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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

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

By

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To my family, particularly to my grandfather António Teixeira Gomes, and to Beatriz and Ana.

ABSTRACT

The aim of this research was to examine the physiological and biochemical bases of color (enzymic browning) and textural changes, two of the main quality attributes limiting the storage life of fresh-cut fruit, and to evaluate the physiological response to current technologies, as modified atmosphere packaging and food additives. Two fruit species were selected as model systems: one highly susceptible to enzymic browning ('Rocha' pear), and another fruit whose shelf-life is limited by softening and juice retention but not does not undergo significant color changes (cantaloupe melon).

The kinetics of respiration as a function of oxygen partial pressure was determined in pear and melon at four temperatures (0-15 ºC) to provide fundamental information to assess the potential benefits of modified atmosphere packaging (MAP), and to design MA packages. Respiratory parameters (maximum respiration rate and apparent K_m) were derived from Michaelis-Menten kinetic models adjusted to the experimental data, and the effect of temperature on the respiration rate was modelled by exponential functions. Apparent K_m values for oxygen uptake in fresh-cut pear and melon were very close to or even lower than the fermentation thresholds at all temperatures, indicating that there is a strong physiological limitation to the reduction of respiration rate of these fresh-cut fruits by lowering oxygen levels. This hypothesis was tested in packaged fresh-cut pear stored at 5 ºC for 20 days. Packages were designed to achieve three equilibrium levels of oxygen partial pressures, high (HO), low (LO) and very-low (VLO) O_2. Changes in quality attributes related to fruit metabolism, namely firmness, titratable acidity, pH, and SSC were not affected by oxygen levels. Water activity and levels of ascorbate during storage were independent of oxygen levels. Oxygen partial pressure inside the packages affected browning and microbial growth, which were reduced under VLO. Sensory analyses

confirmed that panelist perceived the differences in color but did not notice differences in firmness or taste among samples. These results confirmed the theoretical estimation.

The use of additive is likely to alter, intentionally or unintentionally, the surface pH of fresh-cut produce. Considering the wide range of pH optima for enzymes germane to the quality of fresh-cut fruit, it is also anticipated that pH might have differential effects on the various quality attributes. The effects of pH, *per se* and in interaction with calcium formulations, of dipping solutions on enzymic browning (related to polyphenoloxidase activity), on textural changes, and on the disassembly of the cell wall of fresh-cut pear were studied. Pear slices were dipped in a buffer solution at pH 3.0, 5.0 or 7.0 and stored at 4.5 ºC for 13 days. In other experiments, pear slices were dipped in buffer solutions containing 250 mM of calcium ascorbate, lactate, chloride, or propionate, at pH 3.0 or 7.0, and stored at 4.5 ºC for 6 days. pH of the dipping solution significantly affected browning, softening, and microbial growth in fresh-cut pears. Browning and softening were more intense in pears treated at pH 3.0 than at pH 5.0 or 7.0, but microbial growth was lower in slices treated at pH 3.0. Calcium salts also affected texture and color in a salt-specific manner. Discrepancies were observed between the pH dependency of PPO activity and tissue browning. Chemical inhibition of PPO was affected by the pH of the buffer, and chemicals.

In conclusion, the knowledge of the kinetics of respiration rates as a function of oxygen partial pressure is fundamental to the assessment of the potential of low-oxygen atmospheres to reduce respiration and extend shelf-life. MAP is of little benefit to delay physiological changes in fresh-cut pear and pH of the dipping solution can affect, *per se*, the quality of fresh-cut fruit. The choice of calcium additives to prevent undesirable change on visual and sensorial quality of cut produce should consider the pH ranges that provide the expected benefits.

RESUMO

Título da tese: '**Bases fisiológicas das alterações da textura e da cor em fruta minimamente processada utilizando pêra e melão como sistemas modelo: implicações para o desenvolvimento de tecnologias e processos**'.

As frutas e as hortaliças minimamente processadas (ou de IV gama) aliam a conveniência de consumo e os benefícios para a saúde, e representam um segmento de mercado em crescimento nos países desenvolvidos. A manutenção do aroma, textura, cor, nutrientes e segurança alimentar, durante o processamento e ao longo da cadeia de distribuição, são os principais desafios enfrentados pela indústria de produtos minimamente processados. O processamento implica o ferimento dos tecidos vivos e sujeita-os a stresse. O desafio de reconciliar a necessidade de frescura com a resposta fisiológica ao stresse provocado pelo processamento e pelos métodos de conservação, implica um conhecimento profundo da fisiologia dos frutos minimamente processados.

Este trabalho de investigação teve por objectivo obter informação sobre as bases fisiológicas e bioquímicas das alterações da cor (acastanhamento enzimático) e da textura, dois dos principais atributos da qualidade que limitam a vida útil dos frutos minimamente processados, e avaliar a resposta fisiológica às tecnologias disponíveis, nomeadamente embalagem em atmosfera modificada e aditivos alimentares, tendo ainda em conta a estabilidade microbiológica e a qualidade sensorial dos produtos. Seleccionaram-se duas espécies de frutos como sistemas modelo: um susceptível ao escurecimento enzimático (pêra 'Rocha'), e outro fruto cuja vida útil é limitada pelo amolecimento e pela retenção de sumo mas que não sofre variações significativas de cor (melão cantalupe).

A cinética da respiração em função da pressão parcial de oxigénio foi determinada em pêra e melão a quatro temperaturas (0, 5, 10 e 15 ºC), de modo a obter a informação fundamental para inferir sobre os potenciais benefícios da embalagem em atmosfera modificada (MAP, do inglês *Modified Atmosphere Packaging*) e para a concepção de embalagens. Os parâmetros respiratórios (taxa máxima de respiração e K_m aparente) foram derivados de modelos de Michaelis-Menten ajustados aos dados experimentais, e o efeito da temperatura na taxa de respiração foi modelado por funções exponenciais. Os valores de K_m aparente para o consumo de oxigénio em pêra e melão cortados foram muito próximos ou mesmo inferiores aos limiares de fermentação para todas as temperaturas, indicando a existência de uma forte limitação fisiológica a reduções significativas da taxa de respiração destes frutos cortados. Dada esta limitação fisiológica, foi levantada a hipótese de que baixos teores de oxigénio no interior das embalagens teriam um benefício insignificante na redução do metabolismo. Neste caso, a sua optimização seria infrutífera para peras e melões minimamente processados. Esta hipótese foi testada com pêra cortada.

Os dados respiratórios foram utilizados no desenho de embalagens em atmosfera modificada para testar o efeito de baixos teores de oxigénio na redução significativa da actividade metabólica. As embalagens foram desenhadas para permitirem três níveis de pressão parcial de oxigénio: alta (HO) 18 kPa O_2, baixa (LO) 2 kPa O_2 e muito baixa (VLO) 0.5 kPa O_2, com 1-5 kPa CO_2. A pêra cortada foi tratada com ascorbato de cálcio (250 mM), tamponado a pH 3.0 e pH 7.0, embalada e armazenada a 5 ºC durante 20 dias. Os valores (médias ± intervalo confiança a 95%) de oxigénio obtidos experimentalmente foram 16.7±0.2, 1.8±0.2 e 0.25±0.04 kPa com níveis de CO_2 correspondentes de 1.3±0.1, 4.3±0.2 e 6.5±0.4 kPa, respectivamente para os tratamentos HO, LO e VLO. As alterações nos atributos de qualidade de pêra cortada relacionados com a actividade metabólica, como a firmeza, os sólidos solúveis, o pH e a acidez titulável do sumo não foram afectados pelas

concentrações de oxigénio. A actividade da água também não foi afectada pela concentração de oxigénio e a taxa de perda de ascorbato durante o armazenamento foi independente do nível de oxigénio. A pressão parcial de oxigénio no interior das embalagens afectou o acastanhamento e o crescimento microbiano, os quais foram reduzidos sob VLO. As análises sensoriais efectuadas após 8 dias de armazenamento confirmaram que o painel de provadores detectou diferenças na cor, mas não percebeu diferenças na firmeza e no sabor entre os vários tratamentos de oxigénio. O acastanhamento foi mais intenso a pH 3.0 e o crescimento microbiano foi mais rápido a pH 7.0. Os resultados mostram que reduzindo os níveis de oxigénio no interior de embalagens não se conseguem ganhos significativos de qualidade nos parâmetros que dependem da fisiologia da respiração, confirmando a estimativa teórica.

Os aditivos alimentares usados para prevenir as perdas de qualidade devidas a acastanhamento enzimático, amolecimento ou crescimento microbiano, alteram, intencionalmente ou não, o pH da superfície dos produtos minimamente processados. É expectável que o pH interfira com vários atributos de qualidade de frutos cortados, como as reacções de acastanhamento mediadas pela enzima polifenoloxidase, a textura e o metabolismo da parede celular, e o crescimento microbiano. Considerando o amplo intervalo de valores de pH óptimo para as enzimas mais directamente relacionadas com a qualidade de frutos minimamente processados, antecipa-se que o pH possa ter efeitos diferenciais nos vários atributos de qualidade. O efeito do pH da solução de imersão na qualidade de fruta cortada foi estudado em pêra, pela sua susceptibilidade ao acastanhamento (relacionada com a actividade da polifenoloxidase) e ao amolecimento (relacionado com as enzimas da parede celular).

Os efeitos do pH, *per se* e em interacção com as formulações de cálcio, das soluções de imersão foi estudado no escurecimento enzimático, nas alterações de textura, e na

desintegração da parede celular de pêra cortada. As fatias de pêra foram mergulhadas por 60 s em soluções tampão a pH 3.0, 5.0 ou 7.0 e armazenadas por 13 dias a 4.5 ºC. Noutras experiências, as fatias de pêra foram mergulhadas por 60 s em soluções tampão contendo 250 mM de ascorbato, lactato, cloreto, ou propionato de cálcio, a pH 3.0 ou 7.0, e armazenadas a 4.5 ºC por 6 dias. O pH da solução afectou significativamente o acastanhamento, o amolecimento e o crescimento de microrganismos em peras cortadas. O acastanhamento e o amolecimento foram mais intensos em peras tratadas a pH 3.0 do que a pH 5.0 ou 7.0, mas o crescimento microbiano foi menor em fatias tratadas a pH 3.0. Os sais de cálcio também afectaram a textura e a cor, de um modo de específico para cada sal. As maiores variações de cor ocorreram nos tratamentos com os sais propionato e lactato, em que as fatias tratadas a pH 3.0 amoleceram mais e apresentaram maior efluxo de electrólitos do que as fatias tratadas a pH 7.0. O ascorbato de cálcio foi muito eficaz na preservação da cor e na redução do crescimento microbiano independentemente do pH, mas promoveu a solubilização de pectinas e o amolecimento a pH 3.0.

Os efeitos do pH e do substrato fenólico no acastanhamento da superfície de pêra cortada e na actividade da polifenoloxidase (PPO) foram estudados no sentido de relacionar o acastanhamento com a actividade da PPO para vários substratos fenólicos. A maior actividade da PPO obteve-se com catecol a todos os valores de pH testados. Observaram-se discrepâncias entre a dependência do pH para o escurecimento e para a actividade da PPO. Obtiveram-se correlações significativas entre a actividade da PPO e os parâmetros de cor luminosidade (L*) ou diferença métrica de tonalidade (ΔH*) no intervalo de pH entre 3.0 e 8.0, excepto para o ácido clorogénico e o 4-metilcatecol. Com o ácido clorogénico, o mais abundante substrato fenólico para a PPO em pêra ‘Rocha’, o acastanhamento dos tecidos foi maior a pH 3.0 (ΔH* mais alto), mas a actividade da PPO foi menor a este pH. Similarmente a outros substratos fenólicos, o acastanhamento a pH 3.0 foi superior à

correspondente actividade da PPO. Testou-se ainda a inibição química da PPO usando catechol como substrato. A inibição da PPO foi afectada pelo pH da solução tampão e foi mais efectiva quando se utilizou ácido ascórbico, N-acetil-L-cisteína e ascorbato de cálcio. Estes resultados sugerem que o pH dos aditivos para pêra cortada deve ser corrigido para reduzir o acastanhamento potencial.

Em conclusão, a análise da cinética das taxas de respiração em função da pressão parcial de oxigénio é fundamental para avaliar o potencial que as atmosferas com baixa concentração de oxigénio poderão ter na redução da respiração e na extensão da vida útil. A verificação da existência de uma 'atmosfera prática de segurança' deve preceder as tentativas morosas de optimização dos níveis de oxigénio no interior de embalagens em atmosfera modificada para frutos minimamente processados. O pH dos aditivos pode afectar, *per se*, a qualidade dos frutos cortados. A escolha dos aditivos de cálcio para prevenção de alterações visuais e organolépticas indesejáveis de produtos minimamente processados deve considerar os valores de pH compatíveis com os benefícios pretendidos.

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CONTENTS

SYMBOLS AND ABBREVIATIONS

Respiration and modified atmosphere packaging

A	Surface area (cm^2)
E_a	Activation energy (kJ mol^{-1})
FT	Fermentation threshold (kPa)
K_{m,O_2}	Michaelis-Menten constant for the $pO_{2\,pkg}$ at half of $R_{O_2}^{max,T}$ (kPa)
K_{mn,CO_2}	Michaelis-Menten constant for the non-competitive CO_2 inhibition
l	Thickness (cm)
M	Mass (kg)
MAP	Modified atmosphere packaging
p	Partial gas pressure (kPa)
P	Permeability (mmol cm cm^{-2} h^{-1} kPa^{-1})
R	Universal gas constant (0.0083144 kJ mol^{-1} K^{-1})
RMSE	Root mean square error
R_{O_2}, R_{CO_2}	Respiration (consumption, production) rate (mmol kg^{-1} h^{-1})
$R_{O_2}^{max,T}$	Michaelis-Menten constant for maximal value of R_{O_2} (mmol kg^{-1} h^{-1})
RQ	Respiratory quotient
T	Temperature (K)

Subscripts

atm	Outside the package
pkg	Inside the package

Additives and enzymes

AA	Ascorbic acid
Ca-Asc	Calcium ascorbate
Ca-Chl	Calcium chloride
Ca-Lact	Calcium lactate
Ca-Prop	Calcium propionate
4-HR	4-hexylresorcinol
NAC	N-acetyl-L-cysteine
POD	Peroxidase
PPO	Polyphenoloxidase

Quality parameters

a*	Chromaticity coordinate on a green (-a*) to red (+a*) axis
b*	Chromaticity coordinate on a blue (-b*) to yellow (+b*) axis
C*	Chroma, $C^* = [(a^*)^2 + (b^*)^2]$ ½
CFU	Colony forming units [log (CFU g^{-1})]
CIE	Commision Internationale de L'Eclairage
ΔH*	Metric-hue difference, $\Delta H^* = [(\Delta a^*)^2 + (\Delta b^*)^2 - (\Delta C^*)^2]$ ½
ΔE*	Color difference, $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$ ½
EE	Electrolyte efflux (% electrolyte leakage)
EIS	Ethanol-insoluble solids
hº	Hue angle, $h^o = \tan^{-1} (b^*)/(a^*)$
L*	Lightness
SSC	Soluble solids content (º Brix)
TA	Titratable acidity (mmol H^+ L^{-1})

CHAPTER 1

GENERAL INTRODUCTION

1. Minimally processed fruits

Fruit are traditionally perceived as having inherent health benefits, and no marketing effort is needed to clarify consumers (Innova Market Insights, 2010). When inherent goodness and healthy eating joined convenience in the fresh-cut fruit, a new market segment was created. This segment is growing and is expected to continue expanding in the coming years (Innova Market Insights, 2010). "Fresh-cut produce" were defined by the International Fresh-cut Produce Association (now United Fresh Produce Association), as "any fresh fruit or vegetable or any combination thereof that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or prepackaged to offer consumers high nutrition, convenience and value while still maintaining freshness" (Lamikanra, 2002). As consequence of processing, minimally processed fruit are highly perishable compared to intact fruit. Safe and secure fresh-cut produce requires adequate technologies and strict quality-control in every step. Negligent postharvest handling, like poor produce or equipment sanitation, poor water quality, and temperature abuse, increase the chance that the ready-to-eat foods at the point of sale become microbiologically unacceptable and represent a potential health risk to humans (Suslow et al., 2003). Disease outbreaks linked to fresh-cut produce will reflect on consumer confidence, and ultimately on sales, and will depreciate all the efforts made by all other operators in this market segment. In addition to safety, quality attributes related to produce physiology are inherently difficult to manage in the supply chain of fresh-cut produce.

2. Quality of fresh-cut produce

Quality perception is subjective, with diverse attributes being unequally important to different players within the distribution chain (Shewfelt, 1999), but can be defined based on the product intrinsic properties that are important for consumers and market handlers. Quality can be limited by visual (e.g., color), textural (e.g., firmness), microbial, chemical (e.g., nutritional) and sensorial (e.g., flavor) attributes. Postharvest changes lead to product depreciation, and quality parameters should be quantified to meet market demands and limits. Keeping quality (defined as the time until a product becomes unacceptable) will depend on the initial produce quality, and on the rate of quality decrease of individual quality attributes to reach the acceptance limit (Tisjkens and Polderdijk, 1996). Excellent raw material is a prerequisite for excellent starting quality of fresh-cut product, while external storage conditions, such as temperature, modified atmosphere and relative humidity, are assumed to affect only the rate of the degradation reactions (Tisjkens and Polderdijk, 1996).

The loss of cellular integrity and protective tissues during processing of cut produce modifies the metabolism and accelerates quality depreciation. Respiration rates of fresh-cuts are generally higher than those of whole product (Watada et al., 1996; Gorny et al., 2000; Mao et al. 2006; Techavuthiporn et al., 2008). The wound response also includes an increase of ethylene production (Brecht, 1995), an induction of enzymes of phenolic metabolism (Howard and Griffin, 1993; Reyes et al., 2007) and enzymes of membrane and cell wall degradation (Karakurt and Huber, 2003). Processing, and a higher degree of cutting, enhances moisture loss and dehydration of damaged cells (Tatsumi et al., 1991; Cisneros-Zevallos et al., 1995), loss of vitamins and flavor volatiles (Lamikanra and Richard, 2002; Gil et al., 2006; Techavuthiporn et al., 2008), softening (Karakurt and Huber, 2003), browning or other color changes (Ke and Saltveit, 1989; Tatsumi et al.,

1991; Cisneros-Zevallos et al., 1995), and overall degradation rates that result in the shortening of the useful lifespan. Minimally processed fruits become unacceptable because of alterations in one or more of the following attributes (Ahvenainen, 1996; Soliva-Fortuny and Martín-Belloso, 2003), depending on the type of fruit: 1) tissue softening, 2) enzymic browning and other undesirable color changes, 3) microbiological stability, 4) sensory and nutritional depreciation. The evolution of each one of these attributes depends 1) on the physiology of the fruit, 2) on the processing conditions, and 3) on the preservation technologies.

Quality maintenance of fresh produce in the supply chain is the goal of processors, distributors and researchers. Preservation of fresh-cut fruit resorts to good sanitation, gentle processing methods, refrigeration, modified atmosphere packaging, enzymic browning inhibitors, and firming additives (Ahvenainen, 1996; Watada and Qi, 1999; Toivonen and Brummell, 2008).

