information obtained from using low oxygen, 1-MCP and LPE treatments in fresh-cut melon and lead to the general discussion and conclusions, and the identification of future prospects in Chapter 6.


CHAPTER 1
State of the art
CHAPTER 2
Lysophosphatidylethanolamine effects on horticultural commodities: a review
CHAPTER 3
Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons
CHAPTER 4
1-Methylcyclopropene effects on temporal changes in aroma volatiles and phytochemicals of fresh-cut cantaloupe
CHAPTER 5
Lysophosphatidylethanolamine effects on volatiles and quality of fresh-cut Charentais melon
CHAPTER 6
Conclusions and future prospects

The results presented in this thesis have been partially presented in national and international scientific meetings and subjected to peer reviewed publication in international scientific journals and conference proceedings, or have been acepted for publication, as follows:

Chapter 1:

Amaro, A. L. and Almeida, D. P. F. 2008. Preservar o prazer: o problema do aroma dos frutos minimamente processados. Oral communication at the Seminar IV Gama de Produtos Hortofrutícolas: da Fisiologia à Tecnologia, July 30, Alcobaça, Portugal.

Amaro, A. L. and Almeida, D. P. F. 2010. Compostos do aroma e qualidade de fruta IV Gama. Comunicação oral at the Seminar IV Gama Hortofrutícola em Portugal: Investigação e Industrialização, November 19, Foz do Arelho, Portugal.

Amaro, A. L. and Almeida, D. P. F. 2010. Compostos do aroma: fronteira da qualidade. Frutas, Legumes e Flores 114: 54-55.

Chapter 2:

Amaro, A. L. and Almeida, D. P. F. 2012. Lisofosfatidiletanolamina: uma história de modulação química do amadurecimento e senescência. Oral communication at the Seminar “Atualizações sobre Tratamentos Pós-colheita de Produtos Hortofrutícolas” March 15, Batalha, Portugal.

This chapter will be submitted for publication in an international scientific journal as: Amaro, A. L. and Almeida, D. P. F. Lysophosphatidylethanolamine effects on horticultural commodities: a review.

Chapter 3:

Amaro, A.L., Beaulieu, J.C., Stein, R.E. and Almeida, D.P.F. 2010. Volatile and quality changes in fresh-cut cantaloupe and honeydew melons stored in modified atmosphere packaging. In Nunes, C. (Ed). *Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables*, Universidade do Algarve, Faro, pp. 201-206.

Amaro, A. L.; Beaulieu, J. C.; Stein, R. E.; Almeida D. P. F. and Malcata, F. X. 2009. Volatile and quality changes in fresh-cut cantaloupe and honeydew melons stored in modified atmosphere packaging. Oral communication at COST 924 Conference: Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables, January 14-16, Faro, Portugal.

Amaro, A. L.; Beaulieu J. C.; Stein R. E.; Almeida D. P. F. and Malcata, F. X. 2009. Volatile composition and quality of fresh-cut *Cucumis melo* var Cantaloupensis and Inodorus, as affected by modified and controlled atmospheres. Poster presented at 6th International Postharvest Symposium, April 8-12, Antalya, Turkey.

Amaro, A. L.; Beaulieu, J. C.; Stein, R. E.; Almeida, D. P. F and Malcata, F. X. 2010. Volatile composition and quality of fresh-cut *Cucumis melo* var *Cantaloupensis* and *Inodorus* as affected by modified and controlled atmospheres. Acta Horticulturae 877(4): 603-610.

Amaro, A. L.; Beaulieu, J. C.; Grimm, C. C; Stein, R. E. and Almeida, D. P. F. 2012. Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons. Food Chemistry, 130 (1): 49-57.

Chapter 4:

Amaro, A. L.; Fundo, J. F.; Oliveira, A.; Beaulieu, J. C.; Fernández-Trujillo, J. P. and Almeida, D. P. F. 2012. 1-Methylcyclopropene effects on temporal changes in aroma volatiles and phytochemicals of fresh-cut cantaloupe. Journal of the Science of Food and Agriculture (in press).

Chapter 5:

Amaro, A. L.; Fundo, J.; Beaulieu, J. C.; Stein, R. E.; Fernandez-Trujillo, J. P. and Almeida, D.P.F. 2012. Lysophosphatidylethanolamine effects upon volatiles and quality of fresh-cut cantaloupe melon. Acta Horticulturae (in press).

Amaro, A. L.; Fundo, J.; Beaulieu, J. C.; Stein, R. E.; Fernandez-Trujillo, J. P. and Almeida, D. P. F. 2010. Lysophosphatidylethanolamine effects upon volatiles and quality of fresh-cut cantaloupe melon. Oral communication at 24th International Horticultural Congress, 22-27 August, 2010, Lisbon.

This chapter will be submitted for publication in an international scientific journal as: Amaro, A. L.; Fundo, J.; Beaulieu, J. C.; Stein, R. E.; Fernandez-Trujillo, J. P. and Almeida, D. P. F. 2010. Lysophosphatidylethanolamine Effects on Volatiles and Quality of Fresh-cut Charentais Melon.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

State of the art

1.1 Introduction

Fruits and vegetables are major components of a healthy diet. Increased intake of vitamins, antioxidants, minerals, dietary fiber (Gil *et al*., 2006), carotenoids, flavonoids, and other phenolics are recognized benefits from a higher consumption of fruits and vegetables. These fresh produce protect overall health, namely by improving the immune response, helping maintain eyesight, lowering the incidence of certain types of cancer and the risk of heart disease, and preventing degenerative diseases (Kaur and Kapoor, 2001).

Lifestyles of modern consumers, along with the demand for natural, fresh, flavorful, convenient and high quality products with health benefits have raised the production and consumption of fresh-cut fruit (Oms-Oliu *et al*., 2010). The continuous increase in the consumption of fresh-cut produce implies the need to improve supply and distribution systems and to ensure safety and quality of these products. Convenience, therefore, is of paramount importance to sustain consumption of fresh fruits and vegetables by urban consumers. Minimal processing adds to this convenience.

Fresh-cut processing and preservation resort to several hurdles to maintain quality and safety during shelf-life. The study of the effects of processing and preservation technologies on the quality of fresh-cut fruit has emphasized appearance and limited information is available on their impact on aroma volatiles. It is known, though, that shelf-life is shorter when flavor is used as a criterion instead of appearance (Pelayo *et al*., 2003). In addition to current preservation technologies (*e.g.* refrigeration and MAP), emerging and novel technologies need to be assessed for their potential use in fresh-cut preservation. 1-MCP, a known inhibitor of ethylene action, is expected to provide benefits (Toivonen, 2008) but, simultaneously, to change the aroma volatiles in an unknown way. Considering the wound response role of PLD (EC 3.1.4.4) (Wang, 2000), the inhibition of this enzyme is anticipated to

improve membrane integrity, therefore help maintain the quality of fresh-cut fruit. On the other hand, by reducing the availability of free fatty acids (FFA) (Ryu and Wang, 1998) that are catabolized into volatiles by the lipoxygenase (LOX; EC 1.13.11.12) pathway (Sanz *et al*., 1997), the inhibition of PLD is also expected to alter the profile of aroma volatiles. If proven effective, LPE, a reported *in vitro* inhibitor of PLD, may provide a novel technology for the preservation of fresh-cut fruit.

Therefore, the specific objectives of this study were to evaluate the effect of oxygen levels, 1-MCP and LPE on the overall quality, antioxidant phytochemicals, and aroma volatiles of fresh-cut melon. Melon was chosen as model system for these studies because it accounts for a major fraction of the fresh-cut fruit in Europe and America (Rojas-Graü *et al*., 2011), its shelf-life is limited by textural changes and juice retention problems (Toivonen, 2008) likely to be improved by the treatments, and because its aroma is essential for consumer acceptability (Beaulieu, 2006a,b).

1.2. Minimally processed fruits

The International Fresh-cut Produce Association defines a fresh-cut product as fruits or vegetables that have been trimmed and/or peeled and/or cut into 100% usable product that is bagged or pre-packaged.

Different techniques can be used in fresh-cut processing but there are critical common steps to most fresh-cut fruits preparation diagrams. To avoid risks associated with microbial growth and simultaneously ensure quality preservation during storage of fresh-cuts, raw materials have to be carefully inspected, discarded if damaged or injured, and selected for color, size and maturity stage uniformity. The next steps in processing sequence are fruit washing and sanitation, followed by coring, peeling, and size reduction operations. Depending on fruit type and peel characteristics, different washing and sanitizing techniques can be used and new

techniques for maintaining quality and inhibiting undesired microbial growth are demanded in all steps of the production and distribution chain of fresh-cut products. Guidelines for packing fresh or minimally processed fruit and vegetables generally specify a washing or sanitizing step to remove dirt, pesticide residues, and microorganisms responsible for quality loss and decay (Sapers, 2003). Although several sanitizing agents are available for fruits and vegetables, the use of chlorine solutions is the most common method, for its low cost and high effectiveness even at low concentrations (Issa-Zacharia *et al*., 2010). When chlorine use has been forbidden (Germany, Holland and Denmark), hydrogen peroxide and peroxyacetic acid are often used as sanitizing agents (Ölmez and Kretzschmar, 2009). Fruit peeling is usually carried out mechanically but infusion methods for chemical, enzymatic or thermal peeling have also been developed (Toker and Bayindirli, 2003; Barreiro *et al*., 2007; Panchev *et al*., 2011). Peeling, a primary stress experienced by fresh-cut produce, causes major tissue disruption, whereby enzymes and substrates sequestered in different organelles come in contact (Beaulieu and Gorny, 2001). Also, signalling-induced wound responses are initiated and microbiological, enzymatic, and physicochemical reactions simultaneously take place, causing negative quality changes (Artés *et al*., 2007).

In addition to sanitation, removal of unwanted parts, and size reduction, preservation operations are required to maintain the quality and safety of fresh-cut fruit throughout the supply chain.

1.3. Preservation technologies for fresh-cut fruit

The objectives of fresh-cut preservation technologies are to deliver to the consumers a convenient, safe, fresh-like product with adequate shelf-life, and high nutritional and sensory quality (Reyes, 1996). The use of low temperatures before,

during, and after processing is essential for quality preservation (Watada and Qi, 1999). The rate of metabolic reactions that happen in fresh-cut products and result in changes in quality, either chemical or enzymatic, is highly dependent on temperature (Wiley, 1994). Low temperature is an absolute necessity in fresh-cut industry, affecting, simultaneously and in a positive way, respiration rate and metabolic activity (ripening, senescence, and wound response), microbial growth and water loss. Storage temperatures (Bai *et al*., 2003), MAP and CA (O'Conner-Shaw *et al*., 1996; Ayhan *et al*., 1998; Portela and Cantwell, 1998; Qi *et al*., 1999; Bai *et al*., 2003; Oms-Oliu *et al*., 2008a, b), films and coatings (Oms-Oliu *et al*., 2008c; Raybaudi-Massilia *et al*., 2008; Sangsuwan *et al*., 2008), use of ozone gas (Selma *et al*., 2008), hot water treatments (Aguayo *et al*., 2008; Silveira *et al*., 2011), use of calcium dips (Luna-Guzman *et al*., 1999; Luna-Guzman and Barrett, 2000; Saftner *et al*., 2003; Lamikanra and Watson, 2007; Silveira *et al*., 2011), application of inhibitors of specific chemical reactions or metabolic pathways (Gorny *et al*., 2002) and more recently, use of electron beam treatment (Kim *et al*., 2010), UV-C light treatment (Manzocco *et al*., 2011) and intense light pulses (Ramos-Villarroel *et al*., 2012) are some of the techniques utilized to reduce the damage caused by wounding.

These chemical and physical preservation strategies can be presently used, often in combination, to reduce loss of quality and increase safety of fresh-cut fruits and vegetables (Reyes, 1996). However, absolute control of all the undesirable parameter changes that compromise the quality and shelf-life of minimally processed products cannot probably be reached and key issues are still unknown; in particular, the effect of preservation technologies on aroma remain largely ignored. Little sensory research has been published on the flavor quality of fresh-cut melon, or other fresh-cut produce, in contrast with extensive research that has been conducted on whole fruit flavor. Oxygen levels, however, are expected to affect the aroma volatiles in fresh-cut fruit (Kader and Ben-Yehoshua, 2000).

Modified atmosphere packaging

Fresh-cut fruits and vegetables are generally packaged in flexible plastic films or rigid plastic containers, which naturally attain an atmosphere within the package, with modified composition. The influence of O_2 and CO_2 levels on plant metabolic processes has lead to the modification of their natural concentrations for the purpose of extending shelf-life (Beaudry, 1999). MAP implies altering the gases surrounding the commodity to produce the desired composition, different from that of air (Al-Ati and Hotchkiss, 2002), and generally having higher CO_2 and lower O_2 levels. The beneficial effects of high carbon dioxide in storage of fruits and vegetables is generally thought to arise from suppression of respiration (Beaudry, 1999), reduction of ethylene biosynthesis (de Wild *et al*., 2003), and suppression of fungal growth in commodities that tolerate inhibitory levels of CO_2 (Pérez and Sanz, 2001). Low O_2 availability lowers respiration rate and ethylene biosynthesis (Bender *et al*., 2000). Still, the actual possibilities and benefits of reducing respiration rate via reduction of O_2 levels has been questioned in specific commodities (Beaudry, 2000; Gomes *et al*., 2010), including fresh-cut melon (Gomes *et al*., 2012).

Low O_2 atmospheres combined with adequate CO_2 concentrations have been used in fresh-cut fruits, namely melon (O'Connor-Shaw *et al*., 1996; Gorny, 1997; Ayhan and Chism, 1998; Portela and Cantwell, 1998; Qi *et al*., 1999; Bai *et al*., 2001; Bai *et al*., 2003; Oms-Oliu *et al*., 2008a), apple (Gunes *et al*., 2001; Soliva-Fortuny *et al*., 2004), strawberry (Odriozola-Serrano *et al*., 2010), tomato (Aguayo *et al*., 2004a), mango (Beaulieu and Lea, 2003) and pineapple (Marrero and Kader, 2006) reducing respiration and ethylene production rates, inhibiting or delaying enzymatic reactions thus reducing quality losses. Although low oxygen reduces respiration, the inhibition of ethylene action, rather than its inhibition of respiration appears to be the basis by which low oxygen extends the storage life of many crops (Saltveit, 1999). On the other hand, effective and efficient design of MAP systems for fresh-cut produce requires

information as produce respiration rates in various temperature ranges and atmospheres (Gorny, 2001). Exposure to O_2 or CO_2 levels outside the limits of tolerance may lead to anaerobic respiration with the production of undesirable metabolites and other physiological disorders (Soliva-Fortuny *et al*., 2002). The final gas level in a MAP system depends on film permeability and product respiration rate, thus, for a given product of known weight and storage temperature, proper film selection is essential for attaining the desired gas mixture inside the package (Bai *et al*., 2001).

1.4. Physiological wound-induced responses

Wound damage is sensed by sensors/receptors that recognize increased concentrations of endogenous molecules or their fragments, and tissue response involves the production of ethylene and jasmonate (Heil, 2012), increased respiration rate, and membrane deterioration (Watada *et al*., 1990; Wiley, 1994; Watada and Qi, 1999; Toivonen and DeEll, 2002).

1.4.1. Respiration rate

Respiration is a complex metabolic process that releases energy for plant chemical pathways (Fonseca *et al*., 2002). Respiration rate of fresh-cut commodities is, in general, higher than that of the corresponding whole fruits (Watada *et al*., 1996). Aguayo *et al*. (2004b) reported higher ethylene production and respiration rates during storage of fresh-cut melon at 5 ºC when compared to whole fruits. An increase in CO_2 production observed immediately after cutting has been associated with enhanced synthesis of enzymes involved in the respiratory pathway and enhanced ethylene production rate. After this wound-induced respiration peak, usually fresh-cut fruits show a decrease in the production of CO_2 during storage, followed by an increase

close to the end of storage time, attributed to possible microbial growth and general tissue deterioration (Aguayo *et al*., 2003). Respiration rate of fresh-cut melon depends on cultivar (Madrid and Cantwell, 1993), maturity stage (Fonseca *et al*., 2002), processing efficiency and storage temperature (Aguayo *et al*., 2004b). Respiration rate is also affected by MAP or CA (Oms-Oliu *et al*., 2008a,). Low O_2 availability in the package decreases respiration rate of fresh-cut fruits by reducing overall metabolism. Bai *et al*. (2001) reported lower respiration rate of fresh-cut cantaloupe melon stored under flushed modified atmosphere with 4 kPa O_2 and 10 kPa CO_2, when compared with the internal gas mixture attained naturally in the packages. Nevertheless, the magnitude of reduction of respiration rate via reduction of O_2 levels and its practical implications in extending the shelf-life have been questioned in fresh-cut pear (Gomes *et al*. 2010) and melon (Gomes *et al*., 2012).

Generally, studies on the use of modified atmospheres during fresh-cut fruit storage target the respiration reduction and consequent impact on products quality and shelf-life. However, there is a lack of studies that monitor the effect of low oxygen packaging in aroma production, evolution and alteration during fresh-cut fruit storage.

1.4.2. Ethylene production

Ethylene is a simple gaseous two-carbon plant hormone, active in very low amounts (0.01 µL L^{-1}), that plays a key regulatory role in many physiological processes (Saltveit, 1999). Ethylene production in plant tissues results from methionine metabolism. Briefly, ethylene is synthesized from methionine in three steps: conversion of methionine to S-adenosyl-L-methionine (SAM) catalyzed by the enzyme SAM synthetase (SS; EC 2.1.1.13), formation of 1- aminocyclopropane-1-carboxylic acid (ACC) from SAM via ACC synthase (ACS; EC 4.4.1.14) activity, and the conversion of ACC to ethylene, which is catalyzed by ACC oxidase (ACO; EC 1.14.17.4) (Wang *et al*., 2002).

Ethylene can profoundly affect quality of harvested climacteric fruits. Ethylene perception regulates the expression of downstream genes which produce the typical ripening events including color changes, altered sugar metabolism, fruit softening and alterations in texture, increased susceptibility to pathogen infection and volatile compounds synthesis (Flores *et al*., 2002; El-Sharkawy *et al*., 2005). Genes encoding cell wall degradation, ethylene production and pigment biosynthesis enzymes were among the first ethylene-responsive genes which have been isolated from tomato fruit (Bapat *et al*., 2010).

Besides its physiological roles in different developmental stages, ethylene is also a stress plant hormone, whose synthesis is induced in a variety of stress signals, such as mechanical wounding, chemicals, extreme temperatures and pathogens (Johnson and Ecker, 1998). A commonly observed response to fresh-cut processing is enhanced ethylene production (Watada and Qi, 1999). Stress-induced ethylene production is typically controlled by accelerating the conversion of SAM to ACC, suggesting that the expression of ACS is the major target of regulation (Zheng *et al*., 2005). Increases in production of stress ethylene are a result of an increase in ACS activity suggesting that this enzyme can be induced by wounding.

The manipulation of ethylene biosynthesis and perception can be done via genetic, molecular or chemical approaches. Genetic variations in ethylene production rate and in ethylene sensitivity exist within species (Klassen and Bugbee, 2004), and can be explored to obtain less sensitive cultivars. Molecular approaches with transgenic plants have been used to reduce ethylene biosynthesis and action. Strong reduction in ethylene production was achieved through downregulation of ACS and ACO genes in fruit and flowers (Ayub *et al*., 1996; Silva *et al*., 2004; Serek *et al*., 2006) and the mutation in ethylene binding sites created ethylene insensitivity in *Arabidopsis thaliana* and tomato (Chang *et al*., 1993; Tieman *et al*., 2000). Chemicals that inhibit biosynthetic enzymes or block the ethylene receptor are also known. For

example, both ACS and ACO can be inhibited by nitric oxide, ACO can also be inhibited by ethanol and the receptor can be blocked by silver (Singh *et al*., 2007; Asoda *et al*., 2009; Zaharah and Singh, 2011). Among these chemicals, 1-MCP stands out as a powerful tool to maintain fruit quality through ethylene inhibition (Watkins, 2006). The effects of 1-MCP on fresh-cut fruit will be discussed in chapter 4.

1.4.3. Membrane phospholipids and enzyme activity

Functional alterations of membranes occur during ripening, senescence and after wounding, and have been correlated with changes in the lipid metabolism (Thompson *et al*., 1998). Changes in the proportions and composition of lipids within cell membranes, as well as fatty acid remodeling of the phospholipid fraction, have an important role in ripening and senescence stages, changing the physical properties of the lipid matrix and consequently the functional activity of membrane-associated proteins (Paliyath *et al*., 1984).

Minimal processing injures and destabilizes cells, leading to membranes loss of functionality, via chemical and biophysical changes in membrane lipids and proteins (Marangoni *et al*., 1996). PLD, phosphatidic acid phosphatase (EC 3.1.3.4), lipolytic acyl hydrolase (EC 3.1.1.5) and LOX, are lipid-degrading enzymes associated with microsomal membranes. Enhanced by wounding, deterioration of membranes results from the action of these four cascade cell-wall enzymes (Paliyath and Droillard, 1992; Karakurt and Huber, 2003).

PLD is a key enzyme in the metabolism of membrane lipids in that it mediates the first step in accelerated phospholipid breakdown (Wang, 2000), generating lipids that are involved in signal transduction, cell proliferation, signaling pathways, and in senescence. PLD hydrolyzes membranes glycerol-phospholipids at the phosphodiesteric bond terminal, producing phosphatidic acid (PA) and a free hydrophilic head group. PLDs in the cell may be activated differently, hydrolyze

different lipid species, and have unique functions. The varied substrate specificities and preferences of PLD isoforms suggest that activation of different PLDs may result in selective hydrolysis of membrane phospholipids and therefore in different wound-response pathways and reactions. Wounding stimulates an increase in cytoplasmic Ca^{2+}, which promotes translocation of cytosolic PLD to bind microsomal membranes, and such translocation increases PLD-mediated hydrolysis of phospholipids (Ryu and Wang, 1996).

A coordinate activation of PLD and phospholipase A_2 (PLA_2; EC 3.1.1.4) enzymes upon wounding triggers an increased release of FFA in membranes for jasmonic acid (JA) biosynthesis and downstream defense-gene activation (Chapman, 1998). The liberated polyunsaturated fatty acids serve as substrates for LOX that produces activated oxygen and lipid peroxides contributing even further to membrane degradation (Paliyath and Droillard, 1992). Substrates for LOX, such as linoleic and linolenic acids, are common constituents of plant membranes but are not available for enzymes unless they are in the free acid form (Leshem, 1984). LOX is essentially latent until FFA are released, which occurs after mechanical wounding.

LOX catalyzes the peroxidation of FFA, leading to the formation of highly reactive hydroperoxides that can, by themselves, further degrade the membrane bilayer (Matsushita, 1975; Hildebrand *et al*., 1988). The hydroperoxide dehydratase (HPD; E.C 4.2.1.92) pathway results in the formation of allene oxide, which can be hydrolyzed to form ketols or can undergo rearrangement to form JA, an essential compound in wound response and senescence. Through the hydroperoxide lyase (HPL; EC 4.1.2.-) pathway, hydroperoxide linolenic and hydroperoxide linoleic acids are broken-down and lead to the formation of short chain (C_6-C_9) aldehydes, as hexenal or (*Z*)-3-hexenal (reviewed by Feussner and Wasternack, 2002). In further reactions, (*Z*)-3-hexenal can form (*Z*)-3-hexenol, (*E*)-2-hexenol, (*E*)-3-hexenol and (*E*)-2-hexenal.

Considering these metabolic pathways, it is anticipated that inhibition of PLD by LPE would reduce fresh-cut melon membrane lipid degradation and improve quality retention through the suppression of lipid-derived compounds, resulting in a change in aroma volatiles. In support of this hypothesis, transgenic modification of fatty acid biosynthesis in tomato leaves led to significant changes in emitted volatiles (Wang *et al*., 2001). The relevance of fatty acid metabolism for aroma production is further illustrated by observations on CA-induced inhibition of LOX activity in 'Fuji' (Lara *et al*., 2006) and 'Mondial Gala' (Lara *et al*., 2007) apples, leading to abnormal fruit aroma after transfer from hypoxia to air.

1.5. Wound effects on quality attributes

Wound-induced symptoms include discoloration and tissue softening (Toivonen and Brummell, 2008), decline in levels of nutrients (Gil *et al*., 2006), membrane breakdown (Hodges *et al*., 2000) and aroma alteration and development of off-odors (Beaulieu, 2006a).

1.5.1. Color

Color may be defined as the impact of the wavelengths of light in the visual spectrum, from 390 to 760 nm, on the human retina and is one of the major attributes which affect the consumer perception of quality (Toivonen and Brummell, 2008). Color is one of the main attributes that characterizes the freshness of most fresh-cut fruits and vegetables, as consumers take product appearance as a primary criterion in food choice and acceptability, and may even influence taste thresholds, sweetness perception and pleasantness (Clydesdale, 1993).

Fresh-cut processing damages cells and tissues increasing their vulnerability to dehydration and discoloration. Fresh-cut products undergo changes in color due to

different biochemical processes, mainly chlorophyll and carotenoid degradation, and overall activities of phenylalanine ammonia lyase (PAL; EC 4.3.1.24) and often peroxidase (POD; EC 1.11.1.7) and/or polyphenol oxidase (PPO; EC 1.14.18.1) (Saltveit, 2000). Fresh-cut tissue browning is explained by the *de novo* synthesis of phenolic compounds due to increased PAL activity and by the tissue oxidation due to increased POD activity. Fresh-cut melon is not very susceptible to surface browning. The common color changes observed during fresh-cut melon storage are decreases in lightness (L*) and whiteness index, generally attributed to translucency symptoms (Aguayo *et al*., 2004b). In carrots, whitening also is due to lignin formation and surface dehydration of the outer layers (Cisneros-Zevallos *et al*., 1997). In melons, enzymatic color changes are primarily affected by POD enzymes. Lamikanra and Watson (2000) demonstrated that cantaloupe POD activity could be related to the tissue response to increased oxidative stress in the cut fruit.

1.5.2. Texture

Fruit texture relates to the mechanical properties induced by tissue structure (Toivonen and Brummell, 2008), namely cell size, shape and packing, cell-wall thickness and strength, and cell turgor and adhesion status. Texture of fruits and vegetables is often interpreted in terms of firmness, crispiness, juiciness and toughness, where firm and crisp tissues are mostly desired in fresh produce (Toivonen and Brummell, 2008). Disassembly of the primary cell wall, reduction in cell–cell adhesion following dissolution of the pectin-rich middle lamella and degradation of a specific subset of xyloglucan polymers that are tightly bound to cellulose microfibrils are believed to contribute to fruit softening (Wakabayashi, 2000). These modifications in cell wall polymers during ripening involve the co-ordinated and interdependent action of numerous cell wall-modifying enzymes and proteins (Nishiyama *et al*., 2007). β-Galactosidase (β-Gal; EC 3.2.3.23), polygalacturonase

(PG; EC 3.2.1.15), pectin methylesterase (PME; EC 3.1.1.11), xyloglucan endotransglycosylase (EC 2.4.1.207), endo-β-glucanase (β-Glc; EC 3.2.1.4) and expansin are key-enzymes in cell-wall modification and tissue softening (Moreno *et al*., 2008). Cutting operations result in dramatic increase in these processes and loss of firmness, as pectinolytic and proteolytic enzymes exuding from injured cells diffuse into inner cells (Karakurt and Huber, 2003). Although PME activity is not affected, ethylene regulated-cell wall modifications are major contributors to the accelerated cell wall disassembly and increased melon softening (Nishiyama *et al*., 2007). Softening can also be caused by transformation of protopectin to water-soluble pectin, decrease in cellulose crystallinity, and thinning of cell walls, diffusion of sugar to the intercellular spaces, loss of turgor, and ion movement from the cell wall (Toivonen and Brummell, 2008).

Firmness retention is an important quality parameter in fresh-cut fruit products. Increased metabolic and water activities and mixing of intracellular and intercellular enzymes and substrates contribute to texture changes/loss during and after processing (Beaulieu and Gorny, 2001). Fresh-cut melon is very susceptible to softening during storage, even under low temperatures due to enzymatic degradation of the middle lamella of the cell wall and loss of cell adhesion. Also, there appears to be a detrimental trade-off between firmness and acceptable volatiles and flavor/aroma attributes in fresh-cut melon (Beaulieu *et al*., 2004). Cantaloupe cubes prepared from ≥1/2-slip fruit had excellent sensory attributes, texture, and quality compared against less ripe and full slip cubes (Beaulieu *et al*., 2004; Beaulieu and Lea, 2007).

Firmness and resistance to softening in fresh-cut fruit can be increased by the addition of calcium, due to stabilization of membrane systems and formation of Ca pectates increasing the rigidity of the middle lamella and cell walls and retarding PG activity (Poovaiah, 1986). Heat and calcium treatments (Luna-Guzmán and Barrett,

2000; Aguayo *et al*., 2008; Silveira *et al*., 2011) and use of modified atmospheres (Bai *et al*., 2001; Aguayo *et al*., 2007; Oms-Oliu *et al*., 2007) generally allow good firmness maintenance in fresh-cut melon.

1.5.3. Compositional quality

Composition affects several quality attributes, namely, sensory and nutritional attributes such as sweetness, acidity, aroma, firmness, color, vitamins and antioxidants. These are dependent on the physiological stage of the raw material and are also affected by fresh-cut processing. Here we revise the fresh-cut fruit soluble solids, phenolic compounds and carotenoids content and the fresh-cut fruit aroma.

1.5.3.1. Soluble solids content

Consumer's perception of fruit quality is highly correlated with taste, which depends on fruit sugar content and sugar/acid ratio. Fruit sugars can be determined indirectly as soluble solids content (SSC). SSC and aromatic volatiles may both contribute to melon sweetness in terms of human perception, since a number of specific aromatic volatiles in melons are associated to "sweet" aroma description (Saftner *et al*., 2006). Lamikanra *et al*., (2000) found that fresh-cut cantaloupe sugar levels do not vary substantially under refrigerated storage while the reduction in organic acids content is highly influenced by the storage temperature in fresh-cut cantaloupe. Once that fresh-cut melons generally show relative maintenance or small decrease in SSC during storage (Aguayo *et al*., 2008), at harvest, melon pulp should have a minimum of 10% soluble solids concentration for adequate quality (Bianco and Pratt, 1977). These small changes in SSC are attributed to consumption of carbohydrates due to the increased respiration rates (Boyton *et al*., 2006) and due to possible microbial growth (Oms-Oliu *et al*., 2008a).

1.5.3.2. Components with antioxidant activity

Consumers are nowadays more and more concerned with the nutritional qualities of what they eat. Antioxidants play an important role in defending the body against free radical damage, and are associated to lowered risk of cancer, diabetes, cardiovascular and neurological diseases and have been referred as one of the principal reasons for increased consumption of fruits (Prior and Cao, 2000). Fresh-cut fruits are presented as a convenient alternative to supply the dietary needs of fresh food, but wounding can induce changes in antioxidant capacity. Cutting of fruits and vegetables can enhance their total antioxidant capacity (measured using $ABTS^{+}$ or DPPH radical scavenging methods), through increases in wound-induced phenolic levels (Reyes *et al*., 2007). However, wounding, through cells disruption and release of enzymes and substrates, promotes oxidative enzyme-catalyzed processes (Oms-Oliu *et al*., 2008a). Antioxidant capacity of fresh-cut fruits is known to depend on a wide number of compounds, namely flavonoids, phenolic acids, aminoacids, ascorbic acid and tocopherols.

Phenolic compounds occur in all fruits as a diverse group of secondary metabolites with a large range of structures and functions (Robards *et al*., 1999). The phenolic components of fruits constitute a complex mixture of cinnamic acids, flavonoids and catechins (Robards *et al*., 1999). The preservation of the fruit phenolic content has a great impact on the quality of fresh-cuts because of the participation of phenols in enzymatic browning reactions, but also on the nutritional value of fresh-cut products. Wounding may affect phenolic metabolism through ethylene-induced activation of PAL, the key regulatory enzyme in the biosynthesis of phenolic compounds (Ke and Salveit, 1989; Saltveit, 2000), that can result in an increase in antioxidant activity.

Fruits and vegetables are a natural source of carotenoids. Carotenoids are tetraterpenoid pigments, biosynthesized and accumulated in the plastids of leaves,

flowers and fruits, where they contribute to red, orange and yellow colors (reviewed by Cunningham and Gantt, 1998). Two molecules of geranylgeranyl diphosphate (GGDP), the initial precursor for carotenoid biosynthesis, are condensated into phytoene, by phytoene synthase (PS; EC 2.5.1.32), an ethylene up-regulated enzyme (Marty *et al*., 2005). Subsequent sequential desaturations of phytoene lead to the production of lycopene and β-carotene.

The accumulation of carotenoids in fruits occurs concomitantly with changes in pigmentation, firmness, sweetness, acidity, and aroma (Marty *et al*., 2005). The distribution of carotenoids in fruits is subject to considerable variation from one specie to another, such as from the accumulation of an intermediate in the pathway as is the case of red lycopene in ripe tomato, orange β-carotene in orange, and species-specific carotenoids in pepper fruits. Carotenoids are unstable when exposed to oxygen or light (Klein, 1987), all of which may occur when cells are disrupted, and the internal tissue is exposed by cutting. Wound-induced ethylene hastens the oxidation of fatty acids by LOX, during which carotenoids can be degraded by co-oxidation (Biacs and Daood, 2000) leading to a decrease in liposoluble antioxidant capacity in fresh-cut fruits.

Despite these chemical and physiological considerations, minimal processing had almost no effect on the main antioxidant constituents during shelf-life of a variety of fruits (Gil *et al*., 2006) indicating that fresh-cut fruits visually spoil before any significant nutrient loss occurs.

1.6. Aroma

Aroma is determined by the combination of volatile compounds, determinants of fruit sensory quality perceived by the consumer and has therefore to be investigated in detail. Consumers judge quality of fresh-cut fruit on the basis of appearance and freshness at the time of purchase. However, subsequent purchases

depend upon consumer's satisfaction in terms of texture and flavor (Fallik *et al*., 2001; Kourkoutas *et al*., 2006; Beaulieu and Lea, 2007). As an important trait of food quality, fruit aroma has gained increased attention in recent years. The knowledge of flavor and aroma formation pathways, as well as their regulation, is essential for the control of these desirable fruit qualities.

Fruit aroma depends on a complex mixture of a large number of volatile compounds, such as esters, alcohols, aldehydes and sulfur-containing compounds, which contribute to the overall sensory quality of fruit specific to species and cultivar (Sanz *et al*., 1997). Each fruit has a characteristic odor that is determined by the ratio of volatiles and the presence or absence of key compounds is responsible for the fruit aromatic attributes. The contribution of each compound to the specific aroma of each fruit differs according to their perception thresholds, synergism with, and masking of, other components. Some of these compounds are present in very low concentrations in fruit but contribute to the aroma characteristics while others contribute to aroma intensity and aroma quality.

1.6.1. Biosynthesis of volatile compounds

Despite the vast number of chemical structures involved, it has long been known that the large majority of volatile compounds are biosynthesized by a small number of metabolic pathways: conversion of some amino acids like leucine and valine into branched alkyl and acyl compounds of esters and into branched alcohols; production of acids, alcohols, esters and ketones via fatty acid metabolism and enzymatic oxidative splitting of linoleic and linolenic acids into C_6 and C_9 aldehydes (Sanz *et al*., 1997; Defilippi *et al*., 2009). Volatiles important to aroma and flavor are synthesized from aminoacids, membrane lipids and carbohydrates (Sanz *et al*., 1997) and are breakdown products of phytonutrients such as fatty acids, carotenoids, phenolics and terpenoids (Buttery and Ling, 1993).

Esters form the largest group of volatile compounds produced by apple, pear, banana, melon, pineapple and strawberry (Defilippi *et al*., 2009). Volatile esters mainly result from the esterification of alcohols and carboxylic acids, utilizing Co-A moiety or Co-A-ester as the acyl donor (Ueda *et al*., 1997; Shalit *et al*., 2001). The mixture of esters produced by fruit depend on the alcohol acetyltransferase (AAT; EC 2.3.1.84) activity (Ueda *et al*., 1997; Yahyaoui *et al*., 2002), specificity and preference (Dixon and Hewet, 2000; El-Sharkawy *et al*., 2005; Lucchetta *et al*., 2007). Esters are categorized into two classes based on the compound structure (Beaulieu, 2006a). Acetate esters have an acetate ion (acyl group) as the terminal R'_{acid} attached at the carboxylic group via an ester bond, whereas compounds not containing a methyl group at their R' terminus are designated as nonacetate esters (Beaulieu, 2006a; b). When a direct pool of acetyl Co-A is available, esterification of many possible alcohols leads to acetate esters synthesis. Alternatively, AAT will esterify other acyl donors that can arise through decarboxylations or oxidations of pyruvate, amino acids and fatty acids (β-oxidation or LOX-mediated), with available alcohols to form non-acetate esters (Beaulieu, 2006b).

The volatile esters containing a branched alkyl chain originate from leucine (3-methylbutyl and 3-methylbutanoate esters; *e.g*. 3-methylbutyl butanoate and 3-methylbutyl acetate), isoleucine (2-methylbutyl and 2-methylbutanoate esters; *e.g.* ethyl 2-methylbutanoate and 2-methylbutyl acetate), valine (2-methylpropyl and 2-methylpropanoate esters; *e.g.* ethyl 2-methylpropanoate and 2-methylpropyl acetate) and methionine (many sulfur-containing compounds) (Wyllie and Leach, 1990), whereas the aliphatic esters and alcohols are produced from FFA such as linoleic and linolenic acids (Baldwin *et al.*, 2000). The compounds with shorter chains can be generated from fatty acids by β-oxidation (Sanz *et al*., 1997). Both pathways converge in the formation of aldehydes, which are later reduced to alcohols in a reaction carried out by alcohol dehydrogenase (ADH; EC 1.1.1.1) (Manríquez *et al*., 2006). The

combination of different alcohols, arising from either lipids or amino acids derived from degradation of lipids and proteins (Sanz *et al*., 1997), and acyl-CoAs, result in a wide range of esters emitted by the fruit. On the other hand, it is possible that this diversity also arises from the multiplicity of genes encoding AAT enzymes with different specificity (El-Sharkawy *et al*., 2005).

1.6.2. Fresh-cut fruit aroma

Minimal processing may create significant negative changes in aroma. There are also synergistic interactions between numerous factors such as variety, source, season, initial maturity, optimum processing maturity, slicing and cutting equipment, sanitation and chemical treatments, packaging (including MAP) and temperature management that may have negative consequences on sensory quality. Still, minimal processing may also have positive effects, such as the generation of some volatile compounds important in the fruit aroma. In some cases, cell disruption is necessary in order to allow enzyme interaction with aromatic precursor compounds (Buttery, 1993).

Most primary aroma compounds in cut fruits are believed to be products of β-oxidation of fatty acids and secondary compounds that result from fatty acid oxidation via the LOX pathway (Beaulieu and Baldwin, 2002). In cut fruits, there may be increased oxidative reactions (*e.g.* β-oxidation, β-carotene breakdown and LOX activity) that are required to deliver various straight chain fatty acids and cyclic moieties, which condense with alcohols to recycle esters (Beaulieu, 2006a). Production of key volatiles derived from aminoacids increase immediately after processing as a result of increased metabolic activity, and decrease during subsequent storage as glycolysis and tricarboxylic acid cycle pathway become less active as senescence approaches (Beaulieu, 2006a).

Upon wounding, rapid increases in LOX and HPL activities are observed (Myung *et al*., 2006) and a number of volatiles derived from LOX and HPL pathways

may be released. Also, after wounding, fresh-cut fruit total fatty acids increase which may be a consequence of cellular events in which enzymes involved in biosynthesis of fatty acids, fatty acid binding-proteins involved in transfer of fatty acids to membranes, and/or desaturases are activated to fill disrupted membrane bilayers as a wound healing process (Myung *et al*., 2006).

LOXs are a group of enzymatic proteins that can oxidize free polyunsaturated fatty acids, namely linoleic, linolenic, and arachidonic acids, to obtain the corresponding conjugated hydroperoxides, in which the double bonds are in a *cis-trans* configuration (Siedow, 1991). These enzymes catalyze the first reaction of the complex metabolic pathway that leads to the formation of C_6 and C_9 volatile compounds (Feussner and Wasternack, 2002). Through the action of LOX, polyunsaturated fatty acids oxygenation is catalyzed, resulting in a hydroperoxide located at carbon 9 or carbon 13, depending on the isozyme (Riley *et al*., 1996). The resulting fatty acid hydroperoxide [(13*S*)-hydroperoxy-(9Z,11E,15Z)-octadecatrienoic acid] is cleaved by HPL, resulting in the production C_6-C_9 aldehydes. These characteristic volatile flavor compounds are typically associated with breakage or disruption of tissue (Riley and Thompson, 1998). These compounds are subsequently reduced to the corresponding alcohols by ADH, and alcohols may be esterified to acetates or butyrates by AAT.