2.1 Quality attributes and tissue wound response

2.1.1 Texture and cell wall stability

Textural properties, such as firmness, crispness, and juice retention, are very important to the appearance and flavor of fresh-cut fruit. Turgidity, rigidity, and crispness of the tissues depend on cell size, cell wall composition, and are affected by the ability of the parenchyma cells to retain water thus causing turgor pressure (Bourne, 1979; Toivonen and Brummell, 2008). In pear fruit, it is possible to notice grittiness due to the presence of sclereids (collenchyma cells) in the flesh (Bourne, 1979). Texture can be quantified by instrumental methods or assessed by sensorial panels. Instrumental tests usually use puncture (measuring the force required to a probe penetrate into the food to a defined depth,

causing irreversible tissue rupture), or deformation (the food is gently squeezed, and the distance to which the product compresses under a standard force or the force required to compress it to a standard distance is measured) (Szczesniak and Bourne, 1969). Flesh firmness of fresh-cut pear is usually measured by penetration using a firmness tester or a texture analyzer (Gorny et al., 1998; Dong et al., 2000; Oms-Oliu et al. 2006; Soliva-Fortuny et al., 2007); the midpoint between endocarp and skin or the geometric center of the sample is penetrated by a flat-head probe, to one-half of its thickness. The same procedures can be used to assess firmness of fresh-cut cantaloupe or honeydew melons (Luna-Guzman and Barrett, 2000; Aguayo et al, 2004; Lamikanra et al, 2005). Kramer shear tests are believed to be closely related to the human experience of chewing foods. In this technique, the movable blades are attached to the load cell and are forced through the test sample producing a force proportional to the sum of both the shear and compression failure point of the test sample. Shear tests were chosen by Qi et al. (1999), Bai et al. (2001), and Saftner et al. (2003) to measure the firmness of fresh-cut melons. Texture of whole fruit can also be measured by tensile strength, twist tests, and by recording the chewing sounds (Harker et al., 2002). A promising non-destructive technique for pear is based on dynamic resonance frequency of drop impact (Wang et al., 2007). Sensory panelists can assess a wide range of texture attributes including crispness, crunchiness, hardness, juiciness, ease of breakdown, and mealiness (Harker et al., 2002). However, it is often problematic to find a relationship between objective measurements and sensory studies. Puncture tests (using an 11 mm diameter Effegi probe) were better at predicting sensory texture attributes of intact apple, although a panel only perceived a texture variation when firmness differed by more than 6 N (Harker et al., 2002). In addition, these authors separated the mechanism of tissue failure along a range of apple firmnesses: at values higher than 66 N cell fracture (cells break across the equator and release juice) generally

occurred; between 66 and 45 N cells ruptured (cells burst and collapse with separation of the plasma membrane and cell wall), while debonding (neighboring cells separate at the middle lamella without compromising cell integrity) occurred at firmness values lower than 39 N, which was the minimum acceptable firmness. Microscopic images of olive fruit also revealed cell fracture in green fruit and a progressive parenchyma cell separation along the middle lamella region as the fruit soften (Mafra et al., 2001). Soft apples developed a mealy texture, and a critical puncture force threshold for acceptable texture of apples was set above 50 N (Harker et al., 2002). Partially ripe pears (44-58 N) are assumed to be the most suitable for fresh-cut processing (Gorny et al., 2000; Soliva-Fortuny et al., 2004a); by analogy with apple texture, cell rupture and fracture are presumably the predominant phenomena during textural measurements in pear tissue.

Textural deterioration of fresh-cut produce occurs as a result of physiological responses to wounding, water loss, turgor changes, and lignification in case of vegetables (Toivonen and Brummell, 2008). After cutting there is a continuation of ripening-related cell wall disassembly events, enhanced by ethylene, which decrease cell wall strength and reduce intercellular adhesion (Toivonen and Brummell, 2008). A decline in fruit firmness during ripening coincide with partial solubilization of pectic, hemicellulosic and cellulosic polysaccharides within the cell wall matrix, and lead to a progressive loss of cell adhesion and increased cell separation (Mafra et al., 2001). In addition, membrane disruption during fruit ripening (Varela et al., 2007) or during storage of fresh-cuts (Soliva-Fortuny et al., 2002b) cause cellular collapse and exudation, altering tissue organization. Textural changes during fruit ripening and softening are mediated by several enzymes, such as polysaccharide hydrolases, transglycosylases, lyases, and expansins, and the relative role of these enzymes differ among fruit types (for review see Brummell and Harpster, 2001; Toivonen and Brummell, 2008; Li et al., 2010). Polygalacturonase (PG, EC 3.2.1.15)

activity is responsible for pectin depolymerization and solubilization, but PG requires a pectin substrate that is partially demethylesterified by pectin methylesterase (PME, EC 3.1.1.11). Furthermore, PG activity is affected by the pH and mineral composition of the apoplast (Almeida and Huber, 1999). Large increases in the activity of β-galactosidase (β-gal, EC 3.2.1.23), which catalyzes the removal of the terminal nonreducing β-D-galactosyl residues from galactan, accelerate cell wall disassembly and enhance softening of fresh-cuts by changing the accessibility or reactivity of cell wall polysaccharide to other cell wall hydrolases (Toivonen and Brummell, 2008; Li et al., 2010). Endo-1,4-β-D-glucanase (EGase, EC 3.2.1.4), and xyloglucan endotransglycosylase (XET, EC 2.4.1.207), play a role in the degradation of cellulose matrix in ripening fruit and contribute to fruit softening (Li et al., 2010). Karakurt and Huber (2003) found that fresh-cut papaya soften and deteriorate more rapidly than whole papaya, and verified that the activity of α- and β-galactosidases, lipoxygenase, phospholipase D, and polygalacturonase were enhanced in response to cutting. However, transgenic experiments showed that suppression of ripening-related cell wall enzymes have little or no impact on fruit firmness (Brummell and Harpster, 2001). Saladié et al. (2007) calls the attention to the importance of turgor regulation at the cell- and tissue-level to the softening of fleshy fruits, as an additional key factor affecting tissue texture.

2.1.2 Color

Color changes on minimally processed produce include browning, discoloration events, and other color-changing processes specific to particular crops (Toivonen and Brummell, 2008). If enzymes and their substrates are separated within cells, as is the case of polyphenoloxidase and the phenolic substrates, a physical stress or deteriorative process (e.g., wounding or senescence) are necessary to initiate browning reactions (Toivonen and

Brummell, 2008). Wounding of lettuce induces the synthesis of enzymes of phenylpropanoid metabolism, which initiate the conversion of L-phenylalanine to *trans*-cinnamic acid by phenylalanine ammonia lyase (PAL; EC 4.3.1.5). Subsequent synthesis and accumulation of phenolic compounds that can be oxidized by polyphenoloxidase lead to wound-induced browning (Ke and Saltveit, 1989). In many fruit, however, the levels of preexistent phenolic substrates are enough for rapid browning to occur independently of the *de novo* synthesis of phenolic compounds. Enzymic browning reactions on fruits are mainly catalyzed by polyphenoloxidase (PPO; EC 1.14.18.1). PPO causes the oxidation of the *o*-diphenols to *o*-quinones, which then polymerize to produce brown pigments called melanin (Martinez and Whitaker, 1995). Phenol peroxidase (POD; EC 1.11.1.7.) can also mediate browning reactions, namely on pear (Richard-Forget and Gauillard, 1997), melon (Chisari et al., 2008), and lettuce (Degl'Innocenti et al., 2005), although its activity is limited by hydrogen peroxide availability. White discoloration on peeled carrots is considered a result of surface cells dehydration and wetting characteristics of the lignin produced after cutting (Tatsumi et al., 1991; Howard and Griffin, 1993; Cisneros-Zevallos et al., 1995), while chlorophyll degradation is involved in discoloration of green tissues (Fonseca et al., 2005; Toivonen and Brummell, 2008).

Objective color description is based in the numerical expression of three coordinates in the chromaticity diagram (Konica Minolta, 2007): the Cartesian (rectangular) coordinates L*, a*, and b*, or the cylindrical coordinates hue (h^o), lightness (L*), and saturation (syn. chroma, C*). The L*a*b* color space defined by the International Commission on Illumination (CIELAB) is very often used in color studies of horticultural commodities. Lightness (L*) changes from white (+100) to black (0). A decrease in L* value indicates a loss of whiteness (brightness) or darkening. The chromaticity coordinates, a* and b*, vary on a green (-a*) to red (+a*) axis and on a blue (-b*) to yellow (+b*) axis. A more positive

a* value indicates browning, whereas a more positive b* indicates yellowing. As the a* and b* value become more positive (+60) or more negative (-60) the saturation of the color increases (more vivid colors). Chroma values, $C^* = [(a^*)^2 + (b^*)^2]$ ½, measure color saturation and vary between 0 (pale color) and +60 (vivid color). Hue angle is expressed in degrees, $h^o = \tan^{-1} (b^*)/(a^*)$, and is defined as the angle the vector makes with the +a* axis; 0º corresponds to red hue (+a*), 90º to yellow (+b*), 180º to green (-a*) and 270º to blue (-b*). Color difference, $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$ ½, indicates the degree of color difference but not the direction. For difference measurements the metric-hue difference is calculated, $\Delta H^* = [(\Delta E^*_{ab})^2 - (\Delta L^*)^2 - (\Delta C^{*\ 2}]$ ½ = $[(\Delta a^*)^2 + (\Delta b^*)^2 - (\Delta C^*)^2]$ ½). The hue difference (Δh) can also be quantified, and is the simple difference between the hº of the sample and the hº of the target (initial value or reference sample).

Browning intensity on cut surfaces can be assessed by an increase of a* values on pear (Gorny et al., 2000) and apple (Tortoe et al., 2007), and a decrease of L* values (Gunes et al., 2001; Soliva-Fortuny et al., 2002a). Several authors have used the decline in hue angle as an indicator of cut pear browning (Pittia et al., 1999; Dong et al., 2000; Oms-Oliu et al., 2006). On the other hand, browning reactions in melons are nearly inexistent. The small changes in lightness, chroma, and hue measured in fresh-cut cantaloupe, confirmed visually by a sensorial panel, indicate absence of browning (Lamikanra et al., 2000). Nevertheless, Portela and Cantwell (2001) observed a decrease in L* and chroma of melon blunt-cut pieces, which were associated with the appearance of translucency.

2.1.3 Taste, aroma, and nutritional composition

Satisfaction in terms of flavor (taste and aroma) motivates consumers for repeated purchases (Kader, 1999); it is therefore important to consider the wound effects on flavor of fresh-cut fruit. The main pre-cutting factors that influence the naturally occurring flavor of

intact produce are maturity and storage temperature and atmosphere (Beaulieu and Baldwin, 2002). Processing and subsequent storage alter endogenous volatile compounds, but allow synthesis of enzyme-mediated secondary compounds that could have a positive or a negative impact on flavor of fresh-cuts (Beaulieu and Baldwin, 2002). Lamikanra and Richard (2002) observed a drastic decrease of total volatiles of fresh-cut cantaloupe just 24 h after processing, although further decreases were minimal over a period of 7 days at 4 ºC. Aromatic esters were better retained during storage, but enzymic hydrolysis of aliphatic esters was severe, significantly modifying the flavor of the cut cantaloupe (Lamikanra and Richard, 2002). Cut tissue subjected to intense stress, such as sterilization by UV light, also fails to maintain ester compounds (Lamikanra et al., 2002). Wound-induced lipoxygenases (LOX; EC 1.13.11.12) and browning reactions related to oxidation of phenolic compounds (catalyzed by PAL) may contribute to the formation of numerous aldehydes and ketones responsible for off-odors (Beaulieu and Baldwin, 2002). Quality of fresh-cuts based on flavor is assuming increasing importance, and is an area of active research (Beaulieu and Baldwin, 2002).

Consumers are concerned with the nutritive value of foods, i.e., energy, and the content of vitamin, minerals, sugars, amino acid, fat, fiber, and phytochemicals. Produce response to wounding depends on the type of fruit or vegetable tissue. Gil et al. (2006) evaluated nutrient retention in several fresh-cut fruits (pineapples, mangoes, cantaloupes, watermelons, strawberries, and kiwifruits) in comparison with whole fruits stored under the same conditions. These authors observed greater, but variable among species, losses in carotenoids in cut than in whole fruits, and no significant losses in total phenolics throughout the storage. Vitamin C was well preserved in cut fruits that have acid pH but not in cantaloupe melon whose pH > 6.0. In tomato cultivars (pH 4.3-4.5) the content of lycopene, vitamin C and phenolics, and the antioxidant capacity did not differ significantly

between whole and processed fruit (Odriozola-Serrano et al., 2008). On the other hand, ascorbate depletion was higher in cut than in intact broccoli, a difference that is correlated with the higher respiration rate of the former (Techavuthiporn et al., 2008). Reyes et al. (2007) suggested that wound response is influenced by the initial levels of reduced ascorbic acid and phenolic compounds of vegetable tissues. These authors observed a significant increase in soluble phenolic content and antioxidant activity of shredded lettuce, celery, carrot, parsnips and sweetpotato, while in shredded lettuce zucchini, potato and red cabbage a decrease in the same variables was observed. In general, a loss of vitamin C is associated with the increase or maintenance of antioxidant capacity, suggesting that phenolics are the main antioxidant compounds in fresh-cut vegetables. In tissues with low levels of reduced ascorbic acid (e.g., lettuce, carrot, parsnips, and celery), phenolics are possibly synthesized to scavenge reactive oxygen species and compensate for the rapid consumption of ascorbate (Reyes et al., 2007). In contrast, when ascorbate is not limiting, the phenolics synthesized after wounding can be used for the formation of insoluble polymers (e.g., lignin and suberin).

2.1.4 Microbiological safety

Physicochemical properties and nutrient composition of the raw material, together with the handling operations, are major factors determining possible microbial spoilage of fresh-cut produce (Ahvenainen, 1996; Soliva-Fortuny and Martín-Belloso, 2003). Processed produce have a large number of cut cells exposed to air and prone to contamination with bacteria, yeasts and moulds. The rate of growth is dependent upon the produce type, humidity, temperature, surrounding gas composition and type of packaging. Most minimally processed vegetables, and some fruits, provide ideal conditions for the growth of microorganisms because they are low-acidic matrices (pH 5.8-6.0) with high

water content (Ahvenainen, 1996; Soliva-Fortuny and Martín-Belloso, 2003). During storage, an increase in the temperature and carbon dioxide concentration in the package favors a change in the composition of the dominant microflora from fungi to lactic acid bacteria (Ahvenainen, 1996). The production of lactic acid, a decrease in pH, and the loss in soluble solids during storage at 20 °C, but not at 4 ºC, indicates that lactic acid bacteria multiplication in fresh-cut cantaloupe is favored by temperature abuse during processing and storage (Lamikanra et al., 2000). This group of microorganism is responsible for fruit flavor deterioration, mediated by increased lipase production and changes in the amino acids profile (Lamikanra et al., 2000). Comparing sensory results with microbial counts in fresh-cut vegetables it was possible to set a maximum lactic acid bacteria count of 10^6 colony forming units g^{-1} as the microbiological limit of shelf-life (García-Gimeno and Zurera-Cosano, 1997). This value has been considered as satisfactory for total aerobic counts in fresh-cut produce (Gilbert et al., 2000). The microbiological limits are dependent on the type of food product, the processing it has received, the number of indicator organisms, and on the presence of pathogens, and should be revised as new knowledge is accumulated or the production and processing conditions of fresh-cut produce evolve (Gilbert et al., 2000).

2.2 Factors affecting quality changes during processing and storage

2.2.1 Temperature

Refrigeration is the most important technology for quality preservation of fresh fruits and vegetables. The desirable temperature for most fresh-cuts is 0 ºC, but very often preparation, shipment, and storage occur at 5 °C and even at 10 °C (Watada et al., 1996). Elevated temperatures hasten produce deterioration, mainly due to higher respiration rates

(Watada et al., 1996). The Arrhenius' equation has been widely used to describe the temperature dependence of respiration rates in several commodities, e.g., cauliflower (Ratti et al., 1996), cucumber (Schouten et al., 2002), endive (Charles et al., 2005), blueberry (Song et al., 1992; Cameron et al., 1994), pear (Ho et al., 2008), fresh-cut pear (Gomes et al., 2010a), and fresh-cut vegetables (Jacxsens et al., 2000). The influence of temperature on respiration rate was first quantified by the temperature coefficient (Q_{10}) value, the rate by which a process increases when the temperature is raised by 10 °C (Fonseca et al., 2002). The Q_{10} values for respiration of whole fruits and vegetables, in air and in 3% O_2, range from 2 to 3 between 0 and 10 ºC (Exama et al., 1993), but processed vegetables have higher Q_{10} than the intact counterparts (Jacxsens et al., 2000).

The effect of temperature on the growth of microorganisms and/or toxin production is well known. The lag phase (time before an increase in the number of cells is apparent) and generation time of a microbial population depend on temperature: as storage temperature decreases below optimum, the lag phase extends and the rate of growth decreases (IFT, 2003).

2.2.2 Cultivar, ripening stage, and degree of cutting

The correct selection of cultivars for minimal processing is essential to assure quality of the final product (Ahvenainen, 1996). There are differences among mango cultivars in susceptibility to browning, tissue damage, desiccation, spoilage and aroma loss during storage of fresh-cut (Beaulieu and Lea, 2003). 'Conference' pear was preferred by a trained panel who favored the combination of less browning, higher sugar and juice content than in 'Williams' (Arias et al., 2008). Browning intensity and tissue softening of pear slices differ widely among 'Bartlett', 'Bosc', 'Anjou' and 'Red Anjou' pears, with the first variety being the best for fresh-cut processing (Gorny et al., 2000). Between four clingstone

peach cultivars, ‘Romea’ was the one more indicated for processing, given its lower polyphenoloxidase activity and degree of browning (González-Buesa et al., 2011). These examples illustrate the potential for improving the quality of fresh-cut produce via breeding of specific cultivars for processing.

The proper stage of maturity provides acceptable eating quality to the consumer, but at same time fruit should be adequately firm for handling, processing and shipping. The effect of the stage of maturity on quality of fresh-cut pears was studied by Gorny et al. (2000), Soliva-Fortuny et al. (2004a) and Bai et al. (2009). Soft pears can develop translucency related to higher degree of mechanical injury during processing and packaging (Soliva-Fortuny et al., 2004a). Partially ripe pears (44-58 N) preserved tissue firmness and integrity better than ripe fruit, extending marketable shelf-life, and were found to be the most suitable for fresh-cut processing (Gorny et al., 2000; Soliva-Fortuny et al., 2004a). In addition, Bai et al. (2009) suggested delayed-harvest ‘Anjou’ fruits (55 N) for fresh-cut instead of commercially-harvest fruits (66 N). . The former had a desirable crunchiness, higher juiciness, finer texture, and less browning, associated with a more aromatic flavor. Flavor is also an important attribute in the choice of the correct stage of maturity of cantaloupe and mango for processing, even in detriment of a firmer raw material with extended shelf-life (Beaulieu et al., 2004).

Wounding intensity and sharpness of blades affect several quality attributes that limit the shelf-life of cut produce. Slicing methods that minimize cell rupture and tissue exudates postponed off-odor development due to tissue decay, and provide superior appearance and aroma scores of carrot (Barry-Ryan and O’Beirne, 1998). The degree of cutting of the produce is positively correlated with respiration rates (Zhu et al., 2001) and tissue dehydration (Artés-Hernández et al., 2007). Cantaloupe melon blunt-cut pieces had increased ethanol concentrations, off-odor scores, and electrolyte leakage compared to

sharp-cut pieces (Portela and Cantwell, 2001), but low temperature (5 ºC) was able to minimize differences in respiration and ethylene production rates between these two cutting treatments. A higher severity of wounding increased PAL activity and browning in cut lettuce (Ke and Saltveit, 1989).

2.2.3 Post-cutting treatments and packaging

Postharvest treatments to keep the quality of fresh-cut fruits generally include dipping in antioxidant and firming solution, and modified atmosphere packaging (Soliva-Fortuny and Martín-Belloso, 2003; Oms-Oliu et al., 2010). Alternatives methods to control produce deterioration, such as the application of plant extracts and physical treatments (e.g., mild heat treatments, irradiation, high hydrostatic pressure) continue to be studied but, as of yet, their commercial application to fresh-cut fruits and vegetables remains limited.

2.2.3.1 Dipping solutions and other complementary treatments

Solutions of compounds aiming at reducing undesirable color and textural changes, microbial growth, and other degradative reactions are often used in the fresh-cut industry. The preferred method of application of these additives to fresh-cut produce is by dipping the cut pieces into the solution. The choice of the preservative compounds applicable to fresh-cut produce is limited by the odors and tastes imparted by the additives (Soliva-Fortuny and Martín-Belloso, 2003). Another important issue is pH of the dipping solution. Low pH values are usually recommended to prevent microbial proliferation (Parish et al., 2003). Soliva-Fortuny and Martín-Belloso (2003) list some situations where it is advisable to raise pH values, e.g., the development of pinkish-red colored compounds on fruit tissue when cysteine is used under acidic conditions. Besides this effect on color, pH can also be

co-responsible for undesirable changes in firmness of minimally processed fruit (Ponting et al., 1971; Pinheiro and Almeida, 2008).

Browning inhibitors can be classified by their action on PPO enzyme, on adducts, and on the reaction medium (Garcia and Barrett, 2002). Medium acidification using citric acid is a common practice. Higher inhibition of apple PPO is achieved when pH is reduced from 5.0 to 3.6 using carboxylic acids (Janovitz-Klapp et al., 1990). Reductant compounds react with *o*-quinones formed by the PPO-mediated oxidation of phenolics. Ascorbic acid reduces the *o*-quinones back to their precursor diphenols, while thiol-containing amino acids, such as L-cysteine, N-acetyl-L-cysteine and glutathione produce stable and colorless adducts (Garcia and Barrett, 2002). During these reactions, ascorbate is irreversibly oxidized and can lose its reducing power shortly after the treatment (Son et al., 2001). Complexing agents (e.g., cyclodextrins), chelating cooper agents (e.g., polyphosphate), and specific enzyme inhibitors (e.g., 4-hexylresorcinol) are other categories of antibrowning additives (Garcia and Barrett, 2002; Arias et al., 2007). The combination of acidulants, chelators and reductants is often proposed to improve the treatments' effectiveness.

Firming additives containing calcium, such as the calcium salts of ascorbate, chloride, lactate, propionate, and gluconate, can be successfully used in several fresh-cut fruit (Dong et al., 2000; Luna-Guzmán and Barrett, 2000; Saftner et al., 2003; Lamikanra and Watson, 2007; Aguayo et al., 2008, 2010). Calcium chloride dips are the most common treatment (Soliva-Fortuny and Martín-Belloso, 2003), while alternative calcium salts are being tested for similar firming effect without the undesirable bitterness (Luna-Guzmán and Barret, 2000). Calcium is required to membrane and cell wall integrity, and can improve cell adhesion and cell turgor (Mignani et al., 1995; Picchioni et al., 1995) by binding to the negative charges of demethylated galacturonic residues of pectins (Toivonen and Brummell, 2008), therefore improving firmness retention.

Microbial load is a quality criterion determinant of the shelf-life of fresh-cut fruit, and surface sanitization is required for raw materials and, sometimes, for final product. Acidic solutions are used to limit bacterial growth (Bhagwat et al, 2004; Karaibrahimoglu et al., 2004; Simón et al., 2010) and malic acid is effective in repressing fungal growth (Raybaudi-Massilia et al., 2007). Acidification to pH 4.5 is sufficient to prevent microbial proliferation (Parish et al., 2003). The antimicrobial activity of organic acids depends on their low pH and on their structure (Soliva-Fortuny and Martín-Belloso, 2003), since species should be present in the solution as undissociated acids to affect microbial metabolism (Ita and Hutkins, 1991). Reduction in microbial load in response to some calcium salts, namely propionate, chloride, lactate and carbonate, has also been documented (Buta et al., 1999; Saftner et al., 2003; Mao et al., 2006; Aguayo et al., 2008).

Alternative methods are being used to reduce the use of chemical compounds in quality preservation of fresh-cut produce. Natural compounds, namely plant volatiles that play a role in the defense system against decay microorganisms, are generally recognized as safe (Lanciotti et al., 2004). Hexanal, 2-(*E*)-hexenal, hexyl acetate and citrus essential oils, have antimicrobial activity against naturally occurring spoilage species and pathogenic bacteria such as *Escherichia coli*, *Salmonella enteritidis*, and *Listeria monocytogenes* (Lanciotti et al., 2004). Carvacrol and cinnamic acid are effective in delaying spoilage of fresh-cut kiwifruit and honeydew melon (Roller and Seedhar, 2002). Vanillin, a phenolic compound, possesses antimicrobial activity *in vitro* and also when applied to fresh apple slices (Rupasinghe et al., 2006). Recommendation for use of these substances in post-cut dipping solution requires information about their possible odors and flavors or undesirable color changes that might compromise consumer acceptance (Roller and Seedhar, 2002). Food irradiation with UV-C radiation is effective in reducing yeast, mold, and *Pseudomonas* spp. populations in fresh-cut melon (Lamikanra et al., 2005) and aerobic

microorganisms in watermelon, without affecting juice leakage, color, and overall visual quality (Fonseca and Rushing, 2006). Gamma radiation reduces total aerobic plate counts on apple slices, but increases tissue softening and browning (Fan et al., 2005). Dipping fresh-cut celery in ozonated water diminishes PPO activity, respiration rate, and microbial contamination, while sensory quality was improved (Zhang et al., 2005). Mild heat treatments, applied to whole or cut fruit, combined or not with calcium dips, showed good results in preserving firmness of fresh-cut melon (Luna-Guzmán and Barrett, 2000; Lamikanra and Watson, 2007), fresh-cut pear (Abreu et al., 2003) and fresh-cut kiwifruit (Beirão-da-Costa et al., 2006). Mild heat treatments applied to fresh-cut pear were also considered beneficial to color retention, microbial counts, with an acceptable loss of texture (Pittia et al., 1999). However, more severe treatments (increasing heating times and/or temperature) can result in a significant loss of firmness and weight (Pittia et al., 1999), and surface discoloration (Abreu et al., 2003).

2.2.3.2 Modified atmosphere packaging and edible coatings

Modified atmosphere packaging (MAP) and edible coatings can be successfully used to maintain quality of fresh-cut fruits and vegetables (Farber et al., 2003; Olivas and Barbosa-Canovas, 2005; Rojas-Graü et al., 2009; Sandhya, 2010).

A controlled or modified atmosphere is created by altering the normal air composition (79 kPa nitrogen, 21 kPa oxygen, 0.03 kPa carbon dioxide and traces of noble gases). In controlled atmosphere (CA), a known gas mixture is established and maintained under relatively tight control throughout storage. Large quantities of product can be packed, for transportation and storage, in pallet bags and paperboard containers wrapped with a barrier plastic film (bulk packaging), within which product will experience a passively modified atmosphere (Farber et al., 2003). When small amounts of product (intact or fresh-

cut) are packed using a selected film type, an equilibrium atmosphere is reached naturally, as a consequence of the products' respiration and the diffusion of gases through the film (passive MAP). An improvement can be obtained with active MAP, where steady state gas levels inside package are reached faster by the injection of the desired mixture of gases. In addition, a layer of edible materials with barrier properties can be applied onto the surface of a cut-fruit with the objective of modifying the composition of the internal atmosphere or vehicle food additives (antimicrobial, antibrowning, texture enhancers, or nutraceuticals), in order to improve overall product quality during storage (Olivas and Barbosa-Canovas, 2005; Rojas-Graü et al., 2009; Oms-Oliu et al., 2010). Some commercially available CA and MAP systems, polymers for MAP, and edible films are listed in Farber et al. (2003).

Minimally processed fruits and vegetables packed under low oxygen and elevated carbon dioxide can have extended shelf-life and marketing period, since MAP protects from water-loss, contamination and, sometimes, reduces the metabolic rate. Beaudry (1999) discussed the effect of controlled and modified atmospheres on primary and secondary metabolism. Oxygen depletion and elevated CO_2 reduce respiratory activity, oxidative tissue damage or discoloration, ethylene sensitivity, and, therefore, the rate of ripening, the rate of chlorophyll degradation, and other ethylene-dependent phenomena. When subjected to extreme atmospheres, i.e. above the tolerance limits of the commodity, plant responses include fermentation, off-flavor development, and induction of tissue injury. Lowering oxygen levels below ~ 5 kPa affect the enzymes of glycolytic metabolism and aerobic respiration rate is reduced, and, at very low O_2 levels, the decline of energy and carbon skeletons availability induces fermentation (Beaudry, 1999). Inhibition of ethylene synthesis occurs at higher O_2 levels than those required for significant reductions in respiration rate. Farber et al. (2003) reported an oxygen partial pressure of 8 kPa to be sufficient to start reducing ethylene biosynthesis but reductions in respiration rate only

occur below 1 to 5 kPa of oxygen. A study with pea seedlings showed that 50% inhibition (K_m) of the ethylene response occurred at 2.8 kPa O_2 while the K_m for respiration was 0.25 kPa O_2. Low levels (1.55 kPa) of CO_2 were enough to compete with ethylene and inhibit its response during growth of pea stem sections, without affecting respiration rate (Burg and Burg, 1967). The inhibition of wound-induced ethylene production in climacteric fruits depends on CO_2 concentration; elevated CO_2 (~ 20 kPa) inhibits l-aminocyclopropane-1-carboxylate (ACC) synthase and, in some cases, ACC oxidase (Mathooko, 1996). Some studies reported that high levels of CO_2 could reduce, have no effect, or even stimulate respiration (Beaudry, 1999; Fonseca et al., 2002). Low oxygen levels decrease PPO activity (its K_m for O_2 is around 7-10 kPa), and dark pigment generation can be slowed down (Beaudry, 1999). Browning prevention in lettuce resort to fermentative oxygen levels (< 0.5 kPa), but, in this particular product, the resulting off-flavors are less important than the improved appearance (Beaudry, 1999). Poor availability of oxygen generally suppresses the production of flavor-important volatiles, but the effect of CO_2 on aroma volatiles is unclear (Beaudry, 1999).

MAP can also be designed to avoid the optimal O_2 level for microbial growth (21 kPa for aerobes, 0-2 kPa for anaerobes). Oxygen partial pressures below 1 to 2 kPa can stimulate the growth of anaerobic pathogens, and increasing CO_2 levels (below 50 kPa) inhibit spoilage microorganisms but pathogens, such as *Clostridium perfringens*, *C. botulinum* and *L. monocytogenes*, are only minimally affected and can proliferate due to a lack of competition by the indigenous microflora (Farber et al., 2003). Low oxygen levels and moderate to high levels of CO_2 (10-20 kPa) are enough to inhibit the growth of aerobic microorganisms, such as pseudomonas, but lactic acid bacteria can be stimulated under these conditions (García-Gimena and Zurera-Cosano, 1997; Farber et al., 2003). CO_2 has a significant and direct antimicrobial activity, as a result of a reduction in intracellular pH,

changes on enzyme activity, and alteration of overall microbial cellular metabolism (Farber et al., 2003). This inhibition is concentration and temperature dependent; as temperature decreases, the inhibitory power of CO_2 increases (García-Gimena and Zurera-Cosano, 1997). However, very few horticultural commodities cultivars tolerate exposures to CO_2 levels known to have an antimicrobial effect (Kader et al., 1989; Watkins, 2000), which limits the use of CO_2 as an effective antimicrobial agent in fresh-cut produce.

The effect of superatmospheric oxygen levels (CA and MAP) and high CO_2 levels have been tested to prevent quality changes, with varying results. In elevated CO_2 atmospheres (10-20 kPa) tissue necrosis was observed in pear fruit, although softening rates were similar to those of pears kept in atmospheric air (Gorny et al., 2002). In contrast, atmospheres with a high concentration of CO_2 (21 kPa O_2 + 10 kPa CO_2 and 2 kPa O_2 + 10 kPa CO_2) were the most appropriate to preserve color and microbial stability of fresh-cut pear at low temperatures (Arias et al., 2008). High levels of O_2 can be effective in inhibiting enzymic discoloration, preventing anaerobic fermentation reactions, and inhibiting aerobic and anaerobic microbial growth (Farber et al., 2003). Different results were obtained with mango cubes held at 13 ºC, but not at 5 ºC, where 60 kPa oxygen stimulated respiration rate, browning, and growth of mesophilic aerobic bacteria (Poubol and Izumi, 2005). Fresh-cut pears stored in 70 kPa O_2 at 4 ºC were susceptible to enzymic browning and produced objectionable levels of fermentative metabolites but the growth of spoilage microorganisms was reduced (Oms-Oliu et al., 2008b).

MAP effectiveness to preserve fresh-cut quality depends on the temperature. MAP is designed to generate an equilibrium between the product respiration rate and gas permeability of the plastic film, both dependent on temperature (Jacxsens et al., 2000). In the packages designed to obtain very low O_2 atmospheres (near the fermentation threshold) at low temperatures, risks of hypoxia occur when the temperature-related rise in respiration

rate is higher than the increase in gas permeation through the package (Exama et al., 1993). This effect is greater in microperforated packages than in those in which gas flows through the continuous films, since diffusion through holes is relatively temperature-independent (Cameron et al., 1994).

3. Mathematical models relevant to fresh-cut produce

Models are useful tools to describe and predict changes in variables occurring in a system, at equilibrium or under dynamic conditions (as a function of time). Several models have been proposed to describe physiological processes, such as respiratory metabolism and softening, or microbial growth. Respiratory models as a function of gas concentration are needed to assist in the design of modified atmosphere packaging (Fonseca et al., 2002). The oxygen uptake has been described by Michaelis-Menten kinetics (Lee et al., 1991; Cameron et al., 1994; Jacxsens et al., 2000), polynomial (Yang and Chinnan, 1988), and even exponential models (Beaudry et al., 1992). Specific rates of chemical, biochemical and enzymic reactions usually depend exponentially on temperature according to Arrhenius' law (Chang, 1981), and respiratory metabolism is no exception (Fonseca et al., 2002). Respiratory data is also used as an independent variable in different model equations, e.g., to predict softening of whole apples and kiwifruit (Hertog et al., 2001, 2004), color changes (Ishikawa and Hirata, 2001) and ascorbate decrease (Techavuthiporn et al., 2008) in packed fresh broccoli, and spoilage by *Botrytis* in strawberry (Hertog et al., 1999). Firmness loss in whole (Hertog et al., 2004) or cut (Soliva-Fortuny et al., 2002b; Lana et al., 2005) fruit has been described by first-order fractional conversion models, and differential equations have been adopted to describe color changes based on chlorophyll degradation (Schouten et al., 2002). Taoukis and Labuza (1989) proposed that the depreciation of a single quality attribute can be described by one or more of the following

basic mechanisms: i) zero order reactions with a linear kinetics; ii) Michaelis-Menten kinetics; iii) first order reactions having an exponential kinetics; iv) autocatalytic reactions following a logistic kinetics. More recently, Tisjkens and Polderdijk (1996) proposed a generic model for keeping quality (time to cross the quality acceptance limit) of horticultural crops, for single or double limiting attributes, that included the effects of temperature, chilling injury, and different levels of initial quality and of quality acceptance limits.

Mathematical models that predict the rate of growth or decline of spoilage microorganisms under a given set of environmental conditions are helpful in the estimation of the shelf-life of minimally processed produce. Microbial growth curves are often modelled by the logistic or other sigmoid functions such as the Gompertz equation, modified by Zwietering et al. (Soliva-Fortuny et al., 2004b; Raybaudi-Massilia et al., 2007) or re-parameterized to directly include shelf-life as a fitting parameter (Corbo et al., 2006). Best curve adjustment equation was used by García-Gimena and Zurera-Cosano (1997) to describe bacterial growth of minimally processed vegetables as a function of time in order to establish the end of a product's shelf-life. Although kinetic parameters use empirical coefficients, experimentally determined from microbial measurements, they facilitate the assessment of different process technologies in reducing microbial populations (IFT, 2002).

A proper design of MAP for fresh-cut produce is based on mathematical models for O_2 and temperature dependence of aerobic respiration and permeability of a packaging film, combined with the weight of product, package area, and type of film (Cameron et al., 1994; Jacxsens et al., 2000). Matching the intrinsic properties of the produce (respiration rate, optimal modified atmosphere, and minimum O_2 and maximum CO_2 concentrations required) with the package gas environment and temperature, it is possible to create the optimal storage conditions.

Quantification of the dynamics of quality changes throughout storage, processing, and distribution chains will help market operators to make rational options in order to optimize the shelf-life of their fresh-cut products.

4. Aim and outline of this thesis

The aim of this thesis was to contribute to the understanding of the physiological bases of quality changes of fresh-cut fruits, in order to assist in process optimization and technology development. The specific objectives of the research that lead to this thesis were:

1) Obtain experimental data on the respiratory behavior of fresh-cut pear and melon under a range of oxygen partial pressures inside packages in a range of temperatures relevant to the supply-chain of fresh-cut fruits (0, 5, 10 and 15 ºC);
2) Mathematically model the experimental data to determine the respiratory parameters and to assess the physiological limits to decreasing respiration via the reduction of oxygen partial pressure;
3) Test the effectiveness of low oxygen concentrations in modified atmosphere packaging in reducing metabolic activity;
4) Evaluate the effect of pH and calcium additives on textural changes and enzymic browning of fresh-cut 'Rocha' pear and on the underlying mechanisms of cell wall disassembly and PPO activity.

Minimally processed fruits are highly perishable and their quality can be limited by several attributes, as discussed in the General Introduction. Color (enzymic browning) and texture were selected as the dependent variables (quality attributes) for detailed studies of the physiological and biochemical bases for the quality changes, and evaluation of the

effect of current technologies on these attributes. Microbial proliferation and consumer sensory evaluation were not ignored, although not studied with the same detail, because their relation to the physiological processes occurring in cut fruit is indirect. Two fruit species were selected as model systems: pear (cv. 'Rocha') and cantaloupe melon. Pear shelf-life is limited by enzymic browning, while quality in melon fruit is limited by softening and juice release but not by browning reactions.

The General Introduction (**Chapter 1**) summarizes the relevant literature on the changes in quality of fresh-cut produce. Post-cutting treatments of fresh-cut fruits generally include dipping in antioxidant and firming solution, and modified atmosphere packaging (Soliva-Fortuny and Martín-Belloso, 2003). The use of a low O_2 concentration and a high CO_2 concentration, in combination with storage at refrigeration temperatures, is proposed by many researchers as optimal conditions to maintain the quality of fresh-cuts. Current recommendations for modified atmospheres for sliced pear (0.5 kPa O_2 plus <10 kPa CO_2) and cubed cantaloupe (3-5 kPa O_2 plus 6-15 kPa CO_2) (Gorny, 2001) were reassessed based on comprehensive respiration data. Since much of the improvement in MAP have been due to trial-and-error experimentation (Beaudry, 1999), the benefits of MAP were evaluated by studying the physiological behavior of these two fruit types under a range of oxygen levels. As proposed by Beaudry (2000), low oxygen atmospheres could be useful if a sizeable reduction in respiration rate (e.g. 50%) can be reached without the induction of fermentation. The respiratory behavior of fresh-cut pear and melon, as affected by oxygen concentration and temperature, was mathematically described using Michaelis-Menten kinetics and respiratory parameters were derived from the models (**Chapter 2**). After theoretical inference about benefits of MAP (**Chapter 2**), the effectiveness of low oxygen atmospheres in reducing the metabolic activity was tested on packed fresh-cut pear (**Chapter 5**).

Acidifying additives (e.g., ascorbate, citrate) are frequently used in fresh-cut fruit to limit microbial growth, to inhibit polyphenoloxidase (PPO), or to incorporate calcium for firming effect. Based on suggestions that acidic media may promote cell wall degradation (Pinheiro and Almeida, 2008), we explored the possibility that pH of the dipping solutions could be, by itself, a meaningful explanatory variable for the observed effects. **Chapters 3** and **4** addresse the effect of pH of the dipping solution on physiological and biochemical changes on quality attributes of fresh-cut pear. **Chapter 3** reports on the effect of calcium formulations and pH of the dipping solution on cell wall polyssacharides, firmness, and enzymic browning of the cut surface of pear. The effect of pH, phenolic substrate and food additives on PPO activity and tissue browning is addressed in **Chapter 4**. The General Discussion in **Chapter 6** brings it all together and makes a case for physiology-based approaches to process and technology development in fresh-cut fruit. Improving our understanding of the physiological and biochemical bases for the quality of fresh-cut fruits and vegetables will help in the development of more precise, and possibly faster, responses for the gaps felt by system handlers and would enhance one's ability to predict responses to new technologies and processes.

CHAPTER 2

MODELLING RESPIRATION OF PACKAGED FRESH-CUT FRUIT AS AFFECTED BY OXYGEN CONCENTRATION AND TEMPERATURE

CHAPTER 2

2.1 FRESH-CUT 'ROCHA' PEAR

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Abstract

Respiration rates were measured in fresh-cut 'Rocha' pear (*Pyrus communis* L.) stored at four temperatures (0, 5, 10 and 15 ºC) and with oxygen partial pressures ranging from 0 to 18 kPa. Respiratory quotient and ethanol production were used to determine the fermentation threshold. The oxygen concentration effect on the respiration rate was accurately described using Michaelis-Menten kinetics, without non-competitive inhibition by CO_2, and the effect of temperature on the respiration rate was well modelled by exponential functions. The oxygen level at which respiration was half its maximum (apparent K_{m,O_2}) was similar to or only slightly greater than the fermentation threshold. The narrow range of oxygen between K_{m,O_2} and the fermentation threshold, suggests that modified atmosphere packaging technology has a limited applicability toward extension of the shelf-life of fresh-cut 'Rocha' pear.

1. Introduction

Respiration is a central process in fruit metabolism: it provides energy and carbon skeletons for the anabolic reactions that occur during maturation and ripening, and for cell maintenance during storage. Besides its physiological importance, knowledge of fruit respiratory parameters possesses a technological relevance, particularly toward development of modified atmosphere packaging (MAP). Fruit respiration rate is affected by a number of factors, among which temperature and oxygen partial pressure exert strong effects (Fonseca et al., 2002). In fresh-cut fruit wounding enhances the respiration rate as part of the healing and ethylene responses (Brecht, 1995).