There are inconsistencies in the reports regarding aroma formation during fresh-cut melon storage. The transient increase in volatile compounds of fresh-cut cantaloupe melons observed by Beaulieu (2005) followed by a decline in volatile compounds and the post cutting immediate reduction in important aroma compounds observed by Lamikanra and Richard (2002) warrants more research. Fresh-cut melon volatile production can undergo a transient upsurge because skin removal creates secondary compound formation, allows rapid off-gassing, and increases available O_2 for enzymatic action. Through storage, as starch declines and less sugar is

catabolized, less substrate (acetyl-CoA) would be available for continued volatile production (Beaulieu and Lea, 2003).

Climacteric fruit exhibits a characteristic rise in ethylene production accompanied by softening, changes in color and development of aroma. The regulatory role of ethylene in the biogenesis of melon aroma volatiles has been demonstrated through the inhibition of ethylene production by antisense ACO, which resulted in a considerable loss in production of volatiles, mainly esters (Bauchot *et al*., 1998). Flores *et al*. (2002) also investigated the role of ethylene in the aliphatic ester aroma volatiles reduction in antisense ACO transgenic cantaloupe melons. Obando-Ulloa *et al*., (2009) characterized the aroma volatiles associated with the senescence of climacteric and non-climacteric melon fruit. However, these studies were performed using whole melon fruit and additional research is needed to clearly differentiate the effects of wounding and storage on fresh-cut melon with different climacteric behavior.

1.7. 1-MCP as a complementary technology in fresh-cut produce

Reduction of commodity exposure to exogenous ethylene, reduction of ethylene production rate and its action can be achieved through temperature control and atmosphere conditioning (Watada *et al*., 1996; Watkins, 2002). Ethylene perception can be blocked by the use of cyclopropenes. The chemical 1-MCP is a potent antagonist of the action of ethylene, patented in 1996, by Sisler and Blankenship, and approved by the United States Environment Protection Agency (EPA) in 1999, for use in cut flowers, potted flowers, bedding, nursery and foliage plants, and in stored fruits and vegetables. The commercial success of 1-MCP technology resulted from the stable powder formulation of 1-MCP, easily released as a gas when the powder is dissolved in water. Since the discovery of 1-MCP, many studies have demonstrated that, even at low concentrations, 1-MCP can significantly

delay ripening and senescence progress by irreversibly binding to the ethylene receptors, leading to tissues ethylene insensitivity (Blankenship and Dole, 2003; Mao *et al*., 2004; Watkins, 2006; Huber, 2008). 1-MCP is reported as useful in prolonging the quality of various whole fruits including apple (Fan *et al*., 1999a); papaya (Ergun and Huber, 2004), watermelon (Mao *et al*., 2004); tomato (Hurr *et al*., 2005), melon (Ergun *et al*., 2005), nectarine (Bregoli *et al*., 2005); avocado (Zhang *et al*., 2011), and fig (Freiman *et al*., 2012). 1-MCP is also used to maintain quality of fresh-cut fruits derived from treated whole fruits, including apple (Jiang and Joyce, 2002), tomato (Jeong *et al*., 2004), melon (Ergun *et al*., 2007), papaya (Ergun *et al*., 2006), pineapple (Budu and Joyce, 2003) and watermelon (Mao *et al*., 2006; Zhou *et al*., 2006; Saftner *et al*., 2007).

The most common effects of 1-MCP on postharvest quality of horticultural commodities are respiration rate reduction, softness prevention, delay of greenness and titratable acidity loss and total volatile content reduction (reviewed in Watkins, 2006). The knowledge of which factors affect the efficacy of 1-MCP is critical to the optimization of the technology. The control of fruit ripening by 1-MCP is affected by cultivar (Bai *et al*., 2005), maturity stage (Watkins *et al*., 2000), 1-MCP concentration (DeEll *et al*., 2008), temperature and duration of treatment (DeEll *et al*., 2002), application time (DeEll *et al*., 2008), combination with other treatments or technologies (Watkins and Nock, 2012) and delay between harvest and 1-MCP treatment (Watkins and Nock, 2005; DeEll *et al*., 2008). Depending on these factors, ethylene production after 1-MCP application can be variable and may not be inhibited. Beyond its effect on ethylene receptors, 1-MCP is reported to affect the genes encoding ACO and ACS, either lowering their expression or their activity (Defilippi *et al*., 2005). However, reports can be found on ACS increased activity by 1-MCP (Mullins *et al*., 2000).

Results from 1-MCP application to horticultural commodities also include inhibition of physiological senescence disorders (Fan *et al*., 1999b) and reduction of

superficial scald (Zanella, 2003; Lurie and Watkins, 2012), but negative 1-MCP impact on fruits quality can be found in the literature, namely increased fruit decay (Ku *et al*., 1999), flesh browning (Watkins, 2008; Jung and Watkins, 2011), increased susceptibility to external CO_2 injury (Fawbush *et al*., 2008) and reduction of volatiles production (Fan and Mattheis, 1999; Saftner *et al*., 2003; Defilippi *et al*., 2004).

Many volatile compounds biosynthetic pathways are regulated by ethylene (Flores *et al*., 2002). Ethylene regulated the reduction of fatty acids and aldehydes and the esterification step in aliphatic ester pathway (Flores *et al*., 2002). Therefore, 1-MCP is expected to reduce the production of ethylene-dependent volatiles. Most studies of 1-MCP effect on aroma production are centered in apple and report the reduction of esters production (Lurie *et al*., 2002; Defilippi *et al*., 2004; Mattheis *et al*., 2005). However, literature reports different 1-MCP effects on volatile compounds production and the mechanisms of 1-MCP action are still not clear. 1-MCP lowered AAT transcripts and activity in apple (Defilippi *et al*., 2005) and suppressed the expression of four AAT genes in melon (El-Sharkawy *et al*., 2005).

1-MCP application to fresh-cut products

Ethylene production is stimulated by physical injury and its accumulation inside fresh-cut packages affects product quality (Abe and Watada, 1991). 1-MCP has been shown to extend the storage potential of a number of fruits when applied at advanced stages of ripening (Hoeberichts *et al*., 2002; Jiang and Joyce, 2002; Wills and Ku, 2002) which is critical for its application to fresh-cut fruits, necessarily derived from near ripe to ripe fruit (Ergun *et al*., 2005; 2007). Three possible approaches to 1-MCP treatment of fresh-cut fruit can be envisioned: 1-MCP can be applied to whole fruits before processing, applied directly to processed fruit and applied before and after processing, resulting in different responses in respiration, firmness, color, ethylene production and volatile biosynthesis.

The number of studies on 1-MCP application after fruit processing are scarce and reported effects on fresh-cut fruit ethylene production is variable. Exposure to 1-MCP after processing of kiwifruit and mango (Villas-Boas and Kader, 2007) decreased ethylene production rates while in persimmon, 1-MCP treated slices showed similar ethylene production to control slices (Villas-Boas and Kader, 2007). Aguayo *et al*. (2006) and Nimitkeatkai *et al*. (2007) report higher ethylene production rates from strawberry wedges and papaya cubes, respectively, treated with 1-MCP after cutting when compared to those treated before cutting and treated before and after cutting. Jiang and Joyce (2002) showed that 1-MCP applied to apple before cutting is more effective at reducing ethylene production than 1-MCP applied after cutting while Villas-Boas and Kader (2006) report no effect of 1-MCP on fresh-cut banana ethylene production. Jeong *et al*. (2004) found no significant differences in ethylene production maxima between 1-MCP treated fresh-cut tomato slices and untreated controls when 1-MCP treatment was 10 ºC and 15 ºC. These authors also report that when the treatment temperature was 5 ºC, 1-MCP treated fresh-cut tomato slices showed a significant higher ethylene production peak than untreated controls.

It is the lack of consistency that continues to be a challenge in understanding the mode of action of 1-MCP in a wide range of fresh-cut fruits. 1-MCP has been shown to compete with ethylene for the binding site in plant tissue, which prevents ethylene from exerting its physiological action, but 1-MCP effect on wound-induced ethylene is still not clear. Many factors may alter the 1-MCP effects on fresh-cut fruit quality, namely fruit climacteric behavior, cultivar, stage of ripeness and firmness at cutting, as well as storage regime before processing and exposure temperature (Toivonen, 2008). The assumption has been made that 1-MCP binds permanently to receptors present at the time of treatment and any return of ethylene sensitivity is due to appearance of new sites but 1-MCP binding may not be complete (Blankenship and Dole, 2003) and wound-response may lead to a faster synthesis of binding sites. Plant

tissues vary greatly in their ability to regenerate binding sites and receptor sensitivity and regeneration may provide explanations for some differences in response to 1-MCP as well as wound-induced increased ACO gene expression (Bouquín *et al*., 1997).

The different 1-MCP effects on fresh-cut fruit ethylene production allow the anticipation of different 1-MCP effects on fresh-cut fruit quality parameters, namely firmness, color, sugars and acidity. Exposure to 1-MCP slowed softening but did not affect the color of kiwifruit and mango slices (Villas-Boas and Kader, 2007) and fresh-cut bananas (Villas-Boas and Kader, 2006) but treatment with 1-MCP was not effective in preventing softening and browning of fresh-cut persimmons (Villas-Boas and Kader, 2007) and strawberries (Aguayo *et al*., 2006). No significant effects on quality were shown for apples (Jiang and Joyce, 2002) and pineapples (Budu and Joyce, 2003) treated with 1-MCP after cutting. 1-MCP effect on fresh-cut tomato slices depended on treatment temperature and fruit maturity stage (Jeong *et al*., 2004). Studies on 1-MCP effects on fresh-cut fruit sporadically report measures of soluble solids and titratable acidity since that, in most cases, changes in these measures are not consistent and part of the reason for the inconsistency may be explained by the hypothesis of soluble solids and titratable acidity may not be closely linked with ethylene action (Calderón-López *et al*., 2005).

1-MCP inhibition of ethylene action or perception is expected to mediate wounding/defense responses by injured tissue most times decreasing the production of characteristic aroma volatiles (Saltveit, 1999). The 1-MCP effects on temporal changes in aroma volatiles and phytochemicals of fresh-cut cantaloupe are studied and reported in Chapter 3.

CHAPTER 2

Lysophosphatidylethanolamine effects on horticultural commodities: a review

Abstract

LPE is a naturally occurring lipid with regulatory effects in senescence and ripening. When applied exogenously to horticultural crops, LPE affects growth, development, and postharvest longevity. The effects of exogenously applied LPE have been studied in a range of plant organs in more than a dozen horticultural species. The claimed horticultural benefits include delayed leaf senescence, stimulation of ripening in nonclimateric table grape, acceleration of color development and extension of shelf-life in cranberry and tomato, and increased vase life of cut flowers. Responses to LPE application are found to vary dramatically within horticultural commodity, developmental stage and organ type. Effects on ethylene responses are contradictory. LPE inhibits PLD and is reported to affect the activity of enzymes relevant for produce quality, such as PAL and acid invertase (AcInv). The biochemical mode of action of LPE is poorly understood. In particular, a mechanism by which a plant growth regulator might delay senescence of plant organs and accelerate ripening-related changes is not obvious. The horticultural, physiological and biochemical effects of LPE are reviewed in an attempt to highlight the knowledge gaps regarding the putative regulatory role of exogenously applied LPE.

2.1. Introduction

Modulation of plant physiology by bioactive molecules has long been the basis of horticultural techniques. Plant growth regulators have been used in pre and postharvest applications to achieve horticultural objectives such as enhancement of seed germination and stand establishment, improvement of stress resistance, alteration of crop morphology, modulation of flower development and sex expression, and control of fruit set and ripening (Arteca, 1996).

LPE is a membrane glycerolipid whose use as a plant growth regulator has been proposed. The initial studies on the horticultural effects of LPE were driven by the need to improve quality of cranberry fruit (Özgen and Palta, 1999). The first horticultural trials with LPE in cranberry fields aimed at solving the problem of uneven fruit ripening and anticipate harvest to avoid late Fall frosts (Özgen and Palta, 1999). The first evidence of relevant LPE effects on a horticultural commodity was presented in 1989, when LPE was reported to enhance ethylene production (Farag and Palta, 1989a) and improve the effectiveness of ethephon ([*2*-chloroethyl]phosphonic acid) on cranberry fruit (Farag and Palta, 1989b). Two years later, the same authors reported on the effects of LPE on tomato, apple, and cranberry fruit and on tomato foliage (Farag and Palta, 1991a; Farag and Palta, 1991b; Farag and Palta, 1991c; Farag and Palta, 1991d). Subsequently, LPE was reported to, simultaneously, accelerate ripening and enhance the storage life of tomato fruit (Farag and Palta, 1992a, b; Farag and Palta, 1993a).

LPE formulations have been patented and marketed for horticultural applications. LPE formulations have received the approval for use as a plant growth regulator by the United States EPA, and have since been marketed.

Literature reports on the horticultural effects of LPE have accumulated in the last two decades. A range of crop species and plant organs have now been treated with LPE in an attempt to improve postharvest quality and extend shelf-life. The

claimed horticultural benefits include delayed leaf senescence (Farag and Palta, 1993a; Özgen *et al*., 2005a; Hong *et al*., 2009a), stimulation of ripening in nonclimateric table grape (Hong *et al*., 2007), acceleration in color development and extension of shelf life in cranberry (Özgen *et al*., 2004) and tomato (Farag and Palta, 1993b), and increased vase life of cut flowers (Kaur and Palta, 1996; Kaur and Palta, 1997; Snider *et al*., 2003a). Taken together, these horticultural effects appear contradictory: a mechanism by which a plant growth regulator might delay senescence of plant organs and accelerate ripening related changes is not obvious.

This paper reviews the literature on LPE applications on horticultural commodities, summarizes its effects at the horticultural, physiological, and biochemical levels, and attempts a critical assessment of the biochemical and physiological mechanisms underlying the reported effects. The literature germane for the horticultural effects of LPE includes 45 references, distributed as follows: 13 (29% of the total) are primary peer-reviewed publications; 7 (15%) are patents obtained on LPE; 8 (18%) are conference proceedings, and 17 (38%) are abstracts from communications at scientific meetings. Conference proceedings and abstracts were considered relevant for the purposes of this review given the contribution of these sources to build the case for the horticultural effects of LPE.

2.2. Development of LPE as a plant growth regulator

2.2.1. Chemistry and production

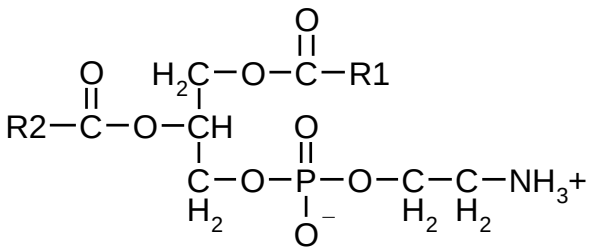


Fig. 2.1. Chemical structure of phosphatidylethanolamine

Phosphatidylethanolamine (PE) is a membrane phospholipid with two fatty acid acyl chains esterified to a glycerol backbone with an ethanolamine head group linked to the glycerol by a phosphate bridge (Vance and Vance, 2008), as shown in Fig. 2.1. PE is present in animals and yeast, as well as in the membranes of all plants. A lysophospholipid is a phospholipid molecule in which only one of the alcohol groups of the glycerol backbone is esterified to a fatty acid. LPE (Fig. 2.2) can be obtained from PE via hydrolysis by PLA_2 (Farag and Palta, 1992). As a breakdown product of PE, LPE is naturally present in plant and animal tissues. Egg yolk and soy lecithin are particularly rich in PE and LPE, and are sources for the commercial manufacturing of LPE.

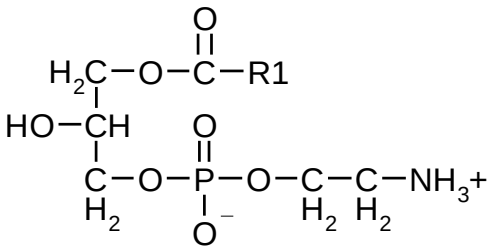


Fig. 2.2. Chemical structure of lysophosphatidylethanolamine

The nature of LPE depends on the acyl chain (14:0, 16:0, 18:0, or 18:1) present at the *sn*-1 position of the glycerol backbone. LPE containing a 14:0 acyl chain is seldom present in plant tissues (Ryu *et al*., 1997). The egg-derived LPE commercially used as a plant bio-regulator has an 18:0 fatty acid esterified to the glycerol backbone (Cowan, 2009). Unless otherwise stated, commercial 18:0-LPE is

the substance whose effects are discussed in this review and is hereafter referred to as LPE.

With the chemical formula $C_{23}H_{46}NO_7P$ and molecular weight of 479.59 g mol^{-1}, LPE has very low solubility in water. Solubility in water is estimated at 1.76 μg mL^{-1} (Wishart *et al*., 2009) and lipid micelles are formed when LPE is added to an aqueous environment. The concentrations required for horticultural applications are often in the range of 100 to 200 μg mL^{-1}, one or two orders of magnitude above the water solubility of LPE.

2.2.2. Industrial property protection

The first patent pertaining the horticultural application of LPE was granted to Palta and Farag in 1992 (US 5126155), and subsequent industrial property protection has been obtained. The initial patent claimed a method to enhance fruit ripening and delay senescence in fruit and other plant tissues using LPE. Ten years later, Palta and Ryu (2002) obtained the US patent 6426105 for the use of 18:1-LPE for the same purpose and also protected lysophosphatidylinositol to retard senescence and enhance fruit ripening. Farag *et al*. (2003) obtained the US patent no. 6559099 on “methods for enhancing plant health, protecting plants from biotic and abiotic stress related injuries and enhancing the recovery of plants injured as a result of such stresses” reporting a method to enhance or accelerate the recovery of injured plants and enhance the germination of seeds and seedling vigor by the treatment with a formulation containing at least one lysophospholipid.

A method for preparing LPE is described in US patent no. 6773902 issued in 2004 (Chung and Yang, 2004). This method comprises hydrolyzing a mixture of phospholipids containing 10-99% of PE with PLA_2 to produce lysophospholipids and the treatment of the resulting lysophospholipids with a solvent made of water and one

or more organic solvents to eliminate impurities and increase the content of LPE. Four years later, Chung *et al*. (2008) obtained the US patent no. 0188683 where a method to make a stable aqueous solution of LPE (0.1-50%) combined with lecithin (0.1-60%) is disclosed. More recently, Chung *et al*. (2010) and Chung *et al*. (2011) registered the US patents no 0255990 and 8030244 on a “method, composition and kit for promoting plant growth and improving plant preservation” with a combination of LPE and silicate salts.

2.2.3. Registration for horticultural applications and marketing

The United States EPA reviewed the toxicological, environmental, and human health risks associated with the use of LPE on horticultural crops and approved its use as a plant growth regulator (biochemical pesticide) in 1995). LPE was initially registered in 2002 and the license for sale granted to Nutra-Park, Inc. (Middleton, Wisconsin, USA). The technical grade active ingredient consisted of 94% or 20% for LPE E94T and LPE-94 20% Aqueous, respectively (EPA). Subsequently, a formulation with a lower concentration of LPE (10%) and 90% of other unspecified ingredients, named LPE-94 10% Aqueous Growth Regulator, was registered for spraying or immersion (EPA). These products were approved for outdoor agricultural use to accelerate ripening and improve the quality of fresh produce, and for use in packing houses to preserve stored produce and cut flowers. Being a natural product applied at low concentrations and naturally degradable, LPE is not toxic to mammals and other organisms and the amounts found in treated food are not expected to cause any adverse effects (EPA).

The commercial production of LPE formulations was assured by Doosan Corporation (Seoul, South Korea). Pure LPE prepared from egg yolk, soy lecithin or porcine brain is also commercially available from Avanti Polar Lipids, (Alabaster, Alabama, USA) and from Sigma Aldrich (St. Louis, Missouri, USA). The actual usage

of LPE by the horticultural industry is unknown. LPE formulations can be applied by spraying in preharvest treatments, by spraying or dipping in postharvest applications, and as a vase solution for cut flowers.

2.3. LPE applications on horticultural commodities

LPE effects have been studied in several plant organs from more than a dozen horticultural commodities. A summary of the horticultural, physiological, and biochemical effects reported in the literature for different organs and species is presented in Table 2.1, with the indication of the application methods and LPE concentrations.

Table 2.1. LPE applications and effects on horticultural commodities

Horticultural commodity and organ	Application method	LPE concentration and other treatment conditions	Horticultural (1), physiological (2), and biochemical (3) effects	References
Apple crop 'McIntosh'	Preharvest spray Postharvest fruit vacuum infiltration	50-100 mg L^{-1}; ethanol (1-2%)	(2) Improved postharvest fruit firmness and color uniformity; increased fruit peel anthocyanin content; stimulated ethylene production; no effect on respiration rate	Farag and Palta, (1991c)
Arabidopsis thaliana	Foliage spray	100 mg L^{-1}	(1) Increased freezing tolerance	Rajashekar *et al*., (2006)
Banana (excised peel)	Postharvest incubation	25, 50 or 100 mg L^{-1}	(2) Decreased ethylene production; delayed firmness loss; decreased ion and protein leakage	Workmaster and Palta, (1996)
Banana fruit	Postharvest dip	500 mg L^{-1}	(1) Extended shelf-life; increased fruit diameter (2) Fresh weight loss unaffected; decreased ion electrolyte leakage	Ahmed and Palta, (2010)

Table 2.1 Continued

Horticultural commodity and organ	Application method	LPE concentration and other treatment conditions	Horticultural (1), physiological (2), and biochemical (3) effects	References
Carnation flowers	Vase solution	10 mg L^{-1}	(2) Delayed fresh weight loss and reduced ion leakage at an early stage of flower opening; no effect of LPE on older (stage VII) carnations	Kaur and Palta, (1996)
Cranberry crop	Preharvest plant spray and postharvest fruit dip	200 mg L^{-1} 50-100 mg L^{-1}	(1) Effects depended on field location	Ozgen and Palta, (1999)
Cranberry crop	Preharvest plant spray	100 or 200 mg L^{-1}; chlorothalonil (6 L ha^{-1})	(1) Increased fruit set; prevention of chlorothalonil toxicity	Ozgen and Palta, (2003)
Cranberry 'Searles' crop	Preharvest spray plant application	200 mg L^{-1}; Sylgard (0.05%); ethanol (5%)	(2) Increased anthocyanin content	Ozgen *et al*., (2004)
Cranberry 'Searles' crop	Preharvest application Postharvest vacuum infiltration or dip	50-100 mg L^{-1}; ethanol (1-2%)	(2) Increased fruit peel anthocyanin content; fruit color uniformity; improved postharvest fruit firmness	Farag and Palta, (1991c)
Cranberry 'Stevens' fruit	Postharvest dip	100 µM of LPE with different acyl chains	(2) Inhibition of ethylene production increased with acyl chain length and the unsaturation of LPE	Ryu *et al*., (1997)
Cantaloupe melon (fresh-cut)	Vacuum infiltration	200 mg L^{-1}	(2) No effect on color, firmness, SSC or ethylene production; negligible effect on respiration rate; lower production of aldehydes	Amaro *et al*., (in press)
Grape 'Thompson Seedless'	Preharvest foliar spray	10 mg L^{-1}	(2) Higher anthocyanin concentration; increased berries SSC, firmness and size	Hong *et al*., (2007)
Impatiens ' Super Elfin', 'Rose' and 'Dazzler'	Bedding plants spray	50; 100 and 200 mg L^{-1}	(1) Higher number of open flowers; faster recovery from water-stress cycles	Snider *et al*., (2003b)

Table 2.1. Continued

Horticultural commodity and organ	Application method	LPE concentration and other treatment conditions	Horticultural (1), physiological (2), and biochemical (3) effects	References
Philodendron cordatum	Incubation of excised leaf discs	50; 150; 200 and 250 µM	(3) No PAL or insoluble Ac Inv inhibition; no effect on ABA induced increase in maloaldehyde or loss of chloroplast pigments	Hong *et al*., (2009a)
Potato 'Russet Burbank'	Growing medium with LPE	50 or 100 mg L^{-1}	(1) Higher number of healthy leaves (2) Higher chlorophyll content; less auxiliary shoot formation	Ozgen *et al*., (2005)
Potato	Potato shoot culture medium	400 mg L^{-1}	(1) Mitigation of calcium deficiency injury (2) Enhanced calcium uptake; promoted root growth	Ahmed and Palta, (2011b)
Radish cotyledons	Incubation	2 mmol L^{-1}; K-phosphate (2mmol L^{-1})	(3) No effect on soluble AcInv activity, glucose and sucrose levels; increased PAL and insoluble AcInv	Hong *et al*., (2009b)
Radish cotyledons	Unknown		(3) Transient induction of AcInv, PAL, endo β-1,3(4)-Glc and POD,suppression of PPO and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity	Cowan *et al*., (2006)
Red pepper crop	Preharvest plant spray	100; 200 or 400 mg L^{-1}; siloxane (0.1 mg L^{-1}); Ethephon (250 mg L^{-1})	(1) Mitigation of Ethephon-induced foliage injury; increased ripened fruit yield (2) Enhanced ethylene production	Kang *et al*. (2003)
Red and green pepper crops	Preharvest plant spray	0 or 50 mg L^{-1}	(1) High marketable yield than controls but similar to that of Ethephon treated plants; reduced chilling injury	Hong and Chung, (2006)
Rose 'Lavande' and 'Sensation'	Vase solution	10% Aqueous solution; 8-hydroxyquinoline citrate; sucrose	(1) Slower flower opening; increased vase life	Snider *et al*., (2003a)
Tomato	Unknown	Unknown	(2) Reduced firmness loss (3) PG activity inhibition	Mangat and Palta, (1995)

Table 2.1. Continued

Horticultural commodity and organ	Application method	LPE concentration and other treatment conditions	Horticultural (1), physiological (2), and biochemical (3) effects	References
Tomato	Incubation of excised pericarp tissue at different maturity stages	Different LPE concentrations in buffer solutions	(2) Increased ethylene production, respiration rate and in green tissue; reduced ethylene production, repiration rate in light red tissue (3) Increased ACO activity in green tissues; reduced ACO activity in light red tissues	Hong *et al*., (2002); Hong, (2006)

2.3.1. Leaves and foliage

LPE has been applied to attached and detached leaves of dicotyledonous species. The reported effects include delay of natural senescence, mitigation of ethylene-induced senescence, and increased tolerance to freezing injury.

Attached tomato leaves (*Solanum lycopersicum* L. 'H7155') sprayed with a solution of LPE (100 mg L^{-1}) containing 1-2 % ethanol showed delayed senescence, as assessed by the lower rates of ethylene and CO_2 production, higher fresh weight and chlorophyll content, and lower electrolyte leakage than untreated controls (Farag and Palta, 1991b). The same effects were observed in detached tomato leaflets allowed to take up an LPE solution through the cut petiole (Farag and Palta, 1991b). Application of LPE (200 mg L^{-1}) prior to a treatment with ethephon (1000 mg L^{-1}) or inclusion of LPE in the ethephon spray reduced foliar injury by ethephon in two-month-old 'Mountain Spring' tomato plants (Özgen *et al.*, 2000). LPE-treated tomato leaves and plants treated with a mixture of LPE and ethephon had lower PLD activity than leaves treated with ethephon alone (Özgen *et al.*, 2000). The protective effect of LPE (100 mg L^{-1}) on ethephon-induced foliage injury was also observed in red pepper (*Capsicum annuum* L.) by Kang *et al*. (2003). LPE solution (100 mg L^{-1}) sprayed on

the foliage of *Arabidopsis thaliana* L. increased freezing tolerance, presumably by inhibiting PLD activity (Rajashekar *et al*., 2006).

2.3.2. Cut flowers and other ornamentals

The effects of LPE on cut flowers were evaluated on snapdragon (*Antirrhium majus* L.), carnation (*Dianthus caryophyllus* L.) and rose (*Rosa hybrida* L.) (Kaur and Palta, 1996; Kaur and Palta, 1997; Snider *et al.*, 2003a). A 24-h pulse treatment of snapdragon with LPE at 25 mg L^{-1} enhanced bud opening, delayed weight loss, lowered ethylene production and reduced ion leakage from the cut flowers during subsequent vase life in deionized water (Kaur and Palta, 1997). Carnation flowers treated with LPE at 10 mg L^{-1} showed the same effects; carnations, on the other hand, responded to the LPE treatment until the developmental stage open brush bud (stage IV) but more developed flowers were not affected (Kaur and Palta, 1996). The use of LPE is suggested as an environmentally safe alternative to silver thiosulphate to inhibit ethylene-induced senescence and flower abscission in cut flowers (Kaur and Palta, 1997).

The effect of LPE on the vase life of cut roses was compared with that of standard industry practices (Snider *et al.*, 2003a). A vase solution composed of LPE, 8-hydroxyquinoline citrate, and sucrose is reported to increase the vase life of cut roses by 30% in relation to the commercial vase solution Cristal Clear® from Floralife (Walterboro, South Carolina, USA). The relative contribution of LPE, the antimicrobial agent 8-hydroxyquinoline and the carbon source sucrose for the observed effects was not addressed. LPE-treated flowers had a higher incidence of bentneck than those maintained in water or Cristal Clear®. Rose cultivars differed in the LPE concentration for optimal effect: best results were obtained in ‘Lavande’ with 10 mg L^{-1} LPE while ‘Sensation’ required 100 mg L^{-1} (Snider *et al.*, 2003a).

LPE has also been applied to impatiens (*Impatiens wallerana* L.) (Snider *et al.*, 2003b). Impatiens from three cultivars ('Super Elfin', 'Rose' and 'Dazzler') sprayed to drip with LPE (50, 100 and 200 mg L^{-1}) and subsequently subjected to water stress cycles consistently had a higher number of open flowers and recovered more quickly from periods of stress than untreated controls. The maximum number of flowers per plant was obtained with LPE at 100 mg L^{-1}. The rate of flower senescence during water stress was similar in impatiens treated with 50 or 100 mg L^{-1} LPE, but recovery after rewatering was better in plants treated with 100 mg L^{-1} (Snider *et al.*, 2003b).

2.3.3. Preharvest applications to fruit crops

LPE effects have been evaluated on a wide range of fruit, including climacteric and nonclimacteric species in preharvest and postharvest applications. The effects of preharvest LPE sprayings have been studied in cranberry (*Vaccinium macrocarpon* L.), apple (*Malus domestica* Borkh.), tomato, grape (*Vitis vinifera* L.), loquat (*Eriobotrya japonica* L.), and red pepper.

'Searles' cranberry and 'McIntosh' apple fruit sprayed with LPE (50-100 mg L^{-1}) two weeks before harvest had higher anthocyanin content in the peel, better color uniformity, and retained higher firmness during storage (Farag and Palta, 1991a). The LPE formulation contained 1-2% ethanol to enhance diffusion across the plant cuticle (Farag and Palta, 1991a). The effect of LPE on anthocyanin accumulation in cranberry was confirmed by other reports (Özgen *et al.*, 2004). Vines of ‘Heinz 7155’ tomato sprayed with LPE (100 mg L^{-1}) 20 days before harvest exhibited accelerated fruit ripening, higher percentage of red fruit, and fruit with improved storability assessed by lower respiration rate during postharvest storage, independently on the maturity stage at harvest (Farag and Palta, 1991b). ‘Thompson Seedless’ table grapes, sprayed with 10 mg L^{-1} LPE, 4 or 6 weeks after fruit set, yielded larger and firmer berries with higher SSC than untreated controls (Hong *et al.*, 2007). In loquat

trees, LPE spraying before bloom results in higher fruit set and anticipated harvest date (Demirkeser *et al.*, 2009). Higher fruit set was also observed in LPE-treated cranberry (Özgen and Palta, 2003). In red pepper treated with three LPE applications (200 mg L^{-1}), the total number of harvested fruit increased by 30%, the yield of red fruit increased by 39%, and the number of fruit with disease symptoms decreased (Kang *et al.*, 2003). When ethephon (250 mg L^{-1}) is combined with LPE treatment the proportion of red fruit increases to 100% (Kang *et al.*, 2003). LPE enhanced ethylene production and yield of ripe red pepper, a nonclimacteric fruit. The LPE solution included a siloxane compound (0.1 mg L^{-1}) used as a wetting agent. 'Bukwang' and 'Nokkwang' bell pepper were foliar-sprayed with LPE (10 mg L^{-1} or 50 mg L^{-1}) and compared with ethephon (1000 mg L^{-1}) (Hong and Chung, 2006). LPE application resulted in higher marketable yield than controls but similar to that of ethephon-treated plants. Foliar application of LPE (10 mg L^{-1} or 50 mg L^{-1}) to bell pepper had no significant effect on marketable yield but the highest LPE concentration was more effective at reducing pitting and decay during cold storage of green peppers (Hong and Chung, 2006).

Preharvest LPE spray can mitigate the adverse effect of the fungicide chlorothalonil in cranberry (Özgen and Palta, 2003). LPE (100 and 200 mg L^{-1}) prevented the reduction in fruit set and yield of cranberry plots sprayed twice, at 20% and 80% bloom, with chlorothalonil (Özgen and Palta, 2003).

The effects of preharvest applications of LPE are influenced by the source of LPE (Özgen *et al.*, 1999), by the formulation (Farag *et al.*, 1989b; Özgen *et al.*, 2004) and even by the trial location (Özgen *et al.*, 1999). Higher anthocyanin content in cranberry was obtained with egg LPE at one location but with soy LPE at another location (Özgen *et al.*, 1999). It has been suggested that the combination of egg and soy LPE is more efficient for cranberry color development than egg LPE alone (Özgen *et al.*, 1999). Spray formulations of LPE often contain adjuvant substances. Ethanol (1

to 5%) has been used in the spray solution to enhance cuticle penetration (Farag *et al.*, 1989b; Farag and Palta, 1991a). Sylgard 309 (0.005%), a silicone based nonionic surfactant, combined with LPE enhances antocyanin concentration in cranberry (Özgen *et al.*, 2004).

2.3.4. Postharvest applications to fruit

Postharvest applications of LPE to fruit or fruit tissues have been studied in a number of conditions. LPE has been applied to harvested tomato, banana (*Musa acuminata* L.), apple, cranberry, orange (*Citrus sinensis* L.), and to fresh-cut cantaloupe (*Cucumis melo* L.). The application methods addressed in the literature include absorption via the fruit peduncle, spraying, dipping, and vacuum infiltration.

A continuous supply of LPE (100 mg L^{-1}) combined with 1-2% ethanol via the peduncle of firm-ripe tomato delayed fruit senescence and lowered the rates of ethylene and CO_2 production (Farag and Palta, 1991b). Tomato fruit allowed to take up a LPE solution (50 mg L^{-1}) via the peduncle had higher pericarp firmness and lower electrolyte leakage than control fruit placed in water (Farag and Palta, 1993a). The extension of shelf-life by LPE was observed in tomato fruit harvested at different maturity stages (Farag and Palta, 1993b). Still, LPE stimulates ripening in mature-green tomato fruit, but inhibits ethylene production in ripe fruit (Hong *et al*., 2002; Hong, 2006), suggesting that LPE effects depend on the developmental stage of climacteric fruit. LPE (50 mg L^{-1}) applied by dipping to drop mature-green tomatoes stimulates ripening of fruit at the MG4 stage, with or without a subsequent ethylene treatment, but had only moderate effect on fruit at the MG1 and MG2 stages, which required exogenous ethylene to ripen (Altwies *et al*., 2002). Excised pericarp tissues from mature-green tomatoes incubated in LPE had higher ethylene production rate and higher ACO activity but the opposite effect was observed in light red fruit (Hong *et al*., 2002; Hong, 2006).

Excised peel pieces of banana incubated for 4 days in LPE concentration ranging from 25 to 100 mg L^{-1} showed reduced leakage of ions and soluble proteins, lower ethylene production rate, and better tissue maintenance (Workmaster and Palta, 1996). These effects were dose-dependent, with better results at 100 mg L^{-1} LPE, although the nature of this dependency was not specified (Workmaster and Palta, 1996). Peel tissue treated with 100 mg L^{-1} remained firm while tissue treated with lower LPE concentrations expanded and lost integrity. Whole banana fruit dipped for 30 min in 500 mg L^{-1} LPE solution had lower ion leakage from the peel and a slightly longer (1 day) shelf-life than untreated fruit (Ahmed and Palta, 2010; Ahmed and Palta, 2011a). The extension of shelf-life of banana fruit by one day was attributed to an improvement of membrane integrity (Ahmed and Palta, 2011a), but the criteria to assess shelf-life are not evident. The authors report that “fruit diameter was reduced significantly more in control than in LPE treated fruits” (Ahmed and Palta, 2011a). The mechanism for this effect of LPE on fruit diameter is not clear since weight loss was not affected by the LPE treatment (Ahmed and Palta, 2010).

Postharvest LPE (50-100 mg L^{-1}) application to apple and cranberry fruit, via vacuum infiltration or dipping, stimulated ethylene production, had no effect on respiration rate, and reduced fruit softening in relation to untreated controls (Farag and Palta, 1991c). In a different trial, postharvest LPE (100 mg L^{-1}) application via dipping reduced ethylene production and respiration rate in cranberry (Özgen *et al.*, 1999). Spraying LPE (480 mg L^{-1}) onto ‘Navel’ oranges, stored at 20 °C, 90% RH, for 10 days, reduced the incidence of peel pitting but increased fruit decay by *ca.* 50% compared with untreated fruit (Alfarez *et al*., 2008). The reduction in pitting was attributed to a putative prevention of membrane deterioration.

The effect of LPE was also investigated in fresh-cut cantaloupe. The treatment of fresh-cut melon cubes with 200 mg L^{-1} LPE did not affect color, firmness, SSC or ethylene production during storage at 5 ºC, and had a negligible effect on the

respiration rate (Amaro *et al*., in press). LPE-treated melon cubes produced similar amounts of volatile esters and alcohols but accumulated smaller amounts of aldehydes than control cubes (Amaro *et al*., in press). The LPE treatment in this study was applied by vacuum infiltration (-5 kPa for 30 s) to assure good cellular contact and controls were infiltrated with water under the same conditions.

2.3.5. Other plant structures

Addition of LPE (50 or 100 mg L^{-1}) to the micropropagation medium reduces the ethylene response of potato (*Solanum tuberosum* L.) plantlets. Plantlets grown in medium containing 50 mg L^{-1} LPE had three times less auxiliary shoots, more than twice the number of healthy leaves and 98% higher chlorophyll content than ethylene-treated controls (Özgen *et al*., 2005). LPE (400 mg L^{-1}) incorporated into the medium of cultures enhanced calcium uptake and reduced calcium deficiency symptoms in potato shoot cultures (Ahmed and Palta, 2011b). LPE is reported to promote root growth in potato shoot cultures (Ahmed and Palta, 2011b).

The effect of LPE on the viability of loquat pollen was studied in tree branches sprayed with 100 mg L^{-1} LPE (Demirkeser *et al*., 2009). Pollen collected from LPE-treated branches had higher viability then pollen from untreated control branches. Incubation of loquat pollen in 100 mg L^{-1} LPE reduced the germination and pollen tube growth rates in relation to untreated pollen (Demirkeser *et al*., 2009).

2.4. Physiological and biochemical effects of LPE

2.4.1. Ethylene and other plant hormones

An interaction between LPE treatments and ethylene biosynthesis may explain some of the horticultural effects. 18:1-LPE reduces the ethylene production rate of

ripe cranberry by 40% (Ryu *et al*., 1997). This inhibitory effect is specific; 14:0-LPE has no effect and 16:0- and 18:0-LPE have only a minor inhibitory effect on ethylene biosynthesis (Ryu *et al*., 1997). LPE is also reported to reduce ethylene production rate in carnation (Kaur and Palta, 1996) and snapdragon flowers (Kaur and Palta, 1997), and in tomato fruit (Farag and Palta, 1993a; Hong, 2006). In other instances, however, the LPE treatment stimulated ethylene production in apple (Farag and Palta, 1991c), banana (Ahmed and Palta, 2011a), cranberry (Farag and Palta, 1991c), tomato (Hong *et al*., 2001) and red pepper (Kang *et al*., 2003). In tomato, the effect of LPE on ethylene production rate depends on the maturity stage (Altwies *et al*., 2002; Hong *et al*., 2002; Hong, 2006). A treatment with LPE increased ethylene production rate in mature-green tomato (Hong *et al*., 2002; Hong *et al*., 2006), a response that can be attributed to increased activity of ACO (Hong *et al*., 2002). In contrast, postclimacteric red-ripe tomato allowed to take up a LPE solution via the peduncle had lower ethylene production rates than untreated fruit (Farag and Palta, 1993a) and pericarp tissue of light red tomatoes incubated in LPE solution showed decreased ethylene production (Hong, 2006) .

A reduction in ethylene production by LPE would provide a means to affect ethylene-regulated processes, such as ripening-related changes in texture and color. On the other hand, despite the evidence that LPE reduces ethylene production rate in cranberry (Ryu *et al*., 1997) an increase in ethylene production rate was credited as the cause of color enhancement induced by LPE in cranberry (Özgen *et al*., 2004). Similarly in apple, anthocyanin content and color enhancement induced by LPE was attributed to enhanced ethylene production (Farag and Palta, 1991c). Surprisingly, LPE-treated apples were firmer than untreated control fruit (Farag and Palta, 1991c).

The involvement of LPE on ethylene biosynthesis is not clear nor is it obvious how the inconsistencies reported in the literature can be interpreted and reconciled. The mechanism by which LPE interferes with ethylene biosynthesis is unknown but

interactions with other plant hormones have been reported. In mung bean (*Vigna radiate* L.) hypocotyls, LPE induced the expression of two ACS genes (Hong *et al*., 2008). ACO activity was enhanced by LPE in mature-green tomato but reduced in ripe fruit (Hong *et al*., 2002; Hong, 2006). Alternatively, it has been suggested that LPE interferes with the ethylene signal transduction pathway (Cowan, 2009).

Auxin-like effects of LPE, such as stimulation of cell elongation and growth of plant organs (Özgen and Palta, 2001; Lee *et al*., 2003; Ahmed and Palta, 2011b) and pollen viability and development (Cechetti *et al*., 2008) have been reported and interaction between LPE action and auxin- and ethylene-dependent pathways suggested (Hong *et al*., 2008). LPE treatment increased reactive oxygen species (ROS) production in mung bean, suggesting the possibility that LPE effects on hormones are mediated by ROS (Hong *et al*., 2008).

2.4.2. Protein structure and enzyme activities

The role of lysophospholipids as molecular chaperones was investigated *in vitro*. Exogenously applied LPE increased the proportion of correctly folded citrate synthase (E.C. 2.3.3.1) and α-glucosidase (EC 3.2.1.20), increased the refolding yield and protected both enzymes from thermal denaturation (Kern *et al.*, 2001).

Several enzyme activities have been examined in plants treated with LPE. In expanding radish (*Raphanus sativus* L.) cotyledons, LPE induced a transient increase in the activities of AcInv (E.C. 3.2.1.26), PAL, endo β-1,3(4)-Glc and to a lesser extent POD, but reduced the activity of polyphenoloxidase (E.C. 1.14.18.1) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (E.C. 1.1.1.34) (Cowan *et al*., 2006). Similar results were obtained in isolated radish cotyledons, where 18:0-LPE enhanced the activity of PAL and insoluble AcInv (Hong *et al*., 2007; Hong *et al*., 2009b). However, attempts to increase the activities of insoluble AcInv and PAL *in vitro* by the direct addition of 18:0-LPE to the enzyme preparations were unsuccessful (Hong *et al*.,

2009a), suggesting that LPE does not interact directly with these proteins but, instead, interferes with signal transduction or gene expression (Alvarez-Venegas *et al*., 2006a; 2006b).

LPE effect on tomato fruit firmness has been attributed to the reduction of PG activity (Farag and Palta, 1992a; Mangat and Palta, 1995). LPE inhibition of PG activity in tomato fruit is maturity-dependent (Mangat and Palta, 1995), but not enough information is disclosed to clarify the nature of the enzyme (endo or exo-PG) or the effect of developmental stage.

Of particular interest is the effect of LPE on PLD. LPE specifically inhibits the activity of partially purified cabbage PLD (presumably PLDα) in a cell-free system (Ryu *et al*., 1997). PLD activity is inhibited in a concentration-dependent way by LPE concentrations ranging from 40 and 200 µM. The inhibition of PLD by LPE is highly specific and increased with length and the unsaturation of the acyl chain (Ryu *et al*., 1997). The strong inhibition of PLD by 18:1-LPE when compared with LPE containing other acyl-chains (14:0, 16:0, and 18:0) suggests that a specific configuration of LPE is required for inhibition (Ryu *et al*., 1997). Only intact LPE molecules are capable of inhibiting PLD; the removal of any of the structural components of LPE renders the molecule completely ineffective or even in the stimulation of PLD activity (Ryu *et al*., 1997). The inhibitory effect of 18:1-LPE was also observed against membrane associated and soluble PLD from cabbage and castor bean leaf tissue (Ryu *et al*., 1997). This inhibition was less pronounced than that of the partially purified cabbage PLD, presumably due to the presence of interfering factors or other forms of PLD less sensitive to LPE (Ryu *et al*., 1997).

The relevance of *in vitro* inhibition of PLD by LPE to the physiological effects has recently been questioned. Hong *et al*. (2009b) argue that the delayed senescence observed after LPE treatments results from the elicitation of plant defense responses, which include increased activity of insoluble AcInv and PAL, a stimulation of anabolic

metabolism, and the deposition of lignin. The interaction of LPE with PA or with downstream targets of PA may explain the effects of exogenous LPE applications on senescence (Cowan, 2009).

2.4.3. Lipid catabolism

The inhibition of lipid breakdown by LPE, resulting in the protection of membrane integrity during ripening or senescence, is expected based on its inhibitory activity against PLD (Ryu *et al*., 1997). The hydrolysis of structural phospholipids and the release of PA are integral of senescence processes (Li *et al*., 2009). PA is formed in response to stress by the hydrolysis of membrane phospholipids by PLD and phospholipase C (PLC; EC 3.1.4.3) and initiates a signaling cascade that leads to senescence. LPE interacts with the PA resulting from PLD catalyzed reactions and with downstream targets of PA thus delaying senescence (Hong *et al*., 2009a). The effect of LPE on abcisic acid (ABA), ACC, and PA-induced changes in malondialdehyde formation and chlorophyll and carotenoid catabolism were compared using leaf discs from *Philodendron cordatum* (Vell.) Kunth. LPE did not affect senescence progression in *Philodendron* leaves and was unable to reverse hormone-induced senescence (Hong *et al*., 2009a).

2.5. Possible modes of action

2.5.1. Tentative physiological model

The physiological and biochemical mechanisms underlying the horticultural effects attributed to exogenously applied LPE are not clear. To explore these mechanisms, two lines of enquiry can be pursued: understand of the regulatory

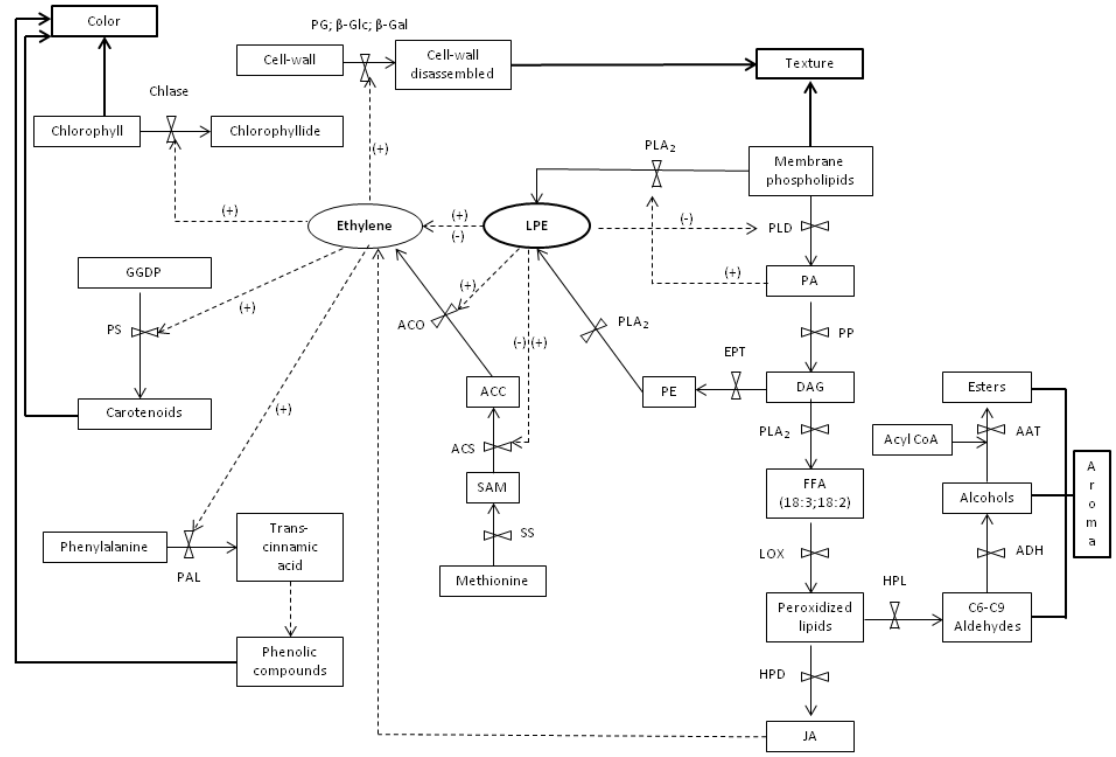
effects of endogenous LPE and identify exogenous LPE effects and possible modes of action.

Endogenous LPE mediates the propagation of wound and stress signals (Lee *et al*., 1997) and its concentration in leaves rises in response to freezing and wounding, with systemic accumulation observed in non-wounded tissue (Lee *et al*., 1997; Welti *et al*., 2002). The temporal coincidence between the increase in endogenous LPE concentration in plants and the onset of abiotic and biotic stress can be altered by exogenous LPE applications. It has been suggested that exogenous LPE mitigates stress responses by attenuating the endogenous PA signal and by inducing defense enzymes (*e.g.*, PAL, extracellular AcInv), resulting in slower progression of stress-induced senescence or programmed cell death (Hong *et al*., 2009a). The mechanism for this putative interference remains unknown.

LPE inhibits PLD *in vitro* (Ryu *et al*., 1997). The effects of LPE on ethylene are not as clear: both enhancement and reduction of ethylene biosynthesis have been documented. A tentative model is proposed for the physiological and horticultural effects of LPE based on the documented biochemical effects on PLD and ethylene (Fig. 2.3).

Disruption of membrane integrity and function are primary events of senescence in plant organs (Marangoni *et al.*, 1996). PLD is a key enzyme in the catabolism of membrane lipids, catalyzing the first step of accelerated phospholipid breakdown (Bargmann and Munnik, 2006). PLD is implicated in the selective degradation of membrane phospholipids in senescing tissues and in the response to several stresses, including wounding (Wang, 2000). PLD hydrolyzes phospholipids at the terminal phosphodiester bond, producing PA which is dephosphorylated by phosphatidate phosphatase (PP; EC 3.1.3.4). The resulting diacylglycerol (DAG) can be deacylated by lypolitic acyl hydrolase (EC 3.1.1.3) (Wang, 2000). Lipids resulting from PLD action are signal molecules involved in cell proliferation and senescence

(Wang, 2000; Wang *et al*., 2002). The free polyunsaturated fatty acids are substrates for LOX that produces activated oxygen and lipid peroxides contributing even further



Plant glowth regulators
Quality attributes
Regulatory valve for carbon flow
Carbon pools
Flow of carbon skeletons
Flow of information (regulation)
Quality effects

Chlase: chlorophyllase; PG: polygalacturonase; β-Glc: β-1,4-glucanase; β-Gal: β-galactosidase; GGDP: geranylgeranyldiphosphate; PS: phytoene synthase; SS: SAM synthetase; ACS: 1-aminocyclopropane-1-carboxylic acid synthase; ACC: 1-aminocyclopropane-1-carboxylic acid; ACO: 1-aminocyclopropane-1-carboxylic acid oxidase; LPE: lysophosphatidylethanolamine; PLA_2: phospholipase A_2; PLD: phospholipase D; PA: phosphatidic acid; PP: phosphatidate phosphatase; DAG: diacylglycerol; EPT: ethanolaminephosphotransferase; FFA: free fatty acids; LOX: lipoxygenase; HPD: hydroperoxide dehydratase; JA: jasmonic acid; HPL: hydroperoxide lyase, ADH: aldehyde dehydrogenase; AAT: alcohol acyltransferase

Fig. 2.3. Tentative physiological model for the horticultural effects of LPE on horticultural commodities.

to membrane degradation (Paliyath *et al*., 2008). LOX catalyzes the peroxidation of FFA, leading to the formation of highly reactive hydroperoxides that damage the membrane bilayer (Matsushita, 1975). The HPD pathway results in the formation of allene oxide, which can be hydrolyzed leading ultimately to JA, a volatile compound involved in senescence (Creelman and Mullet, 1997). Hydroperoxides of linolenic and linoleic acids are broken down by the HPL pathway, leading to the formation of short chain (C_6-C_9) aldehydes, as hexenal or (*Z)*-3-hexenal and (*E)*-2-hexenal (Feussner and Wasternack, 2002). In further reactions, these compounds are reduced to alcohols in a reaction catalyzed by ADH and condensed into esters through a condensation reaction with acyl moieties, utilizing AAT (Beaulieu, 2005). Therefore, PLD inhibition by LPE would result in membrane preservation and in lower levels on lipid-derived volatiles (Fig. 2.3). Under this mechanism, improved texture retention and altered aroma and flavor are the anticipated physiological effects of LPE. Nonetheless, several knowledge gaps remain.

Although protective effects of LPE on membranes are anticipated based on the inhibition of PLD demonstrated *in vitro* (Ryu *et al*., 1997), there is no convincing evidence for the *in vivo* inhibition of PLD action by exogenous LPE. The negative regulation of PLD in still poorly understood in plant systems and doubts persist about the existence of effective, specific, *in vivo* PLD inhibitors (Wang, 2000). In addition, PLD inhibition by exogenous LPE is expected to reduce the level of endogenous LPE due to the decreased levels of PA. PA stimulates PLA_2 activity (Ryu, 2004). The physiological consequences of this mechanism are unclear. The specific role of PLD isoforms and selective phospholipd hydrolyses in senescence, wound, and stress responses remain unclear (Jenkins and Frohman, 2005). Antisense suppression of PLD delays ABA- and ethylene-promoted leaf senescence in *Arabidopsis* (Fan *et al*., 1997) and fruit ripening in tomato (Pinheiro *et al*., 2003). These results suggest that hydrolysis of structural phospholipids and specifically PA production, play a role in

hormone-mediated senescence. Jasmonate, a volatile hormone resulting from the PLD-LOX pathway, enhances ethylene biosynthesis (Creelman and Mullet, 1997), and provides a possible linkage between the effects of LPE on PLD and on ethylene.

Ethylene biosynthesis is well established (Wang *et al*. 2002). The amino acid methionine is converted to SAM, in a reaction catalyzed by SS. SAM is subsequently converted to ACC by ACS, the rate-limiting step of ethylene biosynthesis (Yang, 1985). In the final step, ACC is oxidized by the enzyme ACO to form ethylene. A reduction in ethylene biosynthesis by LPE would provide a means to regulate some ripening and senescence processes. But, as discussed above (4.1), there are several inconsistencies in the reported effects of LPE on ethylene that require further clarification before a reliable model can be built.

Ethylene regulates several physiological and developmental processes in higher plants, including ripening of fruit, abscission, senescence, and responses to wounding and other stresses, by regulating genes expression (Deikman, 1997). Responses of plant organs to endogenous and applied ethylene include fruit cell wall metabolism and softening, enhanced phenylpropanoid metabolism and anthocyanin accumulation, chlorophyll degradation and organ yellowing, and changes in aroma volatiles. The suppression or stimulation of ethylene production by LPE would result in concomitant changes in these physiological responses. For instance, a reduction in ethylene production by a climacteric fruit by LPE is expected to improve firmness retention by the reduction of PG, β-1,4-Glc, and β-Gal activities (Gonzalez-Bosch *et al*. 1996; Nishiyama *et al*., 2007). However, inconsistencies exist in the literature: LPE is often reported to maintain fruit firmness during storage but also to increase endo β-1,3-Glc activity (Cowan *et al*., 2006); a decrease in PG activity has been reported (Mangat and Palta, 1995). Reduced ethylene biosynthesis would also affect color, via a decrease in the level of phenolic pigments due to the reduction in PAL activity (Ecker *et al*., 1987), a decrease in total carotenoids due to the reduction in PS activity

(Marty *et al*., 2002) and to the maintenance of green color due to the reduction in chlorophyllase (Chlase; EC 3.1.1.14) activity (Jacob-Wilk *et al*., 1999). Color improvement in LPE-treated fruits has been attributed to enhanced PAL activity and anthocyanin accumulation (Farag and Palta, 1991c; Özgen *et al*., 2004) or to carotenoid accumulation (Kang *et al*., 2003). Concomitant increases in PAL activity and anthocyanin production was found by Hyodo (1971), suggesting that a rise in PAL activity is required for a high rate of anthocyanin synthesis. Similarly, stimulation of PAL activity is related to the increase of total anthocyanin content in asparagus (Flores *et al*., 2005).

2.5.2. How specific is LPE?

The physical-chemical properties of LPE may be relevant for its effects. LPE, as a lysophospholipid, is an amphipathic molecule with detergent-like properties. Amphipathic substances applied exogenously to plant organs may elicit a wound response and affect enzyme activities and biochemical pathways (Dawson and Hemington, 1967). Like other detergents, LPE interacts with the lipidic and the proteic components of membranes (Hong *et al*. 2009b). Therefore, it is not surprising if the effects of amphipatic lipids on plants are not specific. Neutral lipids, phospholipids, stripped plant oils, or even commercial plant oils inhibit chlorophyll degradation, stabilize membranes, and delay cell senescence in stored apple fruit (Ju *et al*., 2000). A treatment with a corn oil emulsion delays ripening and reduces decay in apples and pears (*Pyrus communis* L.) (Ju and Curry, 2000). Oil-treated apples and pears had lower ethylene production rates in early storage than controls, which resulted in greener, firmer and more acidic fruit after the storage period (Ju and Curry, 2000).

2.5.3. Can excipients explain the treatment effects?

LPE is applied to horticultural commodities in aqueous formulations containing other unspecified ingredients in proportions of up to 90% (EPA). The commercial product formulation contains 10% LPE and is applied at a maximum concentration of 400 mg L^{-1} of active ingredient. The diluted product is applied in aqueous solutions using conventional sprayers. To assure LPE dispersion in the solution for commercial application, the EPA recommends strong agitation. Several preparation methods can be found in the experimental literature. For example, Snider *et al*. (2003a) diluted the 10% aqueous solution with deionized water and heated to 70 °C to increase LPE dispersion, while Ryu *et al*. (1997) prepared the LPE solution by sonicating the powder in water. Still, the preparation mode is not always described. Considering the requirements to obtain a stable and homogeneous LPE solution or suspension, this lack of information can hinder the interpretation of some of the published results.

The addition of ethanol and unspecified detergents is reported in a technical publication to improve the performance of LPE for postharvest dip applications (Özgen and Palta, 1999). The differences in LPE preparation modes and the use of excipients and adjuvants in the formulations may account for some of the reported effects attributed to LPE. Ethanol, per se, can delay ripening-related changes (Pesis, 2005). The application of an aqueous ethanol solution enhanced internal ethylene production and anthocyanin accumulation in grape berries (Chervin *et al*., 2005) and reduced decay of peaches and nectarines (*Prunus persica* L.) (Margosan *et al*., 1997).

The combination of a surfactant (Tween 60) and oil emulsion (corn or soybean) results in lower ethylene production, higher flesh firmness and greener skin color of 'Bartlett' pears than the surfactant alone (Ju and Curry, 2001). In addition, treated fruit had higher levels of SSC and titratable acidity, and produced higher levels of volatiles than non treated controls by the end of cold storage. Dipping fruit in

aqueous surfactant solution can modify sublenticel structure resulting in blurring, smoothing out, or reduction of surface cracking (Roy *et al*., 1996). Surfactant concentration >2% cause tissue discoloration of fruit peel in both apples and pears after only a few days at 0 °C (Ju *et al*., 2001).

2.6. Research questions and needs

The analysis of the published reports on the horticultural effects of LPE shows inconsistencies and generalizations about the effects of LPE on physiological processes remain difficult to establish. Despite the evidence for the regulatory effects of LPE, the specific *in vivo* targets of exogenous LPE remain elusive. The tentative physiological model proposed herein suggests the following research questions, aimed at clarifying the mode of action of LPE: the nature of acyl chain of LPE needs to be known and reported, given the specificity of LPE effects; *in vivo* inhibition of PLD by exogenously applied LPE needs to be demonstrated in horticultural contexts; the relevance of the feedback control of PLA_2 and PLD activity by PA should be explored to identify interaction possibilities. The precise mechanisms by which LPE interferes with ethylene biosynthesis need to be established. The reported effects of LPE on specific enzymes require clarification of whether the effect is direct or indirect. The attribution of the horticultural effects to LPE requires the clarification of the role of excipients or additives present in LPE formulations or added to LPE solutions. All these compounds should be tested, individually and combined, as controls. LPE formulations and preparation methods should be standardized and the properties of the solution (*e.g.*, pH) should be reported.

2.7. Conclusions

Lysophospholipids in general and LPE in particular, are signaling molecules in the biochemical cascades involved in wound response and senescence. However, the horticultural effects of exogenous LPE applications reported in the literature are not always consistent with the putative mode of action of LPE. A coherent mechanism to explain the horticultural effects of LPE action is currently lacking. It is not clear to what extent the horticultural effects attributed to LPE are specific or generic responses to amphipatic molecules. Excipients in the formulation and tensioactive additives, such as oils and ethanol, may explain some of the horticultural effects attributed to LPE.

CHAPTER 3

Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons

Abstract

Cantaloupe and honeydew melon cultivars were processed and stored under a high oxygen passive MAP or reduced oxygen CA (kPa O_2 + 10 kPa CO_2, balance N_2) for 14 days at 5 ºC. Atmosphere did not affect softening rate or SSC and had a negligible effect on color. Volatile compounds known as flavor-important in melons were extracted using stir bar sorptive extraction (SBSE) and quantified via GC-MS. Acetate esters increased more in MAP than in CA. Non-acetate esters increased markedly in both cultivars and storage types. Alcohols were more abundant in honeydew than in cantaloupe, aldehydes decreased during storage in both cultivars and storage types. Lower O_2 availability under CA conditions likely suppressed some of the esters relevant to the aroma of fresh-cut melon. Results suggest that package O_2 levels are more important in determining aroma than other quality attributes of fresh-cut melon, and high O_2 levels may be required to reveal desirable aroma compounds.

3.1. Introduction

Fresh-cut produce provides convenience and value-added to meet the demands of a growing number of consumers for fruits and vegetables. Ease of consumption is a particularly desirable feature in a fruit like melon that is too large to provide a single portion and requires some degree of preparation prior to eating, along with the associated disposal of residues. Convenience, however, comes at the expense of some quality attributes.

The European and American melon markets are dominated by two major cultivar types differing in quality attributes and postharvest behavior (Robinson and Decker-Walters, 1999): the very aromatic, climacteric, and fast senescing cantaloupes and muskmelons (*Cantaloupensis* and *Reticulatus* groups) and the less aromatic, non-climacteric, slow-senescing melons belonging to the Inodorus group.

Fresh-cut processing of melons induces changes in a number of quality attributes: color, sweetness, firmness (Portela and Cantwell, 1998), ethylene and respiration rate (Aguayo *et al*., 2007), microbial load (Ayhan *et al*., 1998; Aguayo, *et al*., 2003), and aroma volatiles (Beaulieu, 2006a; 2006b). The quality attributes of fresh-cut melon depend on the genotype (Aguayo *et al*., 2003; Saftner and Lester, 2009), maturity stage (Beaulieu, 2006a; Simandjuntak *et al*., 1996), ethylene production (Bauchotet *et al*., 1998; Flores *et al.*, 2002; Saftner *et al*., 2007), and storage temperature (Bett-Garber *et al*., 2005). Aroma and sugar are major determinants of melon quality and consumer preference (Beaulieu *et al*., 2003), which deteriorates quickly after fresh-cut processing (Beaulieu, 2006a).

Low O_2 modified atmospheres are considered effective technologies to improve shelf-life of fresh-cut cantaloupe (Bai *et al*., 2001) and honeydew melons (Portela and Cantwell, 1998). But considering the contrasting metabolism of the two melon cultivars, optimal conditions to preserve fresh-cuts prepared from these raw

materials also differ: higher O_2 and CO_2 levels are generally recommended for cantaloupes than for honeydews (Gorny, 2001).

MAP can delay aroma deterioration, but there is also a risk of inducing off-flavors and imbalances in the aroma profile (Mattheis and Fellman, 2000). Despite the beneficial effects of reduced oxygen on respiration rate, color and firmness changes (Aguayo *et al*., 2003), oxygen concentration inside the packages may have a profound, and yet uncharacterized, effect on aroma volatiles. For example, low O_2 storage has been reported to decrease volatile esters biosynthesis in apple (Mattheis *et al*., 1998) and increase some esters in orange (Shaw *et al*., 1992).

Although aroma volatiles have been characterized in whole (Beaulieu, 2006a; Beaulieu and Grimm, 2001; Perry *et al*., 2009; Obando-Ulloa *et al*., 2009) and fresh-cut melons (Beaulieu, 2006a, 2006b; Saftner and Lester, 2009; Beaulieu and Lancaster, 2007), the effects of package O_2 levels on individual compounds concentrations of the different melon genotypes stored for 14 days have not been reported and compared.

This study was designed to evaluate the effect of two low-oxygen atmospheres on volatiles and quality attributes in fresh-cuts prepared from cantaloupe and honeydew melons. Aroma volatiles were extracted with a recent method, SBSE, and the effects of atmosphere and storage time on individual volatiles believed to be flavor-important were assessed.

3.2. Materials and methods

3.2.1. Raw material and fruit processing

An orange fleshed cantaloupe (*Cucumis melo* var. *cantaloupensis,* Sol Real) and a green fleshed honeydew (*Cucumis melo* var. *inodorus*) melon were cooled

overnight to 5º C, prior to processing. Cantaloupes were harvested at commercial maturity (¾–slip, ºBrix ≥ 9) in California and shipped overnight whereas honeydews were bought commercially, and carefully selected for uniformity and maturity based on ground color appearance and waxy development at the stem end (greenish-white external color, waxy stem end), and acceptable ºBrix (≥ 9). Melons were inspected carefully for bruising and compression damage and fruit with no visual defects and uniform in shape and size were selected. Fruits were washed in cold water, dipped in 100 µg L^{-1} of sodium hypochlorite solution for 2 min, rinsed with deionized water and allowed to drain. The skin was uniformly removed using a peeler (CP-44 Muro Corporation, Tokyo, Japan), the blossom and stem ends were discarded, placental tissue and seeds were removed and cubes (ca. 2.5 × 2.5 cm) were prepared by hand with a sharp knife. For both cultivars and storage conditions, ten fresh-cut melon cubes were randomly placed in each package and assessed for quality parameters on days 0, 4, 7 and 14 of storage.

3.2.2. Packaging and storage conditions

Cubes prepared from numerous fruits were randomized before packaging. Fresh-cut fruit subjected to passive MAP treatment were placed in 250 g polypropylene trays (Green-Tek, Edgerton WI, USA) containing ca. 175 g of fresh-cut melon. Trays were overwrapped with a P-Plus Perm 90 microperforated film with an O_2 transmission rate of 5200 $cm^3\ m^{-1}\ d^{-1}\ atm^{-1}$ (Amcor Flexibles, Mundelein, IL, USA) and heat-sealed on the edges with a vacuum packing machine (Koch Kats 100 Basic V/G, ILPRA FoodPack Basic, Kansas City, MO, USA). Fresh-cut melons (ca 175 g) stored under CA were placed in 500 mL glass mason jars, connected to a flow through system (custom built Flow Boards, Post Harvest Research, Davis, CA) with commercially prepared mixed gas cylinders (Praxair Distribution, Geismar, LA) supplying 5 kPa O_2 and 10 kPa CO_2, at a constant rate of 10 mL min^{-1} as measured

with a Mass Flowmeter (Post Harvest Research, Davis, CA). All samples were stored for 14 days at 5 ºC, and analyzed at days 0, 4, 7 and 14 for color, firmness, SSC and volatiles.

3.2.3. Package atmosphere composition

O_2 and CO_2 levels were monitored in the package headspace, by removing a 1-mL sample with a needle via a small piece of adhesive rubber septa strip attached to the MAP film. O_2 and CO_2 concentrations were measured with a Pac Check gas analyzer (Mocon 650 Dual Head Space Analyzer, Mocon, MN, USA).

3.2.4. Color, firmness and SSC

Surface color of the fresh-cut cubes was measured in the CIE L*a*b* color space, with a CR-400 colorimeter (Konica Minolta, Japan), using the D_{65} illuminant. Hue angle (h^o = arctan b*/a*) and chroma [$C^*=(a^{*2}+b^{*2})^{1/2}$] were calculated from the primary a* and b* readings. Two color measurements were performed on random points in each of three cubes from six replicated packages of each cultivar. Mesocarp firmness was measured with a hand-held penetrometer (McCormick, FT327, Alphonsine, Italy) equipped with an 8 mm-probe on three cubes from each replicate. Color and firmness measurements were not made on skin-facing or seed cavity sides of the cubes. SSC was determined on the juice obtained from three cubes from each of the six replicated packages using a digital hand-held refractometer (Atago PAL-1, Tokyo, Japan).

3.2.5. Volatile extraction

Tissue samples were juiced into slurry with a Braun MP80 Juicer (Germany) and the SBSE (Gerstel, Muelheim an der Ruhr, Germany) technique (Baltussen *et al*., 1999) was used to extract the volatiles. Stir bars (polydimethylsiloxane) with 10 mm

length, were conditioned at 300 ºC for 1 hour, and re-conditioned between samplings on an Agilent gas chromatograph (Agilent 6890 GC, Palo Alto, CA, USA) inlet, at 280 ºC, with a constant 50 mL min^{-1} helium flow, for 8 minutes. Stir bars were placed in 10 mL vials containing 1 mL juice for each replicated sample, 4 mL of 62.5 % saturated salt solution, 2-methylbutyl 3-methylbutanoate as internal standard (100 µg L^{-1} final concentration), and sealed with a steel cap fitted with a teflon/silicon septum. Extraction was performed for 1 hour at 37.5 ºC and 850 rpm on a Variomag Multipoint HP15 (Oberschleissheim, Germany) stir plate.

3.2.6. Volatile identification and quantification

After extraction, the stir bars were removed from the vials, rinsed with deionized water, blotted dry on a lint free tissue and placed in glass thermodesorption tubes. The tubes were placed on an autosampler equipped with a Twister Desorption Unit (MPS2-TDU, Gerstel, Inc., Baltimore, Maryland, USA) mounted on an Agilent gas chromatograph (Agilent 6890 GC, Palo Alto, CA, USA). The stir bars were cryofocused (- 60 ºC) at the GC inlet and desorbed under the following conditions: after a 0.4 min delay, the temperature was increased from 35 °C to 280 °C at 200 °C min^{-1} and held for 5 min. The programmable temperature vaporizing inlet (PTV) was held at -60 °C during desorption period in solvent vent mode with a 50 mL min^{-1} helium flow. After a 0.2 min delay, the PTV was switched to splitless mode and the temperature was increased at 12 °C sec^{-1} to 280 °C and held for 3 min. The oven temperature was held at 40 °C for 1 min, increased to 110 °C at 5 °C min^{-1}, increased to 160 °C at 10 °C min^{-1}, increased to 280 °C at 25 °C min^{-1} and held for 1.2 min. Helium carrier gas was used at a velocity of 35 cm sec^{-1} through a DB5 capillary column (30 m × 0.25 mm × 0.25 µm, Agilent, Palo Alto, CA, USA). The column was interfaced to an Agilent 5973 MSD (Palo Alto, CA, USA) through a transfer line held at 280 °C, operated in the electron ionization mode at 70 eV (electron volts) with a

source temperature of 200 ºC, and a continuous scan from m/z (mass to charge ratio) 33 to 300 at 5.24 spectra s^{-1}.

Data were collected with HP Chemstation Software (Agilent Technologies, Wilmington, Delaware, USA, A.03.00) and searched against the Wiley registry of mass spectral data (7th Edition, Palisade Corp., Newfield, N.Y., USA). Propyl acetate, ethyl 2-methyl propanoate, 2-methylpropyl acetate, methyl 2-methyl butanoate, ethyl butanoate, ethyl 2-methyl butanoate, 3-methyl 1-butyl acetate, 2-methyl butyl acetate, ethyl (methylthio) acetate, ethyl hexanoate, hexyl acetate, eucalyptol, ethyl 3-(methylthio) propanoate, benzyl acetate and octyl acetate were obtained from Sigma Aldrich (St. Louis, MO) and (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenyl acetate, (*Z*)-3-octenol, (*Z*)-6-nonenal, (*E*)-2-nonenal, (*E,Z*)-2,6-nonadienol, (*Z*)-6-nonenol, 3,6-nonadienyl acetate and (*E,Z*)-2,6-nonadienyl acetate were obtained from Bedoukian Research (Danbury, CT). Volatile compounds identified and quantified using selected and/or unique ions and a mixed external standard calibration (0.05, 0.1, 1, 10, 50, 100, 500, and 1000 µg L^{-1}) which, were bracketed to deliver linear equations within the range of each compound recovered in melons (Table 3.1). The internal standard was used additionally to examine each run and then each compound´s integrated response was examined via their selective unique target ion (T-ion) and qualifying ion (Q-ion) ratios (Table 3.1).

3.2.7. Statistical analyses

Data were subjected to analysis of variance according to a split-plot design with atmospheric treatment (CA and MAP) as main-plot and time in storage as sub-plot (Gomez and Gomez, 1984). Each genotype was considered a separated experiment. When significantly different, means were separated with Duncan´s multiple range test. Statistical analysis was performed using SPSS software (v. 16.0,

Table 3.1. Integrated and analyzed volatile compounds recovered by stir bar sorptive extraction, gas chromatograph-mass spectrometry

Compound class Compound	Sensory attributes[a]	RT[b] (min)	T-ion[c] (*m/z*)	Q-ion[e] (*m/z*)[d]	CAS[f] no.	Linear Equation	Range (µg kg^{-1})	R^2
Acetate esters								
Propyl acetate	Celery, ethereal, fruity, pear, powerful, raspberry	3.93	43	61	109-60-4	y = 21870x + 119772	1-1000	0.985
2-Methylpropyl acetate	Apple, banana, ethereal, floral, fruity, sweet	4.93	43	56	110-19-0	y = 6927.7x + 2E+06	50-1000	0.982
3-Methyl butyl acetate	Banana, fragant, fruity, pear, sweet	7.27	87	70	123-92-2	y = 2290.6x + 23673	0.05-500	0.996
2-Methyl butyl acetate	Banana, candy, citrus, ether, floral, fresh, fruity, citrus, peanuts, fresh, vegetable	7.33	70	73	624-41-9	y = 7712x + 546102	0.1-1000	0.988
Hexyl acetate	Apple, cherry, floral, pear, wine	11.08	56	84	142-92-7	y = 4931.5x + 137212	0.1-500	0.995
Benzyl acetate	Floral, fruity, fresh, pine, sweet	15.52	108	91	140-11-4	y = 16915x + 1E+06	0.05-1000	0.988
Octyl acetate	Floral, fruity, herbaceous, jasmine	16.70	112	70	112-14-1	y = 969x - 5161.1	1-500	0.993
3,6-Nonadienyl acetate	Fresh, green	18.51	122	107	76649-26-8	y = 3354.5x - 21282	1-500	0.997
(E,*Z*)-2,6-Nonadienyl acetate	Spicy, violet leaf	18.66	122	68	68555-65-7	y = 1747.6x - 22757	10-500	0.997
(*Z*)-3-Hexenyl acetate	Diffusive, fruity, green, strong, unripe banana	10.87	67	82	3681-71-8	y = 9942.4x + 1E+06	0.1-500	0.990
Non-acetate esters								
Ethyl 2-methyl propanoate	Citrus, ethereal, fruity, floral, melon-like, sweet	4.65	71	116	97-62-1	y = 11582x + 12878	0.1-50	1.000
Methyl 2-methyl butanoate	Apple, artificial strawberry, floral, fruity, medicinal, sweet	4.98	88	57	868-57-5	y = 15405x +153649	1-100	0.966
Ethyl butanoate	Banana, banana-pineapple, candy, diffusive, fragrant, fruity ethereal, medicinal, sweet, sick, tutti frutti	5.47	71	88	105-54-4	y = 7624.8x + 569900	0.1-500	0.978
Ethyl 2-methyl butanoate	Cantaloupe-like, floral, fruity, green, melon, powerful, pungent, strawberry, sweet	6.58	102	85	7452-79-1	y = 7103.4x + 201695	0.1-500	0.991

Table 3.1. Continued

Compound class / Compound	Sensory attributes[a]	RT[b] (min)	T-ion[c] (*m/z*)	Q-ion[e] (*m/z*)[d]		CAS[f] no.	Linear Equation	Range (µg kg^{-1})	R^2
Ethyl hexanoate	Apple, banana, brandy, floral, fruity, powerful, wine-like	10.69	88	99		123-66-0	y = 6854.7x + 73786	0.1-500	0.999
Alcohols									
Eucalyptol	Camphoraceous, cool, diffusive, fresh, minty	11.73	108	154		470-82-6	y = 9275.3x + 33889	1-100	0.992
(*Z*)-6-Nonenol	Cucumber, green, green melon, melon, powerful, pumpkin, sweet, waxy	15.71	95	82		35854-86-5	y = 6264.3x + 99001	10-500	0.999
(*E,Z*)-2,6-Nonadienol	Cucumber, fresh, violet leaf	15.57	69	93		28069-72-9	y = 5760.8x + 64479	0.1-500	0.998
(*Z*)-3-Octenol	Powerful, fresh, earth	12.33	110	81		20125-84-2	y = 2729.6x + 22197	1-500	1.000
Aldehydes									
(*Z*)-3-Hexenal	Apple-like, freshly crushed, green, leafy, powerful, Strawberry leaf, wine-like	5.43	69	80		6789-80-6	y = 321.86x + 30388	100-1000	0.957
(*E*)-2-Hexenal	Green leafs, vegetable, Apple	6.69	55	69		6728-26-3	y = 7536.7x + 53960	1-100	0.971
(*Z*)-6-Nonenal	Citrus, cucumber, green, melon-like	13.78	55	122		2277-19-2	y = 3864x + 59304	10-500	0.999
(*E*)-2-Nonenal	Fatty, green, penetrating, waxy, tallowy	15.46	70	96		18829-56-6	y = 4575.8x + 57663	0.05-500	0.999
Sulfur-containing Compounds									
Ethyl (methylthio) acetate	Cucumber, green, grassy	10.22	61	134		4455-13-4	y = 17814x + 87933	0.1-50	0.999
Ethyl 3-(methylthio) propanoate	Fruity, green, grassy	13.69	148	74		13327-56-5	y = 10666x + 242436	10-100	0.989

[a]Compound sensory/aroma attributes contained either within references or in FlavorWorks 2.0 (Anaheim Hills, CA)
[b]RT = retention time *m/z* = mass to charge ratio
[c]T-ion = target aion [e]Q-ion = qualifying ion

SPSS, Chicago, IL, USA). An individual package or jar constituted a true experimental unit which was used as one replicate on each sampling day, per treatment. Data from two separate repeated trials, with three replicated packages each were pooled together for analysis.

3.3. Results

3.3.1. Atmospheric composition

O_2 levels inside the MA packages gradually decreased during the 14-day storage period from normal air concentrations to 11.1 kPa and 14.2 kPa while CO_2 increased to 11.3 kPa and 7.9 kPa, for cantaloupe and honeydew, respectively (Fig. 3.1). The average O_2 partial pressure in MAP during the 14 days of shelf-life was 18.2 kPa and 16.2 kPa for honeydew and cantaloupe, respectively. CA treatment maintained O_2 and CO_2 levels at constant partial pressures of 5 and 10 kPa, respectively. Overall, throughout the storage period, fresh-cut stored in CA were exposed to lower O_2 and higher CO_2 partial pressures than in MAP.

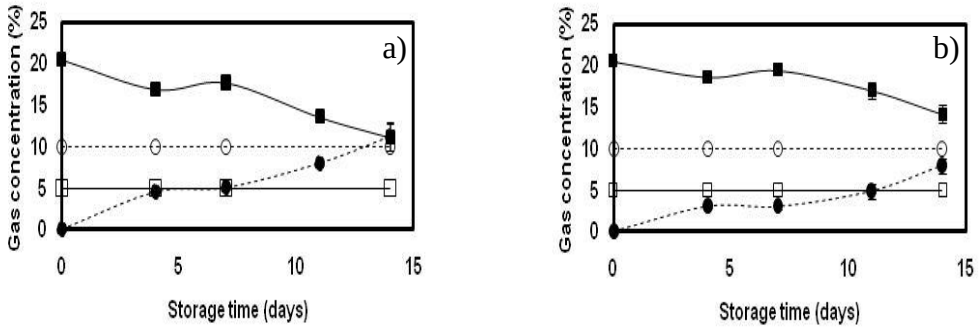


Fig. 3.1. O_2 (■,□) and CO_2 (●,○) concentrations in MAP (■,●) and CA (□,○) of fresh-cut cantaloupe (a) and fresh-cut honeydew (b) melons stored for 14 days at 5ºC. Values are average ± SD (*n*=6). When not seen, error bars fall within symbols.