Fresh-cut processing often uses barrier technologies to modify atmosphere composition, prevent contamination, and reduce water loss (Kader et al., 1989). Since fresh-cut fruit respire, hermetically-sealed barriers used in these convenience products

result in a modified atmosphere inside the package. Since MAP can extend the shelf-life and marketing period of fresh-cut fruit (Bai et al., 2001; Martínez-Ferrer et al., 2002) a great deal of effort has been dedicated to develop and test appropriate packages, and to optimize oxygen and carbon dioxide partial pressures inside the packages (Kader et al., 1989; Fonseca et al., 2002). A number of studies encompassing packaging films and geometries have assessed O_2 concentration versus produce quality, in order to empirically find the best package (Bai et al., 2003; Del Nobile et al., 2007; Soliva-Fortuny et al., 2007; Teixeira et al., 2007). An alternative approach to the trial-and-error, should data be available, is to deduce optimal packaging geometry and film permeability based on the produce respiration rate (Lakakul et al., 1999; Jacxsens et al., 2000), as affected by oxygen concentration and temperature.

Respiration rates of fresh-cut pear were reported for 'd'Anjou', 'Bartlett', 'Bosc' and 'Red Anjou' cultivars stored in air and in 2 kPa oxygen (Gorny et al., 2000). Values range between 0.07-0.22 and 0.27-0.53 mmol CO_2 kg^{-1} h^{-1}, respectively at 0 and 10 ºC, rising to 0.86-1.63 mmol CO_2 kg^{-1} h^{-1} at 20 ºC. However, detailed information on the effect of oxygen concentration on the respiration of fresh-cut pear at different temperatures is still unavailable.

The impact of MAP on fresh-cut produce has been shown to be effective for some commodities; however benefits have not been demonstrated for fresh-cut pear. Further, the requirement for packaging fresh-cut fruit in sealed packages, to maintain produce safety and quality, will result in a modified atmosphere. Therefore, it is essential to quantify the relationship between oxygen concentration and respiration rate for fresh-cut 'Rocha' pear, stored at different temperatures, and to test respiratory models that would allow the design of MAP for fresh-cut pear at temperatures normally found during processing and distribution.

2. Materials and methods

2.1 Plant material

Pears (*Pyrus communis* L. 'Rocha') were harvested in two consecutive years, 2006 and 2007, at commercial maturity (65 N firmness), from orchards located in the Western Region of Portugal (39º11' N, 9º08' W). 'Rocha' pear has a long storage life and raw material is available for fresh-cut processing for up to ten months. Pears used in 2006 had been stored for 6 months at -0.5 ºC, 93-95% RH, and 2.5 kPa O_2 plus 0.7 kPa CO_2. Fruits processed in 2007 had been stored at -0.5 ºC and 93-95% RH in air for 5 weeks. Fruits removed from storage were allowed to ripen and were processed when the flesh firmness reached 47-49 N, since partially ripe pears are the most suitable for fresh-cut processing (Gorny et al., 2000; Soliva-Fortuny et al., 2004a).

2.2 Fresh-cut preparation and packaging

Whole fruits were sanitized with 150 µL L^{-1} NaClO for 2 min, rinsed with tap water and air dried. Pears were cut in wedges with 5-10 mm of thickness, without skin removal. The fresh-cut pear wedges (30 to 265 g) were packed in low-density polyethylene (LDPE) (Dow Chemical Company, Midland, MI) pouches (15×15 cm or 20×20 cm), that were hermetically sealed using an impulse heat sealer. The film thickness used in the experiments ranged from 27.0 to 102.1 µm.

Package size, film thickness, and fruit mass were varied to intentionally develop a range of oxygen concentrations (18 to 0.1 kPa). A gas sampling septum, consisting of a dab of silicone sealant (GE® Silicone, N.Y.) on a short strip of electrical tape (Scotch®, St. Paul, MN), was attached to each pouch.

2.3 Respiration rate determination

A permeable system using LDPE of known permeability was used to obtain the respiratory rate of the enclosed produce. Rates of O_2 uptake (R_{O_2}) and CO_2 production (R_{CO_2}), and respiratory quotient ($RQ = R_{CO_2} / R_{O_2}$), were calculated once steady-state O_2 and CO_2 partial pressures were achieved inside the packages, using the following equations (Beaudry et al., 1992; Joles et al., 1994; Lakakul et al., 1999):

$$R_{O_2} = \frac{\frac{P_{O_2} \times A}{l} \times \left(pO_{2atm} - pO_{2pkg} \right)}{\mathrm{M}} \quad (1)$$

$$R_{CO_2} = \frac{\frac{P_{CO_2} \times A}{l} \times \left(pCO_{2pkg} - pCO_{2atm} \right)}{\mathrm{M}} \quad (2)$$

where R_{O_2} and R_{CO_2} denote respiration rates (mmol kg^{-1} h^{-1}) of O_2 and CO_2, P_{O_2} and P_{CO_2} denote O_2 and CO_2 permeabilities (mmol cm cm^{-2} h^{-1} kPa^{-1}), A is the film area (cm^2), l is the film thickness (cm), $pO_{2\ atm}$, $pO_{2\ pkg}$, $pCO_{2\ atm}$ and $pCO_{2\ pkg}$ are O_2 and CO_2 partial pressures (kPa) outside ($_{atm}$) and inside ($_{pkg}$) the package, and M is the produce mass (kg).

Three replicates of each combination of film thickness, film area, and fruit mass were stored at 0, 5, 10 and 15 ºC.

2.4 Film permeability assessment

Film permeability to O_2 (P_{O_2}) and CO_2 (P_{CO_2}) was measured using an isostatic method (Beaudry et al., 1992; Gavara et al., 1996). The permeation cell contained two

external receiver chambers separated from a middle donor chamber by film samples. The middle chamber was initially flushed (100 mL min^{-1}) with pure N_2 to purge receiver chamber of oxygen. Once receiver chamber oxygen levels were reduced below detection limits, a mixture of 72 kPa O_2 and 29 kPa CO_2 was supplied to the donor chamber. The receiver gas composition was sampled (100 μL), using a glass syringe, at 10 minute intervals and analyzed via sequential O_2 and CO_2 analyzers, using a paramagnetic O_2 detector (Series 1100, Servomex Co., Sussex, UK) and an infrared CO_2 detector (ADC 255-MK3, Analytical Development Co., Hoddesdon, UK), respectively, and connected in series (Lakakul et al., 1999). The diffusion rate of gases through the film was used to calculate the permeability coefficients, at 0.6, 10, 21 and 23 ºC, in controlled temperature chambers. The Arrhenius equation was used to fit the temperature influence on permeability (Beaudry et al., 1992; Joles et al., 1994; Cameron et al., 1995; Lakakul et al., 1999). The pre-exponential factor and activation energies were estimated via regression analysis, after linearization (Beaudry et al., 1992): $\ln (P_i) = - E_a/RT + \ln (P_{0,i})$, where P_i is permeability to O_2 or CO_2 (mmol cm cm^{-2} h^{-1} kPa^{-1}), $P_{0,i}$ is the pre-exponential factor, E_a is activation energy (kJ mol^{-1}), R is ideal gas constant (kJ mol^{-1} K^{-1}) and T is temperature (K).

2.5 O_2, CO_2, and ethanol determination

Headspace gas composition (O_2 and CO_2) in individual packages was monitored daily using a gas analyzer, as described above, until steady-state was attained. The ethanol present in the headspace was sampled at steady-state and determined using a gas chromatograph system (HP-6890, Hewlett Packard, USA), equipped with a mass spectrometry detector.

2.6 Experimental data modelling

Respiration rate (R_{O_2}) was described as a function of O_2 partial pressure by a Michaelis-Menten model (Eq. (3)), as suggested by Lee et al. (1991):

$$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_{2\,pkg}}{K_{m,O_2} + pO_{2\,pkg}} \quad (3)$$

where $R_{O_2}^{\max,T}$ is the maximal value of R_{O_2} (mmol kg^{-1} h^{-1}), $p\mathrm{O}_{2\ \mathrm{pkg}}$ is the oxygen partial pressure inside packages (kPa) and K_{m,O_2} (apparent K_m) is the $p\mathrm{O}_{2\ \mathrm{pkg}}$ at half of $R_{O_2}^{max,T}$ (kPa).

A non-competitive CO_2 inhibition model (Peppelenbos and van't Leven, 1996) was also fitted to the data (Eq. (4)), to account for the possible effect of $p\mathrm{CO}_2$ on O_2 consumption (Kerbel et al., 1988):

$$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_{2\,pkg}}{\left(K_{m,O_2} + pO_{2\,pkg}\right) \times \left(1 + pCO_{2\,pkg} / K_{mn,CO_2}\right)} \quad (4)$$

where K_{mn,CO_2} is the Michaelis-Menten constant for the non-competitive CO_2 inhibition.

Estimation of $R_{O_2}^{\max,T}$ and K_{m,O_2}, based on experimental data, was performed by non-linear regression using the Levenberg-Marquardt method. The estimates obtained at each temperature were used to describe the temperature dependence of $R_{O_2}^{\max,T}$ and K_{m,O_2}.

In addition, the Michaelis-Menten models were fitted to the whole data set at all temperatures (n=129 in 2006 and n=154 in 2007). In both models, $R_{O_2}^{\max,T}$ was an exponential function of temperature, and K_{m,O_2} could be modelled as either an exponential function of temperature or a constant, whereas K_{mn,CO_2} was always a constant (Table 1). The predictions of respiratory parameters, $R_{O_2}^{\max,T}$ and K_{m,O_2}, at 0, 5, 10 and 15 ºC, generated by solving the equations depicted on Table 4, were used to calculate activation energies, through an Arrhenius plot (Eq. (5) and Eq. (6)), and the temperature coefficient (Q_{10}).

$$\ln\left(R_{O_2}^{\max,T}\right) = \ln\left(R_{O_2}^{\max,0}\right) - \frac{E_a}{R}\left(\frac{1}{T}\right) \quad (5)$$

$$\ln\left(K_{m,O_2}\right) = \ln\left(K_{m,O_2}^{0}\right) - \frac{E_a}{R}\left(\frac{1}{T}\right) \quad (6)$$

where $R_{O_2}^{\max,T}$ is the maximal value of R_{O_2} (mmol kg^{-1} h^{-1}), $R_{O_2}^{\max,0}$ and K_{m,O_2}^{0} are the pre-exponential factors, K_{m,O_2} (apparent K_m) is the $p\mathrm{O}_{2\ \mathrm{pkg}}$ at half of $R_{O_2}^{\max,T}$ (kPa), E_a is activation energy (kJ mol^{-1}), R is ideal gas constant (kJ mol^{-1} K^{-1}) and T is temperature (K).

The accuracy of parameters estimation was given by the ratio of the standard error to value estimate. The likelihood of the fit was ascertained by the root mean square error (RMSE) (Yang and Chinnan, 1988).

All statistical analyses were performed using the software package SPSS for Windows v.16.0 (SPSS, Chicago, IL).

3. Results and discussion

3.1 Film permeability

Permeability to O_2 and CO_2 and their temperature dependence are depicted in Figure (1). The predicted permselectivity ($\beta = P_{CO_2} / P_{O_2}$) at 0 ºC was 4.7 and slightly lower (4.4) at 20 ºC. These permselectivities are similar to previous published results by Joles et al. (1994) (4.8 at 20 ºC and 4.9 at 0 ºC) and by Beaudry et al. (1992) (4.4 at 25 ºC and 5.2 at 0 ºC). The activation energy for P_{O_2} was 36.8 kJ mol^{-1} (Table 2), a value similar to those reported elsewhere (Beaudry et al., 1992; Joles et al., 1994; Lakakul et al., 1999), but slightly below the 40-50 kJ mol^{-1} range claimed for LDPE films used in MAP (Cameron et al., 1995). Activation energies for P_{CO_2} were 34.5 kJ mol^{-1} (Table 2), which were essentially identical to the values 35.0 kJ mol^{-1} (Lakakul et al., 1999) and 35.5 kJ mol^{-1} (Beaudry et al., 1992; Joles et al., 1994).

MAP design requires knowledge of permeability coefficients in the range of temperatures found during processing and marketing. Film suppliers usually provide O_2 transmission rate determined at a single temperature (e.g. 23 ºC), often outside the temperature range in the cold chain. Using these values to predict O_2 concentration inside packages at 5 ºC, without correction of film O_2 permeability, would led to gross overestimation.

3.2 Effect of oxygen concentration and temperature on respiration rate

Fruit at the same maturity stage were harvested in two consecutive years and stored for 6 months or 5 weeks, in 2006 and 2007, respectively. Despite the differences in storage durations, respiratory parameters did not differ significantly between years, likely because fruit were processed and analyzed with similar maturities, as assessed by flesh firmness.

Gas equilibrium concentration inside packages was reached by 4-5 days at 15 ºC, 6-8 days at 10 ºC, 7-10 days at 5 ºC, and 10-11 days at 0 ºC. The oxygen uptake rate increased with $pO_{2\ pkg}$ in a manner consistent with saturation kinetics (Figure 2).

Respiration rates of fresh-cut 'Rocha' pear are shown in Table 3. These rates, expressed as CO_2 production and obtained between 0.1 and 18 kPa O_2, were similar to the range of value reported in air and in 2 kPa O_2 for other fresh-cut pear varieties at 0 or 10 ºC (Gorny et al., 2000).

The respiratory quotient (RQ) for aerobic respiration of fresh-cut 'Rocha' pear stored at various temperatures ranged between 1.2 and 1.4 (Table 3), consistent with the usage of organic acids as major respiratory substrates (Fonseca et al., 2002). RQ values reported in the literature for whole 'Bartlett' pear varied from 0.8 at the initial climacteric to 1.4 at fully ripe stage (Biale, 1950). RQ values pertaining to intact 'Conference' pear lie between 0.7 and 0.8 (de Wild and Peppelenbos, 2001; Lammertyn et al., 2003). The reported RQ for fresh-cut 'Conference' pear above 1.7 after one day following processing (Soliva-Fortuny et al., 2007) are likely an analytical artefact or else a clear sign of fermentation.

Respiratory quotients and ethanol levels responded similarly to steady-state oxygen partial pressure (pO_2) (Figure 3), and either variable may be used to determine the lower limit for aerobic respiration (Joles et al., 1994; Cameron et al., 1995). 'Fermentation threshold', the sudden increase in RQ or headspace ethanol concentration occurring when pO_2 dropped below a certain level, ranged between 0.3 and 0.9 kPa (Table 3). At elevated temperatures fermentation occurred at higher pO_2 (Beaudry et al., 1992; Lakakul et al., 1999), as observed for 5 up to 15 ºC (Table 3). Fermentation threshold of whole 'Bartlett' pears varied between 0.3 kPa and 1.7 kPa, at temperatures between 0 and 25 °C (Kader et al., 1989). Temperature has a small influence on CO_2 and O_2 diffusion in 'Conference' pear

as demonstrated by small activation energies obtained by Ho et al. (2006). Since the higher demand for O_2 at elevated temperatures cannot be compensated by diffusion, fermentation occurs at higher pO_2 (Beaudry et al., 1992).

Fresh-cut produce generally need lower O_2 levels than whole fruits (Beaudry, 2000), due to absence of skin or less cuticle that could restrict gas diffusion (Burg and Burg, 1965; Schotsmans et al., 2003; Ho et al., 2006). The minimum O_2 concentration tolerated by mature intact green 'Bartlett' pear at 25 ºC is 1.6 to 1.7 kPa, and by 'Passe Crassane' pear cell cultures is 1.1 to 1.3 kPa (Boersig et al., 1988). The lower oxygen limit reported for sliced pear is 0.5 kPa at 0-5 ºC (Beaudry, 2000; Gorny, 2001). Our results (Table 3) indicate that fresh-cut 'Rocha' pear should be packaged with pO_2 higher than 0.8 kPa at 0 ºC, 0.3 kPa at 5 ºC, 0.8 kPa at 10 ºC and 0.9 kPa at 15 ºC to avoid fermentation. Consistent with the fermentation thresholds reported herein for 'Rocha' pear, 2.5 kPa O_2 was considered acceptable for fresh-cut 'Conference' pear hold at 4 ºC, but 0 kPa O_2 induced visual tissue damage (Soliva-Fortuny et al., 2007).

3.3 Modelling the effect of oxygen and temperature on respiration rate

The respiratory parameters $R_{O_2}^{\max,T}$, K_{m,O_2} and K_{mn,CO_2} were estimated individually at each temperature, and their temperature-dependence subsequently studied with the models presented in Table 1.

Exponential functions gave the best fit of $R_{O_2}^{\max,T}$ in both experiments using the estimates of each temperature (R^2>0.98, not shown). The temperature dependence of $R_{O_2}^{\max,T}$ has been analyzed according to the Arrhenius' law (Hertog et al., 1998; Jacxsens et al., 2000; Lammertyn et al., 2001; Ho et al., 2008). The rise in maximal rate of O_2 uptake with temperature was equivalent to Q_{10} values of 6.5 to 7.8 for data obtained in 2007 (Table

4). Q_{10} values of 3.5 to 4.1 obtained in 2006 (not shown) are similar to those reported for other fresh-cut pear varieties (Gorny et al., 2000). Similarly Q_{10} of fresh-cut vegetables may range from 2.8 to 8.0 (Jacxsens et al., 2000). $E_{a,RO_2}{}^{maxT}$ of whole 'Conference' pear ranged from 64.6 kJ mol^{-1} (Lammertyn et al., 2001) to 80.2 kJ mol^{-1} (Ho et al., 2006); these values are only 48% to 86% of $E_{a,RO_2}{}^{maxT}$ found in this study for fresh-cut 'Rocha' pear (Table 4). Respiration activation energy of common intact produce range between 50 and 89 kJ mol^{-1} (Exama et al., 1993; Fonseca et al., 2002), and can be as high as 135.9 kJ mol^{-1} in fresh-cut vegetables (Jacxsens et al., 2000).

Hertog et al. (1998) examined the temperature dependence of apparent K_m according to the Arrhenius' law and concluded that this variable can be treated as a constant. Non-competitive models published for intact 'Conference' pear (de Wild et al., 1999; Lammertyn et al., 2001, 2003; Ho et al., 2006, 2008) did not make K_{m,O_2} and K_{mn,CO_2} temperature-dependent. A constant K_{m,O_2} was obtained for ten fresh-cut vegetables (Jacxsens et al., 2000) and packaged raspberry fruit for the temperature range of 0 to 20 ºC (Joles et al., 1994), while for blueberry a temperature effect on K_{m,O_2} was found (Song et al., 1992; Cameron et al., 1994). Ho et al. (2006) indicated for cortex tissue of 'Conference' pear a K_{m,O_2} of 1.16 kPa, similar to K_{m,O_2} found for fresh-cut 'Rocha' pear (Table 4). Cytochrome *c* oxidase, which is believed to be the rate determining enzyme in the respiratory pathways, has a high affinity to oxygen (K_{m,O_2} = 0.10-0.15 kPa) and its activity does not change during hypoxia, when pO_2<0.25 kPa (Nanos et al., 1994). However, diffusion limitations can lead to a much higher value of K_{m,O_2} in intact pear (6.2 kPa) than that in protoplasts (≈0.007 kPa), and even higher than in the case of mitochondrial respiration (Lammertyn et al., 2001). Differences in the reported values of K_m can be

accounted for by the joint effect of respiration at the cellular level and gas diffusion through the pear tissue and skin (Lammertyn et al., 2003).

The models in which there was a temperature dependence of K_{m,O_2} were only marginally better than those where K_{m,O_2} was constant (Table 4). Michaelis-Menten and non-competitive Michaelis-Menten models were able to explain more than 93% of the total variability of the data and either model accurately predicted the experimental results (RMSE<0.06). However, the non-competitive Michaelis-Menten model provided an estimate of K_{mn,CO_2} with low accuracy (Table 4). In addition, the K_{mn,CO_2} values were very high (14.3-42.0 kPa), indicating a low inhibitory effect of CO_2 on O_2 consumption (Ho et al., 2008).

To discriminate between different types of inhibition it is necessary to know O_2 consumption at two or more different CO_2 concentrations and at three O_2 concentrations (Peppelenbos and van't Leven, 1996). Since our data did not satisfy these requirements, it is not possible to assume an influence of CO_2 on O_2 consumption. However, given the accuracy of the simpler Michaelis-Menten model and the indications of minimal CO_2 influence, the use of simpler model is preferred in this case (Figure 2).