3.3.2. Color, firmness and SSC

Surface color of fresh-cut cantaloupe and honeydew cubes was not affected by packaging atmospheres. Small, but significant, color changes were observed during storage. Lightness (L*) decreased slightly during storage of cantaloupe and chroma (C*) decreased in both cultivars (Table 3.2).

Firmness of both cultivars decreased during the first 4 to 7 days and remained relatively unchanged for the remaining storage period (Table 3.2). During the initial 4 days after processing, cantaloupe cubes softened at a higher rate (2.0 N day^{-1}) than honeydew cubes (0.9 N day^{-1}), and by the end of the 14 days of storage, firmness values were 63% and 69% of the initial value, for cantaloupe and honeydew cubes, respectively. Atmospheric composition did not affect the softening rate of either cultivar. SSC remained unchanged throughout storage of honeydew but decreased slightly in cantaloupe. Atmospheric composition did not affect SSC (Table 3.2).

Table 3.2. Color, firmness, SSC and selected volatiles in cantaloupe and honeydew cubes during storage at 5 °C under CA (5 kPa O_2 + 10 kPa CO_2, balance N_2) and passive MAP.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
L*	0	68.3	67.4	67.9^{a}	72.2	72.2	72.2^{a}
	4	67.4	67.3	67.4^{ab}	71.9	72.2	72.1^{a}
	7	66.8	66.4	66.6^{b}	71.6	72.9	72.3^{a}
	14	66.5	66.0	66.3^{b}	71.6	70.8	71.2^{a}
	Avg.	67.3^{A}	66.8^{A}		71.8^{A}	72.0^{A}	
C*	0	42.0	42.1	42.1^{a}	28.0	27.2	27.6^{a}
	4	41.2	41.4	41.3^{ab}	26.9	26.6	26.8^{ab}
	7	41.1	40.9	41.0^{b}	25.7	26.5	26.1^{ab}
	14	41.6	40.5	41.1^{b}	26.1	25.4	25.8^{b}
	Avg.	41.5^{A}	41.2^{A}		26.7^{A}	26.4^{A}	

Table 3.2. Continued.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
h°	0	75.8	75.8	75.8^{a}	116.6	117.0	116.8^{a}
	4	75.8	76.0	75.9^{a}	116.7	116.8	116.8^{a}
	7	76.0	76.2	76.1^{a}	116.0	116.8	116.4^{a}
	14	75.6	76.5	76.1^{a}	116.5	116.6	116.6^{a}
	Avg.	75.8^{A}	76.1^{A}		116.5^{A}	116.8^{A}	
Firmness (N)	0	28.7	30.1	29.4^{a}	24.9	21.5	23.2^{a}
	4	25.1	22.1	23.6^{b}	17.2	18.1	17.7^{b}
	7	18.8	20.2	19.5^{c}	15.8	15.0	15.4^{b}
	14	18.2	18.9	18.6^{c}	16.1	15.9	16.0^{b}
	Avg.	22.7^{A}	22.8^{A}		18.5^{A}	17.6^{A}	
SSC (%)	0	10.1	9.6	9.9^{a}	10.6	11.3	11.0^{a}
	4	9.6	9.6	9.6^{ab}	11.7	11.0	11.4^{a}
	7	9.5	9.2	9.4^{ab}	11.4	11.6	11.5^{a}
	14	8.8	9.5	9.2^{b}	11.6	11.6	11.6^{a}
	Avg.	9.5^{A}	9.5^{A}		11.3^{A}	11.4^{A}	
Integrated volatile total[1] ($\mu g\ kg^{-1}$)	0	39499	39435	39467^{b}	14922	15725	15324^{b}
	4	41470	34717	38094^{b}	17852	15468	16660^{b}
	7	39622	43220	41421^{b}	20970	18980	19975^{a}
	14	41044	64887	52966^{a}	18140	24344	21242^{a}
	Avg.	40409^{A}	45565^{A}		17971^{A}	18629^{A}	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.[1]Volatiles comprised by twenty-five integrated and calculated compounds (per Table 1) based on selected ion counts which are not a representation of the total ion counts of a complete chromatogram.

3.3.3. Aroma volatiles

Twenty-five compounds believed to be flavor-important in melon (Beaulieu, 2006b) or with noteworthy abundance and/or flavor importance in other fruits were integrated and used to assess volatile changes in CA and MAP storage. There were qualitative and quantitative effects of atmospheric composition and time in recovered aroma volatiles of both cultivars. Abundance of the 25 integrated volatile compounds in fresh-cut cantaloupe and honeydew melon tested increased during the 14 days of

storage under both atmospheric regimes. By the end of the storage time, the summed volatile concentration was 1.4 times higher than the initial value for both cantaloupe and honeydew melons (Table 3.2). Overall integrated volatile abundance was not significantly affected by atmospheric composition but it was significantly affected by storage time. Total volatile abundance increased significantly by day 14 for cantaloupe cubes and by day 7 for honeydew.

Emission of acetate esters by day 4 of storage and until the end of storage time was 2 to 2.5-fold higher in cantaloupe than in honeydew. A significant increase in acetate esters during storage was observed in cubes from both cultivars in MAP but not in CA. Four of the ten acetate esters quantified (2-methylpropyl acetate, 2-methylbutyl acetate, hexyl acetate, benzyl acetate) accounted for 87 - 93 % of the integrated total (Table 3.3). In both genotypes, levels of most acetate esters were significantly higher in MAP than in CA. The exceptions were benzyl acetate (unchanged), octyl acetate, 3,6-nonadienyl acetate and (*E,Z*)-2,6-nonadienyl acetate (lower in MAP than in CA for cantaloupe).

Table 3.3. Acetate esters concentrations in cantaloupe and honeydew cubes during storage at 5 °C under CA (5 kPa O_2 + 10 kPa CO_2, balance N_2) and passive MAP.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
Propyl acetate ($\mu g\ kg^{-1}$)	0	604	490	547^{b}	91	135	113^{b}
	4	669	367	518^{b}	466	337	402^{a}
	7	408	570	489^{b}	423	506	465^{a}
	14	500	1070	785^{a}	333	611	472^{a}
	Avg.	545^{A}	624^{A}		328^{B}	397^{A}	
2-Methylpropyl acetate ($\mu g\ kg^{-1}$)	0	8764	8890	8827^{a}	1107	1730	1419^{c}
	4	7198	6301	6750^{b}	2302	2811	2557^{b}
	7	5609	8673	7141^{b}	2117	3813	2965^{b}
	14	5711	12267	8989^{a}	1767	5659	3713^{a}
	Avg.	6821^{B}	9033^{A}		1823^{B}	3503^{A}	

Table 3.3. Continued.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
3-Methyl butyl acetate (µg kg^{-1})	0	40	41	41^{c}	18	54	36^{b}
	4	83	35	59^{c}	62	65	64^{b}
	7	698	79	389^{b}	60	77	69^{b}
	14	99	879	489^{a}	60	642	351^{a}
	Avg.	230^{A}	259^{A}		50^{B}	210^{A}	
2-Methyl butyl acetate (µg kg^{-1})	0	8123	9054	8589^{a}	1354	1928	1641^{c}
	4	6918	6917	6918^{b}	2579	3537	3058^{b}
	7	5900	9051	7476^{b}	2678	4264	3471^{b}
	14	5523	11952	8738^{a}	2525	5863	4194^{a}
	Avg.	6616^{B}	9244^{A}		2284^{B}	3898^{A}	
Hexyl acetate (µg kg^{-1})	0	5885	7586	6736^{ab}	404	557	481^{c}
	4	5671	5092	5382^{c}	1660	1651	1656^{b}
	7	5785	6746	6266^{bc}	3076	2376	2726^{a}
	14	5899	10624	8262^{a}	1958	3768	2863^{a}
	Avg.	5810^{B}	7512^{A}		1775^{B}	2088^{A}	
Benzyl acetate (µg kg^{-1})	0	4820	5020	4920^{b}	3289	3550	3420^{a}
	4	4853	5065	4959^{b}	3180	3132	3156^{a}
	7	5696	4900	5298^{b}	3252	3291	3272^{a}
	14	7342	6408	6875^{a}	3138	3502	3320^{a}
	Avg.	5678^{A}	5348^{A}		3215^{A}	3369^{A}	
Octyl acetate (µg kg^{-1})	0	192	652	422^{a}	63	88	76^{b}
	4	181	193	187^{b}	81	105	93^{a}
	7	411	112	262^{ab}	59	65	62^{b}
	14	878	74	476^{a}	53	90	72^{b}
	Avg.	416^{A}	258^{A}		64^{B}	87^{A}	
3,6-Nonadienyl acetate (µg kg^{-1})	0	43	34	38^{a}	118	96	107^{a}
	4	N/D	N/D	-	15	33	24^{b}
	7	19	N/D	19^{b}	17	13	15^{c}
	14	18	N/D	18^{b}	16	N/D	16^{c}
	Avg.	27^{B}	34^{A}		42^{B}	47^{A}	

Table 3.3. Continued.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
E,Z-2,6-Nonadienyl acetate (µg kg^{-1})	0	45	37	41	114	101	108^{a}
	4	N/D	N/D	-	28	N/D	28^{b}
	7	N/D	N/D	-	N/D	N/D	-
	14	N/D	N/D	-	N/D	N/D	-
	Avg.	45^{A}	37^{A}		71^{B}	101^{A}	
Z-3-Hexenyl acetate (µg kg^{-1})	0	2683	2988	2835^{ab}	60	119	89^{d}
	4	1576	2162	1869^{b}	179	241	210^{c}
	7	2365	1141	1753^{b}	1170	608	889^{b}
	14	1887	4148	3017^{a}	663	515	589^{a}
	Avg.	2128^{A}	2610^{A}		518^{A}	371^{B}	
Total acetate esters (µg kg^{-1})	0	31199	34792	32996^{a}	6618	8358	7488^{d}
	4	27149	26132	26641^{b}	10552	11912	11232^{c}
	7	26891	31272	29082^{b}	12852	15013	13933^{b}
	14	27857	47422	37640^{a}	10513	20650	15582^{a}
	Avg.	28274^{B}	34905^{A}		10134^{B}	13983^{A}	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

Also, except for benzyl acetate in honeydew and octyl acetate in both cultivars, storage time had a significant effect on all acetate esters, showing a consistent increase during storage (Table 3.3). 3,6-Nonadienyl acetate and (*E,Z*)-2,6-nonadienyl acetate were found in very small amounts in both cultivars. These compounds were detected in cantaloupe only on day 0. The effect of storage atmosphere for these compounds was subsequently inconclusive due to the differences in initial concentrations. In cantaloupe, (*E,Z*)-2,6-nonadienyl acetate was found at a smaller concentration than honeydew. (*Z*)-3-Hexenyl acetate in cantaloupe was not affected by atmosphere while in honeydew it increased more intensely in CA than in MAP. Storage time had an inconsistent effect on these compounds (Table 3.3).

Table 3.4. Non-acetate esters concentrations in cantaloupe and honeydew cubes during storage at 5 °C under CA (5 kPa O_2 + 10 kPa CO_2, balance N_2) and passive MAP.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
Ethyl 2-methyl propanoate ($\mu g\ kg^{-1}$)	0	56	53	55^{c}	5	15	10^{b}
	4	234	114	174^{b}	43	27	35^{a}
	7	236	210	223^{b}	44	21	33^{a}
	14	157	531	344^{a}	31	34	33^{a}
	Avg.	171^{B}	227^{A}		31^{A}	24^{A}	
Methyl 2-methyl butanoate ($\mu g\ kg^{-1}$)	0	557	490	524^{d}	13	40	27^{c}
	4	1381	1075	1228^{c}	133	195	164^{b}
	7	1209	2170	1690^{b}	310	488	399^{a}
	14	1219	3243	2231^{a}	198	634	416^{a}
	Avg.	1092^{B}	1745^{A}		164^{B}	339^{A}	
Ethyl butanoate ($\mu g\ kg^{-1}$)	0	877	1105	991^{c}	95	164	130^{c}
	4	4931	3017	3974^{b}	2919	1080	2000^{b}
	7	4343	4329	4336^{b}	3646	983	2315^{b}
	14	5081	7424	6253^{a}	3855	2072	2964^{a}
	Avg.	3808^{A}	3969^{A}		2629^{A}	1075^{B}	
Ethyl 2-methyl butanoate ($\mu g\ kg^{-1}$)	0	647	657	652^{c}	16	43	30^{b}
	4	2645	1751	2198^{b}	818	296	557^{a}
	7	2248	2930	2589^{b}	922	317	620^{a}
	14	1866	5124	3495^{a}	623	564	594^{a}
	Avg.	1852^{B}	2616^{A}		595^{A}	305^{B}	
Ethyl hexanoate ($\mu g\ kg^{-1}$)	0	256	397	327^{c}	38	96	67^{b}
	4	4426	2032	3229^{b}	1823	169	996^{a}
	7	3892	2559	3226^{b}	2006	113	1060^{a}
	14	4879	4148	4514^{a}	1383	286	835^{a}
	Avg.	3363^{A}	2284^{A}		1313^{A}	166^{B}	
Total non-acetate esters ($\mu g\ kg^{-1}$)	0	1780	2159	1970^{c}	149	303	226^{c}
	4	12002	6800	9401^{b}	5560	1545	3553^{b}
	7	10483	9818	10151^{b}	6574	1413	3994^{a}
	14	11826	16696	14261^{a}	5861	2922	4392^{a}
	Avg.	9023^{A}	8868^{A}		4536^{A}	1546^{B}	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

Non-acetate esters (Table 3.4) were the second major class of volatiles recovered in fresh-cut melon. In cantaloupe, except for ethyl hexanoate and ethyl butanoate, the recovery of all other non-acetate esters was significantly greater under MAP. In contrast, honeydew melon accumulated more non-acetate esters under CA, except for methyl 2-methyl butanoate. For both atmospheres and cultivars, storage time had a significant effect on the levels of non-acetate esters. By the end of the 14 days of storage, in cantaloupe non-acetate esters increased 6.6 times in CA and 7.7 times in MAP and in honeydew, non-acetate esters increased 39.3 times in CA and 9.6 times in MAP when compared with the initial values (Table 3.4).

Alcohols (Table 3.5) emitted by both cultivars decreased dramatically during the first four days in storage in both regimes. Atmospheric composition had a significant effect on alcohol production by cantaloupe, which decreased more intensely in CA than in MAP. Increased oxygen in CA could have facilitate increased oxidative reactions (*i.e.* β-oxidation and/or LOX-activity) that are required to deliver various straight chain fatty acid moieties, which condense with alcohols to form ester (Beaulieu, 2006a). In honeydew cubes, atmosphere had no effect on total alcohols. In honeydew, storage regime did not affect any of the individual alcohols studied. (*Z*)-3-Octenol was detected in cantaloupe and honeydew cubes at day 0 and decreased thereafter below detection. Storage regime did not affect eucalyptol in both cultivars and had significant but inconsistent effects on (*Z*)-6-nonenol and (*E,Z*)-2,6-nonadienol in cantaloupe (Table 3.5).

Table 3.5. Alcohols concentrations in cantaloupe and honeydew cubes during storage at 5 °C under CA (5 kPa O_2 + 10 kPa CO_2, balance N_2) and passive MAP.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
Eucalyptol (µg kg^{-1})	0	217	201	209^{a}	22	246	134^{a}
	4	149	97	123^{b}	29	15	22^{bc}
	7	178	143	161^{b}	36	23	30^{b}
	14	119	187	153^{b}	9	10	10^{c}
	Avg.	166^{A}	157^{A}		24^{A}	74^{A}	
Z-6-Nonenol (µg kg^{-1})	0	866	606	736^{a}	2394	2018	2206^{a}
	4	95	45	70^{b}	408	657	533^{b}
	7	66	35	51^{b}	376	1050	713^{b}
	14	85	106	96^{b}	727	450	589^{b}
	Avg.	278^{A}	198^{B}		976^{A}	1044^{A}	
E,Z-2,6 Nonadienol (µg kg^{-1})	0	174	115	145^{a}	1933	1890	1912^{a}
	4	25	12	19^{c}	44	43	44^{b}
	7	34	10	22^{c}	28	34	31^{b}
	14	38	32	35^{b}	63	104	84^{b}
	Avg.	68^{A}	42^{B}		517^{A}	518^{A}	
Z-3-Octenol (µg kg^{-1})	0	559	444	502	1135	1132	1134
	4	N/D	N/D	-	N/D	N/D	-
	7	N/D	N/D	-	N/D	N/D	-
	14	N/D	N/D	-	N/D	N/D	-
	Avg.	559^{A}	444^{A}		1135^{A}	1132^{A}	
Total alcohols (µg kg^{-1})	0	1816	1366	1591^{a}	5484	5286	5385^{a}
	4	269	154	212^{b}	481	715	598^{b}
	7	278	188	233^{b}	440	1107	774^{b}
	14	242	325	284^{b}	799	564	682^{b}
	Avg.	651^{A}	508^{B}		1801^{A}	1918^{A}	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

Aldehydes (Table 3.6) decreased during storage under both regimes. The effect of atmosphere on total aldehydes was not conclusive since significant differences between atmospheres were observed on day 0 for most compounds. As

for individual aldehydes, (*E*)-2-nonenal and (*E*)-2-hexenal were better maintained in CA than in MAP. (*Z*)-3-Hexenal was detected only on day 0 in honeydew cubes.

Table 3.6. Aldehydes concentrations in cantaloupe and honeydew cubes during storage at 5 °C under CA (5 kPa O_2 + 10 kPa CO_2, balance N_2) and passive MAP.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
Z-3-Hexenal (µg kg^{-1})	0	N/D	N/D	-	886	163	525
	4	N/D	N/D	-	N/D	N/D	-
	7	N/D	N/D	-	N/D	N/D	-
	14	N/D	N/D	-	N/D	N/D	-
	Avg.	N/D	N/D		886^{A}	163^{B}	
E-2-Hexenal (µg kg^{-1})	0	29	19	24^{ab}	26	40	33^{b}
	4	24	13	19^{b}	31	26	29^{b}
	7	44	21	33^{a}	50	38	44^{a}
	14	N/D	N/D	-	22	N/D	22^{c}
	Avg.	32^{A}	18^{B}		32^{A}	35^{A}	
Z-6-Nonenal (µg kg^{-1})	0	3711	199	1955^{a}	1218	1048	1133^{a}
	4	793	806	800^{b}	932	985	959^{b}
	7	575	937	756^{b}	762	1149	956^{b}
	14	N/D	N/D	-	504	N/D	504^{c}
	Avg.	1693^{A}	647^{B}		854^{A}	1061^{A}	
E-2-Nonenal (µg kg^{-1})	0	885	857	871^{ab}	362	401	382^{a}
	4	865	670	768^{b}	296	248	272^{b}
	7	1034	879	957^{a}	292	259	276^{b}
	14	518	276	397^{c}	441	105	273^{b}
	Avg.	826^{A}	671^{B}		348^{A}	253^{B}	
Total aldehydes (µg kg^{-1})	0	4625	1075	2850^{a}	2492	1652	2072^{a}
	4	1682	1489	1585^{b}	1259	1259	1259^{b}
	7	1653	1837	1745^{b}	1104	1446	1275^{b}
	14	518	276	397^{c}	967	105	536^{c}
	Avg.	2120^{A}	1169^{B}		1456^{A}	1116^{B}	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

Sulfur-containing compounds were also initially found in very low levels in both cultivars and always in lower levels under MAP than in CA for cantaloupe. In honeydew stored under CA, sulfur-containing compounds were only detected on day 0 and decreased below detection after 4 days of storage. Ethyl-3-(methylthio) propanoate increased during storage in cantaloupe but decreased below detection levels in honeydew by day 4 of CA storage and by day 7 of MAP storage (Table 3.7).

Table 3.7. Sulfur-containing compounds concentrations of cantaloupe and honeydew cubes during storage at 5 °C under CA (5 kPa O_2 + 10 kPa CO_2, balance N_2) and passive MAP.

Variable	Days	Cantaloupe			Honeydew		
		CA	MAP	Avg.	CA	MAP	Avg.
Ethyl methylthio acetate (µg kg^{-1})	0	56	22	39^{a}	152	107	129^{a}
	4	69	10	39^{a}	N/D	7	7^{b}
	7	25	2	13^{b}	N/D	1	1^{b}
	14	52	2	27^{a}	N/D	68	68^{b}
	Avg.	50^{A}	9^{B}		152^{A}	45^{A}	
Ethyl 3-methylthio propanoate (µg kg^{-1})	0	23	21	22^{c}	27	19	23^{a}
	4	299	132	216^{b}	N/D	30	30^{a}
	7	292	103	198^{b}	N/D	N/D	-
	14	549	166	358^{a}	N/D	35	35^{a}
	Avg.	290^{A}	105^{B}		27^{A}	28^{A}	
Total Sulfur-containing compounds (µg kg^{-1})	0	79	43	61^{c}	179	126	153^{a}
	4	368	142	255^{b}	N/D	37	37^{b}
	7	317	105	211^{b}	N/D	1	1^{b}
	14	601	168	385^{a}	N/D	103	103^{b}
	Avg.	341^{A}	115^{B}		179^{A}	67^{A}	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

3.4. Discussion

Color, firmness, SSC, and aroma volatiles are major quality attributes of fresh-cut melon that depend on the metabolic activity of the living tissue and could, therefore, be modulated by O_2 partial pressure. Atmospheric composition in passive

MAP evolves over time and low-oxygen levels are often attained in passive MAP only at the end of the shelf-life. The patterns of O_2 and CO_2 evolution inside MAP (Fig. 3.1) have been commonly observed (Aguayo *et al*., 2003; Bai *et al*., 2001). Overall, fresh-cut melons in MAP were exposed to higher O_2 levels throughout the storage period than those stored in CA, where strict gas levels were maintained throughout storage. This difference in O_2 partial pressure had a significant impact on volatiles, but not on color, firmness or SSC.

Color, an extrinsic attribute readily evaluated by the consumer, undergoes little or no change during storage of fresh-cut inodorus (Aguayo *et al*., 2007) or cantaloupe melons (Bai *et al*., 2001). Occasionally, slight color changes are observed *e.g.* a decrease in the lightness of fresh-cut melon during storage (Table 3.2; Saftner *et al*., 2003), but color changes do not limit quality of fresh-cut melons which, are not compromised by microbes.

Softening occurred during storage in both fresh-cut cantaloupe and honeydew melons but the rate and magnitude of these textural changes were not affected by atmosphere. In particular, reduced oxygen did not improve firmness retention, as previously observed in cantaloupe (Madrid and Cantwell, 1993) and honeydew (Portela and Cantwell, 1998).

SSC is an intrinsic quality attribute of melons, which the consumer can only assess by eating the fruit. This quality attribute is closely dependent on the sucrose levels present in whole fruit at harvest (Beaulieu, 2006a) and undergoes little or no change after fresh-cut processing and storage (Aguayo *et al*., 2008). These three quality attributes, color, firmness and SSC, were not significantly affected by the atmospheric compositions used in this study. Even with careful visual, nondestructive monitoring of initial melon quality, maturity differences were observed in both melon types for control day 0 firmness and SSC in CA versus MAP (Table 3.2) but, this did not affect observed trends.

For fresh-cut melons and to guarantee that consumers buy a fresh and safe product, a 10-day shelf-life is desirable in the distribution chain and, in our study, the abundance of total volatile compounds in fresh-cut cantaloupe and honeydew melons increased during 14 days of storage. Aroma volatiles underwent significant changes during storage and individual volatiles proved to be very sensitive to atmospheric composition in both cultivars. The volatile profiles reported herein are consistent with the published characterizations of aroma volatiles in cantaloupe and honeydew melons. Ester (Table 3.3 and 3.4) and aldehyde (Table 3.6) production was higher in cantaloupe than in honeydew (Bauchot *et al*., 1998). For both genotypes, except honeydew on day 0, 2-methylpropyl acetate, ethyl butanoate, 2-methylbutyl acetate, hexyl acetate and benzyl acetate accounted for *ca.* 74-80 % of total volatiles, consistent with previous reports indicating that these are the dominant aroma volatiles in melon (Beaulieu, 2006a; Bauchot *et al*., 1998; Saftner *et al*., 2003). In melons, butyl acetate (although not flavor-important) is often recovered in large quantities. Yet, we could not recover butyl acetate in our analyses because it co-eluted with a siloxane impurity.

The differences between the volatile profiles of both genotypes observed in this study were largely quantitative. However, a few qualitative differences between the genotypes should be noted. The production of sulfur-containing compounds ethyl-(methylthio) acetate and ethyl-3-(methylthio) propanoate was suppressed during storage of fresh-cut honeydew but not in cantaloupe (Table 3.7) and (*Z*)-3-hexenal was only found in fresh-cut honeydew. These two sulfur-containing compounds are believed to have an important role in the overall aroma profile of melon (Bauchot *et al*., 1998; Kemp *et al*., 1972; Wyllie and Leach, 1990) and their suppression, even during short term storage, may limit consumer acceptability of fresh-cut melon.

(*E,Z*)-2,6-Nonadienyl acetate was recently detected for the first time in honeydew melon (Perry *et al*., 2009). We too detected this compound in honeydew and also, at lower levels, in cantaloupe cubes (Table 3.3).

Eucalyptol, suspected of contributing to the characteristic aroma of muskmelon and positively correlated with fruity and sweet taste (Beaulieu and Lancaster, 2007), was higher in cantaloupe than in honeydew. (*Z*)-3-Hexenal, associated to green notes in muskmelon flavor (Schieberle *et al*., 1990, Beaulieu and Grimm, 2001), was only detected in honeydew on day 0 and not detected in cantaloupe by our method. In our experience, the PDMS coating is not highly effective regarding recovery of certain low molecular weight aldehydes and S-compounds.

Differences in genotype and uncontrolled factors such as maturity at harvest, processing, and storage conditions are likely responsible for the diverse patterns of volatiles evolution during storage reported in the literature (Beaulieu, 2005).

Total volatiles recovered have been reported to increase throughout storage of fresh-cut honeydew melon (Saftner *et al*., 2003), decrease in honeydew melon (Portela and Cantwell, 1998) and in both melon genotypes (Saftner and Lester 2009). In our study, total volatiles increase was observed from day 4 to 14 in cantaloupe and throughout the entire storage period in honeydew. The increase in total volatiles during storage of fresh-cut cantaloupe and honeydew melons may be explained by an acceleration of the metabolism as a response to wounding. As respiration rate increases more energy is driven to glycolysis and the tricarboxylic acid cycle, increasing the availability of acetyl-CoA and ultimately resulting in the production of amino-acid precursors to many volatile compounds (Beaulieu, 2006a, 2006b). Much less information is available concerning the effect of storage conditions on volatile classes and individual volatiles. In our study, the effect of storage time was significant for all compound classes and, except for benzyl acetate and ethyl 3-(methylthio) propanoate in fresh-cut honeydew, on all individual volatiles. This increase in total

volatile abundance was mostly due to an increase in non-acetate esters, similar to previously reported when volatile classes were calculated based upon relative percentages (Beaulieu, 2006a).

The increase observed in ester emission during storage is in agreement with previous observations (Beaulieu, 2006a) reporting total esters, non-acetate and acetate esters displaying a transient increase in relative proportion during storage of fresh-cut cantaloupe and honeydew melons packaged in clamshell containers. In our study, emission of acetate esters was not consistent throughout storage. Acetate esters increased during the 14 days of honeydew storage (Table 3.3) but the trend varied in cantaloupe. Non-acetate esters increased consistently during the 14 days of storage (Table 3.4). This class of volatiles contributed significantly for the observed increase in total volatile abundance during storage of cantaloupe in MAP and during storage of honeydew in CA (Table 3.4).

The decrease in alcohols observed during storage (Table 3.5) contrasts with the previous observations (Saftner *et al*., 2003), who reported that most of the increase in total volatile abundance of stored fresh-cut melons was due to the increase of esters and saturated alcohols and with the maintenance of most alcohols during storage fresh-cut cantaloupe storage reported elsewhere (Beaulieu, 2006a). (*E,Z*)-2,6-Nonadienol and (*Z*)-6-nonenol were reported to remain stable or increase during storage (Saftner *et al*., 2003), whereas we observed a decrease of these compounds. (*Z*)-6-Nonenol, a compound associated with pumpkin and squash-like off-odors (Saftner *et al*., 2007), also decreased during storage. This decrease in alcohols in both atmospheres is likely caused by the alcohol esterification catalyzed by alcohol acetyl transferase (AAT), more active in climacteric than in non-climacteric melons (Shalit *et al.*, 2001).

Aldehydes, known to impart “green” and “grassy” notes, decreased during storage, as previously observed in fresh-cut cantaloupe (Beaulieu, 2006a). This

decrease may be attributed to the reduction of aldehydes into branched-chain alcohols, necessary to produce branched-chain acetates through a condensation reaction with various acetyl moieties, utilizing AAT (Beaulieu, 2005). The effect of atmosphere was not conclusive because of the initial differences between atmospheres which may be attributed to the lack of discrimination between intrinsic aldehyde content and aldehyde generation following tissue disruption. (*E*)-2-hexenal, which remained relatively stable during storage, is considered an important compound to the overall melon flavor (Schieberle *et al*., 1990) but may have been generated enzymatically as a consequence of cutting (Fleming *et al*., 1968).

The atmospheric regime during storage differentially affected the changes in individual volatiles and volatile classes. Acetate esters increased at higher rates in MAP than under CA, suggesting that O_2 availability under the CA conditions used in this study (5 kPa), may be suppressive for ester biosynthesis in fresh-cut melon. On the other hand, ethylene biosynthesis and accumulation was likely higher in MAP, a closed system, than under the CA conditions, where ethylene biosynthesis is significantly inhibited when O_2 levels are below 8 kPa (Kader, 1986), and where the small amounts of ethylene produced would be flushed out of the jars. The high levels of butyl, hexyl and 2-methylbutyl acetates (Lara *et al*., 2003) and ethyl butanoante, ethyl 2-methyl butanoate and ethyl 2-methyl propanoate found in MAP are consistent with the higher ethylene availability in MAP since the synthesis of these potent odorants is regulated by ethylene (Bauchot *et al*., 1998; Obando-Ulloa *et al*., 2009). The marked increase of these volatiles might also indicate that a recycling of catabolic ester breakdown products in the presence of alcohols favors non-acetate esters accumulation, as previously speculated (Beaulieu, 2006b). Studies on ethylene inhibition by 1-MCP and downregulation of ACO indicate that ethylene is required for adequate volatile production and contributes to the aroma production in fruit. The transfer of acyl groups to alcohols to form esters was strongly, but not totally, inhibited

in ACO antisense melons (Flores *et al.*, 2002), indicating that this step had both ethylene-dependent and independent components. Also, in agreement with our results, inhibition of LOX in apple stored under low O_2, resulted in lower lipid substrate availability and therefore limited synthesis of esters (Lara *et al*., 2007). Also, after hypoxic storage, the constituent biosynthetic rate of ester formation in apple discs increased as a result of air exposure (Rudell *et al*., 2002).

Atmospheric composition did not have a consistent effect on alcohols, a putative precursor to ester production (Shalit *et al.*, 2001). Higher synthesis of acetate esters in MAP (Table 3.3) was not consistently related to reduced accumulation of volatile alcohols. Also, total aldehydes showed similar concentrations throughout different atmospheric composition.

A significant amount of information and atmosphere recommendations have been generated for fresh-cut fruit products. Package atmosphere recommendations for fresh-cut melons are slightly different for different cultivar types. An atmosphere of 2-4% O_2 and 10-15% CO_2 is recommended for fresh-cut honeydew while for cantaloupe, the recommendation is 3-6% O_2 and 6-15% CO_2 (Gorny, 2001). These atmospheres are reported to enhance shelf-life by reducing softening rate, retention of SSC, and visual quality in fresh-cut cantaloupe and to reduce decay, off-odor, translucency in fresh-cut honeydew (Gorny, 2001). Our results indicate that oxygen availability appears to have a greater effect on aroma volatiles in fresh-cut melon than on other quality attributes. Fresh-cut fruits are mostly stored in clamshell type packages and deli style containers that can become static and form a MAP with low O_2 levels after prolonged storage. Future refinements in the recommendations for the oxygen levels in fresh-cut melon should integrate the effect of atmospheric composition on aroma balance.

3.5. Conclusions

The results presented herein confirm that storage time significantly alters the balance of individual volatiles and volatile classes in the flavorful cantaloupe and in the inodorus honeydew cultivar. Volatile changes during storage are likely to have a stronger impact on the sensorial appraisal of fresh-cut melons than other quality attributes such as color, firmness or SSC, known to affect melon consumer acceptance.

Atmospheric composition had a greater effect on aroma volatiles then on color, firmness or SSC. Although the effect of atmospheric composition on individual volatiles was not always consistent throughout the storage period, acetate esters in both melon cultivars were better maintained under the higher O_2 concentrations used in this study. These results stress the importance of fine-tuning MAP O_2 concentrations for optimal aroma of fresh-cut melon. Moreover, a more balanced profile of fresh-cut melon aroma is likely achieved with higher O_2 concentrations than currently recommended.

CHAPTER 4

1-Methylcyclopropene effects on temporal changes in aroma volatiles and phytochemicals of fresh-cut cantaloupe

Abstract

Orange fleshed cantaloupe melons have intense aroma and flavor but are very perishable during storage life. Fresh-cut processing enhances ethylene-mediated quality losses. Post-cutting 1-MCP application to fresh-cut cantaloupe was evaluated for its effects on quality attributes, phytochemical content and aroma volatiles.

Fresh-cut cantaloupe (*Cucumis melo* var. *cantalupensis* 'Fiesta') cubes treated with 1.0 µL L^{-1} of 1-MCP for 24 h at 5 ºC, packaged in vented plastic clamshells and stored under normal atmosphere at 5 ºC for 9 days, preserved their soluble solids, total phenolics, total carotenoids and β-carotene contents, but significant softening occurred. A significant increase of non-acetate esters and a decrease of aldehydes occurred during storage. Most quality attributes of fresh-cut cantaloupe were unaffected by the treatment with 1-MCP. 1-MCP-treated fresh-cut cantaloupe accumulated higher levels of propyl acetate, 2-methylbutyl acetate, methyl butanoate, methyl 2-methyl butanoate, methyl hexanoate, 2-methylbutyl alcohol, phenethyl alcohol and lower levels of benzyl alcohol and heptanal than untreated controls.

Post-cutting treatment with 1-MCP affected nine of the flavor-important volatiles, particularly those derived from the amino acid isoleucine and phenylalanine, but had no practical effect on phytochemicals or other quality attributes.

4.1. Introduction

Charentais-type cantaloupe melons have an orange flesh rich in carotenoids, intense aroma and taste, but are very perishable. Consumer acceptance of fresh-cut melon declines quickly after processing, largely due to undesirable changes in the aroma volatiles (Beaulieu, 2006a). Fresh-cut cubes prepared from cantaloupes undergo significant softening and changes in volatiles during storage (Amaro *et al.*, 2012). Significant increases in most volatile classes are observed shortly after cutting of cantaloupe (Beaulieu, 2006b) and a transient increase in many flavor-related esters is documented during storage followed by a substantial decline after *ca.* 7 days in storage (Beaulieu, 2006a; Beaulieu, 2006b; Saftner *et al.*, 2006). The concentration of acetate and non-acetate esters, alcohols, aldehydes, and sulfur-containing compounds varied significantly during storage of fresh-cut cantaloupe (Amaro *et al.*, 2012).

Wounding enhances ethylene production in cantaloupe (McGlasson and Prat, 1964). The relationship between ethylene and the synthesis of aroma volatiles has been studied on melons through the use of exogenously applied ethylene (Flores *et al*., 2002; Nishiyama *et al*., 2007), inhibition of ethylene synthesis (Nishiyama *et al*., 2007; Gal *et al*., 2008), and by genetically silencing ethylene biosynthesis in transgenic fruit (Bauchot *et al*., 1998; Flores *et al*., 2002). Collectively, these studies demonstrate that whenever ethylene biosynthesis or its perception is reduced, the overall concentration of volatiles decreases. 1-MCP blocks the ethylene receptors rendering the tissue temporarily insensitive to ethylene (Sisler and Serek, 1997). Although 1-MCP can significantly delay ripening and ethylene-dependent changes in whole climacteric fruits (Blankenship and Dole, 2003), its effects on the quality of fresh-cut fruit are not consistent (Toivonen, 2008).

Melons may to benefit from 1-MCP treatments (Watkins, 2008) given appropriate attention to cultivar, ripening stage, and active ingredient concentration. Nevertheless, the reported effects of 1-MCP on the quality of whole and fresh-cut melon are highly variable. Whole cantaloupe and Galia-type melons treated with 1-MCP showed significant firmness retention during storage (Gal *et al*., 2006; Jeong *et al*., 2007) but 1-MCP applied to whole cantaloupes prior to processing had an inconsistent effect on firmness of the fresh-cut cubes (Jeong *et al*., 2008). In Charentais-type cantaloupe, ethylene affects the synthesis of fatty acid and aldehyde volatiles, whereas alcohol acetylation has ethylene-dependent and ethylene-independent components (Flores *et al*., 2002). It is, therefore, anticipated that the ethylene action inhibitor 1-MCP will modulate the aroma of fresh-cut cantaloupe. The aroma volatiles of whole Galia-type melons treated with 1-MCP were significantly reduced and characterized by a strong “green” note. Meanwhile, a strong ‘acetone’ note, associated with ‘unpleasant’ and ‘non-typical’ aroma, was reported in untreated fruit (Gal *et al*., 2008).

Considering the changes in quality, namely the rapid softening, occurring during storage of fresh-cut cantaloupe, there is potential for modulation of the quality of fresh-cut melons by the post-cutting application of 1-MCP. However, the anticipated changes in aroma volatiles and phytochemical content of fresh-cut Charentais following 1-MCP treatment remain uncharacterized. Therefore, the objective of this work was to describe the changes in quality attributes, phytochemical content, and aroma volatiles during cold storage of fresh-cut cantaloupe and assess whether these changes can be modulated by the post-processing treatment with 1-MCP.

4.2. Materials and methods

4.2.1. Fruit material and processing conditions

Orange fleshed Charentais-type cantaloupes (*Cucumis melo* L. var. *cantalupensis* Naud. 'Fiesta') were harvested at the ¾–slip maturity stage (Jeong *et al*. 2007) and cooled overnight to 5 ºC prior to processing. Fruit were carefully inspected for bruising and compression damage and only fruit without visual defects and uniform in shape and size were selected for processing. Fruit were washed in cold water, dipped in 100 µg L^{-1} NaOCl for 2 min, rinsed with deionized water, and allowed to drain. The rind was removed with a sharp stainless steel knife, the blossom and stem ends discarded, placental tissue and seeds were removed, and the mesocarp prepared in cubes of *ca.* 2.5 cm^3. All cutting tools and containers were sanitized with 70% ethanol and allowed to dry before usage.

4.2.2. 1-MCP treatment and packaging

Fresh-cut melon cubes (5 kg) were sealed inside a 100-L polypropylene container and treated with 1.0 µL L^{-1} 1-MCP (SmartFresh 0.14%, AgroFresh, Philadelphia, PA, USA) for 12 h at 5 °C. The containers were then vented to avoid excessive carbon dioxide and volatile accumulation and the same treatment was repeated for an additional 12 h at the same temperature. Control fresh-cut melon cubes were maintained under the same conditions in the absence of 1-MCP. 1-MCP was generated inside a volumetric flask by injecting water via a rubber stopper to the SmartFresh powder with 0.14% of active ingredient located in the flask. The 1-MCP gas was generated inside the flask for 10 min and the volume required to yield 1.0 µL L^{-1} in the treatment container was extracted from the headspace with a syringe and injected into the sealed plastic container. Calculations of 1-MCP concentration were

based on the free space volume on the sealed treatment container. After treatment, all cubes were packaged in vented clamshells (*ca*. 175 g) and stored at 5 °C for 9 days. To avoid the accumulation of ethylene and CO_2 inside the packages and detrimental ethylene effects (Vilas-Boas and Kader, 2007), clamshells were perforated with single 6 mm vents. CO_2 and O_2 partial pressures throughout storage were measured with a CheckMate II gas analyzer (PBI Dansensor, Ringsted, Denmark).

4.2.3. Ethylene production and respiration rate

1-MCP-treated and control cantaloupe cubes were weighed (*ca*. 50 g) and placed in 150 mL glass jars at 5 ºC. The glass jars were sealed for 2 hours before analyses of the headspace. For ethylene determination, 1 mL gas samples were withdrawn from each glass jar with a syringe and injected in a gas chromatograph (HP 5890, Palo Alto, CA, USA) equipped with a DB-1 column (30 m × 0.25 mm × 0.25 µm, Agilent, Palo Alto, CA, USA) and a FID detector. The carrier gas was helium at 30 mL min^{-1}, the injection port, oven and detector were set at 100 ºC, 35 ºC and 220 ºC, respectively.

For CO_2 determination, 1 mL gas samples were withdrawn from each glass jar with a syringe and injected in a gas chromatograph (HP 5890, Palo Alto, CA, USA) equipped with a Porapak Q 80/100 mesh column (2 m × 3.18 mm × 2 mm, Chrompack, Middelburg, Netherlands), and a TCD detector. The carrier gas was helium at 50 mL min^{-1}, the injection port, oven and detector were set at 175 ºC, 35 ºC and 100 ºC, respectively.

4.2.4. Measurement of color, firmness and SSC

Surface color of the fresh-cut cubes was measured in the CIE L*a*b* color space with a CR-400 colorimeter (Konica Minolta, Osaka, Japan), using the D_{65}

illuminant and observer at 2º. Hue angle (h^o = arctan b*/a*) and chroma [$C^*=(a^{*2}+b^{*2})^{1/2}$] were calculated from the primary a* and b* readings. Two color measurements were performed on the lateral cut surfaces of each of three cubes from four replicated packages of each treatment.

Firmness was measured with a TA-XT2 Plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. The force to drive a cylindrical probe 5 mm in diameter to perforate 5 mm into the tissue at a speed of 1.5 mm s^{-1} was recorded. One firmness measurement was taken on the lateral cut surfaces of each of three cubes from four replicated packages of each treatment.

SSC was determined on the juice obtained from three cubes from four replicated packages using a PAL-1 refractometer (Atago, Tokyo, Japan).

4.2.5. Antioxidant activity and total phenolics

Hydrophilic extracts were obtained by adding 50 mL of 80% methanol to 5 g of fresh-cut melon samples. The mixture was homogenized with an Ultra-Turrax (IKA T18, Wilmington, USA) at 24000 rpm for 30 s and centrifuged at 5000 ×*g* for 10 min at 4 ºC. The supernatant was filtered though a 0.45-µm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and the volume was measured. A 10 mL portion of the extract was evaporated to dryness in a speed-vacuum concentrator (RVC 2-18, Christ, Osterode am Harz, Germany) and the residue suspended in 3 mL of methanol. The extract was filtered through a 0.45-µm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and 10 µL used for HPLC-DAD analysis. The biomass retained in the filter after the hydrophilic extraction was dried overnight at 37 °C, and then suspended in 80% acetone (1:5 w/v) for 30 min to obtain a lipophilic extract. These extracts were performed in triplicate samples and used for antioxidant and phenolic analyses.

Total antioxidant capacity was measured in the hydrophilic and lipophilic extracts through the determination of free radical scavenging effect against the $ABTS^{+}$ [(2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic) acid) diammonium salt] radical, according to the method described by Gião *et al.* (2007). Total antioxidant capacity was quantified by measuring absorbance at 734 nm (UV mini 1240 spectrophotometer, Shimadzu, Tokyo, Japan) with ascorbic acid as standard. Total phenolic compounds were quantified by the Folin-Ciocalteu method (Singleton and Rossi, 1965) using gallic acid as standard.

4.2.6. Extraction and analysis of total carotenoids and β-carotene

Melon cubes (0.6 g) were homogenized in 3 mL of hexane, ethanol and acetate (2:2:1) to perform carotenoid extractions in triplicates. The extraction and saponification of carotenoids was done as described by Wright and Kader (1997). Total carotenoids were quantified by absorbance at 454 nm with a UV mini 1240 spectrophotometer (Shimadzu, Tokyo, Japan) using β-carotene as a standard.

β-Carotene was quantified by HPLC (Waters Series 600, Mildford, MA, USA) equipped with a diode array detector and quaternary pump (Waters, Mildford, MA, EUA). A reverse phase Symmetry® C18 column (250 mm x 4.6 mm i.d., 5 µm particle size and 125 Å pore size) with a length guard column containing the same stationary phase (Symmetry® C18) was operated under isocratic conditions with a mobile phase of acetonitrile (55%), methanol (22%), dicloromethane (11.5%), hexane (11.5%) and ammonium acetate (0.02%) with a flow rate of 1.0 mL min^{-1} during 20 min at 25 ºC. Injection volume was 40 µL and the UV–vis detector was set at 454 nm. β-Carotene was quantified from a calibration curve built with a pure standard (Extrasynthese, Lyon, France).

4.2.7. Volatile extraction

The preparation of samples for volatile extraction by solid-phase microextraction (SPME) was conducted as described by Obando-Ulloa *et al*. (2008). Melon cubes were homogenized for 10 s at 12000 rpm with an Ultra-Turrax (IKA T18 basic, Wilmington, NC, USA) and the resulting juice filtered through a powder funnel and four layers of cheesecloth. Saturated calcium chloride solution (4 mL) was added to 10 mL of juice and the mixture homogenized. Aliquots of the final mixture were collected in 12 mL sterile polypropylene vials and stored at −80 ºC until analysis.

4.2.8. Volatile identification and quantification

Volatile analyses were performed by gas-chromatography-mass spectroscopy as described by Berna *et al*. (2005), Muriel *et al*. (2004) and Obando-Ulloa *et al*. (2008), but using a retention time locked (RTL) method (Agilent Technol. 2008) and some modifications. Phenyl-ethyl alcohol (Sigma-Aldrich, Barcelona, Spain) was used to block the method at 18.5 min.

Juice samples were thawed in a water bath at 35 ºC for 15 min. Aliquots of 2.32 mL mixture containing 20 µL of 0.05% (v/v) phenyl-ethyl alcohol as internal standard were poured into 10 mL glass vials. The vials were sealed using crimp-top caps with TFE/silicone septa seals (Alltech Associates, Inc., Deerfield, IL, USA) and placed in the heat tray of the GC (6890N, Agilent Technologies, Wilmington, DEL, USA) at 35 ºC for 120 min to equilibrate the headspace. The SPME fiber (1-cm long) containing a 50/30 µm divinylbenzene/carboxene on polydimethylsiloxane coating (57329-U DVB/CarboxenTM/PDMS Stable FlexTM Fiber, Supelco, Bellefonte, PA, USA) was preconditioned in the injection port at 250 ºC for 1 h. The needle entered 21 mm into the vial headspace and remained 30 min absorbing volatiles at 35 ºC. After extraction, the volatiles were desorbed from the SPME fiber into the GC injection port

set at 200 ºC for 15 min as a bake-out step (the penetration depth of the needle into the injector was 44 mm). The analyses were conducted with a MPS2 Gerstel Multipurpose sampler coupled to the GC–MS. The injection port operated at 260 ºC in splitless mode. The injector was subjected to a constant pressure of 15.01 psi that was controlled by the Agilent electronic pressure control (EPC). Volatiles were separated on a 30 m × 0.25 mm i.d. × 0.25 µm thickness ultra inert capillary column (HP-5MS, Agilent Technologies, Wilmington, NC, USA) that contained 5% phenyl-methyl silicone as a stationary phase. The carrier gas was helium with a nominal initial flow rate of 1.9 mL min^{-1} (average velocity of 50 cm s^{-1}). The initial oven temperature was 35 ºC, followed by a ramp of 2 ºC min^{-1} up to 75 ºC, and then at 50 ºC min^{-1} to reach a final temperature of 200 ºC, which was held for 5 min. The equilibration time was 0.5 min. The inlet liner used was a 2637505 SPME/direct (Supelco, Bellefonte, PA, USA), 78.5 mm × 6.5 mm × 0.75 mm. Mass spectra were obtained by electron ionization (EI) at 70 eV, and a spectrum range of ion mass captured between 40–450 *m*/*z* was used. The solvent delay was 0.5 min. The detector worked at 230 ºC and in full scan mode, which captured an average of 3.88 scan s^{-1} (sample rate of 2) The total analysis time was 37.5 min due to post-run after samples analysis (10 min at 220 ^{o}C and 26.2 psi). The chromatograms and mass spectra were evaluated using the ChemStation software (G1791CA, Version C.00.00, Agilent Technologies, Wilmington, DEL, USA). The peaks were identified using a mass spectrometer (5973 Network Mass Selective Detector, Agilent Technologies) coupled to the GC by comparison of experimental spectra with those of the National Institute for Standards and Technology (NIST05, search version 2.0) data bank. Forty-eight compounds were identified, integrated and used to characterize 1-MCP-treated and control fresh-cut cantaloupe during storage. Only compounds confirmed in the literature were classified among the following groups of compounds: acetate and non-acetate esters, alcohols,

aldehydes, sulfur-containing compounds and other compounds. Results from the volatile analyses were expressed as the percentage of each compound's integrated area relative to the total integration area of 48 compounds.

4.2.9. Data analyses

Data were subjected to analysis of variance according to a split-plot design with treatment (1-MCP and control) as main-plot and time in storage as sub-plot (Gomez and Gomez, 1984). When significantly different, means were separated by the Duncan's multiple range test at P=0.05. Statistical analysis was performed using SPSS software (v. 17.0, SPSS, Chicago, IL, USA). An individual package constituted an experimental unit which was used as one replicate on each sampling day. Four replicated packages were analyzed.

4.3. Results and discussion

4.3.1. Respiration and ethylene production rates

The respiration rate of fresh-cut cantaloupe declined significantly during storage and was not affected by the post-processing treatment with 1-MCP (Table 4.1). Respiration rate decreased rapidly during the first three days and remained relatively constant at *ca.* 9 mL kg^{-1} h^{-1} of CO_2 during the remaining storage period (Table 4.1). The initially high respiration rate is a consequence of metabolism acceleration following tissue wounding (Aguayo *et al*. 2004b). The reduction in respiration rate reported by Ergun *et al*. (2005) in muskmelon treated with 1-MCP prior to cutting was not observed in this study with post-cutting 1-MCP application.

Average ethylene production decreased by 48% during the first three days and decreased only slightly in the remaining storage period (Table 4.1). The ethylene production rate by fresh-cut cantaloupe reported herein is consistent with previous observations for cantaloupes and muskmelons (Luna-Guzman *et al*., 1999; Aguayo *et al*., 2004b; Ergun *et al*., 2007). This lack of effect of the post-processing 1-MCP treatment on ethylene production rate contrasts with the significant delay in climacteric ethylene production induced by 1-MCP in whole Galia melons (Ergun *et al*., 2005). The trend in ethylene production during storage suggests that post-cutting 1-MCP application did not reduce wound response of fresh-cut cantaloupe in the stage of maturity (¾–slip) most suitable for fresh-cut processing (Jeong *et al*., 2007). Earlier maturity stages are not recommended since flavor and proper orange-flesh color are underdeveloped (Gal *et al*., 2006; Jeong *et al*., 2007).

4.3.2. Color, firmness, and SSC

Surface color of fresh-cut cantaloupe was slightly, but significantly, affected by storage time (Table 4.1). The pattern of color change was not uniform during storage. Changes occurred during the first three days of storage at 5 °C and at the end of the storage period, but color remained unchanged between day 3 and 6 (Table 4.1). Significant color changes have been reported during storage of fresh-cut cantaloupes (Amaro *et al*., 2012), but are generally regarded as negligible from a sensory perspective. Subtle changes in lightness (L*) and chroma can also be an indication of watersoaking (Bai *et al*., 2001), a disorder often observed in fresh-cut melon; still, no evidence of watersoaking was visually detected in these experiments. 1-MCP-treated cantaloupe cubes had a lower L* value than untreated cubes (Table 4.1) and were perceived at the end of storage life (day 9) as having a duller orange color.

Table 4.1. Respiration rate, ethylene production, color, firmness and SSC of control fresh-cut and 1-MCP treated cantaloupe cubes stored for 9 days at 5 ºC.

Variable	Time (days)	Control	1-MCP	Avg.
Respiration rate ($ml\ kg^{-1}\ h^{-1}$)	0	48.5±1.5	48.5±1.5	48.5^{a}
	3	11.8±0.9	11.8±1.8	11.8^{b}
	6	8.9±1.3	8.7±0.6	8.8^{c}
	9	9.0±0.6	9.1±0.4	9.1^{c}
	Avg.	19.8^{A}	19.5^{A}	
Ethylene production ($\mu L\ kg^{-1}\ h^{-1}$)	0	15.4±3.1	23.6 ±3.1	19.5^{a}
	3	9.1±1.3	11.1±2.3	10.1^{b}
	6	9.7±0.4	9.0±1.2	9.4^{b}
	9	7.4±1.3	5.4±1.0	6.4^{b}
	Avg.	10.4^{A}	12.3^{A}	
L*	0	61.8±2.3	61.8 ±2.3	61.8^{a}
	3	57.3±2.8	55.6 ±3.6	56.5^{b}
	6	57.3±2.8	55.5 ±3.6	56.4^{b}
	9	55.2±3.3	49.6 ±3.9	52.4^{c}
	Avg.	57.9^{A}	55.6^{B}	
C*	0	41.8±1.5	41.8 ±1.5	41.8^{a}
	3	36.1±1.8	36.2 ±2.7	36.1^{b}
	6	36.1±1.8	36.2 ±2.7	36.1^{b}
	9	33.7±2.3	30.5 ±2.4	32.1^{c}
	Avg.	36.9^{A}	36.2^{A}	
h°	0	71.7±0.4	71.7 ±0.4	71.8^{a}
	3	73.0±0.6	72.5 ±0.7	72.7^{b}
	6	73.0±0.6	72.5 ±0.7	72.7^{b}
	9	73.7±0.9	73.3 ±0.6	73.5^{c}
	Avg.	72.8^{A}	72.5^{A}	
Firmness (N)	0	5.9±1.1	5.1±1.1	5.5^{a}
	3	5.1±1.2	4.9±1.5	5.0^{a}
	6	3.5±0.7	3.8±1.3	3.7^{b}
	9	2.0±0.7	3.0±1.0	2.5^{b}
	Avg.	4.1^{A}	4.2^{A}	
SSC (%)	0	11.6±0.1	11.6±0.1	11.6^{a}
	3	13.6±0.3	13.8±0.3	13.7^{a}
	6	13.6±0.5	13.7±0.5	13.6^{a}
	9	13.4±0.3	13.0±0.2	13.2^{a}
	Avg.	13.1^{A}	13.0^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

In contrast, the treatment with 1-MCP applied prior to cutting did not affect color of fresh-cut cantaloupe melon (Jeong *et al*., 2008).

Significant softening was also observed in this experiment with fresh-cut cantaloupe during 9 days of storage, but firmness was not affected by the treatment with 1-MCP (Table 4.1). Despite the initial differences in firmness, the average softening rate of 1-MCP-treated cubes were statistically similar to that of untreated controls (Table 4.1). The effect of 1-MCP on melon firmness is more consistent in intact whole fruit than in fresh-cut (Jeong *et al*., 2008).

The average SSC of fresh-cut melon cubes was 13% and remained constant during storage irrespective of the treatment (Table 4.1). Melon SSC undergoes minor changes during postharvest storage of whole fruit or fresh-cut fruit (Beaulieu, 2006a).

4.3.3. Antioxidant metabolites

The antioxidant capacity and total phenolics in the lipophilic extracts were very low. Therefore, the concentrations obtained in the lipophilic and hydrophilic extracts were added and the content derived from the sum of both extracts is presented (Table 4.2).

The initial antioxidant activity, expressed as ascorbic acid equivalents on a fresh weight basis, was 153.3 µg g^{-1} and decreased by 43% during the 9-day storage period irrespective of the treatment used (Table 4.2). The initial phenolic content, expressed as gallic acid equivalents of a fresh weight basis, was 243.8 µg g^{-1} and remained constant during storage with no effect of the 1-MCP treatment (Table 4.2). Wound-induced phenolic compounds occur in some plant tissues (Reyes *et al*., 2007), but are not relevant in fresh-cut cantaloupe (Gil *et al*., 2006 and Table 4.2).

Total carotenoid content of fresh-cut cantaloupe was 60.9 µg g^{-1} fw. No significant changes in total carotenoids occurred occur during storage (Table 4.2), consistent with the observations of Gil *et al*. (2006). β-Carotene, the main carotenoid in orange-fleshed cantaloupes (Gil *et al*., 2006; Saftner and Lester, 2009; Fleshman *et*

Table 4.2. Antioxidant capacity, total phenolics, total carotenoids and β-carotene content of control fresh-cut and 1-MCP treated cantaloupe cubes stored for 9 days at 5 ºC.

Variable	Time (days)	Control	1-MCP	Avg.
Antioxidant activity ($\mu g\ g^{-1}$)	0	153.3±18.0	153.3±18.0	153.3[a]
	3	96.5±14.2	98.5 ±2.2	97.5[b]
	6	78.5±9.6	109.1±7.3	93.8[b]
	9	77.2±11.4	97.1±13.1	87.2[b]
	Avg.	101.4[A]	114.5[A]	
Total phenolics ($\mu g\ g^{-1}$)	0	243.8±50.4	243.8±50.4	243.8[a]
	3	227.3±47.5	251.4±29.8	239.4[a]
	6	224.3±8.0	197.9±19.0	211.1[a]
	9	232.1±29.4	240.5±9.5	236.3[a]
	Avg.	231.9[A]	233.4[A]	
Total carotenoids ($\mu g\ g^{-1}$)	0	60.9±3.2	60.9±3.2	60.9[a]
	3	60.1±4.2	63.3±4.6	61.7[a]
	6	52.7±4.2	61.5±6.7	57.1[a]
	9	54.8±3.6	60.7±5.3	57.8[a]
	Avg.	57.1[A]	61.6[A]	
β – carotene ($\mu g\ g^{-1}$)	0	24.5±1.6	24.5 ±1.6	24.5[a]
	3	25.3±2.5	25.1 ±1.0	25.2[a]
	6	18.3±2.2	24.9 ±6.0	21.6[a]
	9	18.7±2.5	24.5±3.9	21.6[a]
	Avg.	21.7[A]	24.8[A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

al., 2011), remained at constant levels during storage (Table 4.2). The treatment with 1-MCP did not affect total carotenoid or β-carotene content (Table 4.2).

4.3.4. Aroma volatiles

Forty-eight volatile compounds were identified in fresh-cut cantaloupe. Esters and alcohols were the most abundant volatile classes, with aldehydes and sulfur-containing compounds present at lower concentrations (Table 4.3). The aldehydes decreased during storage whereas sulfur-containing compounds and non-acetate esters increased (Table 4.3). The volatile profiles reported herein are generally consistent with the previous characterizations of aroma volatiles in cantaloupes stored in passive MAP at 5 °C (Amaro *et al*., 2012). Integrated total volatile abundance

changed during storage, increasing in the first three days and remaining relatively constant thereafter (Table 4.3).

Table 4.3. Chromatogram mean total integrated volatiles in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Acetate esters	0	9.83E+08	9.97E+08	9.90E+08[a]
	3	1.04E+09	1.09E+09	1.07E+09[a]
	6	1.03E+09	1.04E+09	1.04E+09[a]
	9	1.02E+09	6.96E+08	8.58E+08[a]
	Avg.	1.02E+09[A]	9.56E+08[A]	
Non-acetate esters	0	7.47E+08	8.81E+08	8.14E+08[b]
	3	1.33E+09	1.31E+09	1.32E+09[a]
	6	1.25E+09	1.31E+09	1.28E+09[a]
	9	1.15E+09	1.33E+09	1.24E+09[a]
	Avg.	1.12E+09[A]	1.21E+09[A]	
Alcohols	0	8.38E+07	9.79E+07	9.09E+07[a]
	3	1.11E+08	1.08E+08	1.10E+08[a]
	6	1.06E+08	1.02E+08	1.04E+08[a]
	9	1.18E+08	1.04E+08	1.24E+08[a]
	Avg.	1.05E+08[A]	1.03E+08[A]	
Aldehydes	0	1.07E+08	1.02E+08	1.05E+08[a]
	3	8.48E+07	6.65E+07	7.57E+07[b]
	6	5.27E+07	3.24E+07	4.26E+07[c]
	9	2.65E+07	3.48E+07	3.07E+07[c]
	Avg.	6.78E+07[A]	5.89E+07[A]	
Sulfur-containing compounds	0	1.35E+07	1.47E+07	1.41E+07[a]
	3	2.00E+07	1.75E+07	1.88E+07[a]
	6	2.44E+07	1.63E+07	2.04E+07[a]
	9	2.91E+07	1.80E+07	2.36E+07[a]
	Avg.	2.18E+07[A]	1.66E+07[A]	
Other compounds	0	1.60E+07	2.21E+07	1.91E+07[a]
	3	1.59E+07	2.36E+07	1.98E+07[a]
	6	2.22E+07	2.78E+07	2.50E+07[a]
	9	2.67E+07	2.32E+07	2.50E+07[a]
	Avg.	2.02E+07[A]	2.42E+07[A]	

Table 4.3. Continued.

Variable	Time (days)	Control	1-MCP	Avg.
Total Integrated Volatiles	0	1.95E+09	2.12E+09	2.08E+09^{b}
	3	2.60E+09	2.62E+09	2.61E+09^{a}
	6	2.48E+09	2.53E+09	2.51E+09^{a}
	9	2.37E+09	2.20E+09	2.29E+09^{ab}
	Avg.	2.35E+09^{A}	2.37E+09^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Among the 28 esters quantified, the most abundant were the acetate esters ethyl acetate and 2-methylbutyl acetate and the butanoate esters, methyl 2-methylbutanoate, ethyl butanoate, ethyl 2-methylbutanoate, which accounted for *ca.* 50-58 % of total volatiles (Table 4.4), consistent with reports indicating that these are the predominant aroma volatiles in climacteric melons (Beaulieu, 2006a; Bauchot *et al*., 1998; Obando-Ulloa *et al*., 2008). Although not a flavor-important volatile in melon (Beaulieu, 2006b), butyl acetate was produced in large quantities and increased at the end of the storage period (Table 4.4).

After 9 days in storage, the concentration of total non-acetate esters was 52% higher than the initial concentration but was not affected by 1-MCP (Table 4.3). Total alcohols remained statistically constant during storage (Table 4.3). This trend in total alcohols observed in this study contrasts with a previous report, in a different cultivar 'Sol Real' under MAP, of a strong decrease in alcohols during storage (Amaro *et al*., 2012). Alcohols were the second highest contributors to the increase in total volatile abundance during storage (Table 4.3). Consistently, the increase of esters and saturated alcohols and the maintenance of most alcohols have been reported during storage of fresh-cut melons (Saftner *et al*., 2003; Beaulieu, 2006a).

All aldehydes integrated in this study, particularly hexanal known to impart "green" and "grassy" notes, decreased during storage in both treatments (Table 4.5), consistent with other reports in cantaloupe melon (Beaulieu, 2006a).

The total concentration of sulfur-containing compounds remained statistically unaltered during storage and was not affected by 1-MCP (Table 4.3).

Table 4.4. Chromatogram mean relative areas for acetate esters in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Methyl acetate	0	73	83	78[a]
	3	77	101	89[a]
	6	81	86	84[a]
	9	69	78	74[a]
	Avg.	75[A]	87[A]	
Ethyl acetate	0	397	310	354[b]
	3	507	385	446[ab]
	6	530	414	472[a]
	9	518	394	456[a]
	Avg.	488[A]	376[A]	
Propyl acetate	0	21	27	24[a]
	3	22	29	26[a]
	6	25	25	25[a]
	9	25	30	28[a]
	Avg.	23[B]	28[A]	
Isobutyl acetate	0	99	106	103[a]
	3	78	101	90[a]
	6	61	59	60[b]
	9	56	53	55[b]
	Avg.	74[A]	80[A]	
Butyl acetate	0	21	15	18[b]
	3	20	21	21[b]
	6	26	23	25[b]
	9	33	35	34[a]
	Avg.	25[A]	24[A]	
3-Methylbutyl acetate	0	6	5	6[b]
	3	7	7	7[ab]
	6	7	7	7[ab]
	9	9	10	8[a]
	Avg.	7[A]	7[A]	
2-Methylbutyl acetate	0	153	183	168[a]
	3	99	166	133[b]
	6	99	114	107[b]
	9	91	138	115[b]
	Avg.	111[B]	150 [A]	

Table 4.4. Continued.

Variable	Time (days)	Control	1-MCP	Avg.
Octyl acetate	0	3	3	3[a]
	3	3	3	3[a]
	6	3	2	3[a]
	9	4	2	3[a]
	Avg.	3[A]	3[A]	
Hexyl acetate	0	18	16	17[a]
	3	15	10	13[a]
	6	18	8	13[a]
	9	31	11	21[a]
	Avg.	21[A]	11[A]	
Methyl benzene Acetate	0	22	64	43[a]
	3	18	45	32[ab]
	6	16	30	23[b]
	9	34	29	32[ab]
	Avg.	45[A]	42[A]	
Ethyl benzene Acetate	0	13	6	10[a]
	3	6	5	6[ab]
	6	4	2	3[b]
	9	6	5	10[a]
	Avg.	7[A]	5[A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Ethyl (methylthio) acetate and ethyl 3-(methylthio) propanoate (Table 4.6) are believed to have an important role in the overall aroma profile of melon (Bauchot *et al*., 1998; Kemp *et al*., 1972; Wyllie and Leach, 1990) and their suppression, even during short term storage, may limit consumer acceptability of fresh-cut melon.

The volatile compounds methyl acetate, propyl acetate, octyl acetate, hexyl acetate (Table 4.4), methyl 2-methyl propanoate, methyl butanoate, methyl 2-methyl butanoate, methyl pentanoate, ethyl tiglate, 2-methyl propyl butyrate (Table 4.7), 2-methylbutyl alcohol, phenethyl alcohol (Table 4.8), acetaldehyde, benzaldehyde (Table 4.7) and ethyl (methylthio) acetate (Table 4.6) were not affected by storage time.

Treatment with 1-MCP had no significant effect on total volatile production (Table 4.3) and on several of the individual compounds.

Table 4.5. Chromatogram mean relative areas for aldehydes in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Acetaldehyde	0	11	11	11[a]
	3	13	12	13[a]
	6	11	12	12[a]
	9	9	13	11[a]
	Avg.	11[A]	12[A]	
Hexanal	0	65	53	59[a]
	3	45	43	44[b]
	6	29	11	20[b]
	9	9	15	12[c]
	Avg.	37[A]	44 [A]	
Heptanal	0	8	5	7[a]
	3	6	4	5[ab]
	6	7	2	5[ab]
	9	2	2	2[b]
	Avg.	6[A]	3[B]	
Benzaldehyde	0	4	5	5[a]
	3	5	4	5[a]
	6	5	4	5[a]
	9	5	5	5[a]
	Avg.	5[A]	5 [A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

However, the production of 9 of the 48 volatiles identified was significantly affected by 1-MCP during storage of fresh-cut cantaloupe.

Among the volatiles significantly enhanced by the treatment with 1-MCP, 2-methylbutyl acetate, methyl butanoate, methyl 2-methyl butanote and 2-methylbutyl alcohol are derived from the amino acid isoleucine, whereas propyl acetate and phenethyl alcohol have phenylalanine as precursor (Mathieu *et al*., 2009; Van Moerkercke *et al*., 2009). The reason for this specific effect of 1-MCP is unknown. On

the other hand, since the levels of benzyl alcohol, a compound also derived from phenylalanine, were reduced by 1-MCP (Table 4.8), indicate that the effect is exerted

Table 4.6. Chromatogram mean relative areas for sulfur-containing compounds in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Ethyl methylthio	0	1	1	1[a]
Acetate	3	2	1	2[a]
($\mu g.kg^{-1}$)	6	2	1	2[a]
	9	2	3	3[a]
	Avg.	2[A]	2 [A]	
Methyl 3-	0	1	1	1[b]
Methylthio	3	1	1	1[b]
Propanoate	6	1	1	1[b]
	9	2	2	2[a]
	Avg.	1 [A]	1 [A]	
Ethyl 3-	0	13	11	12[b]
Methylthio	3	15	12	14[b]
Propanoate	6	18	14	16[b]
	9	29	22	26[a]
	Avg.	19[A]	15 [A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

on biosynthetic or catabolic events, downstream of the precursor amino acid. Also, methyl hexanoate and heptanal, volatiles derived from fatty acids, were increased or reduced by 1-MCP, respectively (Table 4.4).

In 1-MCP-treated cantaloupe cubes, (*Z*)-6-nonenol, a compound associated with pumpkin and squash-like off-odors (Saftner *et al*., 2007), was present throughout storage period while in control cubes, this compound was not detected after day 3 (Table 4.8). Eucalyptol, positively correlated with fruity and sweet taste (Beaulieu and Lancaster, 2007), was better maintained in 1-MCP-treated cubes after day 3 (Table 4.8). ADH is not affected by 1-MCP treatment in apple, but alcohol acyl-transferase (AAT) is regulated by ethylene (Defilippi *et al*., 2005; Luchetta *et al*., 2007). The suppression of AAT by 1-MCP may explain the higher levels of some alcohols in 1-MCP-treated fruit reported herein (Table 4.8) and elsewhere (Balbontín *et al*., 2007).

AAT catalyses the conversion of alcohols into esters. Since esters levels in fresh-cut cantaloupe were *ca.* 2-fold higher than alcohols, the downregulation of AAT by 1-MCP can have an impact on individual alcohols (Table 4.8) without measurable effects on total esters (Table 4.3). 1-MCP applied to whole papaya (Balbontín *et al*., 2007) and whole Galia-type melon (Gal *et al*., 2008) may have a stronger impact on these volatiles than the post-processing 1-MCP application, since the same volatile trend was observed in the fresh-cut melon, albeit mitigated (Table 4.3, 4.8).

1-MCP application to fruit tends to reduce volatile production, but not all compounds are inhibited in the same degree (Fan and Mattheis, 1999; Lurie *et al*., 2002). In Galia-type melon, 1-MCP enhances the production of methyl acetate and methyl 2-methylbutanoate but suppresses that of ethyl acetate and ethyl hexanoate (Gal *et al*., 2008). Levels of methyl 2-methylbutanoate were also increased by the 1-MCP treatment in this experiment (Table 4.7).

Table 4.7. Chromatogram mean relative areas for nonacetate esters in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Methyl propionate	0	82	67	75^{ab}
	3	89	77	83^{ab}
	6	104	66	85^{a}
	9	73	65	69^{b}
	Avg.	87^{A}	69^{A}	
Methyl 2-methyl propanoate	0	11	11	11^{a}
	3	12	12	12^{a}
	6	11	11	11^{a}
	9	9	13	11^{a}
	Avg.	11^{A}	12^{A}	
Ethyl propanoate	0	20	20	20^{b}
	3	28	28	28^{a}
	6	32	27	30^{a}
	9	25	31	28^{a}
	Avg.	27^{A}	27^{A}	

Table 4.7. Continued.

Variable	Time (days)	Control	1-MCP	Avg.
Methyl Butanoate	0	15	19	17[a]
	3	13	31	22[a]
	6	19	27	23[a]
	9	15	26	21[a]
	Avg.	16[B]	26[A]	
Ethyl 2-methyl Propanoate	0	30	21	26[b]
	3	39	28	34[a]
	6	31	23	27[b]
	9	22	21	22[b]
	Avg.	31[A]	23[A]	
Methyl 2-methyl Butanoate	0	72	103	88[a]
	3	79	139	109[a]
	6	85	128	107[a]
	9	73	153	113[a]
	Avg.	77[B]	131[A]	
Ethyl Butanoate	0	114	95	105[d]
	3	199	192	196[c]
	6	288	230	259[b]
	9	228	335	282[a]
	Avg.	207[A]	213[A]	
Methyl Pentanoate	0	5	4	5[a]
	3	6	5	6[a]
	6	4	5	5[a]
	9	4	6	5[a]
	Avg.	5[A]	5[A]	
Ethyl 2-methyl Butanoate	0	195	182	189[b]
	3	322	279	301[a]
	6	336	262	299[b]
	9	256	331	294[b]
	Avg.	277[A]	264[A]	
Ethyl Pentanoate	0	14	11	13[c]
	3	26	21	24[a]
	6	24	18	21[ab]
	9	14	19	17[b]
	Avg.	20[A]	17[A]	

Table 4.7. Continued.

Variable	Time (days)	Control	1-MCP	Avg.
Methyl hexanoate	0	11	32	22[a]
	3	18	40	29[a]
	6	12	21	17[b]
	9	14	23	19[b]
	Avg.	14[B]	29[A]	
Ethyl tiglate	0	1	1	1[a]
	3	2	2	2[a]
	6	3	2	3[a]
	9	2	2	2[a]
	Avg.	2[A]	2[A]	
2-Methyl propyl butyrate	0	0	1	1[a]
	3	1	1	1[a]
	6	1	0	1[a]
	9	1	1	1[a]
	Avg.	1[A]	1[A]	
Ethyl hexanoate	0	145	152	156[b]
	3	289	209	249[a]
	6	204	183	194[ab]
	9	171	238	205[a]
	Avg.	202[A]	196[A]	
Methyl benzoate	0	1	1	1[b]
	3	2	2	2[b]
	6	2	1	2[b]
	9	1	4	4[a]
	Avg.	2[A]	2[A]	
4-Heptenoic acid, ethyl ester	0	3	7	5[b]
	3	11	7	9[ab]
	6	12	5	9[ab]
	9	11	8	10[a]
	Avg.	9[A]	7[A]	
Ethyl benzoate	0	18	18	15[c]
	3	36	36	30[b]
	6	32	32	31[b]
	9	120	120	87[a]
	Avg.	52[A]	52[A]	

Values are means (n=6). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Untreated cantaloupe cubes had higher levels of ethyl acetate than 1-MCP treated melon cubes and ethyl hexanoate was not affected by 1-MCP (Table 4.7), in contrast

with the trends reported in whole Galia melon (Gal *et al*., 2008). The same study indicated that whole melons showed relatively high concentrations of hexanal, associated with “green note”, which was induced by 1-MCP (Gal *et al*., 2008). Hexanal was abundant in both 1-MCP treated and control cubes (Table 4.5).

The inhibition of butyl and hexyl acetates production by 1-MCP is nearly complete when the precursor Na-hexanoate is present but there is only a 50% inhibition when hexanol is available (Flores *et al*., 2002). In contrast, with these results in whole cantaloupe fruit, butyl acetate production was very similar in 1-MCP treated and control cantaloupe cubes (Table 4.4).

The precursor ester ethyl hexanoate and hexanol were available in very similar concentrations in both 1-MCP treated and control melon cubes (Table 4.4 and 4.8). As suggested by Flores *et al*. (2002) and Balbontín *et al*. (2007), the esterification of alcohols is, at least in part, ethylene independent and regulated by additional factors. The residual ethylene level produced by fresh-cut cantaloupe may have been sufficient to support a large portion of the esterification step.

Table 4.8. Chromatogram mean relative areas for alcohols in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Ethyl Alcohol	0	47	27	37^{b}
	3	62	33	48^{ab}
	6	64	40	52^{a}
	9	55	54	55^{a}
	Avg.	57^{A}	39^{A}	
2-Methylbutyl Alcohol	0	8	10	9^{a}
	3	8	10	9^{a}
	6	7	9	8^{a}
	9	3	11	7^{a}
	Avg.	7^{B}	10^{A}	

Table 4.8. Continued.

Variable	Time (days)	Control	1-MCP	Avg.
Hexanol	0	4	4	4^{b}
	3	4	9	7^{ab}
	6	7	9	8^{ab}
	9	11	19	15^{a}
	Avg.	7^{A}	10^{A}	
3-Octenol	0	2	2	2^{a}
	3	2	2	2^{a}
	6	2	1	2^{a}
	9	1	1	1^{b}
	Avg.	2^{A}	2^{A}	
Eucalyptol	0	7	10	9^{b}
	3	15	11	13^{a}
	6	8	9	9^{b}
	9	7	10	9^{b}
	Avg.	9^{A}	10^{A}	
Benzyl alcohol	0	2	7	5^{c}
	3	16	8	12^{b}
	6	20	9	15^{a}
	9	17	11	14^{a}
	Avg.	14^{A}	9^{B}	
Octanol	0	1	1	1^{b}
	3	4	2	3^{a}
	6	3	2	3^{a}
	9	2	2	2^{a}
	Avg.	3^{A}	2^{A}	
Phenethyl alcohol	0	2	3	3^{a}
	3	3	3	3^{a}
	6	3	5	4^{a}
	9	3	5	4^{a}
	Avg.	3^{B}	4^{A}	
(*Z*)-3-Nonenol	0	3	2	3b
	3	5	4	5^{a}
	6	5	3	4^{ab}
	9	3	4	4^{ab}
	Avg.	4^{A}	3^{A}	

Table 4.8. Continued.

Variable	Time (days)	Control	1-MCP	Avg.
(*E,Z*)-3,6-Nonadienol	0	1	2	2^{c}
	3	1	2	2^{c}
	6	3	4	4^{b}
	9	5	4	5^{a}
	Avg.	3^{A}	3^{A}	
(*Z*)-6-Nonenol	0	4	9	7
	3	8	14	11
	6	N/D	8	-
	9	N/D	10	-
	Avg.	6^{A}	10^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan's Multiple Range Test.

Table 4.9. Chromatogram mean relative areas for other compounds in control fresh-cut and 1-MCP treated cantaloupe cubes during storage at 5 °C.

Variable	Time (days)	Control	1-MCP	Avg.
Acetophenone	0	14	13	14^{a}
	3	11	14	13^{ab}
	6	10	14	12^{b}
	9	10	13	12^{b}
	Avg.	11^{A}	14^{A}	
2,3-Butanedioldiaceate	0	2	2	2^{b}
	3	6	4	5^{ab}
	6	10	5	8^{ab}
	9	16	6	11^{a}
	Avg.	11^{A}	4^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

4.4. Conclusions

Post-processing application of 1-MCP to cantaloupes did not affect the metabolic rate, the physicochemical quality attributes or the concentration of phytochemicals. Therefore, post-processing 1-MCP application to fresh-cut cantaloupe seems ineffective to modulate the physical and compositional quality attributes of this cultivar harvested in an optimum stage of maturity. However, 1-MCP

altered the volatile profile of fresh-cut cantaloupe during cold storage, with a specific impact on propyl acetate, 2-methylbutyl acetate, methyl butanoate, methyl 2-methyl butanoate, methyl hexanoate, 2-methylbutyl alcohol, phenethyl alcohol, benzyl alcohol and heptanal. The volatiles derived from the amino acids isoleucine and phenylalanine were enhanced by 1-MCP. The sensorial perception of these changes deserves further studies.

CHAPTER 5

Lysophosphatidylethanolamine effects on volatiles and quality of fresh-cut Charentais melon

Abstract

Wounding of plant tissues increases the hydrolysis of membrane phospholipids mediated by PLD. Subsequent reactions originate flavor-related aldehydes, alcohols and esters. LPE is a naturally occurring phospholipid capable of inhibiting PLD *in vitro*. The effect of LPE on the quality and volatile profile of fresh-cut melon was investigated. Charentais-type melons (*Cucumis melo* L. 'Fiesta') were processed into cubes, vacuum-infiltrated with 200 mg L^{-1} of LPE, packaged in plastic clamshells and stored at 5 ºC for 9 days. LPE significantly reduced the activity of PLD one day after the treatment but the effect was transient. LPE did not affect color, firmness, SSC and ethylene production and had a negligible effect on the respiration rate of melon cubes. Antioxidant capacity, total phenolics, total carotenoids, and β-carotene were not affected by the LPE treatment. LPE did not affect the major classes of volatiles in melon – esters and alcohols – but reduced the accumulation of aldehydes after the first day of storage. In conclusion, LPE did not help in the quality retention of fresh-cut melon but had a specific inhibitory effect on volatile aldehydes in the first three days of storage.

5.1. Introduction

Membrane integrity is of paramount importance to maintain the quality of fresh-cut fruit. Tissue disruption caused by processing and subsequent senescence compromises cellular compartmentation with the associated metabolic disruption leading to severe quality loss (Watada and Qi, 1999; Toivonen and Dell, 2002; Wiley, 1994). The activities of membrane hydrolases are greatly enhanced by wounding (Karakurt and Huber, 2003). Therefore, fresh-cut processing enhances lipase activity in fruit, as demonstrated for papaya (Karakurt and Huber, 2003). PLD is a wound-activated enzyme that hydrolyses the ester bond between the head group and the PA of phospholipids and plays an important role in membrane degradation (Ryu and Wang, 1996; Bargmann and Munnik, 2006). Subsequent action of PLA and PLC generates FFA (Ryu and Wang, 1998), DAG and PA (Wang *et al*., 2000). The oxidation of the free linoleic and linolenic acids by LOX results in the production of C_6 and C_9 aldehydes and alcohols known to contribute to the aroma and flavor of several plant tissues (Baldwin *et al*., 2000, Pérez *et al*., 1999, Rowan *et al*., 1999). In addition to its involvement in wound response, PLD plays a role in several other physiological processes including fruit ripening, senescence, stress response, and cellular signaling (Munnik *et al*., 1998). Therefore, any strategy involving PLD inhibition in fresh-cut fruit is anticipated to enhance membrane integrity and to alter the aroma profile via the reduction of specific volatiles, such as hexanal and nonanal.