3.4 Respiratory responses to low oxygen and implications for MAP

Respiratory behavior as a function of oxygen concentration provides a basis to deduce the potential benefits of using MAP technologies in a given commodity. Low oxygen atmospheres could be useful if a sizeable reduction in respiration rate (e.g. 50%) can be reached without the induction of fermentation (Beaudry, 2000). In cases where K_{m,O_2} is much higher than fermentation threshold, a reduction in pO_2 slows down metabolic activities without an increase in the RQ and fermentative compounds. The range

of pO_2 between K_{m,O_2} and fermentation threshold is termed 'safe working atmosphere' (Beaudry, 2000). On the other hand, if K_{m,O_2} is lower than the fermentation threshold any attempt to reduce O_2 could lead to fermentation. Fresh-cut 'Rocha' pear had a very narrow (0.01 to 1.71 kPa) safe working atmosphere at the temperatures tested (Table 5). Low oxygen atmospheres did not offer any reduction in metabolic activity without the danger of inducing anaerobiosis, especially between 0 ºC and 10 ºC, temperatures normally found during storage and marketing. These data are consistent to the findings compiled by Gorny (2001), who also reported a poor efficacy of modified atmosphere for fresh-cut pear at 0-5 ºC.

In conclusion, respiration of fresh-cut 'Rocha' pear as function of oxygen concentration and temperature can be accurately predicted through Michaelis-Menten kinetics without inhibition by CO_2. These data provide a foundation whereby sealed packages can be designed to avoid fermentative metabolism of fresh-cut 'Rocha' pear. However, given the respiratory kinetics of fresh-cut 'Rocha' pear, it is anticipated that low-oxygen packaging is of little or no benefit in slowing down metabolism in this convenience produce.

Tables

Table 1. Models used to describe the effects of oxygen concentration and temperature on the respiration rate of fresh-cut 'Rocha' pear.

Model label [a]	**Model equation**	**Model parameters**		
		$R_{O_2}^{max,T}$	K_{m,O_2}	K_{mn,CO_2}
MM	$R_{O_2} = \frac{R_{O_2}^{max,T} \times pO_{2pkg}}{K_{m,O_2} + pO_{2pkg}}$	a × e $^{(b \times T)}$	q × e $^{(r \times T)}$	–
MM_k	$R_{O_2} = \frac{R_{O_2}^{max,T} \times pO_{2pkg}}{K_{m,O_2} + pO_{2pkg}}$	a × e $^{(b \times T)}$	Constant	–
MMNC	$R_{O_2} = \frac{R_{O_2}^{max,T} \times pO_{2pkg}}{\left(K_{m,O_2} + pO_{2pkg}\right) \times \left(1 + pCO_{2pkg} / K_{mn,CO_2}\right)}$	a × e $^{(b \times T)}$	q × e $^{(r \times T)}$	Constant
$MMNC_k$	$R_{O_2} = \frac{R_{O_2}^{max,T} \times pO_{2pkg}}{\left(K_{m,O_2} + pO_{2pkg}\right) \times \left(1 + pCO_{2pkg} / K_{mn,CO_2}\right)}$	a × e $^{(b \times T)}$	Constant	Constant

[a] MM: Michaelis-Menten equation (Eq. (3)); MMNC: Michaelis-Menten equation with non-competitive inhibition by CO_2 (Eq. (4)). MM_k and $MMNC_k$ have constant K_{m,O_2}.

Table 2. Equations used to predict permeability as a function of temperature (K) and activation energies for the films used in the experiments.

Predicting equation (mmol cm cm^{-2} h^{-1} kPa^{-1})	E_a (kJ mol^{-1})	R^2
P_{O_2} = 0.067 × e $^{[-4423 / T]}$	Ea_{O_2} = 36.8	0.930
P_{CO_2} = 0.118 × e $^{[-4153 / T)]}$	Ea_{CO_2} = 34.5	0.923

Table 3. Experimental rates of O_2 uptake and CO_2 production, respiratory quotient ($RQ=R_{CO_2}/R_{O_2}$) and fermentation threshold (FT) for fresh-cut 'Rocha' pear stored at various temperatures.

Temperature (ºC)	R_{O_2} range (mmol kg^{-1} h^{-1})	R_{CO_2} range (mmol kg^{-1} h^{-1})	RQ [a]	FT (kPa)
0	0.02-0.06	0.04-0.08	1.37±0.02	0.75
5	0.03 -0.18	0.07-0.22	1.38±0.03	0.25
10	0.04-0.49	0.13-0.60	1.26±0.02	0.75
15	0.09-0.95	0.23-1.19	1.18±0.01	0.88

[a] RQ are the mean (±SE) of the experimental values measured at oxygen concentrations ranging from 18 kPa to the fermentation threshold (Figure 3).

Table 4. Models predictions of $R_{O_2}^{\max,T}$, K_{m,O_2} and K_{mn,CO_2} as a function of temperature (K) and models corresponding accuracies.

Model [a]	$R_{O_2}^{max,T}$ (mmol kg^{-1} h^{-1})	K_{m,O_2} (kPa)	K_{mn,CO_2} [b] (kPa)	R^2	RMSE	Q_{10} $R_{O_2}^{maxT}$	E_a [c] $R_{O_2}^{maxT}$	E_a [c] K_{m,O_2}
MM	$2.40\times10^{-26}\times e^{0.205T}$	$4.26\times10^{-30}\times e^{0.238T}$		0.963	0.043	7.8	134.2	155.8
MM_k	$3.84\times10^{-24}\times e^{0.187T}$	2.07		0.927	0.057	6.5	122.4	_
MMNC	$1.38\times10^{-24}\times e^{0.191T}$	$1.28\times10^{-30}\times e^{0.242T}$	41.99 (77%)	0.966	0.039	6.8	125.0	158.4
$MMNC_k$	$2.43\times10^{-19}\times e^{0.149T}$	1.27	14.26 (42%)	0.948	0.048	4.4	97.5	_

[a] MM - Michaelis Menten model; MMNC - Michaelis Menten model with non-competitive inhibition by CO_2.

[b] Values within parenthesis indicate parameter accuracy (ratio of standard error to estimated value).

[c] Activation energies (E_a) of respiratory parameters were calculated using Arrhenius plots (Eq. (5) and Eq. (6)).

Table 5. Michaelis-Menten model (MM) respiratory parameters for fresh-cut 'Rocha' pear stored at various temperatures.

Temperature (ºC)	**Fermentation threshold (kPa)**	K_{m,O_2} **(kPa)**	**Safe working atmosphere (kPa)**
0	0.75	0.07	No
5	0.25	0.24	No
10	0.75	0.79	0.04
15	0.88	2.59	1.71

Figures

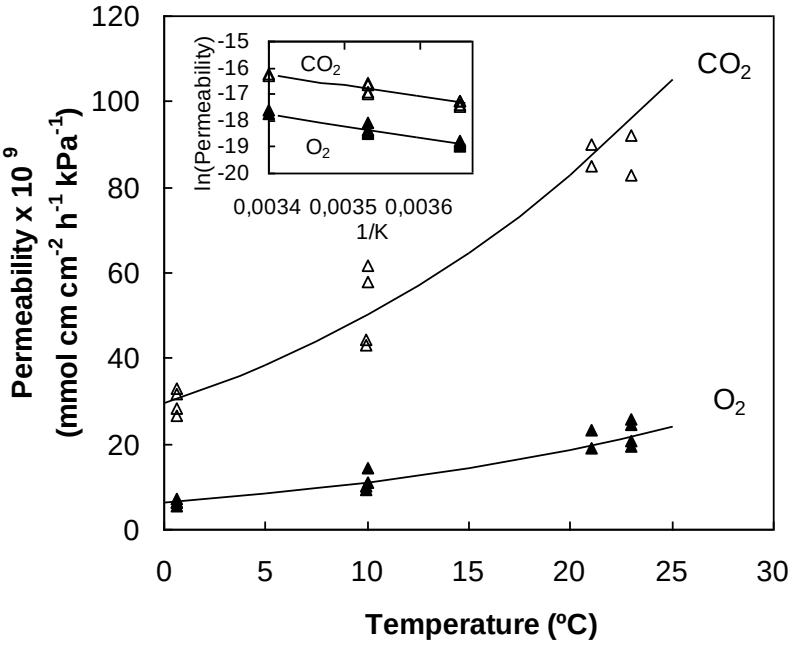

Figure 1. Effect of temperature on film permeability to O_2 (solid symbols) and CO_2 (open symbols). Lines based on predicting equations presented in Table 2. Inset: Arrhenius plot of gases permeabilities.

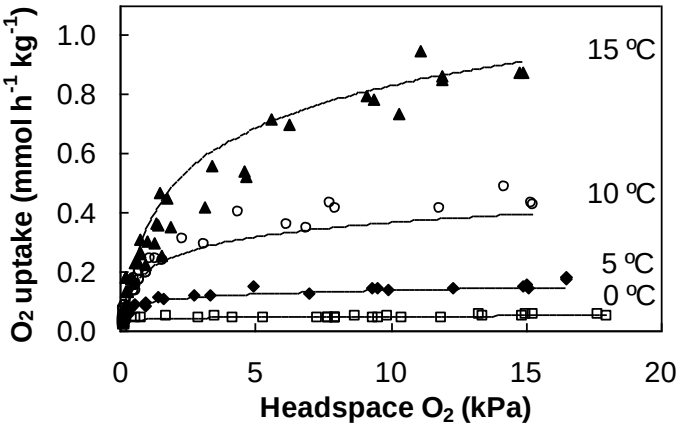

Figure 2. Effect of steady-state O_2 partial pressure and storage temperature on the rate of O_2 uptake of 'Rocha' pear slices in sealed LDPE packages at 0 ºC (□), 5 ºC (♦), 10 ºC (o) and 15 ºC (▲) fitted with a Michaelis-Menten model.

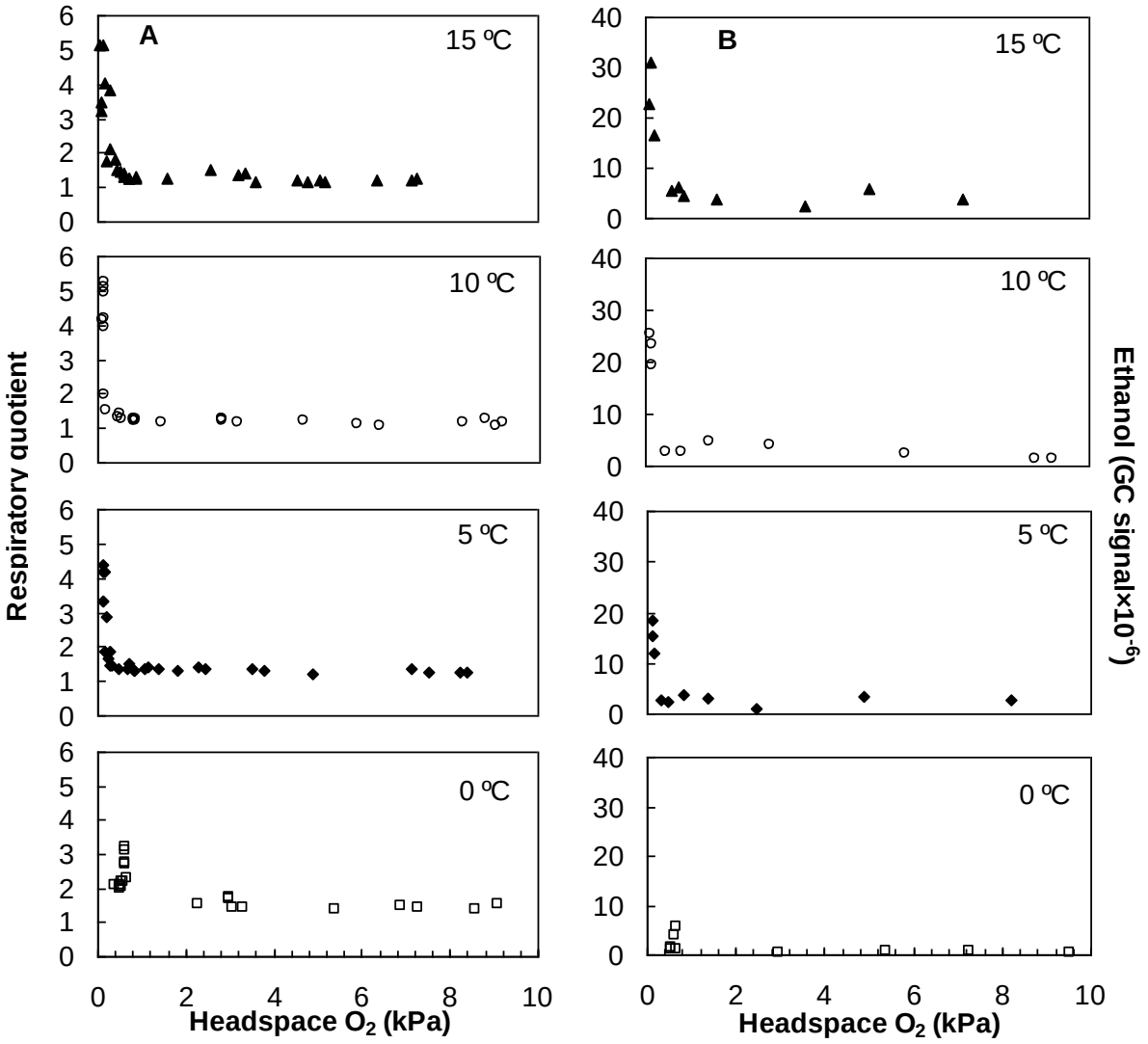

Figure 3. Respiratory quotient (A) and headspace ethanol (B) as a function of steady-state O_2 of packaged fresh-cut 'Rocha' pear.

CHAPTER 2

2.2 FRESH-CUT CANTALOUPE MELON

Part of this chapter was published as:

Gomes, M.H., Beaudry, R.M., Almeida, D.P.F. and Malcata, F.X. 2008. Respiration of fresh-cut cantaloupe melon fruit as a function of package oxygen concentration and temperature. In Avances en maduración y post-recolección de frutas y hortalizas. Oria, R., Val, J. and Ferrer, A. (Eds). P. 616−622. Editorial Acribia. Zaragoza, Espanha. ISBN: 978-84-200-111-0.

Abstract

The aim of this work was to characterize the respiratory behaviour of fresh-cut melon under modified-atmosphere packaging at various temperatures to generate useful information for development of appropriate packaging conditions. Cantaloupe melon (*Cucumis melo* var. *cantalupensis* cv. Olympic Gold) cubes were packaged and stored at 0, 5, 10 and 15 ºC. Packages attained gas equilibrium after 5 days at 10 ºC, 6 days at 5 ºC and 10 days at 0 ºC. In cubes stored at 15 ºC decay started before steady-state gas levels were reached. Respiration rates were measured and respiratory quotient calculated once steady-state O_2 and CO_2 partial pressures were achieved inside the packages. O_2 uptake increased with temperature and oxygen partial pressure ($pO_{2\ pkg}$), according to a Michaelis-Menten kinetics described by $R_{O_2} = [(R_{O_2}^{max,T} \times pO_{2\ pkg}) / (K_{m,O_2} + pO_{2\ pkg})]$. Respiratory parameters were modelled as an exponential function of temperature: $R_{O_2} = [(1.34\times10^{-17} \times e^{(0.131\times T)} \times pO_{2\ pkg}) / (1.15\times10^{-24} \times e^{(0.193\times T)} + pO_{2\ pkg})]$ (R^2=0.95), Q_{10} = 3.7 and $E_{a\ R_{O_2}^{max,T}}$ = 84 kJ mol^{-1}. A good fit to the experimental data was also obtained considering K_{m,O_2} as constant: $R_{O_2} = [(4.36\times10^{-14} \times e^{(0.102\times T)} \times pO_{2\ pkg}) / (0.358 + pO_{2\ pkg})]$ (R^2=0.93), Q_{10} = 2.8 and $E_{a\ R_{O_2}^{max,T}}$ = 66 kJ mol^{-1}. These results provide fundamental information to predict package permeability and steady-state $pO_{2\ pkg}$ required to prevent anaerobic conditions during shelf-life of fresh-cut cantaloupe. The kinetics of respiration as a function of pO_2 suggests that no significant reductions in respiration rate of fresh-cut cantaloupe can be achieved by lowering O_2 levels.

1. Introduction

Melons are large fruit whose preparation requires slicing and disposal of the rind and seeds. Therefore, convenience of consumption is valued in this fruit and, not surprisingly, fresh-cut melons account for a major part of the growing fresh-cut fruit market (Offner, 2011). Fresh-cut processing invariably involves tissue wounding with the concomitant healing response. Wound response in plant tissues is mediated by ethylene and often involves increased respiration. Enhancement of respiration rate after cutting of cantaloupe mesocarp has been documented (McGlasson and Pratt, 1964) although the steady-state respiration rates of cut melon pieces can be similar to those of whole fruit under refrigeration (Watada et al., 1996; Aguayo et al., 2004).

Respiration rates have been reported for fresh-cut melons (cantaloupe and others cultivar types) at various temperatures (Watada et al., 1996; Gorny, 1998; Aguayo et al., 2004), and were predicted under non-equilibrium gases concentrations for an inodorus-type melon ('Piel de Sapo') stored at 4 ºC, with initial oxygen partial pressures of 2.5, 21, and 70 kPa (Oms-Oliu et al., 2008a). The oxygen consumption and CO_2 production were calculated using differential mass balance equations (the first derivative of oxygen partial pressure inside packages as function of time is incorporated in equations 1 and 2, point 2.3), where the O_2 and CO_2 concentrations on the headspace were fitted with a Weibull model and a logistic model, respectively (Oms-Oliu et al., 2008a). During the first ten days of storage at 4 ºC the respiration rates of cut 'Piel de Sapo' ranged from 0.13 mmol CO_2 kg^{-1} h^{-1} to 0.83 mmol CO_2 kg^{-1} h^{-1} (Oms-Oliu et al., 2008a), similar to the values reported by Gorny (1998). For the same storage period at 5 ºC, Aguayo et al. (2004) measured respiration rates of 0.16 to 0.25 mmol CO_2 kg^{-1} h^{-1} for both cantaloupe and inodorus melons, values 1.5 to 2 times higher than those obtained at 0 ºC.

Modified atmosphere packaging (MAP) often complements refrigeration as an additional hurdle to help maintaining the quality and food safety of fresh-cut fruit. The practical benefits of MAP are considered relevant for fresh-cut cantaloupe, with favorable gas concentrations ranging from 3 to 5 kPa O_2 and 6 to 15 kPa CO_2 (Gorny, 1998). Recommended gas compositions for MAP of fresh-cut produce in general and fresh-cut melon in particular, have been established based on few published references (Gorny, 1998) and on experiments with a limited number of combinations of O_2 and CO_2 concentrations (Bai et al., 2001; Oms-Oliu et al., 2007a). Optimal packaging geometry and film permeability to achieve the target gas levels can be deducted from the respiration rates (Lakakul et al., 1999; Jacxsens et al., 2000). However, despite the benefits observed in the few reports on MAP of fresh-cut melon (Bai et al., 2001, 2003; Oms-Oliu et al., 2007a, 2008), an observation of the European and American markets reveals that most operators do not aim at optimizing gas compositions inside the packages given that anaerobiosis is prevented. The discrepancy between the potential benefits of optimal MAP reported in the literature and the apparent lack of adoption of this knowledge by the industry may be due to deficient knowledge transfer. Alternatively, the putative benefits of optimal MAP in fresh-cut melon fail to materialize in actual supply-chains, and the efforts to achieve optimal MAP conditions have little or no economic benefit. Physiological limitations to the reduction of respiration rate by MAP must also be considered.

The reduction in respiration by MAP depends on the kinetics of the respiration rate as a function of O_2 partial pressure at given temperatures. In whole fruit, significant differences in the kinetic parameters are found among species (Hertog et al., 1999; Beaudry, 2000). Postharvest treatment of apple with the ethylene action inhibitor 1-methylcyclopropene, inducing changes in the ripening stage, significantly alters the kinetic parameters of respiration as a function of O_2 substrate (Beaudry, 2000). The kinetics of

respiration vs. O_2 concentration in fresh-cut 'Rocha' pear suggests that no significant reduction in respiration rate can be achieved via MAP (Gomes et al., 2010a); consistent with this physiological limitation, no significant improvement of metabolism-dependent quality parameters was observed in fresh-cut pear under various MAP conditions (Chapter 5). Therefore, the fundamental knowledge of respiratory parameters as affected by O_2 partial pressure is essential to establish the physiological limits of the tissue, to predict the benefits of low-oxygen, and, eventually, to design packages aimed at targeted atmosphere conditions.

The objective of this study was to determine the effect of steady-state oxygen concentration on the respiration kinetics of fresh-cut cantaloupe at various temperatures, to provide detailed information to assess the benefits of MAP and assist in the design of adequate packages for this convenient produce.

2. Material and methods

2.1 Plant material and processing conditions

Orange-fleshed cantaloupe melon (*Cucumis melo* L. subsp. *melo* var. *cantalupensis* Naud. cv. Olympic Gold) fruit with an average weight of 2.5±0.2 kg and 11.7±1.5% (w/w) soluble solids were used in the experiments.

Whole fruit were rinsed with tap water, sanitized with 150 µL L^{-1} NaClO for 2 min, and air-dried. The rind was removed by hand with a sharp knife and the flesh was cut into trapezoidal sections *ca*. 2 × 2.5 cm wide.

2.2 Packaging and storage conditions

Melon pieces were placed in perforated polyethylene terephtalate (PET) clamshells of 964.5×10^{-6} m^3 and packed inside low-density polyethylene (LDPE, Dow Chemical Company, Midland, MI, USA) pouches (18.5 × 19.0 cm, or 18.5 × 19.5 cm), which were hermetically sealed using a heat sealer. Several combinations of film surface area and film thickness, and a range of tissue masses varying from 0.04 to 0.36 kg per pouch, were used to assure a wide series of steady-state gas concentrations within the packages. The film thickness used in the experiments ranged from 28.1×10^{-4} to 77.7×10^{-4} cm, and film permeabilities (Table 1) were calculated as described in Gomes et al. (2010a). Three replicates of each combination of film thickness, film area and fruit mass were stored at 0, 5, 10, and 15 ºC.

2.3 Determination of respiration rate

The gas composition of the headspace in individual packages was monitored daily until steady-state was reached. Gas was withdrawn from packages via a silicon sampling septum (Gomes et al., 2010a). Gases were measured using a paramagnetic O_2 detector (Series 1100, Servomex Co., Sussex, UK) and an infrared CO_2 detector (ADC 255-MK3, Analytical Development Co., Hoddesdon, UK) connected in series. Rates of O_2 uptake (R_{O_2}) and CO_2 (R_{CO_2}) production were calculated from Eq. 1 and Eq. 2 using steady-state O_2 and CO_2 partial pressures, package permeability and fruit weight (Beaudry et al., 1992; Lakakul et al., 1999). Respiratory quotient ($RQ = R_{CO_2} / R_{O_2}$) was computed from the calculated respiration rates.

$$R_{O_2} = \frac{\frac{P_{O_2} \times A}{l} \times \left(pO_{2atm} - pO_{2pkg} \right)}{\mathrm{M}} \quad (1)$$

$$R_{CO_2} = \frac{\frac{P_{CO_2} \times A}{l} \times \left(pCO_{2pkg} - pCO_{2atm} \right)}{\mathrm{M}} \quad (2)$$

where R_{O_2} and R_{CO_2} are respiration rates (mmol kg^{-1} h^{-1}) for O_2 and CO_2, P_{O_2} and P_{CO_2} are the O_2 and CO_2 permeabilities (mmol cm cm^{-2} h^{-1} kPa^{-1}), A is the film area (cm^2), l is the film thickness (cm), $pO_{2\ atm}$, $pO_{2\ pkg}$, $pCO_{2\ atm}$ and $pCO_{2\ pkg}$ are O_2 and CO_2 partial pressure (kPa) outside ($_{atm}$) and inside ($_{pkg}$) the package, and M is the fruit mass (kg).

2.4 Experimental data modelling

Respiration rate (R_{O_2}) was described as a function of O_2 partial pressure by a Michaelis-Menten model (Eq. 3), as described by Lee et al. (1991).

$$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_{2pkg}}{K_{m,O_2} + pO_{2pkg}} \quad (3)$$

where $R_{O_2}^{\max,T}$ is the maximal rate of R_{O_2} (mmol kg^{-1} h^{-1}), $pO_{2\ pkg}$ is the oxygen partial pressure inside packages (kPa) and K_{m,O_2} is $pO_{2\ pkg}$ at half the value of $R_{O_2}^{\max,T}$ (kPa).

The Michaelis-Menten model was adjusted to the data from temperatures between 0 ºC and 10 ºC (n=66), but not at 15 ºC since fruit started to decay before steady-state gas

levels were reached. The maximal respiration rate was found to be an exponential function of temperature ($R_{O_2}^{max,T}$ = a × e $^{(b \times T)}$), and the Michaelis-Menten constant was modelled as either an exponential function of temperature (K_{m,O_2} = q × e $^{(r \times T)}$) or a constant. Parameter estimates were obtained by non-linear regression, using the Levenberg-Marquardt method. The model predictions of respiratory parameters (Table 3) were computed at 0, 5, and 10 ºC, and were used to calculate activation energies, through an Arrhenius plot (Gomes et al., 2010a), and the temperature coefficient (Q_{10}).

Accuracy of the parameters estimates was calculated as the ratio between standard error and estimated value. Differences between the simulated and the experimental results were ascertained by the root mean square error (RMSE) (Yang and Chinnan, 1988).

All statistical analyses were performed using the software package SPSS for Windows v.16.0 (SPSS, Chicago, IL, USA).

3. Results and discussion

3.1 Effect of oxygen concentration and temperature on respiration rate

Packages with fresh-cut melon attained gas equilibrium after 5 days at 10 ºC, 6 days at 5 ºC and 10 days at 0 ºC, but steady-state was not reached at 15 ºC before the tissue started to decay. Respiration rates (R_{O_2} and R_{CO_2}) experimentally determined between 0.1 and 16.2 kPa O_2 (Table 2) are within the range reported in the literature for fresh-cut cantaloupe (Gorny, 1998; Aguayo et al., 2004). Respiration rates of fresh-cut cantaloupe melon reported at 0 ºC, 5 ºC, 10 ºC and 20 ºC are 0.09-0.36, 0.13-0.71, 0.27 and 1.34 mmol CO_2 kg^{-1} h^{-1}, respectively (Gorny, 1998).

RQ of fresh-cut cantaloupe melon stored at various temperatures ranged from 1.6 at 0 ºC to 3.1 at 15 ºC (Table 2, Fig. 1). The RQ values observed are higher than the theoretical value of 1.3 for the oxidation of organic acids (Kader et al., 1989). The higher RQ values obtained in our study at suboptimal temperatures are likely a sign of slight fermentation at steady-state oxygen concentrations. This is not uncommon in fresh-cut melons, where the loss of malic acid at 20 ºC can be related to malo-lactic fermentation by the lactic acid bacteria (Lamikanra et al., 2000). Also, in fresh-cut 'Piel de Sapo' melon stored at 4 ºC under 21 kPa O_2 and 2.5 kPa O_2 + 7 kPa CO_2, the RQ increases during storage in association with ethanol production (Oms-Oliu et al., 2008a). In addition, the raise in CO_2 production observed in fresh-cut honeydew after day 3 at 10 ºC and day 6 at 5 ºC was attributed to tissue deterioration (Qi et al., 1999).

Fermentation occurred at higher pO_2 as the storage temperature increased from 0 to 10 ºC (Table 2; Fig. 1), consistent with previous observations in other fruit (Beaudry et al., 1992; Lakakul et al., 1999). To avoid anaerobic respiration in honeydew melon, pO_2 must be increased from 2 kPa O_2 at 5 ºC to 4 kPa O_2 at 10 ºC (Qi et al., 1999). Our results indicate that fresh-cut cantaloupe melon should be packaged with pO_2 higher than 0.7 kPa at 0 ºC, and 1.3 kPa at 5 ºC and 10 ºC to prevent fermentation. At such oxygen concentrations, carbon dioxide levels were 7.6, 11.3, and 14.0 kPa at 0, 5, and 10 ºC (data not shown), respectively. Packaging atmospheres commonly considered beneficial to the quality of cantaloupe cubes require higher levels of O_2, usually around 3-5 kPa O_2 combined with 6-15 kPa CO_2 (Gorny, 1998). Similar gas mixtures have been tested by other authors with fresh-cut cantaloupe or honeydew melon, e.g., 4 kPa O_2 + 10 kPa CO_2 (Bai et al., 2001), 5 kPa O_2 + 5 kPa CO_2 (Bai et al., 2003), 2.5 kPa O_2 + 7 kPa CO_2 (Oms-Oliu et al., 2007a), 2-4 kPa O_2 + 10 kPa CO_2 (Qi et al., 1999).

3.2 Modelling the effect of oxygen concentration and temperature on respiration rate

Oxygen uptake rate increased with temperature and $p\mathrm{O}_{2\mathrm{pkg}}$ in a way consistent with Michaelis-Menten kinetics (Fig. 1). Respiratory parameters were modelled as exponential functions of temperature (Cameron et al., 1994). Alternatively, K_{m,O_2} was treated as constant coupled with $R_{O_2}^{\max,T}$ as an exponential function of temperature (Hertog et al., 1998; Jacxsens et al., 2000). These models provided a good fit to the experimental data (Fig. 1) as assessed by the RMSE (Table 3). The fit was slightly poorer (RMSE=0.013) when K_{m,O_2} was taken as constant ($\mathrm{MM_k}$), and a deviation from experimental data was observed at high $\mathrm{O_2}$ partial pressures at 10 ºC (Fig. 1).

Maximal $\mathrm{O_2}$ uptake rate is highly dependent on the temperature. The activation energy was estimated at 66 to 84 kJ $\mathrm{mol^{-1}}$ (Table 3) and the Q_{10} was 2.8 to 3.7 (Table 3). Q_{10} values of 3.3-3.6 for cubes of muskmelon and honeydew melons have been reported in the interval 0-10 ºC (Watada et al., 1996) and a Q_{10} of 3.1 can be computed for whole cantaloupe melon from the data compiled by Exama et al. (1993). Activation energy of intact produce generally range from 50 to 89 kJ $\mathrm{mol^{-1}}$ (Exama et al., 1993; Fonseca et al., 2002), and is often higher for fresh-cut produce (Jacxsens et al., 2000; Gomes et al., 2010a).

3.3 Respiratory responses to low oxygen and implications for modified atmosphere packaging

A positive difference between K_{m,O_2} for $\mathrm{O_2}$ uptake and the fermentation threshold, named ‘safe working atmosphere’ (Beaudry, 2000), can be taken as an indicator that significant decreases in metabolic activity can be obtained via the reduction in $p\mathrm{O_2}$. To

reduce the respiration rate of fresh-cut cantaloupe by 50% pO_2 must be <0.6 kPa, values lower than the fermentation threshold of 0.7 kPa at 0 ºC (Table 4; Fig. 1). Therefore, a safe working atmosphere does not exist for fresh-cut cantaloupe, and limited extension of shelf-life is anticipated via the reduction of pO_2. A similar response was found in packed strawberry fruits (Hertog et al., 1999) and fresh-cut 'Rocha' pear (Gomes et al., 2010a) suggesting that low-oxygen MAP provides little or no benefit to these commodities.

The potential benefits of MAP reported for fresh-cut cantaloupe (Gorny, 1998) are likely not due to the low O_2 levels but can be explained by the high pCO_2, reduced water loss, and lower microbial contamination due to the package barrier.

In conclusion, the respiratory parameters reported herein suggest that there is a physiological limitation to the extension of shelf-life of fresh-cut cantaloupe via the reduction of pO_2, justifying the limited attention of the industry operators to the optimization of MAP for pO_2, as long as the tissue is under aerobic conditions. However, further investigation is needed to clarify the interaction between CO_2 and O_2 levels and to validate the hypothesis proposed.

Tables

Table 1. Film permeability to O_2 and CO_2 at various temperatures.

Temperature (ºC)	**Permeability to O_2** (mmol cm cm^{-2} h^{-1} kPa^{-1})	**Permeability to CO_2** (mmol cm cm^{-2} h^{-1} kPa^{-1})
0	6.23×10^{-09}	2.94×10^{-08}
5	8.33×10^{-09}	3.86×10^{-08}
10	1.10×10^{-08}	5.03×10^{-08}
15	1.45×10^{-08}	6.48×10^{-08}

Table 2. Experimental rates of O_2 uptake and CO_2 production, respiratory quotient and fermentation threshold for packaged fresh-cut cantaloupe melon stored at various temperatures.

Temperature (ºC)	**R_{O_2} range (mmol kg^{-1} h^{-1})**	**R_{CO_2} range (mmol kg^{-1} h^{-1})**	**Respiratory quotient** [a]	**Fermentation threshold (kPa)**
0	0.03-0.07	0.07-0.12	1.63±0.03	0.74
5	0.04-0.11	0.13-0.20	1.94±0.07	1.33
10	0.06-0.19	0.26-0.43	2.43±0.11	1.35
15	0.09-0.80	0.62-2.39	3.12±0.05	1.07

[a] RQ are the mean ±SE of the experimental values measured at oxygen concentrations ranging from 16.2 kPa to the fermentation threshold (Fig. 1).

Table 3. Model best estimates of $R_{O_2}^{max,T}$ and K_{m,O_2} for fresh-cut cantaloupe melon as a function of temperature (K) and corresponding model accuracies.

Model [a]	$R_{O_2}^{max,T}$ **(mmol kg^{-1} h^{-1})**	K_{m,O_2} **(kPa)**	R^2	**RMSE** [b]	Q_{10}	$E_{a\,R_{O_2}^{max,T}}$ [c] **(kJ mol^{-1})**	$E_{a\,K_{m,O_2}}$ [c] **(kJ mol^{-1})**
MM	$1.34\times10^{-17}\ e^{0.131\times T}$	$1.15\times10^{-24}\ e^{0.193\times T}$	0.949	0.011	3.7	84.3	124.2
MM_k	$4.36\times10^{-14}\ e^{0.102\times T}$	0.358	0.929	0.013	2.8	65.6	-

[a] MM – Michaelis-Menten model with variable K_{m,O_2}; MM_k – Michaelis-Menten model with constant K_{m,O_2}.

[b] RMSE between the experimental (exp) and computed (comp) values were calculated using the equation: $\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left[(\text{exp})^{i} - (\text{comp})^{i}\right]^2}$, n = 66.

[c] Activation energies (E_a) of respiratory parameters calculated using Arrhenius plots (the logarithm of $R_{O_2}^{max,T}$ or K_{m,O_2} was plotted against the inverse of temperature).

Table 4. Parameter best estimates for fresh-cut cantaloupe melon stored at various temperatures.

Temperature (ºC)	**Fermentation threshold** (kPa)	**MM**		**MM_k**	
		K_{m,O_2} (kPa)	**Safe working atmosphere** (kPa)	K_{m,O_2} (kPa)	**Safe working atmosphere** (kPa)
0	0.735	0.090	**No**	0.358	**No**
5	1.330	0.236	**No**	0.358	**No**
10	1.346	0.620	**No**	0.358	**No**

MM – Michaelis-Menten model with variable K_{m,O_2}; MM_k – Michaelis-Menten model with constant K_{m,O_2}.

Figures

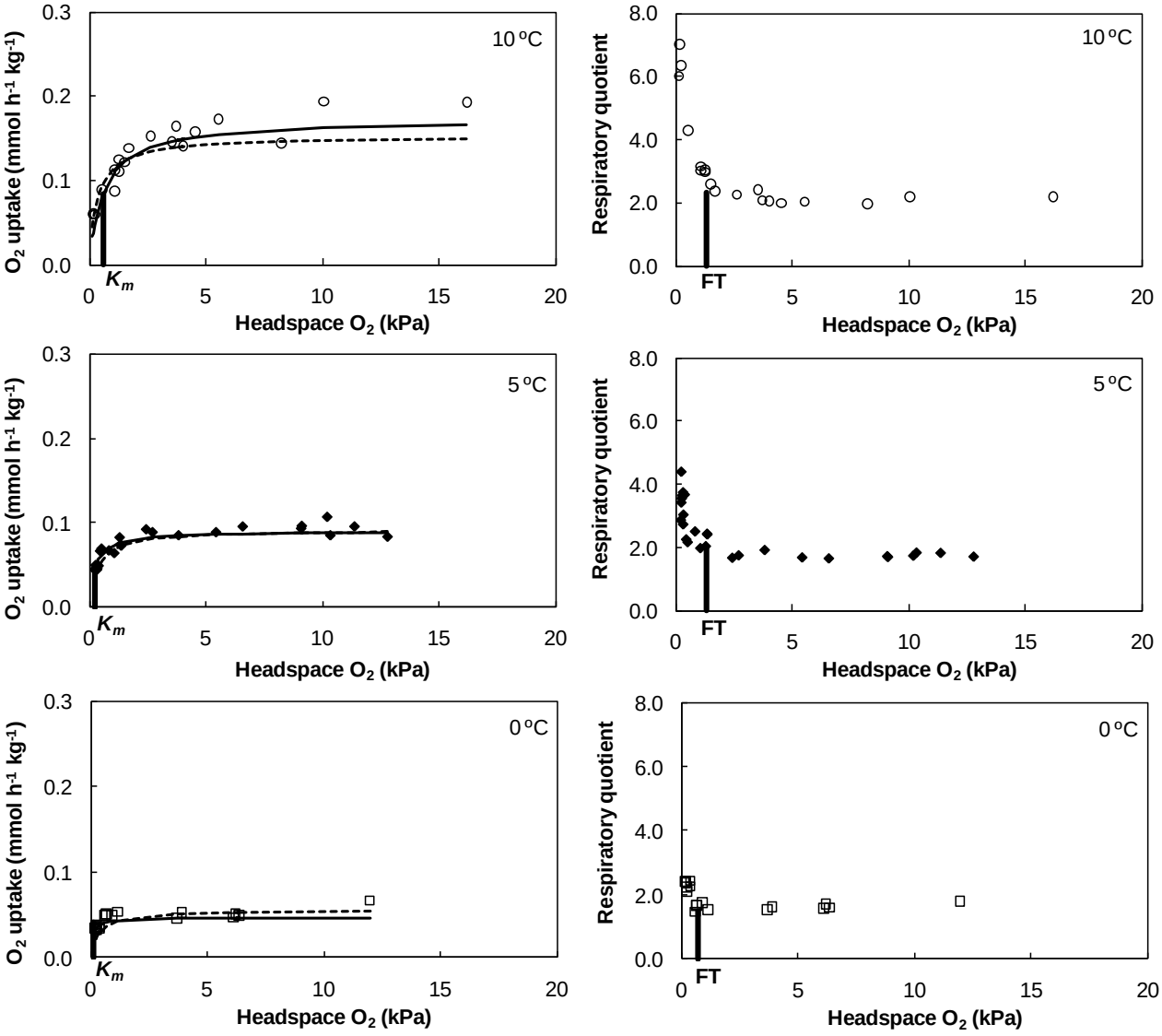

Figure 1. Effect of steady-state O_2 partial pressure and storage temperature on the rate of O_2 uptake (left) by cantaloupe pieces in sealed packages and corresponding respiratory quotient (right). On the left, solid lines represent the MM model fit, while dashed lines represent the MM_k model fit. The oxygen partial pressures at which respiration reaches half its maximum (apparent K_m) and the fermentation threshold (FT) are indicated with a vertical bar.

CHAPTER 3

EFFECTS OF HYDROGEN ION CONCENTRATION AND CALCIUM ADDITIVES ON THE QUALITY RETENTION OF FRESH-CUT 'ROCHA' PEAR

Published as:

Gomes, M.H., Fundo, J.F., Santos, S., Amaro, A.L. and Almeida, D.P.F. 2010. Hydrogen ion concentration affects quality retention and modifies the effect of calcium additives on fresh-cut 'Rocha' pear. Postharvest Biology and Technology 58: 239−246.

Abstract

'Rocha' pear (*Pyrus communis* L.) was used as a model system to assess the effect of pH of dipping solutions on quality retention of fresh-cut fruit and its interaction with calcium additives. Pear slices were dipped for 60 s in a buffer solution at pH 3.0, 5.0 or 7.0 and stored at 4.5 ºC for 13 days. In other experiments, pear slices were dipped for 60 s in buffer solutions containing 250 mM of calcium ascorbate, lactate, chloride, and propionate, at pH 3.0 or 7.0, and stored at 4.5 ºC for 6 days. Browning and softening were more intense in slices dipped in a solution at pH 3.0 than at pH 5.0 or 7.0, but microbial growth was lower in slices treated at pH 3.0. The effect of calcium additives depended on the anion and significant interactions between the effects of calcium salt and pH were observed. Calcium ascorbate was very effective in preserving color and reducing microbial growth irrespective of pH, but enhanced pectin solubilization and tissue softening at pH 3.0. Slices treated with 250 mM calcium propionate or calcium lactate were softer and had higher electrolyte efflux when treated at pH 3.0 than at pH 7.0. Calcium lactate enhanced browning and reduced microbial growth at pH 3.0 but did not affect color or microbial counts at pH 7.0. All calcium treatments enhanced electrolyte leakage. pH of the dipping solution can affect, *per se*, the quality of fresh-cut fruit. The choice of calcium additives to prevent undesirable changes on visual and sensorial quality of cut produce should consider the pH ranges that provide the expected benefits.