LPE is a naturally occurring phospholipid that results from the hydrolysis of PE by PLA (Palta and Farag, 1992). LPE has been shown to inhibit PLD activity *in vitro* (Ryu *et al*., 1997) and to reduce ethylene biosynthesis in fruit and flowers (Ryu *et al*., 1997; Kaur and Palta, 1997; Ozgen *et al*., 2005a). LPE application to horticultural commodities has shown a wide range of beneficial effects. LPE improves the color of apple (Farag and Palta, 1991a), cranberry (Farag and Palta, 1993a; Ozgen *et al*., 2005a), red pepper (Kang *et al*., 2003), and tomato (Ozgen *et al*., 2004), helps

firmness retention in apple (Farag and Palta, 1991a), delays senescence of leaves (Farag and Palta, 1991b; Ozgen *et al*., 2005b, Hong *et al*., 2009) and flowers (Kaur and Palta, 1997), improves grape ripening (Hong, 2008), counters the undesirable effects of ethephon (2-chloroethylphosphonic acid, an ethylene releasing compound) on tomato leaves and fruits (Farag and Palta, 1993b), protects cranberry fruits and leaves from injury by chlorothalonil-based fungicides (Ozgen and Palta, 2003) and inhibits PG activity (Mangat and Palta, 1995).

The effect of LPE on fresh-cut fruit is unknown. However, given its inhibitory effects on PLD and ethylene biosynthesis (Ryu *et al*., 1997), it is hypothesized that significant quality improvements can be achieved by LPE treatment, via the reduction of membrane degradation and the negative effects of ethylene. In addition, a change in aroma volatiles caused by the suppression of lipid-derived compounds is anticipated. LPE is a safe natural compound registered by the U.S. EPA to control ripening and enhance storage life of fresh produce (EPA); if proven effective, LPE would provide a good tool to help retain the quality of fresh-cut fruit.

The objective of this study was to assess the effectiveness of LPE as a modulator of quality in fresh-cut melon, including its effects on the volatile profile. A fast senescing and aromatic cultivar of cantaloupe melon with soft flesh was used to evaluate the effectiveness of LPE. Cantaloupe melon accounts for a high share of the fresh-cut fruit market, is valued by its aroma profile, and juice leakage often limits quality.

5.2. Materials and methods

5.2.1. Fruit material and processing conditions

Charentais-type melons (*Cucumis melo* L. var. *cantalupensis* Naud. 'Fiesta') were harvested at the ¾–slip maturity stage and cooled overnight to 5 ºC prior to processing. Fruits with no visual defects and uniform in shape and size were selected for processing. Fruit were washed with cold water, dipped for 2 min in 100 µg L^{-1} NaOCl, rinsed in deionized water, and allowed to drain. A sharp stainless steel knife, previously sanitized with 70% ethanol, was used to remove the fruit rind, blossom and stem ends, placental tissue and seeds and finally prepare cubes with 2.5 cm side.

5.2.2. LPE treatment and packaging

To perform the LPE treatment, fresh-cut melon cubes were sealed inside a 5 L Erlenmeyer flask and vacuum-infiltrated for 30 s at 5 kPa, with 200 mg L^{-1} of LPE. Control melon cubes were infiltrated with MiliQ-water under the same conditions. After treatment, all cubes were packaged in vented clamshells (*ca*. 175 g), previously sanitized with 70% ethanol, and stored at 5 °C for 9 days. Clamshells had been perforated with 6 mm vents to avoid the detrimental effects of ethylene and the accumulation of CO_2 inside the packages. To make sure that CO_2 and O_2 partial pressures inside the packages were close to those on the atmosphere, a CheckMate II gas analyzer (PBI Dansensor, Ringsted, Denmark) was used at each sampling day. Four replicate packages were sampled on different storage times (0, 3, 6 and 9 days).

5.2.3. Ethylene production and respiration rate

Ethylene and CO_2 production rates were measured in a closed system. Melon cubes (*ca*. 50 g) were sealed inside 150 mL glass jars for 2 h, after which time a 1 mL gas sample was withdrawn from the headspace of each jar through a silicone septum. Ethylene was determined in a gas chromatograph (HP 5890, Palo Alto, CA, USA) equipped with a DB-1 column (30 m × 0.25 mm × 0.25 µm, Agilent, Palo Alto, CA, USA) and a FID detector. The carrier gas was helium at 30 mL min^{-1}, the injection

port, oven and detector were set at 100 ºC, 35 ºC and 220 ºC, respectively. The CO_2 concentration was determined with a gas chromatograph (HP 5890, Palo Alto, CA, USA) equipped with a Porapack Q 80/100 mesh column (2 m x 3.18 mm x 2 mm, Chrompack, Middelburg, Netherlands), and a TCD detector. The carrier gas was helium at 50 mL min^{-1} , the injection port, oven and detector were set at 175 ºC, 35 ºC and 100 ºC, respectively.

5.2.4. Color, firmness and SSC

Two color measurements were performed on the lateral cut surfaces of each of three cubes from four replicated packages of each treatment. Color was measured in the CIE L*a*b* color space with a CR-400 colorimeter (Konica Minolta, Osaka, Japan), using the D_{65} illuminant and observer at 2º. Hue angle (h^o = arctan b*/a*) and chroma [$C^*=(a^{*2}+b^{*2})^{1/2}$] were calculated from the primary a* and b* readings. Firmness was determined using TA-XT2 Plus Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 kg load cell. A cylindrical 5 mm diameter probe perforated 5 mm into the tissue, at a speed of 1.5 $mm.s^{-1}$, and force/deformation curves were analyzed for peak force. One firmness measurement was taken on the lateral cut surfaces of each of three cubes from four replicated packages of each treatment.

SSC was determined on the juice obtained from three cubes using a PAL-1 refractometer (Atago, Tokyo, Japan).

5.2.5. Antioxidant activity and total phenolics

Antioxidant activity of melon cubes was assessed by the determination of free radical scavenging effect against the $ABTS^+$ [(2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic) acid) diammonium salt] radical in hydrophilic and lipophilic extracts, as described by Gião *et al*. (2007). Hydrophilic extracts were obtained by

homogenization of fresh-cut melon sample in 50 mL of methanol (80%) in an Ultra-Turrax (IKA T18, Wilmington, USA) at 24000 rpm for 30 seconds followed by centrifugation at 5000 ×*g* for 10 min at 4 ºC. The supernatant was filtered though a 0.45-µm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and the volume recorded. A 10 mL portion of the extract was evaporated to dryness in a speed-vacuum concentrator (RVC 2-18, Christ, Osterode am Harz, Germany) and the residue suspended in 3 mL of methanol. The extract was then filtered through a 0.45-µm cellulose acetate filter (Orange Scientific, Braine-l'Alleud, Belgium) and 10 µL used for HPLC-DAD analysis. After the hydrophilic extraction, the biomass residue was dried overnight at 37 °C and extracted with acetone (80%; 1:5 w/v) to obtain the lipophilic extract. These extracts were performed in triplicate samples.

Total antioxidant capacity was quantified by measuring absorbance at 734 nm (UV mini 1240 spectrophotometer, Shimadzu, Tokyo, Japan) with ascorbic acid as a standard. Total phenolic compounds were quantified by the Folin-Ciocalteu method (Singleton and Rossi, 1965) using gallic acid as a standard.

5.2.6. Extraction and analysis of total carotenoids and β-carotene

Triplicate melon cube (0.6 g) samples were homogenized in 3 mL of hexane, ethanol and acetate (2:2:1) to perform the carotenoid extractions and subsequent saponification as described by Wright and Kader (1997). Total carotenoids were quantified using β-carotene as a standard and by absorbance at 454 nm using a UV mini 1240 spectrophotometer (Shimadzu, Tokyo, Japan).

β-Carotene was identified and quantified using an external standard calibration curve built with purified standard (Extrasynthese, Lyon, France). An HPLC (Waters Series 600, Mildford MA, USA) equipped with a diode array detector and quaternary pump (Waters, Massachussets, EUA) equipped with a reverse phase Symmetry® C18 column (250 x 4.6 mm i.d., 5 µm particle size and 125 Å pore size) and a guard

column containing the same stationary phase (Symmetry® C18) was operated under isocratic conditions, with a mobile phase of acetonitrile (55%), methanol (22%), dicloromethane (11.5%), hexane (11.5%) and amonium acetate (0.02%) with a flow rate of 1.0 ml min^{-1} during 20 minutes at 25 ºC. Injection volume was 40 µL and the UV–visible detector was set at 454 nm.

5.2.7. Phospholipase D activity

PLD was extracted as described by Karakurt and Huber (2003). Fresh-cut melon cubes were homogenized (10 g) in 10 mL of extraction buffer containing 50 mM Tris-HCl (pH 8.0), 0.5 M sucrose, 10 mM KCl, 1 mM EDTA, 0.5 mM phenylmethylsulfonylfloride (PMSF) and 2 mM dithiothreitol (DTT). The homogenate was centrifuged at 15 000 × *g* for 60 min and the supernatant used for enzyme assay. The PLD activity was measured as described by Gupta and Wold (1980). Ca-acetate (0.9 mL, 50 mM, pH 5.6) containing 25 mM nitrophenylphosphorylcholine (NPPC) was mixed with 0.1 mL (0.04 units) of acid phosphatase dissolved in the same buffer along with 0.3 mL of the cell-free extract. After 60 min incubations at 37 °C, 0.1 mL of 50 mM NaOH was added and nitrophenol content was spectrophotometrically determined at 400 nm.

5.2.8. Volatile extraction

The preparation of samples for volatile extraction by SPME was conducted as described by Obando-Ulloa *et al*. (2008). Melon cubes were homogenized for 10 s at 12000 rpm with an Ultra-Turrax (IKA T18 basic, Wilmington, NC, USA) and the resulting juice filtered through a powder funnel and four layers of cheesecloth. Saturated calcium chloride solution (4 mL) was added to 10 mL of juice and the mixture homogenized. Aliquots of the final mixture were collected in 12 mL sterile polypropylene vials and stored at −80 ºC until analysis.

5.2.9. Volatile identification and quantification

Volatile analyses were performed by gas-chromatography-mass spectroscopy as described by Berna *et al*. (2005) and Muriel *et al*. (2004). Juice samples were thawed in a water bath at 35 ºC for 15 minutes. Aliquots of 5 mL mixture containing 10 µL of phenyl-ethyl alcohol 0.1% v/v (Acros Organics, Morris Plains, NJ, USA) as internal standard were poured into 20 mL glass vials. The vials were sealed using crimp-top caps with TFE/silicone septa seals (Alltech Associates, Inc., Deerfield, IL, USA) and placed in the heat tray of the GC (6890N, Agilent Technologies, Wilmington, DE, USA) at 35 ºC for 2 h to equilibrate the headspace. The SPME fiber (1-cm long) containing a 50/30 µm divinylbenzene/carboxene on polydimethylsiloxane coating (57329-U DVB/CarboxenTM/PDMS Stable FlexTM Fiber, Supelco, Bellefonte, PA, USA) was preconditioned in the injection port at 250 ºC for 1 h. The needle entered 22 mm into the vial headspace and remained 30 min absorbing volatiles at 35 ºC. After extraction, the volatiles were desorbed from the SPME fibre into the GC injection port set at 280 ºC for 3 min as a bake-out step. The analyses were conducted with a MPS2 Gerstel Multipurpose sampler coupled to the GC–MS. The injection port was operated at 280 ºC in splitless mode and subjected to a pressure of 80 psi. Volatiles were separated on a 30 m×0.25 mm i.d.×0.25 µm thickness capillary column (HP-5MS, Agilent Technologies, Wilmington, NC, USA) that contained 5% phenyl-methyl silicone as a stationary phase. The carrier gas was helium with a flow rate of 1.5 mL min^{-1}. The initial oven temperature was 35 ºC, followed by a ramp of 2 ºC min^{-1} up to 75 ºC, and then at 50 ºC min^{-1} to reach a final temperature of 250 ºC, which was held for 5 min. The inlet liner used was a 2637505 SPME/direct (Supelco), 78.5mm×6.5mm×0.75 mm. Mass spectra were obtained by electron ionization (EI) at 70 eV, and a spectrum range of 40–450 *m*/*z* was used. The detector worked at 230 ºC and in full scan with data acquisition and ion mass captured between 30 and 300

amu. The total analysis time was 27.5 min. The chromatograms and mass spectra were evaluated using the Chem-Station software (G1791CA, Version C.00.00, Agilent Technologies, Wilmington, DE, USA). The peaks were identified using a mass spectrometer (5973 Network Mass Selective Detector, Agilent Technol.) coupled to the GC by comparison of experimental spectra with those of the National Institute for Standards and Technology (NIST05, search version 2.0) data bank. Forty-seven compounds were identified, integrated and used to characterize 1-LPE-treated and control fresh-cut cantaloupe during storage. Results from the volatile analyses were expressed as the percentage of each compound's integrated area relative to the total integration area of 47 compounds.

5.2.10. Data analyses

Data were subjected to analysis of variance according to a split-plot design with treatment (LPE and control) as main-plot and time in storage as sub-plot (Gomez and Gomez, 1984). When significantly different, means were separated by the Duncan's multiple range test. Statistical analysis was performed using SPSS software (v. 17.0, SPSS, Chicago, IL, USA). An individual package constituted an experimental unit which was used as one replicate on each sampling day. Data from four replicated packages were analyzed.

5.3. RESULTS

5.3.1. Phospholipase D activity

PLD activity in untreated control cantaloupe cubes remained at the initial levels during the first day after processing and decreased to 16% of the initial value between the first and the third day in storage, remaining low for the remaining storage period

(Fig. 5.1). PLD activity in LPE-treated tissue decreased immediately after the treatment, but after the third day in storage similar activity levels were observed in LPE-treated and untreated tissue (Fig. 5.1).

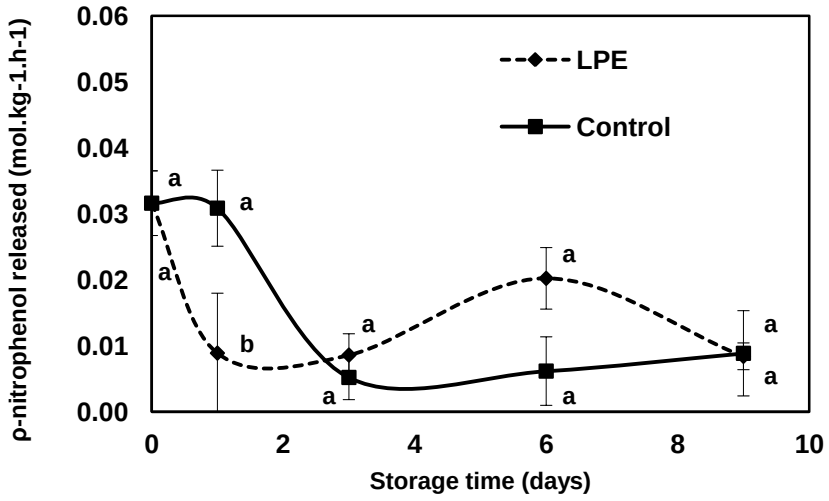


Fig. 5.1. PLD activity in LPE-treated and control fresh-cut melon during storage at 5 ºC. Data expressed as mol nitrophenol equivalents produced per kg of fresh weight. Values are means ± SD (n=4). Values followed by the same letter are not significantly different at α=0.05 by the ANOVA test.

5.3.2. Respiration and ethylene production rates

Respiration rate of LPE-treated and control melon cubes decreased rapidly during the first three days in storage, from 48.5 to 8 mL CO_2 kg^{-1} h^{-1}, remained relatively constant during the following 3 days of storage and increased slightly by the end of the storage time. Respiration of fresh-cut melon was not affected by LPE treatment but there was a significant effect of storage time on CO_2 production rate (Table 5.1).

Average ethylene production decreased by ca. 56% during storage and was not affected by the treatment with LPE (Table 5.1).

5.3.3. Color, firmness and SSC

Slight but significant decreases in lightness and chroma and an increase in hue angle occurred during the 9-day storage period (Table 5.1). Changes in color during storage of fresh-cut melon cubes were not affected by the treatment with LPE. The treatment with LPE did not significantly alter the firmness changes during storage of fresh-cut melon (Table 5.1). During storage, average softening rate of fresh-cut melon was 0.2 N day^{-1}. SSC was not affected by LPE remaining relatively stable during storage (Table 5.1).

Table 5.1. Respiration and ethylene production rates, color, firmness and SSC of controls and LPE-treated fresh-cut cantaloupe stored for 9 days at 5 ºC.

Variable	Time (days)	Control	LPE	Avg.
Respiration rate ($ml.kg^{-1}.hr^{-1}$)	0	48.5±1.5	48.5±1.5	48.5^{a}
	3	10.0±0.4	7.3±0.8	8.7^{c}
	6	8.4±0.4	7.2±0.6	7.8^{bc}
	9	12.3±2.4	12.6±1.5	12.5^{b}
	Avg.	19.8^{A}	18.9^{A}	
Ethylene production rate ($\mu L.kg^{-1}.h^{-1}$)	0	16.7±0.1	18.5±3.4	17.6^{a}
	3	15.5±1.5	12.5±2.3	14.0^{b}
	6	12.7±2.3	11.2±1.9	12.0^{b}
	9	8.3±2.2	7.2±1.4	7.8^{c}
	Avg.	13.3^{A}	12.4^{A}	
L*	0	61.8±2.3	61.8±2.3	61.8^{a}
	3	56.2±2.0	51.9±3.5	54.1^{b}
	6	50.7±3.2	47.2±3.6	49.0^{b}
	9	50.1±3.2	47.2±3.6	48.7^{c}
	Avg.	54.7^{A}	52.0^{A}	
C*	0	41.8±1.5	41.8±1.5	41.8^{a}
	3	36.7±2.4	32.5±3.1	34.6^{b}
	6	30.2±2.5	25.9±2.5	28.1^{b}
	9	30.0±2.5	25.9±2.5	28.0^{c}
	Avg.	34.7^{A}	31.5^{A}	

Table 5.1. Continued.

Variable	Time (days)	Control	LPE	Avg.
h°	0	71.6±0.4	71.7±0.4	71.7[a]
	3	71.9±0.7	73.5±0.9	72.7[b]
	6	73.7±0.8	75.9±1.0	74.8[b]
	9	74.0±0.8	75.9±1.0	75.0[c]
	Avg.	72.8[A]	74.3[A]	
Firmness (N)	0	4.9±0.9	4.3±1.0	4.6[a]
	3	3.7±0.7	3.3±1.2	3.5[b]
	6	2.9±0.6	2.9±0.8	2.9[c]
	9	2.6±0.5	2.2±0.7	2.4[d]
	Avg.	3.5[A]	3.2[A]	
SSC (%)	0	11.6±0.2	11.6±0.2	11.6[a]
	3	13.1±0.5	13.3±0.5	13.2[a]
	6	12.2±0.1	12.8±0.5	12.5[a]
	9	12.7±0.1	13.3±0.3	13.0[a]
	Avg.	12.8[A]	12.8[A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

5.3.4. Antioxidant capacity and phytochemicals

Total antioxidant capacity of fresh-cut cantaloupe, as determined by the $ABTS^+$ assay, decreased significantly in the first three days of cold storage and remained relatively constant during the remaining storage period (Table 5.2). There was no significant effect of LPE on total antioxidant capacity (Table 5.2).

Total phenolic content of fresh-cut melon decreased significantly at the end of the 9-day storage period, but the temporal changes in total phenolics were not affected by LPE (Table 5.2).

Total carotenoid and β-carotene content were maintained during the storage period, with no effect of the LPE treatment (Table 5.2).

Table 5.2. Antioxidant capacity, total phenolics, total carotenoids and β-carotene content of controls and LPE-treated fresh-cut cantaloupe stored for 9 days at 5 ºC.

Variable	Time (days)	Control	LPE	Avg.
Antioxidant activity ($\mu g\ g^{-1}$ fresh weight)	0	123.0±9.6	134.4±2.8	128.7^{a}
	3	69.7±3.8	85.4±4.3	77.6^{b}
	6	74.9±6.0	72.7±7.4	73.8^{b}
	9	87.8±22.6	75.5±2.8	81.7^{b}
	Avg.	88.9^{A}	92.0^{A}	
Total phenolics ($\mu g\ g^{-1}$)	0	213.0±30.4	266.9±63.1	240.0^{a}
	3	255.3±47.5	129.8±79.4	192.6^{ab}
	6	248.4±36.5	170.7±6.7	209.6^{ab}
	9	52.4±37.6	199.8±93.1	126.1^{b}
	Avg.	192.3^{A}	191.8^{A}	
Total carotenoids ($\mu g\ g^{-1}$)	0	55.7±3.4	55.7±3.4	55.7^{a}
	3	55.4±2.1	58.1±3.9	56.8^{a}
	6	50.2±3.2	49.4±2.1	49.8^{b}
	9	62.8±1.9	53.6±3.8	58.2^{a}
	Avg.	56.0^{A}	54.2^{A}	
β - carotene ($\mu g\ g^{-1}$)	0	25.0±1.7	25.0±1.7	25.0^{a}
	3	20.8±1.4	26.3±3.1	23.6^{a}
	6	19.9±3.4	18.6±3.5	19.3^{a}
	9	27.1±0.7	20.2±1.1	23.7^{a}
	Avg.	23.2^{A}	22.5^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

5.3.5. Aroma volatiles

A total of 47 compounds were identified, quantified and grouped into classes. Changes in total volatile abundance during storage were not significant (Table 5.3). Immediately after processing, acetate esters represented the major class of volatiles (56%) produced by fresh-cut Charentais melon, followed by non-acetate esters (28%), alcohols (8%), aldehydes (7%) and sulfur-containing compounds (0.4%). The production of acetate and non-acetate esters increased in the first 3 days of storage

Table 5.3. Chromatogram mean total integrated areas for volatile groups in controls and LPE-treated fresh-cut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Acetate Esters	0	1.46E+09	1.46E+09	1.46E+09^{b}
	3	1.55E+09	1.68E+09	1.62E+09^{a}
	6	1.70E+09	1.58E+09	1.64E+09^{a}
	9	1.45E+09	1.30E+09	1.38E+09^{b}
	Avg.	1.54E+09^{A}	1.51E+09^{A}	
Non-acetate Esters	0	7.41E+08	7.41E+08	7.41E+08^{b}
	3	1.68E+09	1.97E+09	1.83E+09^{a}
	6	2.12E+09	2.20E+09	2.16E+09^{a}
	9	1.74E+09	2.17E+09	1.96E+09^{a}
	Avg.	1.57E+09^{A}	1.77E+09^{A}	
Alcohols	0	2.17E+08	2.17E+08	2.17E+08^{a}
	3	1.36E+08	1.33E+08	1.35E+08^{b}
	6	1.41E+08	1.33E+08	1.37E+08^{b}
	9	1.31E+08	1.52E+08	1.42E+08^{ab}
	Avg.	1.56E+08^{A}	1.59E+08^{A}	
Aldehydes	0	1.69E+08	1.69E+08	1.69E+08^{a}
	3	1.16E+08	6.19E+07	8.90E+07^{b}
	6	1.26E+08	7.37E+07	9.99E+07^{b}
	9	3.73E+07	1.41E+07	2.57E+07^{c}
	Avg.	1.12E+08^{A}	7.97E+07^{A}	
Sulfur-Containing Compounds	0	1.09E+07	1.08E+07	1.09E+07c
	3	2.18E+07	1.58E+07	1.88E+07^{b}
	6	2.87E+07	2.79E+07	2.83E+07^{ab}
	9	4.13E+07	2.80E+07	3.47E+07^{a}
	Avg.	2.57E+07^{A}	2.06E+07^{B}	
Other Compounds	0	3.32E+07	3.32E+07	3.32E+07^{a}
	3	3.33E+07	3.28E+07	3.31E+07^{a}
	6	4.81E+07	3.59E+07	4.20E+07^{a}
	9	6.05E+07	2.82E+07	4.44E+07^{a}
	Avg.	4.38E+07^{A}	3.25E+07^{A}	
Total integrated volatiles	0	2.60E+09	2.60E+09	2.60E+09^{b}
	3	3.50E+09	3.86E+09	3.68E+09^{a}
	6	4.12E+09	4.02E+09	4.07E+09^{a}
	9	3.40E+09	3.67E+09	3.54+09^{ab}
	Avg.	3.41E+09^{A}	3.54E+09^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

and during storage of fresh-cut melon (Table 5.3). Sulfur-containing compounds, on the other hand, increased during storage (Table 5.3). The treatment with LPE did not affect the aggregate abundance of any of the volatile classes (Table 5.3).

Among the nine acetate esters identified, only methyl acetate was significantly affected by both LPE and storage time. LPE-treated melon cubes had lower levels of methyl acetate during the entire storage period (Table 5.4).

Table 5.4. Chromatogram mean relative areas for acetate esters in controls and LPE-treated fresh-cut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Methyl acetate	0	59	59	59^{b}
	3	128	116	122^{a}
	6	123	119	121^{a}
	9	119	84	102^{ab}
	Avg.	108^{A}	95^{B}	
Ethyl acetate	0	69	69	69^{b}
	3	428	363	396^{a}
	6	353	405	379^{a}
	9	376	385	381^{a}
	Avg.	307^{A}	306^{A}	
Propyl acetate	0	10	10	10^{b}
	3	22	26	24^{a}
	6	17	27	22^{a}
	9	22	34	28^{a}
	Avg.	18^{A}	24^{A}	
Isobutyl acetate	0	140	140	140^{a}
	3	104	126	133^{b}
	6	83	77	80^{b}
	9	59	50	55^{c}
	Avg.	97^{A}	98^{A}	
Butyl acetate	0	38	38	38^{b}
	3	27	43	35^{b}
	6	46	52	49^{a}
	9	47	43	45^{ab}
	Avg.	40^{A}	44^{A}	

Table 5.4. Continued.

Variable	Time (days)	Control	LPE	Avg.
3-Methylbutyl acetate	0	1	1	1^{b}
	3	6	6	6^{a}
	6	7	5	6^{a}
	9	8	6	7^{a}
	Avg.	6^{A}	5^{A}	
2-Methylbutyl acetate	0	182	182	182^{a}
	3	151	208	180^{b}
	6	143	161	152^{b}
	9	120	117	119^{c}
	Avg.	149^{A}	167^{A}	
Octyl acetate	0	11	11	11^{a}
	3	4	6	5^{b}
	6	3	5	4^{b}
	9	3	6	5^{b}
	Avg.	5^{A}	7^{A}	
Hexyl acetate	0	41	41	41^{a}
	3	19	37	28^{b}
	6	30	36	33^{b}
	9	31	48	40^{b}
	Avg.	30^{A}	41^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

Ethyl acetate, present at low concentrations at the processing day, increased significantly during storage but this trend was not affected by LPE (Table 5.4). Methyl acetate, ethyl acetate, propyl acetate and 3-methylbutyl acetate increased during the first 3 days in storage and remained relative constant thereafter, whereas isobutyl acetate, 2-methylbutyl acetate, octyl acetate and hexyl acetate decreased during storage (Table 5.4).

The production of total non-acetate esters increased during storage with trend similar to that of acetate esters (Table 5.3). Several individual non-acetate esters also increased significantly during the first 3 days after processing: methyl propanoate, ethyl propanoate, ethyl 2-methyl propanoate, ethyl butanoate, ethyl 2-methyl butanoate, methyl hexanoate, ethyl pentanoate and ethyl hexanoate (Table 5.5).

Table 5.5. Chromatogram mean relative areas for non-acetate esters in controls and LPE-treated fresh-cut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Methyl Propanoate	0	42	42	42[b]
	3	97	84	91[a]
	6	76	88	82[a]
	9	86	82	84[a]
	Avg.	75[A]	74[A]	
Methyl 2-methyl Propanoate	0	11	11	11[a]
	3	15	13	14[a]
	6	12	12	12[a]
	9	10	12	11[a]
	Avg.	12[A]	12[A]	
Ethyl Propanoate	0	8	8	8[b]
	3	27	25	26[a]
	6	24	29	27[a]
	9	24	28	26[a]
	Avg.	21[A]	23[A]	
Methyl Butanoate	0	34	34	34[a]
	3	38	49	44[a]
	6	68	65	67[a]
	9	51	41	46[a]
	Avg.	48[A]	47[A]	
Ethyl 2-methyl Propanoate	0	8	8	8[b]
	3	29	22	26[a]
	6	19	22	21[a]
	9	19	20	20[a]
	Avg.	19[A]	18[A]	
Methyl 2-methyl Butanoate	0	73	73	73[a]
	3	132	142	137[a]
	6	128	141	135[a]
	9	108	143	126[a]
	Avg.	110[A]	125[A]	
Ethyl Butanoate	0	35	35	35[c]
	3	216	238	227[b]
	6	239	351	295[a]
	9	270	351	311[a]
	Avg.	190[A]	244[A]	

Table 5.5. Continued.

Variable	Time (days)	Control	LPE	Avg.
Methyl pentanoate	0	6	6	6[a]
	3	6	6	6[a]
	6	8	7	8[a]
	9	4	5	5[a]
	Avg.	6[A]	6[A]	
Ethyl 2-methyl butanoate	0	29	29	29[b]
	3	263	234	249[a]
	6	239	265	252[a]
	9	229	278	254[a]
	Avg.	190[A]	202[A]	
Ethyl heptanoate	0	N/D	N/D	-
	3	3	2	3[a]
	6	2	2	2[a]
	9	2	3	3[a]
	Avg.	4[A]	4[A]	
Methyl hexanoate	0	16	16	16[c]
	3	37	59	48[a]
	6	28	43	36[ab]
	9	27	36	32[bc]
	Avg.	27[A]	39[A]	
Propyl propanoate	0	2	2	2[a]
	3	3	3	3[a]
	6	3	4	4[a]
	9	N/D	4	-
	Avg.	3[A]	3[A]	
Butyl butyrate	0	2	2	2[a]
	3	1	2	2[a]
	6	1	1	1[a]
	9	N/D	2	-
	Avg.	1[A]	2[A]	
Ethyl pentanoate	0	3	3	3[c]
	3	19	19	19[b]
	6	10	14	12[bc]
	9	23	21	23[a]
	Avg.	14[A]	14[A]	
5-Heptenoic Acid, ethyl Ester (E)	0	2	2	2[a]
	3	3	4	4[a]
	6	2	4	3[a]
	9	3	8	6[a]
	Avg.	3[A]	5[A]	

Table 5.5. Continued.

Variable	Time (days)	Control	LPE	Avg.
Ethyl hexanoate	0	29	29	29[b]
	3	121	252	187[a]
	6	174	220	197[a]
	9	112	299	206[a]
	Avg.	109[A]	200[A]	
Ethyl 3-hexenoate	0	N/D	N/D	-
	3	2	2	2[a]
	6	2	2	2[a]
	9	4	2	3[a]
	Avg.	3[A]	2[A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

Storage time had no effect on methyl 2-methyl propanoate, methyl butanoate, methyl 2-methyl butanoate, methyl pentanoate, ethyl heptanoate, propyl propanoate, butyl butyrate, (E)-5-heptenoic acid, ethyl ester and ethyl 3-hexenoate (Table 5.5). The treatment with LPE had no significant effect on individual non-acetate esters.

Levels of alcohols changed during storage with the opposite trend observed for non-acetate esters, decreasing during the first 3 days of storage and remaining relatively constant thereafter (Table 5.3). Storage duration significantly affected the changes in ethyl alcohol, pentanol, hexanol, 3-octenol, and eucalyptol levels (Table 5.6). LPE treated cubes showing better maintenance of (*Z*)-3-nonenol concentration during storage, but other alcohols were not affected by the treatment (Table 5.6).

The production of aldehydes decreased during the entire storage period in LPE-treated and control melon cubes. LPE had no significant effect on the overall aldehyde production during storage life (Table 5.3) but, significantly lower aldehyde levels were emitted from LPE-treated melon cubes on day 3 (Table 5.3).

Table 5.6. Chromatogram mean relative areas for alcohols in controls and LPE-treated fresh-cut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Ethyl Alcohol	0	17	17	17[b]
	3	33	25	29[ab]
	6	30	30	30[ab]
	9	39	38	39[a]
	Avg.	30[A]	28[A]	
Methyl 2-butyl Alcohol	0	11	11	11[a]
	3	9	13	11[a]
	6	9	11	10[a]
	9	9	12	11[a]
	Avg.	10[A]	12[A]	
Pentanol	0	2	2	2[a]
	3	1	1	1[b]
	6	1	1	1[b]
	9	1	N/D	-
	Avg.	1[A]	1[A]	
Hexanol	0	26	26	26[a]
	3	13	10	12[b]
	6	12	13	13[b]
	9	9	20	15[b]
	Avg.	15[A]	17[A]	
Heptanol	0	N/D	N/D	-
	3	N/D	1	-
	6	1	1	1
	9	N/D	1	-
	Avg.	1[A]	1[A]	
3-Octenol	0	3	3	3[a]
	3	2	3	3[a]
	6	2	1	2[b]
	9	1	1	1[b]
	Avg.	2[A]	2[A]	
Eucalyptol	0	16	16	16[a]
	3	10	13	12[b]
	6	9	11	10[b]
	9	8	11	10[b]
	Avg.	11[A]	13[A]	

Table 5.6. Continued.

Variable	Time (days)	Control	LPE	Avg.
Benzyl	0	5	5	5[a]
Alcohol	3	6	5	6[a]
	6	3	4	4[a]
	9	2	4	3[a]
	Avg.	4[A]	5[A]	
Octanol	0	2	2	2[a]
	3	2	3	3[a]
	6	2	4	3[a]
	9	2	3	3[a]
	Avg.	2[A]	3[A]	
(*Z*)-3-Nonenol	0	5	5	5[a]
	3	3	4	4[a]
	6	4	4	4[a]
	9	1	3	3[a]
	Avg.	3[B]	4[A]	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

Hexanal was the most abundant aldehyde while pentanal, benzaldehyde and nonanal were found in residual concentrations. These four aldehydes were significantly affected by storage time but not affected by the LPE treatment (Table 5.7). Although the effect of LPE on hexanal was not significant, a consistent reduction of this aldehyde was observed in tissue treated with LPE, on day 3 and thereafter (Table 5.7).

Total integrated sulfur-containing compounds were significantly affected by LPE and storage time. Control melon cubes showed higher production of sulfur-containing compounds when compared with the relative maintenance of these compounds in LPE treated cubes (Table 5.3).

Table 5.7. Chromatogram mean relative areas for aldehydes in controls and LPE-treated freshcut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Acetaldehyde	0	7	7	7^{a}
	3	12	10	11^{a}
	6	9	11	10^{a}
	9	9	10	10^{a}
	Avg.	9^{A}	10^{A}	
Pentanal	0	8	8	8
	3	5	N/D	-
	6	N/D	N/D	-
	9	N/D	N/D	-
	Avg.	-	-	
Hexanal	0	45	45	45^{a}
	3	51	27	39^{b}
	6	48	32	40^{b}
	9	15	4	10^{c}
	Avg.	40^{A}	27^{A}	
Heptanal	0	6	6	6^{a}
	3	8	4	7^{a}
	6	7	5	6^{a}
	9	3	2	3^{a}
	Avg.	6^{A}	4^{A}	
Benzaldehyde	0	4	4	4^{ab}
	3	4	3	4^{ab}
	6	5	4	5^{a}
	9	3	2	3^{b}
	Avg.	4^{A}	3^{A}	
Nonanal	0	4	4	4^{a}
	3	4	4	4^{a}
	6	3	3	4^{a}
	9	3	3	3b
	Avg.	4^{A}	4^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

Significant increases of methyl 3-(methylthio) propanoate and ethyl 3 (methylthio) propanoate were observed during storage. Ethyl (methylthio) acetate was found in residual levels in both treatments and only at the day of processing (Table 5.8).

Table 5.8. Chromatogram mean relative areas for sulfur-containing compounds in controls and LPE-treated fresh-cut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Ethyl methylthio Acetate	0	1	1	1
	3	N/D	N/D	-
	6	N/D	N/D	-
	9	N/D	N/D	-
	Avg.	-	-	
Methyl 3-Methylthio Propanoate	0	1	1	1^{c}
	3	1	1	1^{c}
	6	2	2	2^{b}
	9	2	2	3^{a}
	Avg.	2^{A}	2^{A}	
Ethyl 3-methylthio propanoate	0	2	2	2^{c}
	3	11	7	9^{b}
	6	11	13	12^{ab}
	9	20	14	24^{a}
	Avg.	11^{A}	9^{A}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test. N/D, not detected.

Table 5.9. Chromatogram mean relative areas for other compounds in controls and LPE-treated fresh-cut cantaloupe during storage at 5 °C.

Variable	Time (days)	Control	LPE	Avg.
Acetophenone	0	12	12	12^{a}
	3	15	13	14^{a}
	6	12	11	12^{a}
	9	14	12	13^{a}
	Avg.	13^{A}	12^{A}	
2,3-Butanedioldiaceate	0	1	1	1^{b}
	3	5	5	5^{b}
	6	11	9	10^{a}
	9	19	5	12^{a}
	Avg.	9^{A}	5^{B}	

Values are means (n=4). Values followed by the different letters within columns and within lines are significantly different ($p<0.05$) according to Duncan´s Multiple Range Test.

Acetophenone levels were not affected by the LPE treatment or storage time while 2,3-butanedioldiacetate was significantly affected by LPE treatment. By the end of storage time, LPE-treated melon cubes showed lower levels of 2,3-butanedioldiacete than untreated cubes (Table 5.9).

5.4. Discussion

The response to plant tissues to wounding involves increased PLD activity (Ryu and Wang, 1996). PLD is a wound-activated enzyme that hydrolyses the ester bond between the head group and the PA of phospholipids (Wang, 2001), therefore compromising membrane integrity. Polyunsaturated fatty acids, particularly linoleic and linolenic acid, are precursors of aroma volatiles, namely hexanal, (*Z)*-3-hexenal, (*Z)*-3-nonenal and (*Z)*-3- and (*Z)*-6-nonadienal (Tijet at al., 2001). The inhibition of PLD is therefore anticipated to improve membrane integrity and change the profile of aroma volatiles in fresh-cut melon. LPE containing an 18:1 fatty acid esterified to the glycerol backbone inhibits PLD activity *in vitro* (Ryu *et al*., 1997). When applied by vacuum infiltration to fresh-cut cantaloupe, LPE reduced PLD activity in the first day after cutting (Fig. 5.1). Melon fruit have different PLD isoforms (Whitaker and Lester, 2006) whose contribution for the activity trend reported in Fig. 5.1 is unknown. The overall decreasing trend in PLD activity during the late period of storage has been attributed to the increase in membrane viscosity caused by the degradation of phospholipids (Yapa *et al*., 1986).

LPE ranging from 40 and 200 µM, specifically inhibits the activity of partially purified cabbage PLD (presumably PLDα) in a cell-free system and in a concentration-dependent way (Ryu *et al*., 1997). The inhibition of PLD by LPE is highly specific and increased with length and the unsaturation of the acyl chain (Ryu *et al.*, 1997). The strong inhibition of PLD by 18:1-LPE when compared with LPE containing other acyl-chains (14:0, 16:0, and 18:0) suggests that a specific configuration of LPE is required for inhibition (Ryu *et al*., 1997). The inhibition of PLD by LPE *in vivo* has been questioned (Cowan, 2009). Although Ryu *et al*. (1997) conclude that endogenous LPE is a catabolite of hormone-activated PLA_2 and that likely serves as a second messenger in plants to modulate PLD activity, Cowan (2009) points out that corroborative evidence for the above conclusion was not readily

forthcoming as several reports refuted the existence of PLD inhibitors and in particular that of exogenous LPE in this role and that pharmacological studies demonstrated that PLD activity or more accurately PA, plays a pivotal role in cytokinin signaling and thus, in senescence delay. We observed a limited and transient inhibition of PLD in melon cubes vacuum-infiltrated with 200 mM LPE one day after the treatment with subsequent activity evolution similar in treated and untreated melon cubes (Fig. 5.1).

LPE has been reported to delay fruit senescence and extend shelf-life in tomato (Farag and Palta, 1993a), cranberry (Özgen *et al*., 2004), red and green pepper (Hong and Chong, 2006) and grape (Hong, 2008). In addition to the inhibition of PLD, this effect has been attributed to a decrease in ethylene biosynthesis or reduced ethylene action induced by LPE (Hong, 2006; Cowan, 2009). However, we observed no effect of LPE on ethylene production or on the overall metabolic rate of fresh-cut melon, as assessed by the respiration rate or compositional changes (Table 5.1).