1. Introduction

Fresh-cut produce are very perishable even when processed and marketed with the best technology available. Preservation of fresh-cut fruit resorts to refrigeration and modified atmosphere packaging to reduce metabolic rate, water loss, and microbial growth (Lamikanra, 2002; Farber et al., 2003). Additional hurdles, such as acid additives to lower

the pH, are often used to reduce microbial growth (Karaibrahimoglu et al., 2004; Simón et al., 2010). In some fruit and vegetables, additives are also required to improve firmness and to prevent enzymic browning (Toivonen and Brummell, 2008).

Textural properties, such as firmness and juice retention, are very important to the appearance and flavor perception of fresh-cut fruit. A few water-soluble calcium salts (ascorbate, chloride, lactate, propionate, and gluconate), and even the less soluble calcium carbonate, have been successfully used to reduce softening in fresh-cut fruit (Dong et al., 2000; Luna-Guzmán and Barrett, 2000; Saftner et al., 2003; Aguayo et al., 2008). Calcium enhances the mechanical strength of the cell wall (Mignani et al., 1995) and reduces the autolytic release of pectins from cell walls (Wehr et al., 2004) by binding to the negative charges of demethylated galacturonic residues of pectins (Toivonen and Brummell, 2008). In addition, calcium can stabilize cell membranes by delaying galactolipid breakdown (Picchioni et al., 1995). Calcium additives are also thought to inhibit endopolygalacturonase (PG; EC 3.2.1.15) activity (Poovaiah, 1986), although evidence suggests that the effect of calcium is not on the catalytic activity of PG but, instead, on the solubilization of the products of PG-mediated hydrolysis (Almeida and Huber, 2007).

Enzymic browning is a major limiting factor of shelf-life on some fresh-cut fruit and vegetables. Tissue browning following wounding is a result of oxidative reactions mediated by polyphenoloxidase (PPO; EC 1.14.18.1). Phenolic compounds oxidized by PPO into *o*-quinones polymerize leading to the formation of brown pigments (Martinez and Whitaker, 1995). Several categories of additives are used to prevent or reduce enzymic browning in susceptible produce, including carboxylic acids (e.g., citric acid), chelators, reductants (e.g., ascorbate), thiol-containing compounds (e.g., cysteine, glutathione) or specific enzyme inhibitors such as 4-hexylresorcinol (Martinez and Whitaker, 1995; Arias et al., 2007).

The use of additives is likely to alter, intentionally or unintentionally, the surface pH of fresh-cut produce. pH is a measure of hydrogen ion concentration, a chemical variable that interferes with many quality attributes of fresh-cut fruit: it affects PPO activity (Siddiq et al., 1994; Arias et al., 2007), modulates cell wall metabolism and texture (Knee, 1982; Chun and Huber, 1998; Gorny et al., 2002; Pinheiro and Almeida, 2008), and interferes with microbial growth (Bhagwat et al, 2004; Karaibrahimoglu et al., 2004). Despite the effects of pH *per se* and the interaction between pH and calcium additives on several quality attributes of fresh-cut fruit (Ponting et al., 1971; Pinheiro and Almeida, 2008), these remain largely ignored in the literature. The pH of the coatings and dipping solutions used to vehicle the additives is often not reported by authors and the role of pH is seldom recognized as an explanatory variable for the effects of dipping or coating treatments.

This study was designed to evaluate the effect of pH *per se* and its interaction with calcium additives on quality attributes of fresh-cut pear. Treatments were applied by dipping according to the standard practice in fresh-cut industrial processing. Pear was chosen as a model fruit due to its susceptibility to browning and softening.

2. Materials and methods

2.1 Plant material

Pear (*Pyrus communis* L. 'Rocha') fruit were harvested at commercial maturity from an orchard in the Oeste Region, Portugal, selected by hand for uniform size, washed and stored at -0.5 ºC, 90-92% relative humidity. All the fruit used in the experiments were drenched prior to storage with 636 mg L^{-1} diphenylamine and 375 mg L^{-1} imazalil, the standard postharvest treatments used by the 'Rocha' pear industry. Fruit removed from the cold rooms were allowed to ripen at 20 ºC to an edible texture and then cooled to 4 ºC

before processing. Whole fruit firmness was measured after skin removal with a penetrometer mounted on a stand drill and equipped with an 8 mm probe. Fruit used as raw material for the pH experiments were processed with flesh firmness of 57 ± 1 N and pears with 58 ± 2 N were used in the experiment with calcium additives. Both experiments were performed in duplicate with similar results.

2.2 Minimal processing and storage conditions

Fruit were processed at 10 ºC in a sanitized cold room and handled with gloved hands during processing. Whole fruit were surface sanitized with 2 mM sodium hypochlorite (pH 6.5) for 2 min and rinsed with tap water before processing. Pears were cut by hand into longitudinal slices (*ca.* 10–20 mm thick) with a sharp stainless steel knife. After cutting, the pear slices were dipped for 60 s in the treatment solutions, and allowed to drain for 2 min before being packed.

After the dipping treatments, slices were randomly distributed in vented plastic (polyethylene terephthalate) clamshells with 500 cm^3 containing ten slices each (*ca*. 200 g). The containers were covered but a circular perforation (5 mm in diameter) in the lid assured that the atmospheric composition inside the package was not modified. The vent was plugged with cotton to reduce microbial contamination. O_2 and CO_2 concentrations inside the package were monitored throughout storage with a gas analyzer (CheckMate II, PBI Dansensor, Ringsted, Denmark) to assure that no relevant deviation from normal atmospheric level occurred. The containers were stored at 4.5 ± 0.5 ºC for 13 days in the experiment to assess the effect of pH and for 6 days in the experiment designed to evaluate the interaction between pH and calcium salts.

2.3 Buffers and calcium solutions

The effect of pH *per se* was evaluated in a buffer solution prepared with 100 mM citric acid-200 mM sodium phosphate and pH adjusted to 3.0, 5.0 or 7.0.

Different buffers were required in the experiment designed to evaluate the interaction between pH and calcium to assure the complete solubility of calcium salts. Two buffers were used for each pH to allow the distinction between the effects of pH and the chemical composition of the buffer. Buffer solutions at pH 3.0 were 100 mM citric acid-100 mM sodium citrate and 100 mM citric acid-200 mM sodium phosphate. At pH 7.0 the buffers were 100 mM Mops-NaOH and 100 mM Tris-100 mM HCl. In each of these buffer solutions, four calcium salts – ascorbate, chloride, lactate and propionate – were dissolved to a final concentration of 250 mM of calcium (1% Ca^{2+}). Buffer solutions without calcium salt were used as controls.

2.4 Color measurement

Color of the cut surface was measured in the CIE L*a*b* color space with a Konica-Minolta CR-400 chromameter (Osaka, Japan) equipped with a D_{65} illuminant and the observer at 2º. Lightness (L*), chroma (C*) and hue angle (hº) and the metric-hue difference between the initial reading and the observation date (ΔH*) were analyzed. Metric-hue difference was calculated as $\Delta H^* = \sqrt{\left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2 - \left(\Delta C^*\right)^2}$, where a* and b* are the Cartesian color coordinates. One measurement was made in each of six slices sampled from three replicated clamshells per pH treatment or on 30 randomly selected slices per buffer × pH × calcium salt treatment

2.5 Firmness assessment

Firmness was measured using a TA-XT2 Plus texture analyzer (Stable Micro Systems, Surrey, UK). In the experiment designed to evaluate the effect of pH *per se*, firmness was evaluated by compression. Disks (10 mm diameter) were excised from the central region of a pear wedge and cut to a uniform thickness of 10 mm. Disk firmness (compression force) was measured using a 30 mm diameter flat-plate probe (Pinheiro and Almeida, 2008) travelling at a speed of 1.5 mm s^{-1} for 4 mm. One measurement was made in each of six slices sampled from three replicated clamshells. In the experiments with calcium additives, firmness was measured by puncture, the method commonly used to assess firmness in fresh-cut pear (Gorny et al., 2000, 2002; Soliva-Fortuny et al., 2004a). Maximum peak force required to force a 3 mm flat head probe 5 mm into de slice with a travel speed of 1.5 mm s^{-1} was registered. One measurement was made on 30 slices per treatment and a paired measurement in the same slice was made after six days in storage to reduce sample variation.

2.6 Electrolyte efflux

Representative slices from three clamshells per treatment were sampled after 6 days in storage. Disks with 13 mm diameter and 5 mm thickness were excised from pear slices with a cork-borer, rinsed with an isotonic mannitol solution and blot dried. Four disks (*ca.* 2.5 g) were immersed in 25 mL of 700 mM mannitol and incubated for 2 h at 20 ºC with agitation. Electrical conductivity of the incubation solution was measured with a Con 510 meter (Eutech Instruments, Nijkerk, The Netherlands) immediately after immersion of disks (EC_0) and after the 2 h incubation period (EC_f). After incubation, the solution containing the disks was frozen for 24 h then thawed and boiled for 30 min and cooled to room temperature before the measurement of total electrical conductivity (EC_t). Electrolyte

leakage (EL) was expressed as percentage of total tissue electrolytes [$EL = \left(EC_f - EC_0\right) / \left(EC_t - EC_0\right) \times 100$].

2.7 Soluble solids content, pH and titratable acidity

Three samples per treatment and storage time were homogenized, filtered through a cellulose filter and used to measure soluble solids content (SSC), juice pH, and titratable acidity (TA). SSC was measured with a Palette PR-32 digital refractometer (Atago, Tokyo, Japan). pH was measured using a Ion 510 pH meter (Oakton, Vernon Hills, IL, USA). Titration was conducted with a 0.1 M NaOH to pH 8.1 and TA expressed as mmol H^+ per liter of juice.

2.8 Microbial load

Random samples of *ca*. 10 g of pear slices were taken from three packages per treatment and homogenized for 2 min in 90 mL of sterile peptone buffered water (Sigma, Steinheim, Germany) in a stomacher 400 circulator (Seward, Worthing, UK). Dilutions were made in peptone water, as needed for plating onto plate count agar (PCA, Merck, Darmstadt, Germany). Plates were incubated at 30 ºC for 3 days for the count of mesophilic aerobic bacteria and at 4.5 ºC for 10 days for psychrophilic bacteria. Microbial counts were reported as $\log_{10}$ colony forming units per gram of fresh sample weight [log (CFU g^{-1})].

2.9 Pectin extraction and determination of uronic acids

Fruit samples from three packages treated with the citrate-phosphate buffer at pH 3.0 or with the Tris-HCl buffer at pH 7.0, and stored for 6 days, were frozen at -20 ºC for pectin extraction. Untreated slices at day 0 were used as controls. Ethanol-insoluble solids (EIS) were isolated from the fruit flesh after peel removal as described (Pinheiro and

Almeida, 2008). Total pectins were determined in the EIS as described by Ahmed and Labavitch (1977). EIS (20 mg) were incubated for 6 h at room temperature in 7 mL of 20 mM sodium acetate, pH 6.9, containing 1000 U hog pancreatic α-amylase (Fluka, Buchs, Switzerland) per gram of EIS to remove starch. The suspension was filtered through a Whatman GF/C glass fiber filter under aspiration and uronic acids quantified in the filtrate were considered water-soluble pectins. After recovery of water-soluble pectins, ice-cooled ethanol was added to the solid residue and the α-amylase was inactivated at 100 ºC for 15 min under reflux. The suspension was filtered through a GF/C glass fiber filter, the residue washed with ice cooled acetone and oven-dried at 34 ºC for 12 h to further extraction. The residue was suspended in 7 mL of 50 mM sodium acetate containing 50 mM 1,2-cyclohexylenedinitrilotetraacetic acid (CDTA), pH 6.5, and incubated for 6 h at room temperature to obtain the chelator-soluble fraction. After the extraction of CDTA-soluble pectins, the residue was suspended in 50 mM sodium carbonate containing 20 mM $NaBH_4$ for two periods of 12 h. The suspension was filtered through a Whatman GF/C glass microfiber filter. Uronic acids in each fraction were quantified using the *m*-phenylphenol method with D-galacturonic acid as a standard (Filisetti-Cozzi and Carpita, 1991).

2.10 Data analysis

The experiments designed to evaluate the effect of pH *per se* were conducted in a completely randomized design with 18 (color and firmness) or three (other variables) replicates per treatment. Data were analyzed by one-way ANOVA with pH as a fixed factor.

The experiments to evaluate the interaction between pH and calcium salts were designed as a factorial design with three factors: buffer solution, pH, and calcium salt. Color and firmness measurements were made on 30 individual slices per treatment and

other determination performed on three composite samples. A three-factor ANOVA model was adopted in an exploratory analysis to evaluate the buffer effect. In subsequent analyses a two-factor ANOVA model was used with pH and calcium salt as fixed factors, considering the two buffer solution with the same pH as replicates. When significant main effects coincided with significant interaction between pH and calcium salt, separation of mean for the calcium salts was performed separately for each pH level with Duncan's multiple range test at α = 0.05. All data analyses were performed with the statistical software SPSS 16.0 for Windows (SPSS, Chicago, USA).

3. Results

3.1 Effect of pH on the quality of fresh-cut pear

The effect of the dipping solution pH on the quality of fresh-cut pear was evaluated in slices dipped for 60 s in 100 mM citric acid-200 mM sodium phosphate at pH 3.0, 5.0 or 7.0. pH had significant and consistent effects on color, firmness, and juice pH (Table 1), and microbial growth (Table 2). No consistent differences due to pH treatment were observed on SSC or TA (Table 1).

Slice browning was characterized by a decrease in hue angle (Table 1), an increase in chroma, and little or no change in lightness (not shown). Browning was slower and less intense in pear slices treated at pH 7.0 than at pH 5.0 or 3.0, as indicated by the smaller variation in hue angle.

Firmness of fresh-cut pear decreased during the first 6 to 9 days at 4.5 ºC and remained relatively unchanged for the remaining storage period (Table 1). During the first 6 days pear slices softened at a rate of 4.4, 3.2, or 1.7 N day^{-1}, for pH 3.0, 5.0, and 7.0, respectively.

SSC remained relatively unchanged during storage. Slices treated at pH 3.0 generally had higher SSC than samples treated at pH 5.0 or 7.0 (Table 1) but it is not clear whether these initial differences were induced by the treatment or were due to random variation among samples. Initial juice pH was significantly affected by the pH of the dipping solution and increased by 0.1 to 0.2 units during the 13-day storage period. Slices dipped in solutions at pH 7.0 had significant higher juice pH throughout the storage period (Table 1). pH of the dipping solution had no significant effects on TA until day 13. Average TA was 25 mmol H^+ L^{-1} immediately after the treatment and decreased during storage to 18 to 22 mmol H^+ L^{-1} by day 13 (Table 1).

Total mesophilic and psychrophilic bacteria counts increased during storage at a rate that was significantly lower in slices treated at pH 3.0, than in pears treated with higher pH (Table 2).

3.2 Effect of the buffer's chemical nature

A second experiment was designed to evaluate the effect of calcium salts on quality attributes of fresh-cut 'Rocha' pear and their interaction with pH. Quality was assessed after 6 days in storage, the time when the effects of pH on color and firmness were most evident (Table 1) with acceptable microbial quality (Table 2).

Statistical analyses revealed that the chemical nature of the buffer solution had a significant effect on the variation of color coordinates L*, C* and h^o, and on electrolyte leakage (not shown). The effect of buffer on firmness and microbial load was not significant (not shown). The *P*-values for the main effects of calcium and pH were always lower than the *P*-values for buffer. Subsequent statistical analyses adopted the ANOVA model for a two-way factorial design with pH and calcium as factors and the different

buffers were considered as replicates. Therefore, the dependent variables were averaged over the two buffers to assess the effects of pH and calcium salts.

3.3 Effect of pH and calcium salts on color

Consistently with the results from the previous experiment, pH significantly affected browning (Table 3). The effect of pH and calcium salt on surface color were exerted within 1 h after the dipping treatment as can be seen by the significant effects of both factors at day 0. Surface color of pear slices during 6 days at 4.5 ºC changed less in slices treated with solutions at pH 7.0 than in slices treated at pH 3.0 (Table 3). Calcium salt also affected color changes in fresh-cut 'Rocha' pear and a significant interaction between the effects of calcium salts and pH was observed (Table 3). Calcium ascorbate was very effective in reducing color changes at both pH treatments, almost eliminating browning during the 6 day storage period. Calcium chloride was more effective in reducing color changes in relation to control at pH 3.0 than at pH 7.0. Calcium lactate enhanced browning at pH 3.0 and was similar to control at pH 7.0, whereas calcium propionate enhanced browning at pH 3.0 but reduced color changes at pH 7.0 (Table 3).

3.4 Effect of pH and calcium salts on firmness

Firmness, as assessed by resistance to penetration by a 3 mm probe, generally increased during the storage period. pH significantly affected flesh firmness, since slices dipped in pH 7.0 buffers were firmer than those treated with buffers at pH 3.0 (Table 4). Significant effects of calcium salts and interaction between calcium salt and pH were also observed. Calcium salts had no effect on firmness changes in slices treated with solutions at pH 7.0 although calcium ascorbate induced softening at pH 3.0 (Table 4).

3.5 Effect of pH and calcium salts on electrolyte efflux

Electrolyte leakage after 6 days at 4.5 ºC was higher in control slices dipped in solutions at pH 3.0 than at pH 7.0 (Table 4). The presence of calcium salts in the buffer solutions induced higher efflux of electrolytes, an effect that was more evident at pH 3.0 than pH 7.0 (Table 4). Electrolyte leakage of slices treated with calcium propionate at pH 3.0 was particularly high (Table 4), likely due to membrane disintegration leading to visible surface deterioration (not shown) and intense browning (Table 3).

3.6 Effect of pH and calcium salts on microbial counts

The microbial load of the fresh-cut slices remained lower than 10^6 CFU g^{-1} during the 6-day storage period. Mesophilic aerobic bacteria count was, on average, one order of magnitude higher at pH 7.0 (10^5 CFU g^{-1}) than at pH 3.0 (10^4 CFU g^{-1}). Calcium ascorbate reduced microbial counts in relation to control at both pH values, whereas calcium chloride had no effect on the microbial load (Table 4). Calcium lactate was effective in reducing the microbial counts at pH 3.0 but not at pH 7.0. Calcium propionate was more effective at pH 7.0 than at pH 3.0 (Table 4).

3.7 Effect of pH and calcium salts on pectin solubility

Pectin solubilization was significantly affected by pH and calcium salts *per se* and by the interaction between these two factors (Table 5). EIS from pears treated with the acidic buffer had significantly more water- and alkali-soluble pectin and lower levels of insoluble pectins than EIS from pears treated with solutions at pH 7.0 (Table 5). Calcium ascorbate significantly increased the levels of water-soluble pectins at pH 3.0, but not at pH 7.0. As compared to controls treated with buffer solutions without calcium, the levels of CDTA-soluble pectins were increased by the calcium salts at pH 3.0 but not at pH 7.0

(Table 5). Neutral solutions of calcium salts maintained higher levels of insoluble pectin than neutral solution without calcium.

4. Discussion

Additives are used in fresh-cut fruit to enhance firmness retention, reduce enzymic browning, and delay microbial growth (Lamikanra, 2002). The usual vehicles for these additives are aqueous solutions where fresh-cut fruit are dipped. The spontaneous pH of these solutions depends on the chemical nature of the additive, the number of additives and technological adjuvants in the formulation, and on the quality and buffering capacity of the water. The pH may or may not be intentionally adjusted to improve the formulations efficacy.