The inhibition of catabolic reactions (stimulated by ethylene and PLD), by LPE was expected to improve the quality and extend shelf-life of fresh-cut Charentais-melon when compared to untreated control fruit. Yet, the transient inhibition of PLD activity observed herein was not related to improvements in the quality attributes color, firmness, SSC (Table 5.1) or to changes in the antioxidant activity, content of phenolics or carotenoids (Table 5.2). The lack of effect of LPE on the firmness of fresh-cut melon reported herein contrasts with the reduced softening observed in whole apples (Farag and Palta, 1991c), cranberries (Farag and Palta, 1991c) tomatoes (Farag and Palta, 1993b), grapes (Hong *et al*., 2007) and bananas (Ahmed and Palta, 2011).

The LPE effects reported in the literature are often conflicting and difficult to reconcile. LPE is reported to reduce ethylene production rate in tomato (Farag and Palta, 1993a) and cranberry (Ryu *et al*., 1997), however in other instances, LPE

stimulated ethylene production in apple (Farag and Palta, 1991c), banana (Ahmed and Palta, 2011), cranberry (Farag and Palta, 1991c), tomato (Hong *et al*., 2001) and red pepper (Kang *et al*., 2003). LPE induced the expression of two ACS (EC 4.4.114) genes in mung bean (*Vigna radiata*) hypocotyl (Hong *et al*., 2008) and ACO activity was enhanced by LPE in mature-green tomato but reduced in ripe fruit (Hong, 2006). Also, LPE is reported to maintain fruit firmness during storage but also to increase endo β-1,3(4)-Glc activity (Cowan *et al*., 2006) and decrease PG activity (Mangat and Palta, 1995).

Additionally, a change in aroma volatiles caused by the suppression of lipid-derived compounds was anticipated. The total integrated volatile peak areas showed that fresh-cut melon volatiles went through a transient increase followed by a decrease by the end of storage time. Transient increases in total volatiles during storage were also reported in fresh-cut cantaloupe (Beaulieu, 2005) and fresh-cut honeydew (Saftner *et al*., 2003) melons and can be interpreted as a result of processing fruits at a slightly immature stage for fresh-cut storage and, consequently, volatiles increased as fruits finished their climacteric and associated ethylene production (Beaulieu, 2005) or/and as a result of increased volatile precursors availability due to increased post-cutting metabolism acceleration, oxidation reactions and LOX activity (Beaulieu, 2006). Despite the variability of the data, a reduction in aldehydes level on LPE-treated melon cubes by day 3 is consistent with the inhibitory effect of LPE on PLD. These results suggest that initial PLD inhibition by LPE may have reduced the production of polyunsaturated fatty acids, and therefore the formation of short-chain (C_6 and C_9) aldehydes observed at day 3 of storage.

The vacuum infiltration method used in this experiment created conditions in the mesocarp tissue that are not typical of fresh-cut fruit. However, vacuum infiltration assures that more cells are in contact with the bioactive LPE than conventional application methods, such as dipping or spraying. Therefore, the comparative results

reported herein show that LPE treatment applied to fresh-cut Charentais melon did not have a specific effect on quality attributes or shelf life extension.

5.5. Conclusions

In conclusion, the anticipated benefits of LPE on the quality of fresh-cut melon were not confirmed by this study. The inhibitory effect of LPE on PLD was transient and likely irrelevant to improve the overall quality of fresh-cut melon. In conclusion, LPE did not improve the quality of fresh-cut melon but showed a specific effect on aldehydes.

CHAPTER 6

General conclusions

6.1. General conclusions

Fresh-cut technology aims at minimizing damages caused by wounding and at the reduction of the rate of quality losses. Processing injury triggers a number of physiological responses that may affect fresh-cut fruit sensory and nutritional quality, through changes in color, texture, sweetness, antioxidants and aroma. The reports on the use of low-oxygen MAP are abundant; less is known about the effects of 1-MCP on the overall quality of fresh-cut fruit and no information is available concening the technological potential for LPE. On the other hand, although changes in overall quality have been characterized in fresh-cut melon, much less is known about the effects of processing technologies on aroma volatiles and their evolution during storage of fresh-cut fruits.

Storage time and oxygen availability inside fresh-cut melon packages are important factors in determining the concentratrion of aroma volatiles, in both climacteric and non-climacteric melons. The results show that storage time significantly affected all compound classes and, except for benzyl acetate and ethyl 3-(methylthio) propanoate in fresh-cut honeydew, all individual volatiles. The volatile compounds that were significantly affected by atmosphere modification are flavor-important volatiles in melon. Except for benzyl acetate, eucalyptol and (Z)-3-octenol, O_2 availability affected all individual volatile compounds during storage of fresh-cut cantaloupe and honeydew melons. Low O_2 availability suppresses esters biosynthesis while alcohols and aldehydes are not consistently affected. These changes in the aroma balance may alter the overall consumer's perception of quality and influence the acceptance of fresh-cut melon products. These finding open a new venue for optimization of MAP recommendations aimed at optimizing aroma instead of other quality attributes, unaffected by atmospheric composition.

1-MCP was ineffective at reducing fresh-cut melon ethylene production and respiration rate failing or help maintain quality during storage. Nevertheless, the

results show that 1-MCP affected some isoleucine and phenylalanine-derived volatile compounds, suggesting that 1-MCP affects the biosynthetic pathways that lead to the conversion of these aminoacids into branched alkyl and acyl esters and into branched alcohols. As with oxygen levels, aroma volatiles seem to be more sensitive to this postharvest technology applied to fresh-cut melon than other quality attributes.

The use of LPE as technological tool to improve the quality of fresh-cut melon has not been developed, despite the potential benefits of PLD inhibition. The anticipated LPE inhibition of PLD activity and consequent reduction on fatty acid-derived volatile compounds was observed in the first three days of storage. Although LPE did not alter significantly the overall fresh-cut melon aroma production, a decrease in aldehydes production on day three of storage seems to be related to the initial and transient PLD inhibition by LPE. These results strain the need to re-assess LPE as a plant growth regulator and specifically as a PLD inhibitor and re-evaluate the impact of available LPE scientific information.

In conclusion, there is opportunity to use oxygen levels to modulate aroma volatiles in frehs-cut melon, and likely in other fresh-cut fruit. 1-MCP seems to be of little use and little potential for the technological usage of LPE in fresh-cut melon is envisioned, based on our results.

6.2. Future prospects

The research reported herein raised new questions and new opportunities for further research:

- Since atmosphere modification altered the volatile production of both fresh-cut melon cultivars and given that melon flavor-important volatiles were affected, a natural follow up step is to evaluate the effects of different

oxygen levels during fresh-cut melon storage on the consumer's sensory perception and preference;

- Modified atmosphere packages can, therefore, designed for aroma optimization, and that concept must be validated;
- From the integration of the results of 1-MCP application to fresh-cut melon arises the doubt of which is the better fruit maturity stage at processing and at 1-MCP treatment in order to ensure 1-MCP effect on ethylene reduction;
- There is also the need to assess the effect of 1-MCP on wound-induced increased ACO and ACS activities and to determine if processing induces the appearance and renewal of ethylene binding sites; this biochemical approach will help distinguish between the effects of ripening-related and wound-related ethylene effects;
- The transient inhibitory effect of LPE on PLD activity observed in this study suggests the need of a demonstration of a significant *in vivo* inhibition of PLD by LPE and of an evaluation of the effect of LPE on linoleic and linolenic acids levels;
- The negative feedback control of endogenous LPE production after wounding needs to be clarified as well as the possible factors that control/affect this process;
- The precise mechanisms by which LPE interferes with ethylene biosynthesis should be identified;
- The effects of LPE on specific enzymes require clarification as well as the role of excipients or additives present in LPE formulations or added to LPE solutions on the reported results.

REFERENCES

REFERENCES

Abe, K. and Watada, A. E. 1991. Ethylene absorbent to maintain quality of lightly processed fruits and vegetables. *Journal of Food Science*, 56: 1493-1496.

Agilent Technologies. 2008. Retention time locking with the MSD productivity ChemStation. Technical overview, Available at: http://www.chem.agilent.com/Library/technicaloverviews/Public/5989-8574EN.pdf

Aguayo, E., Allende, A. and Artés, F. 2003. Keeping quality and safety of minimally fresh processed melon. *European Food Research and Technology*, 216: 494-499.

Aguayo, E., Escalona, V. and Artés, F. 2004a. Quality of fresh-cut tomato as affected by type of cut, packaging, temperature and storage time. *European Food Research and Technology*, 219: 492-499.

Aguayo, A. E., Escalona, V. and Artés, F. 2004b. Metabolic behavior and quality changes of whole and fresh processed melon. *Journal of Food Science*, 69: 148-155.

Aguayo, E., Janjasithorn, R. and Kader A. A. 2006. Combined effects of 1-methylcyclopropene, calcium chloride dip, and/or atmosphere modification on quality changes of fresh-cut strawberries. *Postharvest Biology and Technology,* 40: 269-278.

Aguayo, E., Escalona, V. H. and Artés, F. 2007. Quality of minimally processed *Cucumis melo* var. saccharinus as improved by controlled atmosphere. *European Journal of Horticultural Science*, 72: 39-45.

Aguayo, E., Escalona, V. H. and Artés, F. 2008. Effect of hot water treatment and various calcium salts on quality of fresh-cut Amarillo melon. *Postharvest Biology and Technology*, 47: 397-406.

Ahmed, Z. F. A. and Palta, J. P. 2010. Lysophosphatidylethanolamine, a natural phospholipid, may retard senescence and improve the shelf life of banana fruit. *HortScience*, 45: 66. (Abstr.).

Ahmed, Z. F. A., Palta, J.P. 2011a. A natural lipid, lysophosphatidylethanolamine, may promote ripening while reducing senescence in banana fruit. HortScience 46, 273. (Abstr.).

Ahmed, Z. F. A. and Palta, J. P. 2011b. Hormone-like effect of a natural lipid, lysophosphatidylethanolamine, can mitigate calcium deficiency injury in potato shoot cultures. *HortScience*, 46: 196. (Abstr.).

Al-Ati, T. and Hotchkiss, J. H. 2002. Application of packaging and modified atmosphere to fresh-cut fruits and vegetables. In: *Fresh-Cut Fruits and Vegetables, Science, Technology and Market.* Lamikanra, O. (Eds.), pp. 305-338. CRC Press, Boca Raton, Florida, USA.

Alfarez, F., Lluch, Y. and Burns, J. K. 2008. Phospholipase A_2 and postharvest peel pitting in citrus fruit. *Postharvest Biology and Technology*, 49: 69-76.

Altwies, J., Guelzow, M., Hong, J. H., Rademacher, M. and Towse, J. 2002. Potential use of lysophosphatidylethanolamine, a natural lipid, as a post-harvest dip to accelerate ripening of mature green tomatoes packed for fresh-market. *Acta Horticulturae*, (Abstr.).

Alvarez-Venegas, R., Sadder, M., Hlavacka, A., Baluska, F., Xia, Y., Lu, G., Firsov, A., Sarath, G., Moriyama, H., Dubrovsky, J. G. and Avramova, Z. 2006a. The Arabidopsis homolog of trithorax, ATX1, binds phosphatidylinositol 5-phosphate, and the two regulate a common set of target genes. *Proceedings of the National Academy of Sciences*, 103: 6049-6050.

Alvarez-Venegas, R., Xia, Y., Lu, G. and Avramova, Z. 2006b. Phosphoinositide 5-phosphate and phosphoinositide 4-phosphate trigger distinct specific responses of Arabidopsis genes. *Plant Signaling Behavior*, 1: 140-151.

Alves, R. E., Filgueiras, H. A. C., Almeida, A. S., Machado, F. L. C., Bastos, M. S. R., Lima, M. A. C., Terao, D., Silva, E. O., Santos, E. C., Pereira, M. E. C. and Miranda, M. R. A. 2005. Postharvest use of 1-MCP to extend storage life of melon in Brazil - current research status. *Acta Horticulturae,* 682: 2233-2237.

Amaro, A. L., Beaulieu, J. C., Grimm, C.C, Stein, R. E. and Almeida, D. P. F. 2012. Effect of oxygen on aroma volatiles and quality of fresh-cut cantaloupe and honeydew melons. *Food Chemistry,* 130: 49-57.

Amaro, A. L., Fundo, J., Beaulieu, J. C., Stein, R. E., Fernández-Trujillo, J. P. and Almeida, D. P. F. Lysophosphatidylethanolamine effects upon volatiles and quality of fresh-cut Galia melon. *Acta Horticulturae*. (in press).

Arteca, R. N. 1996. Plant growth substances. Principles and applications. Chapman and Hall. New York.

Artés, F., Gómez, P. and Artés-Hernández, F. 2007. Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. *Food Science and Technology International*, 13: 177-188.

Asoda, T., Terai, H., Kato, M. and Suzuki, Y. 2009. Effects of postharvest ethanol vapor treatment on ethylene responsiveness in broccoli. *Postharvest Biology and Technology*, 52: 216-220.

Ayhan, Z., Chism, G. W. and Richter, E. R. 1998. The shelf life of minimally processed fresh-cut melons. *Journal of Food Quality*, 21: 29-40.

Ayub, R., Guis, M., BenAmor, M., Gillot, L., Roustan, J. P., Latche, A., Bouzayen, M. and Pech, J. C. 1996. Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. *Nature Biotechnology*, 14: 862-866.

Bai, J., Saftner, R. A., Watada, A. E., and Lee, Y. S. 2001. Modified atmosphere maintains quality of fresh-cut cantaloupe (*Cucumis melo* L.). *Journal of Food Science*, 66: 1207-1211.

Bai, J., Saftner, R. A. and Watada, A. E. 2003. Characteristics of fresh-cut honeydew (*Cucumis xmelo* L.) available for processors in winter and summer and its quality maintenance by modified atmosphere packaging. *Postharvest Biology and Technology*, 28: 349-359.

Bai, J. H., Baldwin, E. A., Goodner, K. L., Mattheis, J. P. and Brecht, J. K. 2005. Response of four apple cultivars to 1-methylcyclopropene treatment and controlled atmosphere storage. *HortScience*, 40: 1534-1538.

Balbontín, C., Gaete-Eastman C., Vergara M., Herrera R. and Moya-León M. A. 2007. Treatment with 1-MCP and the role of ethylene in aroma development of mountain papaya fruit. *Postharvest Biology and Technology,* 43: 67-77.

Baldwin, E. A., Scott, J. W., Sheemaker, C. K. and Schuch, W. 2000. Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control and important aroma components. *HortScience*, 35: 1013-1022.

Baltussen, E., Sandra, P., David, F. and Cramers, C. A. 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples, theory and principles. *Journal of Microcolumn Separations*, 11: 737-747.

Bapat, V. A., Trivedi, P. K., Ghosh, A., Sane, V. A., Ganapathi, T. R. and Nath, P. 2010. Ripening of fleshy fruit: molecular insight and the role of ethylene. *Biotechnology Advances,* 28: 94-107.

Bargmann B. O. and Munnik, T. 2006. The role of phopholipase D in plant stress responses. Current Opinion in Plant Biology, 9: 515-522.

Barreiro, J. A., Sandoval, A. J., Rivas, D. and Rinaldi, R. 2007. Application of a mathematical model for chemical peeling of peaches (*Prunus persica* L.) variety Amarillo Jarillo. *LWT - Food Science and Technology*, 40: 574-578.

Bauchot, A. D., Mottram, D. S., Dodson, A. T., and John, P. 1998. Effect of aminocyclopropane-1-carboxyl acid oxidase antisense gene on the formation of

volatile esters in Cantaloupe Charentais melon (cv. *Védrandais*). *Journal of Agricultural and Food Chemistry*, 46: 4787-4792.

Beaudry, R. M. 1999. Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. *Postharvest Biology and Technology*, 15:293-303.

Beaudry, R. M. 2000. Responses of horticultural commodities to low oxygen: limits to the expanded use of modified atmosphere packaging. *HortTechnology*, 10: 491-500.

Beaulieu, J. C. 2005. Within-season volatile and quality differences in stored fresh-cut cantaloupe cultivars. *Journal of Agricultural and Food Chemistry*, 53: 8679-8687.

Beaulieu, J. C. 2006a. Volatile changes in cantaloupe during growth, maturation, and in stored fresh-cuts prepared from fruit harvested at various maturities. *Journal of the American Society for the Horticultural Science*, 131: 127-139.

Beaulieu, J. C. 2006b. Effect of cutting and storage on acetate and nonacetate esters in convenient, ready-to-eat fresh-cut melons and apples. *HortScience,* 41: 65-73.

Beaulieu, J. C. and Gorny, J. R. 2001. Fresh-cut fruits. In: *The commercial storage of fruits, vegetables, and florist and nursery stocks. USDA Handbook 66*. Gross, K., Saltveit, M. E., Wang, C.Y. (Eds.), pp. 1-49. USDA, Washington, D.C, USA.

Beaulieu, J. C. and Grimm, C. C. 2001. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. *Journal of Agricultural and Food Chemistry*, 49: 1345-1352.

Beaulieu, J. C. and Lea, J. M. 2003. Volatile and quality changes in fresh-cut mangos prepared from firm-ripe and soft-ripe fruit, stored in clamshell containers and passive MAP. *Postharvest Biology and Technology*, 30: 15-28.

Beaulieu, J. C., Lea, J. M., Peralta-Inga, Z. and Eggleston, G. 2003. Sugar and organic acid variations in commercial cantaloupes and their inbred parents. *Journal of the American Society for the Horticultural Science*, 128: 531-536.

Beaulieu, J. C., Lea, J. M., Ingram, D. A. and Bett-Garber, K. L. 2004. Effect of harvest maturity on the sensory characteristics of fresh-cut cantaloupe. *Journal of Food Science*, 69: S250-S258.

Beaulieu, J. C. and Lancaster, V. A. 2007. Correlating volatile compounds, sensory attributes, and quality parameters in stored fresh-cut cantaloupe. *Journal of Agricultural and Food Chemistry*, 55: 9503-9513.

Beaulieu, J. C. and Lea, J. M. 2007. Quality changes in cantaloupe during growth, maturation, and in stored fresh-cuts prepared from fruit harvested at various maturities. *Journal of the American Society for Horticultural Science,* 132:720-728.

Beaulieu, J.C. and E. Baldwin, 2002. Flavor and aroma of fresh-cut fruits and vegetables. In: *Fresh-Cut Fruit and Vegetables: Science, Technology and Market*, Lamikanra, O. (Eds.), pp: 391-425. CRC Press, London, UK.

Bender, R. J., Brecht, J. K., Sargent, S. A. and Huber, D. J. 2000. Mango tolerance to reduced oxygen levels in controlled atmosphere storage. *Journal of the American Society for Horticultural Science*, 125 : 707-713.

Berna, A. Z., Buysens, S., Di Natale, C., Grun, I. U., Lammertyn, J. and Nicolai, B. M. 2005. Relating sensory analysis with electronic nose and headspace fingerprint MS for tomato aroma profiling. *Postharvest Biology and Technology*, 36: 134-155.

Bett-Garber, K., Lamikanra, O., Lester, G. E., Ingram, D. A. and Watson, M. A. 2005. Influence of soil type and storage conditions on sensory qualities of fresh-cut cantaloupe (*Cucumis melo*). *Journal of Science and Food Agriculture,* 85: 825-830.

Biacs, P.A. and Daood, H.G. 2000. Lipoxygenase-catalysed degradation of carotenoids from tomato in the presence of antioxidant vitamins. *Biochemical Society Transactions*, 28: 839-845.

Bianco, V. V. and Pratt, H. K. 1977. Compositional changes in muskmelon during development and in response to ethylene treatment. *Journal of the American Society of Horticultural Science*, 102: 127-133.

Blankenship, S. M. and Dole, J. M. 2003. 1-Methylcyclopropene: a review. *Postharvest Biology and Technology*, 28: 1-25.

Bouquín, T. B., Lasserre, E., Pradier, J., Pech, J. and Balague, C. 1997. Wound and ethylene induction of the ACC oxidase melon gene CM-ACO1 occurs via two direct and independent transduction pathways. *Plant Molecular Biology*, 35: 1029-1035.

Boynton, B. B., Welt, B. A., Sims, C. A., Balaban, M. O., Brecht, J. K. and Marshall, M. R. 2006. Effects of low-dose electron beam irradiation on respiration, microbiology, texture, color, and sensory characteristics of fresh-cut Cantaloupe stored in modified atmosphere. *Journal of Food Science*, 71: 149-155.

Bregoli, A. M., Ziosi, V., Biondi, S., Rasori, A., Ciccioni, M., Costa, G. and Torrigiani, P. 2005. Postharvest 1-mehylcyclopropene application in ripening control of 'Stark Red Gold' nectarines: temperature-dependent effects on ethylene production and biosynthetic gene expression, fruit quality, and polyamine levels. *Postharvest Biology and Technology*, 37: 111-121.

Budu, A. S. and Joyce, D. C. 2003. Effect of 1-methylcyclopropene on the quality of minimally processed pineapple fruit. *Australian Journal of Experimental Agriculture*, 43: 177-184.

Buttery, R.G. 1993 Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. In: *Flavor Science. Sensible Principles and Techniques, ACS Professional Reference Book*. Acree, T. E. and Teranishi, R. (Eds.), pp. 259–286. American Chemical Society, Washington, DC, USA.

Buttery, R. G., and Ling, L. C. 1993. Enzymatic production of volatiles in tomatoes. In: *Flavor Precursors.* Schreier, P. and Winterhalter, P. (Eds.), pp.137-146. Allured Pub. Co., Wheaton, IL, USA.

Calderón-López, B., Bartsch, J. A., Lee, C. Y. and Watkins, C. B. 2005. Cultivar effects on quality of fresh-cut apple slices from 1-methylcyclopropene (1-MCP) treated apple fruit. *Journal of Food Science*, 70: 114-118.

Caprioli, I., Lafuente, M. T., Rodrigo, M. J., and Mencarelli, F. 2009. Influence of postharvest treatment on quality, carotenoids and abscisic acid content of stored “Spring Belle” peach (*Prunus persica*) fruit. *Journal of Agricultural and Food Chemistry,* 57: 7056-7063.

Cecchetti, V., Altamura, M. M., Falasca, G., Constantino, P. And Cardarelli, M. 2008. Auxin regulates *Arabidopsis* anther dehiscence, pollen maturation, and filament elongation. *Plant Cell*, 20: 1760-1774.

Chang, C., Kwok, S. F., Bleecker, A. B. and Meyerowitz, E. M. 1993. *Arabidopsis* ethylene-response gene ETR1: similarity of products to two-component regulators. *Science*, 262*:* 539-544*.*

Chapman, K. D. 1998. Phospholipase D activity during plant growth and development and in response to environmental stress. *Trends in Plant Science*, 3: 419-426.

Chervin, C., Westercamp, P. and Monteils, G. 2005. Ethanol vapours limit Botrytis development over postharvest life of table grapes. *Postharvest Biology and Technology*, 36: 319-322.

Chung, G. H. and Yang, Y. L. 2004. Method for preparing lysophosphatidylethanolamine. *United States Patents, Patent Number*: 6773902. Date of Patent: August 10, 2004.

Chung, G. H., Hong, J. H. and Yang, Y. L. 2008. Stable water soluble composition containing lysophosphatidylethanolamine and lecithin. *United States Patent*

Application, Patent Application Number: 20080188683. Date of Patent Application: August 7, 2008.

Chung, G. H., Hong, J. H. and Yang, Y. L. 2011. Method, composition and kit for promoting plant growth and improving plant preservation. *United States Patents, Patent Number:* 8030244*.* Date of Patent: October 4, 2011.

Chung, G. H., Hong, J. H., Yang, Y. L., Oh, Y. R. and Koh, U. C. 2010. Method, composition and kit for promoting plant growth and improving plant preservation. *United States Patent Applications, Patent Aplication Number:* 20100255990*.* Date of Patent Application: October 7, 2010.

Cisneros-Zevallos, L., Saltveit, M., and Krochta, J. 1997. Hygroscopic coating control surface white discoloration of peeled (minimally processed) carrots during storage. *Journal of Food Science*, 62: 363-367.

Clydesdale, F. M. 1993. Color as a factor in food choice. *Critical Reviews in Food Science and Nutrition*, 33: 83-101.

Cowan, A. K. 2009. Plant growth promotion by 18:0-*lyso*-phosphatidylethanolamine involves senescence delay. *Plant Signaling Behavior*, 4: 324-327.

Cowan, A. K., Leung, C. and Santori, C. H. 2006. Lyso-phosphatidylethanolamine (LPE) as a plant bioregulator. *Acta Horticulturae*, 727: 527-536.

Creelman, R. A. and Mullet, J. E. 1997. Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48: 355-381.

Cunningham, F. X. Jr. and Gantt, E. 1998. Genes and enzymes of carotenoid biosynthesis in plants*. Annual Review of Plant Physiology and Plant Molecular Biology,* 49*:* 557-583*.*

Dawson, R. M. C. and Hemington, N. 1967. Some properties of purified phospholipase D and specially the effect of amphipathic substances. *Biochemistry Journal*, 102: 76-86.

de Wild, H. P. J., Otma, E. C. and Peppelendos, H. W. 2003. Carbon dioxide action on ethylene biosynthesis of preclimacteric and climacteric pear fruit. *Journal of Experimental Botany*, 54: 1537-1544.

DeEll, J. R., Murr, D. P., Porteous, M. D. and Rupasinghe, H. P. V. 2002. Influence of temperature and duration of 1-methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biology and Technology*, 24: 349-53.

DeEll, J. R., Ayres, J. T. and Murr, D. P. 2008. 1-Methylcyclopropene concentration and timing of postharvest application alters the ripening of 'McIntosh' apples during storage. *HortTechnology*, 18: 624-630.

Defilippi, B. G., Kader, A. A. and Dandekar, A. M. 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica* Borkh) fruits. *Journal of Agricultural and Food Chemistry,* 52: 5694-5701.

Defilippi, B. G., Kader, A. A. and Dandekar, A. M. 2005. Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. *Plant Science*, 168: 1199-1210.

Defilippi, B. G., Manríquez, D., Luengwilai, K. and González-Agüero, M. 2009. Chapter 1 Aroma volatiles: Biosynthesis and mechanisms of modulation during fruit ripening. *Advances in Botanical Research*, 50: 1-37.

Deikman, J. 1997. Molecular mechanisms of ethylene regulation of gene transcription. *Physiologia Plantarum*, 100: 561–566.

Demirkeser, T. H., Polat, A. A. and Caliskan, O. 2009. Effect of natural lipid on flowering, pollination traits and fruit set on loquad (*Eriobotrya japonica* Lindl.). *Asian Journal of Chemistry*, 21: 113-120.

Dixon, J. and Hewett, E. W. 2000. Factors affecting apple aroma/flavor volatile concentration: a review. *New Zealand Journal of Crop and Horticultural Science,* 28: 155-173.

Ecker, J. R. and Davis, R. W. 1987. Plant defense genes are regulated by ethylene. *Proceedings of the National Academy of Sciences*, 84: 5202-5206.

El-Sharkawy, I., Manríquez, D., Flores, F. B., Regad, F., Bouzayen, M., Latché, A. and Pech, J. C. 2005. Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. *Plant Molecular Biology*, 59: 343-360.

EPA. United State Environmental Protection Agency biopesticides registration action document, Lysophosphatidylethanolamine (LPE) (PC Code 105120).

Ergun, M. and Huber, D.J. 2004. Suppression of ethylene perception extends shelf-life and quality of 'Sunrise Solo' papaya fruit at both pre-ripe and ripe stages of development. *European Journal of Horticultural Science*, 69: 184-192.

Ergun, M., Jeong, J. W., Huber, D. J. and Cantliffe, D. J. 2005. Suppression of ripening and softening of 'Galia' melons by 1-methylcyclopropene applied at preripe or ripe stages of development. *HortScience,* 40:170-175.

Ergun, M., Huber, D. J., Jeong, J. and Bartz, J.A. 2006. Extended shelf-life and quality of fresh-cut papaya derived from ripe fruit treated with the ethylene antagonist 1-methylcyclopropene. *Journal of the American Society for Horticultural Science*, 131: 97-103.

Ergun, M., Jeong, J., Huber, D. J. and Cantliffe, D. J. 2007. Physiology of fresh-cut 'Galia' (*Cucumis melo* var. reticulatus) from ripe fruit treated with 1-methylcyclopropene. *Postharvest Biology and Technology*, 44: 286-292.

Fallik, E., Tuvia-Alkali, S., Horev, B., Copel, A., Rodov, V., Aharoni, Y., Ulrich, D. and Schulz, H. 2001. Characterization of aroma volatiles in 'Galia' melon after prolonged storage. *Postharvest Biology and Technology*, 22: 85-91.

Fan, L., Zheng, S. and Wang, X. 1997. Antisense suppression of phospholipase D alpha retards abscisic acid- and ethylene-promoted senescence of postharvest Arabidopsis leaves. *Plant Cell*, 9: 2183-2196.

Fan, X., and Mattheis, J. P. 1999. Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. *Journal of Agricultural and Food Chemistry,* 47: 2847-2853.

Fan, X., Blankenship, S. M. and Mattheis, J. P. 1999a. 1-Methylcyclopropene inhibits apple ripening. *Journal of the American Society for Horticultural Science*, 124: 690-695.

Fan, X., Mattheis, J. P. and Blankenship, S. M. 1999b. Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. *Journal of Agricultural Food Chemistry*, 47: 3063-3068.

Farag, K. M. and Palta, J. P. 1989a. Stimulation of ethylene production by urea, thidiazoron, and lysophosphatidylethanolamine and possible sites of this stimulation. *Proceedings of the 16th Annual Meeting of the American Society for Plant Physiology*.

Farag, K. M., Palta, J. P. 1989b. Enhancing effectiveness of ethephon on cranberry fruit by natural products, (ethanol, urea and lysophosphatidylethanolamine (LPE)). *Proceedings of the 16th Annual Meeting of the American Society for Plant Physiology.*

Farag, K. M. and Palta, J. P. 1991a. Use of lysophosphatidylethanolamine, a natural lipid, as an aid for fruit ripening and improving keeping quality. *Proceedings of the 17th Annual Meeting of the Plant Growth Regulation American Society*, 135–137.

Farag, K. M., Palta, J. P. 1991b. Use of lysophosphatidylethanolamine, a natural lipid, to delay tomato fruit and leaf senescence. *HortScience*, 26: 67. (Abstr.)

Farag, K. M. and Palta J. P. 1991c. Enhancing ripening and keeping quality of apple and cranberry fruits using lysophosphatidylethanolamine, a natural lipid. *HortScience*, 26: 67. (Abstr.)

Farag, K. M. and Palta J. P. 1991d. Improving postharvest keeping quality of vine-ripened tomato fruits with a natural lipid. *HortScience*, 26: 778. (Abstr.)

Farag, K. M. and Palta J. P. 1992. Plant and fruit treatment with lysophosphatidylethanolamine. *United States Patents, Patent Number:* US 5126155. Date of Patent: June 30, 1992.

Farag, K. M. and Palta, J. P. 1992a. Evidence for a specific inhibition of the activity of polygalacturonase by lysophosphatidylethanolamine in tomato fruit tissue: Implication for enhancing storage stability and reducing abscission of the fruit. *Plant Physiology*, 99: 54. (Abstr.)

Farag, K. M. and Palta, J. P 1992b. Lysophosphatidylethanolamine, a natural lipid enhances ripening and improves keeping quality of tomato fruit without damage to leaves. *Proceedings of the 18th Annual Meeting of Plant Growth Regulation Society of America*, 9-10.

Farag, K. M. and Palta, J. P. 1993a. Use of lysophosphatidylethanolamine, a natural lipid, to retard tomato leaf and fruit senescence. *Physiologia Plantarum*, 87: 515-521.

Farag, K. M. and Palta, J. P. 1993b. Use of natural lipids to accelerate ripening and enhance storage life of tomato fruit with or without ethephon. *HortTechnology*, 3: 62-65.

Farag, K. M., Palta, J. P. and Ryu, S. B. 2003. Methods for enhancing plant health, protecting plants from biotic and abiotic stress related injuries and enhancing the recovery of plants injured as a result of such stresses. *United States Patents, Patent Number:* 6559099. Date of Patent: May 6, 2003.

Fawbush, F., Nock, J. F. and Watkins, C. B. 2008. External carbon dioxide injury and 1-methylcyclopropene (1-MCP) in the 'Empire' apple. *Postharvest Biology and Technology*, 48: 92-98.

Feussner, I. and Wasternack, C. 2002. The lipoxygenase pathway. *Annual Review of Plant Biology*, 53: 275-297.

Fleming, H. P, Cobb, W. Y., Etchells, J. L. and Bell, T. A. 1968. The formation of carbonyl compounds in cucumbers. *Journal of Food Science*, 33: 572-576.

Fleshman, M. K., Lester, G. E., Riedl, K. M., Kopec, R. E., Narayanasamy, S., Curley, R. W. Jr., Schwartz, S. J., and Harrison E. H. 2011. Carotene and novel apocarotenoid concentrations in orange-fleshed *Cucumis melo* melons: determinations of β-carotene bioaccessibility and bioavailability. *Journal of Agricultural and Food Chemistry,* 59: 4448-4454.

Flores, F., El Yahyaoui, F., de Billerbeck, G., Romojaro, F., Latché, A., Bouzayen, M., Pech, J. C. and Ambid, C. 2002. Role of ethylene in the biosynthetic pathway of aliphatic ester aroma volatiles in Charentais melons. *Journal of Experimental Botany*, 53: 201-206.

Flores, F. B., Oosterhaven, J., Martinez-Madrid, M. C. and Romojaro, F. 2005. Possible regulatory role of phenylalanine ammonia-lyase in the production of anthocyanins in asparagus (*Asparagus officinalis* L.). *Journal of Science of Food and Agriculture*, 85: 925–930.

Fonseca, S. C., Oliveira, F. A. R. and Brecht, J. K. 2002. Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, 52: 99-119.

Freiman, Z. E., Rodov, V., Yablovitz, Z., Horev, B. and Flaishman, M. A. 2012. Preharvest application of 1-methylcyclopropene inhibits ripening and improves keeping quality of 'Brown Turkey' figs (*Ficus carica* L.). *Scientia Horticulturae*, 138: 266-272.

Gal, S., Alkalai-Tuvia, S., Elkind, Y., and Fallik, E. 2006. Influence of different concentrations of 1-methylcyclopropene and times of exposure on the quality of 'Galia'-type melon harvested at different stages of maturity. *Journal of Horticultural Science and Biotechnology,* 81: 975–982.

Gal, S., Altkalai-Tuvia, S., Pertzelan, Y., Elkind, Y., Ravid, U., and Fallik, E. 2008. Sensory evaluation of 'Galia'-type melons treated with 1-methylcyclopropene after prolonged storage. *Journal of Horticultural Science and Biotechnology,* 3: 589-594.

Gião, M. S., González-Sanjosé, M. L., Rivero-Pérez, M. D., Pereira, C. I., Pintado M.E and Malcata F. X. 2007. Infusions of Portuguese medicinal plants: dependence of final antioxidant capacity and phenol content on extraction features. *Journal of the Science of Food and Agriculture*, 87: 2638-2647.

Gil, M. I., Aguayo, E. and Kader, A. A. 2006. Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food Chemistry*, 54: 4284-4296.

Gomes, M. H., Beaudry, R. M., Almeida, D. P. F. and Malcata, F. X. 2010. Modelling respiration of packaged fresh-cut 'Rocha' pear as affected by oxygen concentration and temperature. *Journal of Food Engineering*, 96: 74-79.

Gomes, M. H. 2012. Physiological bases of texture and color changes in fresh-cut fruit using pear and melon as model systems: implications for the development of technologies and processes. PhD Thesis, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical procedures for agricultural research.* John Wiley and Sons, New York.

Gonzalez-Bosch, C., Brummell, D. A. and Bennet, A. B. 1996. Diferential expression of two endo-1,4-β-glucanase genes in pericarp and locules of wild-type and mutant tomato fruit. *Plant Physiology*, 111: 1313-1319.

Gorny, J. R. 1997. Summary of CA and MA requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. In: *Proceedings of 7th International Controlled Atmosphere Conference, 5th Postharvest Outreach Program*. J.R. Gorny (Eds.), pp. 30-66. University of California, Davis, CA, USA.

Gorny, J. R. 2001. A summary of CA and MA requirements and recommendations for fresh-cut (minimally processed) fruits and vegetables. In: *Postharvest Horticulture Series*, University of California, Davis. No. 22A, 95-145.

Gorny, J. R., Hess-Pierce, B., Cifuentes, R. A. and Kader, A. A. 2002. Quality changes in fresh-cut pear slices as affected by controlled atmospheres and chemical preservatives. *Postharvest Biology and Technology*, 24: 271-278.

Gunes, G., Watkins, C. B. and Hotchkiss, J. H. 2001. Physiological responses of fresh-cut apple slices under high CO_2 and low O_2 partial pressures. *Postharvest Biology and Technology*, 22: 197-204.

Gupta, M. N. and Wold, F. 1980. A convenient spectrophotometric assay for phospholipase D using ρ-nitrophenyl-phosphocholine as substrate. *Lipids*, 15: 594-596.

Heil, M. 2012. Damaged-self recognition as a general strategy to injury detection. *Plant signalling and Behavior*, 7: 1-5.

Hildebrand, D. F., Hamilton-Kemp, T. R., Legg, C. S. and Bookjans, G. 1988. Plant lipoxygenases: occurrence, properties and possible functions. *Current Topics Plant Biochemistry and Physiology*, 7: 201-219.

Hodges, D. M., Forney, C. F. and Wismer, W. 2000. Processing line effects on storage attributes of fresh cut spinach leaves. *HortScience*, 35: 1308-1311.

Hoeberichts, F. A., Van-Der Plas, L. H. W. and Woltering, E. J. 2002. Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. *Postharvest Biology and Technology*, 26: 125-133.

Hong, J. H. 2006. Lysophosphatidylethanolamine enhances ripening and prolongs shelf life in tomato fruit: contrasting effect on mature green *vs* red tomatoes. *Horticulture, Environment and Biotechnology*, 47: 55-58.

Hong, J. H. 2008. Lysophosphatidylethanolamine treatment stimulates ripening in table grape. *Korean Journal of Horticultural Science and Technology*, 2: 139-143.

Hong, J. H., Arya, M. A., Altwies, J. R. and Palta, J. P. 2001. Stimulation of ripening of fresh-market tomato fruit by a postharvest dip in lysophosphatidylethanolamine (LPE), a natural lipid. *Journal of the American Society for Horticultural Science*, 36: 332. (Abstr.)

Hong, J. H., Altwies, J. R., Guelzow, M. and Palta, J. P. 2002. The influence of lysophosphatidylethanolamine, a natural lipid, ethylene production and ACC oxidase activity on mature green vs. red tomatoes, *Acta Horticulturae*. (Abstr.)

Hong, J.H. and Chung, G. 2006. Effects of application of lysophosphatidylethanolamine on marketable yield and storability of red pepper (*Capsicum annuum L.)*. *Horticulture, Environment and Biotechnology*, 47: 243-246.

Hong, J. H., Hwang, H. K., Chung, G. H. and Cowan, A.K. 2007. Influence of lysophosphatidylethanolamine application on fruit quality of Thompson seedless grapes. *Journal of Applied Horticulture*, 9: 112-114.

Hong, J. H., Hwang, S. K. and Chung, G. H. 2008. Influence of lysophosphatidylethanolamine on reactive oxygen species, ethylene biosynthesis, and auxin action in plant tissues. *Korean Journal of Horticultural Science*, 26: 209-214.

Hong, J. H., Chung, G. and Cowan, A. K. 2009a. Delayed leaf senescence by exogenous lyso-phosphatidylethanolamine: towards a mechanism of action. *Plant Physiology and Biochemistry*, 47: 526-534.

Hong, J. H., Chung, G. and Cowan, A. K. 2009b. Lysophosphatidylethanolamine-enhanced phenylalanine ammonia-lyase and insoluble acid invertase in isolated radish cotyledons. *Plant Growth Regulation*, 57: 69-78.

Huber, D. J. 2008. Suppression of ethylene responses through application of 1-methylcyclopropene: A powerful tool for elucidating ripening and senescence mechanisms in climacteric and nonclimacteric fruits and vegetables. *HortScience*, 43: 106-111.

Hurr, B. M., Huber, D. J. and Lee, J. H. 2005. Differential responses in color changes and softening of 'Florida 47' tomato fruit treated at green and advanced ripening stages with the ethylene antagonist 1-methylcyclopropene. *HortTechnology,* 15: 617-622.

Hyodo, H. 1971. Phenylalanine ammonia lyase in strawberry fruits. *Plant Cell Physiology*, 12: 989-991.

Issa-Zacharia, A., Kamitani, Y., Muhimbula, H. S. and Ndabikunze, B. K. 2010. A review of microbiological safety of fruits and vegetables and introduction of electrolyzed water as an alternative to sodium hypochloride solution. *African Journal of Food Science*, 4: 778-789.

Jacob-Wilk, D., Holland, D., Goldschmidt, E. E., Riov, J. and Eyal, Y. 1999. Chlorophyll breakdown by chlorophyllase: isolation and functional expression of the *Chlase1*gene from ethylene-treated *Citrus* fruit and its regulation during development. *Plant Journal*, 20: 653-661.

Jenkins, G. M. and Frohman, M. A. 2005. Phospholipase D: a lipid centric review. *Cellular and Molecular Life Sciences*, 62: 2305-2316.

Jeong, J., Brecht, J. K., Huber, D. J. and Sargent, S. A. 2004. 1-Methylcyclopropene (1-MCP) for maintaining texture quality of fresh-cut tomato. *HortScience*, 39: 1359-1362.

Jeong, J., Lee, J., and Huber, D. J. 2007. Softening of Athena cantaloupe (*Cucumis melo* L. var reticulatus) fruit at three harvest maturities in response to the ethylene antagonist 1-methylcyclopropene. *HortScience,* 42: 1231-1236.

Jeong, J., Brecht J. K., Huber, D. J., and Sargent, S. A. 2008. Storage life and deterioration of intact cantaloupe (*Cucumis melo* L. var. reticulates) fruit treated with 1-methylcyclopropene and fresh-cut cantaloupe prepared from fruit treated with 1-methylcyclopropene before processing. *Hortscience,* 43: 435-438.

Jiang, Y. and Joyce, D. C. 2002. 1-Methycyclopropene treatment effects on intact and fresh-cut apple. *Journal of Horticultural Science and Biotechnology*, 77: 19-21.

Johnson, P. R. and Ecker, J. R. 1998. The ethylene gas signal transduction pathway: A molecular perspective. *Annual Reviews of Genetics*, 32: 227-254.

Ju, Z. and Curry, E. A. 2000. Stripped corn oil emulsion alters ripening, reduces superficial scald, and reduces core flush in 'Granny Smith' apples and decay in 'd'Anjou' pears. *Postharvest Biology and Technology*, 20: 185-193.

Ju, Z. and Curry, E. A. 2001. Plant oil emulsions prevent senescent scald and core breakdown and reduce fungal decay in ‘Bartlett’ pears. *Journal of the American Society for Horticultural Science*, 126: 358-363.

Ju, Z., Duan, Y. and Ju, Z. 2000. Mono-, di-, and tri-acylglycerols and phospholipids from plant oils inhibit scald development in ‘Delicious’ apples. *Postharvest Biology and Technology*, 19: 1-7.

Jung, S. K. and Watkins, C. B. 2011. Involvement of ethylene in browning development of controlled atmosphere-stored ‘Empire’ apple fruit. *Postharvest Biology and Technology*, 59: 219-226.

Kader, A. A. 1986. Biochemical and physiological basis for effects of controlled atmospheres on fruits and vegetables. *Food Technology*, 4:, 99-100, 102-104.

Kader, A. A. and Ben-Yehoshua, S. 2000. Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 20: 1-13.

Kang, C. K., Yang, Y. L., Chung, G. H. and Palta, J. P. 2003. Ripening promotion and ethylene evolution in red pepper (*Capsicum annuum*) as influenced by newly developed formulations of a natural lipid, lysophosphatidylethanolamine. *Acta Horticulturae*. 28: 317-322.

Karakurt, Y. and Huber, D. J. 2003. Activities of several membrane and cell-wall hydrolases, ethylene biosynthetic enzymes, and cell-wall polyuronide degradation during low-temperature storage of intact and fresh-cut papaya (*Carica papaya*) fruit. *Postharvest Biology and Technology*, 28: 219-229.

Kaur, C. and Kappor, H. C. 2001. Antioxidants in fruits and vegetables – the millennium's health. *International Journal of Food Science and Technology*, 36: 701-725.

Kaur, N. and Palta, J.P. 1996. Prolonging the vase life of snapdragons and carnations with a natural lipid, lysophosphatidylethanolamine. *HortScience,* 31: 636. (Abstr.)

Kaur, N. and Palta, J. P. 1997. Postharvest dip in a natural lipid, lysophoaphatidylethanolamine, may prolong vase life of snapdragon flowers. *Hortscience*, 32: 888-890.

Ke, D. and Salveit, M. E. 1989. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Physiologia Plantarum*, 76: 412-418.

Kemp, K. R., Knavel, D. E. and Stolz, L. P. 1972. Cis-6-nonenal, a flavor component of muskmelon fruit. *Phytochemistry*, 11: 3321-3322.

Kern, R., Joseleau-Petit, D., Chattopadhyay, M.K. and Richarme, G. 2001. Chaperone-like properties of lysophospholipids. *Biochemical and Biophysical Research Communications*, 289: 1268-1274.

Kim, J., Moreira, R. and Castell-Perez, E. 2010. Simulation of pathogen inactivation in whole and fresh-cut cantaloupe (*Cucumis melo*) using electron beam treatment. *Journal of Food Engineering*, 97: 425-433.

Kita M., Kato M., Ban Y., Honda C., Yaegaki H., Ikoma Y., and Moriguchi T. 2007. Carotenoid accumulation in Japanese apricot (*Prunus mume* Siebold and Zucc.): molecular analysis of carotenogenic gene expression and ethylene regulation. *Journal of Agricultural and Food Chemistry,* 55: 3414–3420.

Klassen, S. P. and Bugbee, B. 2004. Ethylene synthesis and sensitivity in crop plants. *HortScience,* 39: 1546-1552.

Klein, B. P. 1987. Nutritional consequences of minimal processing on fruits and vegetables. *Journal of Food Quality*, 10: 179-193.

Kourkoutas, D., Elmore, J. S. and Mottram, D. S. 2006. Comparison of the volatile compositions and flavour properties of cantaloupe, Galia and honeydew muskmelons. *Food Chemistry*, 97:95-102.

Ku, V. V. V., Wills, R. B. H. and Ben-Yehoshua, S. 1999. 1-Methylcyclopropene can differentially affect the postharvest life of strawberries exposed to ethylene. *Horticultural Science*, 34: 119-120.

Lamikanra, O. and Watson, M. A. 2000. Cantaloupe melon peroxidase: characterization and effects of additives on activity. *Molecular Nutrition and Food Research*, 44: 168-172.

Lamikanra, O., Chen, J. C., Banks, D. and Hunter, P. A. 2000. Biochemical and microbial changes during the storage of minimally processed cantaloupe. *Journal of Agricultural and Food Chemistry*, 48: 955-5961.

Lamikanra, O. and Richard, O. A. 2002. Effect of storage on some volatile aroma compounds in fresh-cut cantaloupe melon. *Journal of Agricultural and Food Chemistry,* 50: 4043-4047.

Lamikanra, O. and Watson, M. A. 2007. Mild heat and calcium treatment effects on fresh-cut cantaloupe melons during storage. *Food Chemistry*, 102: 1383-1388.

Lara, I., Miró, R. M., Fuentes, T., Sayez, G., Graell, J. and López, M. L. 2003. Biosynthesis of volatile aroma compounds in pear fruit stored under long-term controlled-atmosphere conditions. *Postharvest Biology and Technology*, 29: 29-39.

Lara, I., Graell, J., López, M. L. and Echeverria, G. *2006*. Multivariate analysis of modifications in biosynthesis of volatile compounds after CA storage of 'Fuji' apples*. Postharvest Biology and Technology,* 39*:*19–28*.*

Lara, I., Echeverria, G., Graell, J. and Lopez, M. L. 2007. Volatile emission after controlled atmosphere storage of Mondial Gala apples (*Malus domestica*), Relationship to some involved enzyme activities. *Journal of Agricultural and Food Chemistry*, 55: 6087-6095.

Lee, H. Y., Bahn, S. C., Kang, Y-M., Lee, K. H., Kim, H. J., Noh, E. K., Palta, J. P., Shin, J. S. and Ryu, S. B. 2003. Secretory low molecular weight phospholipase A_2 plays important roles in cell elongation and shoot gravitropism in Arabidopsis. *Plant Cell*, 15: 1990-2002.

Lee, S., Suh, S., Kim, S., Crain, R. C., Kwak, J. M., Nam, H. G. and Lee, Y. 1997. Systemic elevation of phosphatidic acid and lysophospholipid levels in wounded plants. *Plant Journal*, 12: 547-556.

Leshem, Y. 1984. Ca^{2+}: calmodulin-induced and ethylene mediated phospholipid catabolism as a mode of plant senescence. In: *Structure, Function and Metabolism of Plant Lipids.* Siegenthaler, P. A., Eichenberger, W. (Eds.), pp. 181-188. Elsevier Science Publishers, Amsterdam, The Netherlands.

Li, M., Hong, Y. and Wang, X. 2009. Phospholipase D- and phosphatidic acid-mediated signaling in plants. *Biochimica et Biophysica Acta*, 1791: 927-935.

Lucchetta, L., Manríquez, D., El-Sharkawy, I., Flores, F. B., Sanchez-Bel, P., Zouine, M., Ginies, C., Bouzayen, M., Rombaldi, C., Pech, J. C. and Latché, A. 2007 Biochemical and catalytic properties of three recombinant alcohol acyltransferases of melon. Sulfur-containing ester formation, regulatory role of CaSH in activity and sequence elements conferring substrate preference. *Journal of Agricultural and Food Chemistry*, 55: 5213-5220.

Luna-Guzman, I., Cantwell, I. M., and Barrett, D. M. 1999. Fresh-cut cantaloupe: effects of CaCl2 dips and heat treatments on firmness and metabolic activity. *Postharvest Biology and Technology,* 17: 201-213.

Luna-Guzmán, I. and Barrett, D.M. 2000. Comparison of calcium chloride and calcium lactate effectiveness in maintaining shelf stability and quality of fresh-cut cantaloupes. *Postharvest Biology and Technology*, 19: 61-72.

Lurie, S., Pre-Aymard, C., Ravid, U., Larkov, O. and Fallik, E. 2002. Effect of 1-methylcyclopropene on volatile emission and aroma in cv. Anna apples. *Journal of Agricultural and Food Chemistry*, 50: 4251-4256.

Lurie, S. and Watkins, C. B. 2012. Superficial scald, its etiology and control. *Postharvest Biology and Technology*, 65: 44-60.

Madrid, M. and Cantwell, M. 1993. Use of high CO_2 atmospheres to maintain quality of intact and fresh-cut melon. *Proceedings of 6th International Controlled Atmosphere Research Conference*, NRAES-71, Ithaca, NY, 736–745.

Mangat, N. K. and Palta, J. P. 1995. Inhibition of polygalacturonase in tomato pericarp tissue by lysophosphatidylethanolamine: Implications in fruit shelf-life. *HortScience*. 30: 889. (Abstr.).

Manríquez, D., El-Sharkawy, I., Flores, F. B., Regad, F., Bouzayen, M., Latché, A. and Pech, J. C. 2006. Fruit-specific gene expression and biochemical characteristics of two highly divergent alcohol dehydrogenases of melon. *Plant Molecular Biology*, 61: 675-685.

Manzocco, L., Da Pieve, S. and Maifreni, M. 2011. Impact of UV-C light on safety and quality of fresh-cut melon. *Innovative Food Science and Emerging Technologies*, 12: 13-17.

Mao, L., Karakurt, Y. and Huber, D. J. 2004. Incidence of water-soaking and phospholipid catabolism in ripe watermelon (*Citrullus lanatus*) fruit: induction by ethylene and prophylactic effects of 1-methylcyclopropene. *Postharvest Biology and Technology*, 33: 1-9.

Mao, L., Jeong, J., Que, F. and Huber, D. J. 2006. Physiological properties of fresh-cut watermelon (*Citrullus lanatus*) in response to 1-methylcyclopropene and post-processing calcium application. *Journal of the Science of Food and Agriculture,* 86: 46-53.

Marangoni, A. G., Palma, T. and Stanley, D. W. 1996. Membrane effects in postharvest physiology. *Postharvest Biology and Technology*, 7: 193-217.

Margosan, D. A., Smilanick, J. L., Simmons, G. F. and Delmer, J. H., 1997. Combination of hot water and ethanol to control postharvest decay of peaches and nectarines. *Plant Disease*, 81: 1405-1409.

Marrero, A. and Kader, A. A. 2006. Optimal temperature and modified atmosphere for keeping quality of fresh-cut pineapples. *Postharvest Biology and Technology*, 39: 163-168.

Marty, I., Bureau, S., Sarkissian, G., Gouble, B., Audergon, J. M. and Albagnac, G. 2005. Ethylene regulation of carotenoid accumulation and carotenogenic gene expression in colour-contrasted apricot varieties (*Prunus armeniaca*). *Journal of Experimental Botany*, 56: 1877-1886.

Mathieu, S., Dal Cin, V., Fei, Z., Li, H., Bliss, P., Taylor, M. G., Klee, H. J., and Tieman, D. M. 2009. Flavour compounds in tomato fruits: identification of loci and potential pathways affecting volatile composition. *Journal of Experimental Botany,* 60: 325-337.

Matsushita, S. 1975. Specific interactions of linoleic acid hydroperoxides and their secondary degraded products with enzyme proteins. *Journal of Agricultural and Food Chemistry*, 23: 150-154.

Mattheis, J. P., Buchanan, D. A. and Fellman, J. K. 1998. Volatile compounds emitted by Gala apples following dynamic atmosphere storage. *Journal of the American Society for the Horticultural Science*, 123: 426-432.

Mattheis, J. and Fellman, J. K. 2000. Impacts of modified atmosphere packaging and controlled atmospheres on aroma, flavor, and quality of horticultural commodities. *Hortechnology,* 10: 507-510.

Mattheis, J. P., Fan, X. and Argenta, L. C. 2005. Interactive responses of 'Gala' apple fruit volatile production to controlled atmosphere storage and chemical inhibition of ethylene action. *Journal of Agricultural and Food Chemistry*, 53: 4510-4516.

Moreno, E., Obando, J. M., Dos-Santos, N., Fernández-Trujillo, J. P., Monforte, A. J. and Garcia-Mas, J. 2008. Candidate genes and QTLs for fruit ripening and softening in melon. *Theoretical and Applied Genetics*, 116: 589-602.

Mullins, E. D., McCollum, T. G. and McDonald, R. E. 2000. Consequences on ethylene metabolism of inactivating the ethylene receptor sites in diseased non-climacteric fruit. *Postharvest Biology and Technology*, 19: 155-164.

Munnik, T., Irvine, R. F. and Musgrave, A. 1998. Phospholipid signaling in plants. *Biochimica et Biophysica Acta*, 1389: 222-272.

Muriel, E.; Antequera, T.; Petrón, M. J.; Martín D. and Ruiz, J. Y. 2004. Volatile compounds on the surface and within Iberian dry-cured loin. *European Food Research Technology*, 219: 445–451.

Myung, K., Hamilton-Kemp, T. R. and Archbold, D. D. 2006. Biosynthesis of *trans*-2-hexenal in response to wounding in strawberry fruit. *Journal of Agricultural and Food Chemistry*, 54: 1442-1448.

Nimitkeatkai, H., Kakaew, P. and Kanlayanarat, S. 2007. Quality of fresh-cut ripe papaya treated with 1-methylcyclopropene. *Acta Horticulturae*, 804: 433-438.

Nishiyama, K., Guis, M., Rose, J. K. C., Kubo, Y., Bennett, K. A., Wangjin, N., Kato, K., Ushijima, K., Nakano, R., Inaba, A., Bouzayen, M., Latche, A., Pech, J. and Bennett, A. B. 2007. Ethylene regulation of fruit softening and cell wall disassembly in Charentais melon. *Journal of Experimental Botany*, 58: 1281-1290.

O'Connor-Shaw, R. E., Roberts, R., Ford, A. L. and Nottingham, S. M. 1996. Changes in sensory quality of sterile cantaloupe dice stored in controlled atmosphere. *Journal of Food Science*, 61: 847-851.

Obando-Ulloa, J. M., Moreno, E., García-Mas, J., Nicolai, B., Lammertyn, J., Monforte, A. J. and Fernández-Trujillo, J. P. 2008. Climacteric or non-climacteric behavior in melon fruit. 1. Aroma volatiles. *Postharvest Biology and Technology*, 49: 27–37.

Obando-Ulloa, J. M., Nicolai, B., Lammertyn, J., Bueso, M. C., Monforte, A. J., and Fernández-Trujillo, J.P. 2009. Aroma volatiles associated with the senescence of climacteric or non-climacteric melon fruit. *Postharvest Biology and Technology*, 52, 146-155.

Odriozola-Serrano, I., Soliva-Fortuny, R. and Martín-Belloso, O. 2010. Changes in bioactive composition of fresh-cut strawberries stored under superatmospheric oxygen, low-oxygen or passive atmospheres. *Journal of Food Composition and Analysis*, 23: 37-43.

Ölmez, H. and Kretzschmar, U. 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology*, 42: 686-693.

Oms-Oliu, G., Soliva-Fortuny, R. and Martín-Belloso, O. 2007. Effect of ripeness on the shelf-life of fresh-cut melon preserved by modified atmosphere packaging. *European Food Research Technology*, 225: 301-311.

Oms-Oliu, G., Martínez, R. M. R., Soliva-Fortuny, R. and Martín-Belloso, O. 2008a. Effect of superatmospheric and low oxygen modified atmospheres on shelf-life extension of fresh-cut melon. *Food Control*, 19: 191-199.

Oms-Oliu, G., Soliva-Fortuny, R. and Martín-Belloso, O. 2008b. Modeling changes of headspace gas concentrations to describe the respiration of fresh-cut melon under low or superatmospheric oxygen atmospheres. *Journal of Food Engineering*, 85: 401-409.

Oms-Oliu, G., Soliva-Fortuny, R. and Martín-Belloso, O. 2008c. Using polysaccharide based edible coatings to enhance quality and antioxidant properties of fresh-cut melon. *LWT-Food Science Technology*, 41: 1862-1870.

Oms-Oliu, G., Rojas-Graü, M. A., González, L. A., Varela, P., Soliva-Fortuny, R., Hernando, M. I. H., Munuera, I. P., Fiszman, S. and Martín-Belloso, O. 2010. Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. *Postharvest Biology and Technology*, 57: 139-148.

Özgen, M. And Palta, J.P. 1999. Using natural lipids to accelerate ripening (uniform color development) and promote shelf life of cranberries. Wisconsin State Growers Association. Summer growers meeting and field day, 33-34.

Özgen, M., Palta, J.P. and Ryu, S. B. 2000. Mitigation of Ethephon injury to tomato plants by a natural lipid, lysophosphatidylethanolamine (LPE): influence on the activity of phospholipase D (PLD). *HortScience*, 35: 503 (Abstr.).

Özgen, M. and Palta, J. P. 2001. Use of lysophosphatidyletahnolamine (LPE), a natural lipid, to mitigate undesirable effects of a fungicide (Bravo) on cranberries. *HortScience,* 36: 579. (Abstr).

Özgen, M. and J.P. Palta. 2003. A natural lipid, lysophosphatidylethanolamine (LPE), can mitigate adverse effect of fungicide, chlorothalonil, on fruit set and yield in cranberries. *Acta Horticulturae*, 628: 747-752.

Özgen, M., Farag, K. M., Özgen, S. and Palta, J. P. 2004. Lysophosphatidylethanolamine accelerates color development and promotes shelf-life of cranberries. *HortScience,* 40: 127-130.

Özgen, M., Park, S. and Palta, J. P. 2005. Mitigation of ethylene-promoted leaf senescence by a natural lipid, lysophosphatidylethanolamine. *HortScience* 40, 1166-1167.

Paliyath. G., Poovaiah, B. W, Munske, G. R. and Magnuson, J. 1984. Membrane fluidity in senescing apples: Effects of temperature and calcium. *Plant Cell Physiology*, 25:1083-1087.

Paliyath. G. and Droillard, M. J. 1992. The mechanisms of membrane deterioration and disassembly during senescence. *Plant Physiology and Biochemistry*, 30: 789-812.

Paliyath, G., Tiwari, K., Yuan, H. and Whitaker, B. D. 2008. Structural deterioration in produce: phospholipase D, membrane deterioration, and senescence. In: *Postharvest biology and technology of fruits, vegetables and flowers.* Paliyath, G., Murr, D. P., Handa, A. K. and Lurie, S. (Eds.), pp. 195-239. Wiley-Blackwell Ed.

Palta, J.P. and Ryu, S.B. 2002. Use of lysophosphatidylethanolamine (18-1) and lysophosphatidylinositol to retard senescence and to enhance fruit ripening**.** *United States Patents, Patent Number:* US 6426105. Date of Patent: July 30, 2002**.**

Panchev, I. N., Kirtchev, N. A. and Dimitrov, D. D. 2011. Possibilities for application of laser ablation in food technologies. *Innovative Food Science and Emerging Technologies*, 12: 369-374.

Pelayo, C., Ebeler, S. E. and Kader, A. A. 2003. Postharvest life and flavor quality of three strawberry cultivars kept at 5 ºC in air or air + 20 kPa CO_2. *Postharvest Biology and Technology*, 27: 171-183.

Pérez, A. G., Sanz, C., Olìas, R. and Olìas, J. M. 1999. Lipoxygenase and hydroperoxide lyase activities in ripening strawberry fruits. *Journal of Agricultural and Food Chemistry*, 47: 249-253.

Pérez, A. G. and Sanz, C. 2001. Effect of high-oxygen and high-carbon dioxide atmospheres on strawberry flavor and other quality traits. *Journal of Agricultural and Food Chemistry*, 49: 2370-2375.

Perry, P. L., Wang, Y. and Lin, J. M. 2009. Analysis of honeydew melon (*Cucumis melo* var. Inodorus) flavor and GC/MS identification of (*E,Z*)-2,6-nonadienyl acetate. *Flavour and Fragrance Journal*, 24, 341-347.

Pesis, E. 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. *Postharvest Biology and Technology*, 37: 1-19.

Pinheiro, R. G., Almquist, K. C., Novotna, Z. and Paliyath, G. 2003. Developmental regulation of phospholipase D in tomato fruit. *Plant Physiology and Biochemistry*, 41: 223-240.

Poovaiah, B. W. 1986. Role of calcium in prolonging storage life of fruits and vegetables. *Food Technology*, 40: 86-89.

Portela, S. I. and Cantwell, M. I. 1998. Quality changes in minimally processed honeydew melons stored in air or controlled atmosphere. *Postharvest Biology and Technology*, 14: 351-357.

Prior, R. L. and Cao, G. H. 2000. Analysis of botanicals and dietary supplements for antioxidant capacity: a review. *Journal of AOAC International*, 83: 950-956.

Qi, L., Wu, T. and Watada, A. E., 1999. Quality changes of fresh-cut honeydew melons during controlled atmosphere storage. *Journal of Food Quality*, 22: 513-521.

Rajashekar, C.B., Zhou, H., Zhang, Y., Li, W. and Wang, X. 2006. Suppression of phospholipase Dα1 induces freezing tolerance in *Arabidopsis*: Response of cold responsive genes and osmolyte accumulation. *Journal of Plant Physiology*, 163: 916-926.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O. and Soliva-Fortuny, R. 2012. Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, 69: 32-39.

Raybaudi-Massilia, R. M., Mosqueda-Melgar, J. and Martín-Belloso, O. 2008. Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. *International Journal of Food Microbiology*, 121: 313-327.

Reyes, V. G. 1996. Improved preservation systems for minimally processed vegetables. *Food Australia*, 48: 87-90.

Reyes, L. F., Villareal, J. E. and Cisneros-Zevallos, L. 2007. The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry*, 101: 1254-1262.

Riley, J. C. M., Willemot, C. and Thompson, J. E. 1996. Lipoxygenase and hydroperoxide lyase activities in ripening tomato fruit. *Postharvest Biology and Technology*, 7: 97-107.

Riley, J. C. M. and Thompson, J. E. 1998 Ripening-induced acceleration of volatile aldehyde generation following tissue disruption in tomato fruit. *Physiologia Plantarum*, 104: 571-576.

Robards, K., Prenzler, P. D., Tuvker, G., Swatsitang, P. and Glover, W. 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*, 66: 401-436

Robinson, R. W. and Decker-Walters, D. S. 1999. Cucurbits. In: *Cambridge, CAB International.* 226 p.

Rojas-Graü, M. A.; Garner, E. and Martín-Belloso, O. 2011. The fresh-cut fruit and vegetables industry: current situation and market trends. Chapter 1. In: *Advances in fresh-cut fruits and vegetables processing.* Martín-Belloso, O. and Soliva-Fortuny, R. (Eds.). CRC Press, Boca Raton, Florida, USA.

Rowan, D. D., Allen, J. M., Fielder, S. and Hunt, M. B. 1999. Biosynthesis of straight chain ester volatiles in red delicious and Granny Smith apples using deuterium-labelled precursors. *Journal of Agricultural and Food Chemistry*, 47: 2553-2556.

Roy, S., Conway, W. S., Buta, J. G., Watada, A. E., Sams, C. E. and Wergin, W. P. 1996. Surfactants affect calcium uptake from postharvest treatment of 'Golden Delicious' apples. *Journal of the American Society for Horticultural Science*, 121:1179-1184.

Rudell, D. R., Mattinson, D. S., Mattheis, J. P., Wyllie, S. G. and Fellman, J. K. 2002. Investigations of aroma volatile biosynthesis under anoxic conditions and in different tissues of "Redchief Delicious" apple fruit (*Malus domestica* Borkh). *Journal of Agricultural and Food Chemistry*, 50: 2627-2632.

Ryu, S. and Wang, X. 1996. Activation of phospholipase D and the possible mechanism of activation in wound-induced lipid hydrolysis in castor bean leaves. *Biochimica et Biophysica Acta*, 1303: 243-250.

Ryu, S. B., Karlsson, B. H., Ozgen, M. and Palta, J. P. 1997. Inhibition of phospholipase D by lysophosphatidylethanolamine, a lipid-derived senescence retardant. *Proceedings of the National Academy of Sciences,* 94: 12717-12721.

Ryu, S. and Wang, X. 1998. Increase in free linolenic and linoleic acids associated with phospholipase D-mediated hydrolysis of phospholipids in wounded castor bean leaves. *Biochimica et Biophysica Acta*, 1393: 193-202.

Ryu, S.B. 2004. Phospholipid derived signaling mediated by phospholipase A in plants. *Trends Plant Science*, 9: 229-235.

Saftner, R. A., Bai, J., Abbott, J. A., and Lee Y. S. 2003. Sanitary dips with calcium propionate, calcium choride, or a calcium amino acid chelate maintain quality and shelf stability of fresh-cut honeydew chunks. *Postharvest Biology and Technology*, 29: 257-269.

Saftner, R. A., Abbott, J. A., Lester, G. and Vinyard, B. 2006. Sensory and analytical comparison of orange-fleshed honeydew to cantaloupe and green-fleshed honeydew for fresh-cut chunks. *Postharvest Biology and Technology*, 42: 150-160.

Saftner, R., Luo, Y., McEvoy, J., Abbott, J. A., and Vinyard, B. 2007. Quality characteristics of fresh-cut watermelon slices from non-treated and 1-methylcyclopropene- and/or ethylene-treated whole fruit. *Postharvest Biology and Technology*, 44: 71-79.

Saftner, R., and Lester, G. E. 2009. Sensory and analytical characteristics of a novel hybrid muskmelon fruit intended for the fresh-cut fruit. *Postharvest Biology and Technology*, 51: 327-333.

Saltveit, M. E. 1999. Fresh-cut product biology. In: *5th Annual Workshop on Fresh-Cut Products: Maintaining Quality and Safety*. University of California, Davis, pp.1-10.

Saltveit, M. E. 2000. Wound-induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biology and Technology*, 21: 61-69.

Sangsuwan J., Rattanapanone, N. and Rachtanapun, P. 2008. Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple. *Postharvest Biology and Technology*, 49: 403-410.

Sanz, C., Olias, J. M. and Perez, A. G. 1997. Aroma biochemistry of fruits and vegetables. In: *Phytochemistry of fruits and vegetables.*Tomás-Barberán, F. A. and Robins, R. J. (Eds.), pp. 125-155. Clarendon Press, Oxford, UK.

Sapers, G. M. 2003. Washing and sanitizing raw materials for minimally processed fruit and vegetable products. In*: Microbial safety of minimally processed foods.* Novak, J. S., Sapers, G. M. and Juneja V. K. (Eds.), pp. 221-253. New York: CRCPress, Boca Raton, Florida, USA.

Schieberle, P., Ofner, S. and Grosch, W. 1990. Evaluation of potent odorants in cucumbers (*Cucumis sativus*) and muskmelons (*Cucumis melo*) by aroma extract dilution analysis. *Journal of Food Science*, 55: 193-195.

Selma, M. V., Ibáñez, A. M., Allende, A., Cantwell, M. and Suslow, T. 2008. Effect of gaseous ozone and hot water on microbial and sensory quality of cantaloupe and potential transference of *Escherichia coli* O157:H7 during cutting. *Food Microbiology*, 25: 162-168.

Serek, M., Woltering, E. J., Sisler, E. C., Frello S. and Sriskandarajah, S. 2006. Controlling ethylene responses in flowers at the receptor level. *Biotechnology Advances*, 24: 368-381.

Shalit, M., Katzir, N., Tadmor, Y., Larkov, O., Burger, Y., Shalekhet, F., Lastochkin, E., Ravid, U., Amar, O., Edelstein, M., Karchi, Z. and Lewinsohn, E. 2001. Acetyl-CoA, alcohol acetyltransferase activity and aroma formation in ripening melon fruits. *Journal of Agricultural and Food Chemistry*, 49: 794-799.

Shaw, P. E., Moshonas, M. G., Nisperoscarriedo, M. O. and Carter R. D. 1992. Controlled-atmosphere treatment of freshly harvested oranges at elevated

temperature to increase volatile flavor components. *Journal of Agricultural and Food Chemistry*, 40: 1041-1045.

Siedow, J. N. 1991. Plant lipoxygenase: structure and function. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42: 145-188.

Silva, J. A., da Costa, T. S., Lucchetta, L., Marini, L. J., Zanuzo, M. R., Nora, L., Nora, F. R., Twyman, R. M. and Rombaldi, C. V. 2004. Characterization of ripening behavior in transgenic melons expressing an antisense 1-aminocyclopropane-1-carboxylate (ACC) oxidase gene from apple. *Postharvest Biology and Technology*, 32: 263-268.

Silveira, A. C., Aguayo, E., Chisari, M. and Artés, F. 2011. Calcium salts and heat treatment for quality retention of fresh-cut 'Galia' melon. *Postharvest Biology and Technology*, 62: 77-84.

Simandjuntak, V., Barrett, D. M. and Wrolstad, R. E. 1996. Cultivar and maturity effects on muskmelon (*Cucumis melo*) colour, texture and cell wall polysaccharide composition. *Journal of Science and Food Agriculture,* 71: 282-290.

Singh, R., Singh, P., Pathak, N., Singh, V. K. and Dwivedi, U. N. 2007. Modulation of mango ripening by chemicals: physiological and biochemical aspects. *Plant Growth Regulation*, 53: 137-145.

Singleton, V. L. and Rossi, J. A. J. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*,16: 144-158.

Sisler, E. C. and Blankenship, S. M. 1996. Methods of counteracting an ethylene response in plants. *United States Patents, Patent Number:* US 5,518,988. Date of Patent: May 21, 1996.

Sisler, E.C., and Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level. *Physiologia Plantarum,* 100: 577-582.

Snider, A., Palta, J. P. and Peoples, T. 2003a. The potential use of lysophosphatidylethanolamine (LPE), a natural lipid, to lengthen the vase life of short-lived 'Lavande' and 'Sensation' roses. *Acta Horticulturae*, 624: 419-426.

Snider, A., Palta, J. P. and Peoples, T. 2003b. Use of lysophosphatidylethanolamine (LPE), a natural lipid, to enhance opening and retention of flowers on bedding plants experiencing water stress during retail sales. *Acta Horticulturae*, 628: 849-853.

Soliva-Fortuny, R. C., Oms-Oliu, G. and Martín-Belloso, O. 2002. Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. *Journal of Food Science*, 67: 1958-1963.

Soliva-Fortuny, R. C., Elez-Martínez, P. and Martín-Belloso, O. 2004. Microbiological and biochemical stability off resh-cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technologies*, 5: 215-224.

Thompson, J. E., Froese, C. D., Madey, E., Smith, M. D. and Hong, Y. W. 1998. Lipid metabolism during plant senescence. *Progress in Lipid Research*, 37, 119-141.

Tieman, D. M., Taylor, M. G., Ciardi, J. A. and Klee, H. J. 2000. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. *Proceedings of the National Academy of Sciences*, 97: 5663-5668.

Tijet, N., Schneider, C., Muller, B.L. and Brash, A.R. 2001. Biogenesis of volatile aldehydes from fatty acid hydroperoxides: molecular cloning of a hydroperoxide lyase (CYP74C) with specificity for both the 9- and 13-hydroperoxides of linoleic and linolenic acids. *Archives of Biochemistry and Biophysics*, 386: 281-289.

Toivonen, P. M. A. and DeEll, J. R. 2002. Physiology of fresh-cut fruits and vegetables. In: *Fresh-cut fruits and vegetables. Science, technology and market.* Lamikanra, O. (Eds.), pp. 91-123. CRC Press LLC, Boca Raton, Forida, USA.

Toivonen, P. M. A. 2008. Application of 1-methylcyclopropene in fresh-cut/minimal processing systems. *HortScience*, 43: 102-105.

Toivonen, P. M. and Brummell, D. A. 2008. Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology*, 48: 1-14.

Toker, I. and Bayindirli, A. 2003. Enzymatic peeling of apricots, nectarines and peaches. *LWT-Food Science and Technology*, 36: 215-221.

Ueda, Y., Fujishita, N. and Chachin, K. 1997. Presence of alcohol acetyltransferase in melons (*Cucumis melo* L). *Postharvest Biology and Technology*, 10: 121-126.

Van Moerkecke, A., Schauvinhold, I., Pichersky, E., Haring, M. A., and Schuurink, R. C. 2009. A plant thiolase involved in benzoic acid biosynthesis and volatile benzenoid production. *The Plant Journal,* 60: 292-302.

Vance, E. and Vance, J. E. 2008. *Biochemistry of lipids, lipoproteins and membranes*. Elsevier, Amesterdam, The Netherlands.

Villas-Boas, E. V. de B. and Kader, A. A. 2007. Effect of 1-methycyclopropene (1-MCP) on softening of fresh-cut kiwifruit, mango and persimmon slices. *Postharvest Biology and Technology*, 43: 238-244.

Wakabayashi, K. 2000. Changes in cell wall polysaccharides during fruit ripening. *Journal of Plant Research*, 113: 231-237.

Wang, X. 2000. Multiple forms of phospholipase D in plants: the gene family, catalytic and regulatory properties, and cellular functions. *Progress in Lipid Research*, 39: 109-149.

Wang, C., Zien, C. A., Afitlhile, M., Welti, R., Hildebrand, D. F. and Wang, X. 2000. Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. *The Plant Cell,* 12: 2237-2246.

Wang, C., Xing, J., Chin, C., Ho, C. and Martin, C. E. 2001. Modification of fatty acids changes the flavor volatiles in tomato leaves. *Phytochemistry*, 58: 227-232.

Wang, K. L. C., Li, H. and Ecker, J. R. 2002. Ethylene biosynthesis and signaling networks. *The Plant Cell*, 14: S131-S151.

Watada, A. E., Abe, K., and Yamuchi, N. 1990. Physiological activities of partially processed fruits and vegetables. *Food Technology*, 44: 116-122.

Watada, A. E., Ko, N. P. and Minott, D. A. 1996. Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology*, 9: 115-125.

Watada, A. E. and Qi, L. 1999. Quality of fresh-cut produce. *Postharvest Biology and Technology,* 15: 201-205.

Watkins, C. B., Nock, J. F. and Whitaker, B. D. 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biology and Technology*, 19: 17-32.

Watkins, C. B. 2002. Ethylene synthesis, mode of action, consequences and control. In: *Fruit quality and its biological basis.* Knee, M. (Eds.). Sheffield Academic Press, pp. 180-224. Boca Raton, Florida, USA.

Watkins, C. B. and Nock, J. F. 2005. Effects of delays between harvest and 1-methylcyclopropene treatment, and temperature during treatment, on ripening of air-stored and controlled-atmosphere-stored apples. *HortScience,* 40: 2096-2101.

Watkins, C. B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, 24: 389-409.

Watkins, C.B. 2008. Overview of 1-methylcyclopropene trials and uses for edible horticultural crops. *HortScience*, 43: 86-94.

Watkins, C. B. and Nock, J. F. 2012. Rapid 1-methylcyclopropene (1-MCP) treatment and delayed controlled atmosphere storage of apples. *Postharvest Biology and Technology,* 69: 24-31.

Welti, R., Li, W., Li, M., Sang, Y., Biesiada, H., Zhou, H-E., Rajashekar, C. B., Williams, T. D. and Wang, X. 2002. Profiling membrane lipids in plant stress

responses: role of phospholipase Dα in freezing-induced lipid changes in *Arabidopsis*. *Journal of Biological Chemistry*, 277: 31994-32002.

Whitaker, B. D. and Lester, G. E. 2006. Cloning of phospholipase Dα and lipoxygenase genes CmPLDα1 and CmLOX1 and their expression in fruit, floral, and vegetative tissues of 'Honey Brew' hybrid honeydew melon. *Journal of the American Society for Horticultural Science*, 131: 544-550.

Wiley, R. C. 1994. *Minimally processed refrigerated fruits and vegetables.* Chapman and Hall, London, UK.

Wills, R. B. H. and Ku, V. V. V. 2002. Use of 1-MCP to extend the time to ripen of green tomatoes and postharvest life of ripe tomatoes. *Postharvest Biology and Technology*, 26: 85-90.

Wishart, D. S., Knox, C., Guo, A. C., Eisner, R., Young, N., Gautam, B., Hau, D. D., Psychogios, N., Dong, E., Bouatra, S., Mandal, R., Sinelnikov, I., Xia, J., Jia, L., Cruz, J. A., Lim, E., Sobsey, C. A., Shrivastava, S., Huang, P., Liu, P., Fang, L., Peng, J., Fradette, R., Cheng, D., Tzur, D., Clements, M., Lewis, A., De Souza, A., Zuniga, A., Dawe, M., Xiong, Y., Clive, D., Greiner, R., Nazyrova, A., Shaykhutdinov, R., Li, L., Vogel, H. J. and Forsythe, I. 2009. HMDB: a knowledgebase for the human metabolone. *Nucleic Acid Research*, 37 (Database issue): D603-610.

Workmaster, B. A. A. and Palta, J. P. 1996. Using banana peel for bioassay development to evaluate the retardation of senescence by lysophosphatidylethanolamine, a natural lipid. *HortScience,* 31: 604. (Abstr.)

Wright, K. P. and Kader, A. A.. 1997. Effect of slicing and controlled-atmosphere storage on the ascorbate content and quality of strawberries and persimmons. *Postharvest Biology and Technology*,10: 39-48.

Wyllie, S. G. and Leach, D. N. 1990. Aroma volatiles of *Cucumis melo* cv Golden Crispy. *Journal of Agricultural and Food Chemistry,* 38: 2042-2044.

Yahyaoui, F. E. L., Wongs-Aree, C., Latché, A., Hackett, R., Grierson, D. and Pech J. C. 2002. Molecular and biochemical characteristics of a gene encoding an alcohol acyltransferase involved in the generation of aroma volatile esters during melon ripening. *European Journal of Biological Chemistry*, 269: 2359-2366.

Yang, S. F. 1985. Biosynthesis and action of ethylene. *HortScience,* 20 : 41-45.

Yapa, P. A. J., Kawasaki, T. and Matsumoto, H. 1986. Changes of some membrane-associated enzyme activities and degradation of membrane phospholipids in cucumber roots due to Ca^{2+} starvation. *Plant Cell Physiology*, 27: 223–232.

Zaharah, S. S. and Singh, Z. 2011. Mode of action of nitric oxide in inhibiting ethylene biosynthesis and fruit softening during ripening and cool storage of 'Kensington Pride' mango. *Postharvest Biology and Technology*, 62: 258-266.

Zanella, A. 2003. Control of apple superficial scald and ripening-a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. *Postharvest Biology and Technology*, 27:69-78.

Zhang, Z. K., Huber, D. J. and Rao, J. P. 2011. Ripening delay of mid-climacteric avocado fruit in response to elevated doses of 1-methylcyclopropene and hypoxiamediated reduction in internal ethylene concentration. *Postharvest Biology and Technology*, 60: 83-91.

Zheng, Q., Nakatsuka, A., Taira, S. and Itamura, H. 2005. Enzymatic activities and gene expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase in persimmon fruit. *Postharvest Biology and Technology*, 37: 286-290.

Zhou, B., McEvoy, J. L., Luo, Y., Saftner, R. A., Feng, H. and Beltran, T. 2006. 1-Methylcyclopropene counteracts ethylene-induced microbial growth on fresh-cut watermelon. *Journal of Food Science*, 71: 180-184.