Enzymic browning is often the limiting factor of shelf-life in fresh-cut prepared from susceptible fruit, such as pear. PPO has a relatively high affinity to oxygen and PPO-mediated oxidation is a fast process leading to objectionable browning after cutting (Toivonen and Brummell, 2008). Therefore, refrigeration and modified atmosphere packaging *per se* are ineffective in completely preventing browning in fresh-cut fruit for market-oriented applications. To overcome this limitation, fresh-cut processors using fruit susceptible to enzymic browning must resort to antibrowning additives. 'Rocha' pear slices had less browning when treated with neutral solutions than in acidic solutions (Tables 1 and 3). A similar effect of pH was observed in fresh-cut pear by Sapers and Miller (1998). Susceptibility to browning is generally attributed to PPO activity and to the concentration of phenolic substrates (Coseteng and Lee, 1987, Amiot et al., 1995). Maximum activity of pear PPO occurs at pH 4.3 in 'Blanquilla' (Espín et al., 1998), 4.5 in 'Conference' (Arias et al., 2007), and 5.0 in 'Bosc' (Siddiq et al., 1994), with very low activity reported at pH 3.0 and above pH 5.5. Optimal pH for 'Rocha' pear PPO activity is expected to occur within

the same range. Therefore, the pH profile for *in vitro* PPO activity cannot totally explain the color response to the dipping solution pH. This lack of correlation may be caused by possible differences between enzyme activity in vitro and enzyme action in vivo, as reported for other enzymes (Fry, 2004). In addition to the effects on PPO, pH also affects the nature and stability of the *o*-quinones formed by PPO (Guyot et al., 1995). The contribution of peroxidase (EC 1.11.1.7.) for pear enzymic browning (Richard-Forget and Gauillard, 1997) may also add to the lack of correlation between the effect of pH on browning and on PPO activity.

Calcium salts *per se* also affected the color of fresh-cut pear (Table 3). Calcium ascorbate, due to the antioxidant effect of the anion, was very effective in reducing discolorations. The effectiveness of ascorbate as an antibrowning agent is not due to a direct interaction with PPO (Arias et al., 2007); instead, ascorbate acts by reducing the *o*-quinones back to their precursor diphenols (Toivonen and Brummell, 2008). Calcium chloride also reduced color changes (Table 3), an effect that has been previously observed in fresh-cut pear (Rosen and Kader, 1989; Gorny et al., 1998; Sapers and Miller, 1998). An interaction between the effects of calcium salts and pH was evident for calcium lactate and calcium propionate. Calcium lactate had no effect at pH 7.0 and actually enhanced browning at pH 3.0 compared with control samples. Gorny et al. (2002) also found no effect of calcium lactate on pear browning. Therefore, if calcium lactate is to be used as a source of calcium for fresh-cut pear it should be combined with a reductant to prevent browning (Gorny et al., 1998, 2002; Dong et al., 2000).

When firmness was measured by compression (Table 1), significant softening was observed during storage. In the experiments designed to evaluate the interaction between pH and calcium, firmness, as assessed by puncture, increased during storage. Increases in firmness of fresh-cut pear have been observed when puncture methods are used (Gorny et

al., 1998, 2000, 2002; Dong et al., 2000; Soliva-Fortuny et al., 2002b). This increase in firmness can be caused by the partial dehydration of the cut surface and the development of an abrasive surface texture (Gorny et al., 2000), by the heterogeneous distribution of scleride cells in the pear tissue, or by maturation differences among individual pieces (Lesage and Destain, 1996).

Effects of calcium and pH on firmness were reported by Ponting et al. (1971) in apple slices. These authors observed that acid dipping solutions induced softening (pH 1.0 to 2.0) or had no effect (pH 3.0 to 4.0), whereas neutral to alkaline (pH 6.5 to 9.0) solutions hardened the apple slices. Calcium chloride increased the firmness of the apple slices at pH values of 4.0-5.0 and 9.0 (Ponting et al., 1971). Apple slices dipped in solutions at pH 3.0 were softer than slices dipped in a solution at pH 7.0 and were perceived as such by a sensory panel (Abbott et al., 2004). Similar conclusions were reported by Lee et al. (2003).

Calcium additives increase firmness in several fresh-cut fruit, including pear (Rosen and Kader, 1989; Gorny et al., 1998; Sapers and Miller, 1998; Dong et al., 2000), apple (Lee et al., 2003), melon (Saftner et al., 2003; Aguayo et al., 2008) and watermelon (Mao et al., 2006). We observed no increases in firmness with a 60 s dip in calcium chloride, lactate or propionate, but significant softening was induced by calcium ascorbate at pH 3.0 (Table 4). Loss of firmness and surface deterioration of fresh-cut pear induced by ascorbic acid has been observed (Dong et al., 2000; Gorny et al., 2002; Oms-Oliu et al., 2006). Other acids, e.g., citrate, also induce softening (Lee et al., 2003). At least in part, this effect of ascorbate is due to pH (Table 4). When pH is adjusted to 7.0 a solution of ascorbic acid (2.0%), calcium lactate (1.0%) and cysteine (0.5%) is more effective in reducing softening than the same solution at pH 3.7 or calcium lactate alone (Gorny et al., 2002). However, a mechanism specific to ascorbic acid and unrelated to the acidic pH *per se* cannot be ruled out. Calcium ascorbate at pH 3.0 substantially increased the amount of water-soluble

pectins in relation to calcium ascorbate at pH 7.0, controls without calcium, and other calcium salts (Table 5). This pH-dependent increase in pectin solubility can be due to nonenzymic cleavage of the polysaccharide by hydroxyl radicals generated by ascorbate, which could explain the enhanced softening induced by this anion (Dumville and Fry, 2003).

Electrolyte leakage (Table 4) and pectin solubilization (Table 5) were higher in pear slices dipped in solutions at pH 3.0 than in slices treated at pH 7.0. Increased disassembly of the pectic matrix and membrane disturbance at pH 3.0 can explain the higher softening as assessed by compression (Table 1) although, except for calcium ascorbate, no changes were observed when firmness was measured by puncture (Table 4). In tomato pericarp disks firmness was lower at pH 4.0 than at pH 7.0, and softening was well correlated with pectin disassembly (Pinheiro and Almeida, 2008). Calcium also interferes with pectin metabolism (Mignani et al., 1995; Pinheiro and Almeida, 2008) but the effect is pH-dependent. Calcium additives at pH 3.0 increased the levels of calcium-bounded pectins solubilized by CDTA and, with the exception of calcium ascorbate, reduced the levels of water-soluble pectins at the end of the storage period (Table 5). Treatment of peach fruit with 187.5 mM calcium lactate and calcium propionate (pH unknown) decreased calcium bound in the water-insoluble pectin fraction and increased calcium levels in the water-soluble pectic fraction, due to surface damage that resulted in reduced tissue firmness compared to a treatment with 62.5 mM of calcium (Manganaris et al., 2007). The higher electrolyte leakage of calcium-treated slices, especially at pH 3.0 (Table 4), cannot be explained only by the efflux of applied calcium as suggested by Mao et al. (2006). Instead, membrane dysfunction, evident as watersoaking in slices treated with calcium propionate at pH 3.0 (not shown), could be caused by high ion concentration, as reported by Manganaris et al. (2007).

Microbial load is a major quality criterion determinant of the shelf-life of fresh-cut fruit. In addition to the sanitation procedures used before and following cutting, additives can be used to reduce microbial growth in fresh-cut fruit. Microbial counts $<10^6$ CFU g^{-1} are considered satisfactory for fresh-cut produce (Gilbert et al., 2000). These levels were reached after 10 days (Table 2) in our first trial and were not reached during the 6-day duration of the second experiment (Table 4). Microbial growth was significantly reduced in pear slices treated at pH 3.0 as compared with pH 5.0 or 7.0 (Tables 2 and 4). Similarly, the survival of food-borne pathogens on fresh-cut apples is lower when the slices are treated at pH < 3.0 as compared with pH 7.0 (Bhagwat et al, 2004; Karaibrahimoglu et al., 2004). The antimicrobial effect of some calcium salts has been documented (Saftner et al., 2003; Karaibrahimoglu et al., 2004; Mao et al., 2006; Aguayo et al., 2008, 2010). Calcium chloride showed no antimicrobial effect at pH 3.0 or 7.0, calcium ascorbate reduced microbial counts at both pH values, whereas the effect of calcium lactate and calcium propionate was pH-dependent (Table 4), indicating that the anion is responsible for the antimicrobial activity of calcium salts. An interaction between pH and calcium salt for antimicrobial activity is expected when the salt is derived from an organic acid, given the effect of pH on the dissociation of acids. The germicidal effect of these organic calcium salts cannot be attributed only to the concentration of hydrogen ions. Undissociated acid species affect microbial metabolism and account for most of the pH effect on the inhibition of bacterial growth (Ita and Hutkins, 1991). At pH 3.0, ascorbate, lactate, and propionate exist predominantly as undissociated acids, since all these substances have pK_a>3.0, leading to lower microbial growth rates than at pH 7.0.

In addition to the effects on physicochemical and microbial quality attributes, calcium salts also affect the taste of fresh-cut fruit. Calcium chloride has been reported to have an undesirable bitter taste when applied to fresh-cut fruit and it has been suggested

that calcium lactate can be an alternative source of calcium with no bitterness (Luna-Guzmán and Barrett, 2000). Calcium propionate can impart off-flavors to fresh-cut melon at 48 mM (Aguayo et al., 2008), but no off-flavors were detected at 40 mM (Saftner et al., 2003).

The results reported herein show that pH *per se* affects quality retention of fresh-cut 'Rocha' pear. Color and firmness are better preserved when slices are dipped in a solution at pH 7.0 but microbial growth is reduced with a treatment at pH 3.0. pH also modified the effect of calcium additives on color, firmness, membrane competence, microbial growth, and pectin disassembly. The effects of calcium chloride are less affected by pH than those of other calcium salts. Calcium ascorbate, very effective in preventing browning and reducing bacterial growth, enhanced pectin solubilization and tissue softening at pH 3.0. The effects of calcium lactate and calcium propionate are very pH-dependent. The use of acidic solutions of calcium ascorbate, lactate, or propionate is not recommended to prevent tissue damage.

In conclusion, pH is a very important explanatory variable for the effects of surface treatments in fresh-cut fruit that must be considered in research and in industrial practice.

Tables

Table 1. Effect of pH on hue angle, firmness, soluble solids content (SCC), juice pH and titratable acidity (TA) of 'Rocha' pear slices treated with a solution at pH 3.0, 5.0 or 7.0 and stored at 4.5 ºC for 13 days. Mean values (n=18 for hue angle and firmness, and n=3 for other parameters), within columns, followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

Factor	Days in storage				
	0	**3**	**6**	**10**	**13**
Hue angle (º)					
pH 3.0	95.3 [b]	90.7 [b]	89.1 [c]	89.1 [b]	87.1 [b]
pH 5.0	97.4 [a]	94.4 [a]	92.8 [b]	92.2 [a]	91.8 [a]
pH 7.0	96.5 [a]	94.9 [a]	95.0 [a]	92.9 [a]	92.2 [a]
Firmness (N)					
pH 3.0	97.5 [a]	83.4 [b]	70.9 [c]	81.7 [a]	71.6 [a]
pH 5.0	96.7 [a]	88.6 [a,b]	77.3 [b]	73.7 [a]	78.5 [a]
pH 7.0	98.0 [a]	93.0 [a]	87.5 [a]	79.0 [a]	77.9 [a]
SCC (º Brix)					
pH 3.0	12.8 [a]	13.6 [a]	13.3 [a]	11.7 [a]	12.5 [a]
pH 5.0	11.8 [b]	12.4 [b]	11.8 [b]	12.3 [a]	11.4 [c]
pH 7.0	12.1 [b]	11.4 [c]	11.7 [b]	12.2 [a]	12.0 [b]
Juice pH					
pH 3.0	4.5 [c]	4.4 [b]	4.5 [c]	4.7 [b]	4.7 [a]
pH 5.0	4.6 [b]	4.6 [b]	4.7 [b]	4.7 [b]	4.7 [a]
pH 7.0	4.7 [a]	4.8 [a]	4.8 [a]	4.8 [a]	4.8 [a]
TA (mmol H^+ L^{-1})					
pH 3.0	24.6 [a]	21.4 [a]	22.5 [a]	19.9 [a]	18.3 [b]
pH 5.0	28.7 [a]	22.5 [a]	23.4 [a]	21.2 [a]	21.3 [a]
pH 7.0	22.5 [a]	21.4 [a]	22.6 [a]	23.3 [a]	21.9 [a]

Table 2. Mesophilic and psychrophilic counts [log (CFU g^{-1})] on pear slices treated with a solution at pH 3.0, 5.0 or 7.0 and stored at 4.5 ºC for 13 days. Mean values (n=3), within columns, followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

Factor	**Days in storage**				
	0	**3**	**6**	**10**	**13**
Mesophilic					
pH 3.0	2.7 [a]	2.9 [b]	4.8 [a]	4.3 [b]	5.7 [a]
pH 5.0	2.2 [a]	4.1 [a]	5.5 [a]	6.2 [a]	5.8 [a]
pH 7.0	2.5 [a]	4.0 [a]	5.7 [a]	6.5 [a]	6.4 [a]
Psychrophilic					
pH 3.0	2.4 [a]	2.9 [a]	3.1 [b]	4.7 [b]	5.6 [b]
pH 5.0	2.6 [a]	4.0 [a]	5.6 [a]	6.0 [a]	5.7 [b]
pH 7.0	2.8 [a]	4.2 [a]	5.5 [a]	6.3 [a]	6.4 [a]

Table 3. Effect of pH and calcium salt on color coordinates lightness (L*), chroma (C*) and hue angle (hº) of 'Rocha' pear slices after the dipping treatment (day 0) and after 6 days at 4.5 ºC with the corresponding metric-hue difference (ΔH*). Mean values (*n*=60), within columns, followed by the same small or capital letter are not significantly different at α=0.05 by the Duncan's multiple range test.

Factor	Color coordinate						ΔH*
	L*		C*		hº		
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	
pH 3.0							
Calcium salt							
None	78.2 ^{b}	75.5 ^{c}	13.0 ^{a}	16.5 ^{b}	101.5 ^{a}	89.8 ^{c}	3.0 ^{c}
Ascorbate	79.0 ^{a}	80.0 ^{a}	13.2 ^{a}	13.6 ^{d}	101.4 ^{a}	101.7 ^{a}	0.5 ^{e}
Chloride	78.2 ^{b}	78.0 ^{b}	13.1 ^{a}	14.9 ^{c}	102.0 ^{a}	95.0 ^{b}	1.7 ^{d}
Lactate	78.6 ^{a,b}	71.1 ^{d}	12.0 ^{b}	19.6 ^{a}	102.0 ^{a}	85.6 ^{d}	4.4 ^{b}
Propionate	78.3 ^{b}	67.7 ^{e}	12.1 ^{b}	19.4 ^{a}	101.8 ^{a}	81.5 ^{e}	5.4 ^{a}
pH 7.0							
Calcium salt							
None	78.2 ^{B}	77.7 ^{B}	13.4 ^{A,B}	15.1 ^{B}	101.9 ^{A}	93.9 ^{C}	2.0 ^{A}
Ascorbate	78.7 ^{A,B}	80.7 ^{A}	13.5 ^{A,B}	15.6 ^{A,B}	101.0 ^{B}	101.2 ^{A}	0.4 ^{D}
Chloride	78.5 ^{A,B}	77.4 ^{B}	13.0 ^{B}	16.2 ^{A}	100.6 ^{B}	94.2 ^{C}	1.6 ^{B}
Lactate	78.9 ^{A}	77.4 ^{B}	12.9 ^{B}	16.2 ^{A}	100.3 ^{B}	92.0 ^{D}	2.1 ^{A}
Propionate	78.3 ^{B}	77.8 ^{B}	14.0 ^{A}	16.4 ^{A}	99.4 ^{C}	97.2 ^{B}	0.6 ^{C}
Significance (*P*-value)							
pH	0.444	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Calcium (Ca)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
pH × Ca	0.375	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4. Effect of pH and calcium salt on firmness, electrolyte leakage and aerobic mesophilic counts of 'Rocha' pear slices during 6 days at 4.5 ºC. Mean values (n=60 for firmness and n=6 for other parameters), within columns, followed by the same small or capital letter are not significantly different at α=0.05 by the Duncan's multiple range test.

Factor	**Firmness** (N)			**Electrolyte leakage** (%)	**Mesophilic count** [log (CFU g^{-1})]
	Day 0	**Day 6**	**Δ(%)**	**Day 6**	**Day 6**
pH 3.0					
Calcium salt					
None	10.1 ^{d}	11.3 ^{a}	113 ^{a}	14.0 ^{c}	3.6 ^{a}
Ascorbate	11.7 ^{a,b}	9.4 ^{b}	82 ^{b}	33.1 ^{b}	1.0 ^{b}
Chloride	11.8 ^{a}	12.1 ^{a}	105 ^{a}	29.5 ^{b}	3.4 ^{a}
Lactate	10.8 ^{c,d}	11.2 ^{a}	106 ^{a}	39.7 ^{b}	1.5 ^{b}
Propionate	11.0 ^{b,c}	12.0 ^{a}	109 ^{a}	53.7 ^{a}	2.6 ^{a,b}
pH 7.0					
Calcium salt					
None	10.0 ^{C}	11.3 ^{A}	117 ^{A}	9.6 ^{B}	5.3 ^{A}
Ascorbate	11.1 ^{A,B}	12.3 ^{A}	113 ^{A}	17.3 ^{A}	3.5 ^{B}
Chloride	11.3 ^{A}	12.1 ^{A}	110 ^{A}	18.5 ^{A}	4.0 ^{A,B}
Lactate	10.3 ^{B,C}	12.2 ^{A}	121 ^{A}	14.8 ^{A}	5.2 ^{A}
Propionate	10.5 ^{B,C}	12.5 ^{A}	124 ^{A}	17.0 ^{A}	3.2 ^{B}
Significance (*P*-value)					
pH	0.009	<0.001	<0.001	<0.001	0.011
Calcium (Ca)	<0.001	<0.001	<0.001	<0.001	0.005
pH × Ca	0.935	<0.001	<0.001	<0.001	0.035

Table 5. Effect of pH and calcium salt on the solubility of pectins from 'Rocha' pear slices during 6 days at 4.5 ºC. Mean values (n=3), within columns, followed by the same small or capital letter are not significantly different at α=0.05 by the Duncan's multiple range test.

Factor	Uronic acids (mg g^{-1} EIS)				
	Total	**Water soluble**	**CDTA soluble**	**Carbonate soluble**	**Insoluble**
Initial value	174	25	23	40	85
pH 3.0					
Calcium salt					
None	199 ^{a}	25 ^{b}	17 ^{b}	56 ^{a}	102 ^{a}
Ascorbate	168 ^{a}	36 ^{a}	40 ^{a}	29 ^{b}	63 ^{b,c}
Chloride	176 ^{a}	9 ^{d}	39 ^{a}	30 ^{b}	99 ^{a}
Lactate	175 ^{a}	17 ^{c}	40 ^{a}	63 ^{a}	55 ^{c}
Propionate	177 ^{a}	9 ^{d}	44 ^{a}	57 ^{a}	66 ^{b}
pH 7.0					
Calcium salt					
None	172 ^{B,C}	19 ^{B}	55 ^{A}	37 ^{A,B}	61 ^{D}
Ascorbate	172 ^{B,C}	15 ^{C}	43 ^{B}	36 ^{B,C}	79 ^{C}
Chloride	185 ^{A,B}	23 ^{A}	18 ^{D}	27 ^{C}	117 ^{A}
Lactate	168 ^{C}	19 ^{B}	30 ^{C}	45 ^{A}	75 ^{C}
Propionate	188 ^{A}	8 ^{D}	51 ^{A,B}	30 ^{B,C}	99 ^{B}
Significance					
(*P*-value)					
pH	0.649	0.002	0.084	<0.001	<0.001
Calcium (Ca)	0.059	<0.001	<0.001	<0.001	0.002
pH × Ca	0.012	<0.001	<0.001	<0.001	<0.001

CHAPTER 4

EFFECTS OF PH, PHENOLIC SUBSTRATE AND FOOD ADDITIVES ON POLYPHENOLOXIDASE ACTIVITY AND TISSUE BROWNING OF FRESH-CUT 'ROCHA' PEAR

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Abstract

Cell rupture in fresh-cut processing allows phenolic substrates and polyphenoloxidase (PPO), previously sequestered in different organelles, to react leading to undesirable color development. However, it is not always clear how PPO activity and phenolic content relate to tissue browning. Our objective was to study the effect of pH, phenolic substrates, and food additives on PPO activity and on tissue browning in fresh-cut 'Rocha' pear.

Substrates 4-methyl catechol, caffeic acid, (+)-catechin hydrate, catechol, chlorogenic acid, dopamine hydrochloride, and pyrogallol, were prepared in citric acid-phosphate buffer at pH ranging from 3.0 to 8.0. PPO activity was assayed by measuring the rate of increase in absorbance at 420 nm wavelength at 25 ºC. Chemical inhibition of PPO was tested using catechol as substrate, and buffered solutions containing 250 mM of Ca^{2+} vehicled by four salts – ascorbate, chloride, lactate and propionate –, 5.7 mM of ascorbic acid, 6.1 mM of N-acetyl-L-cysteine and 0.3 mM of 4-hexylresorcinol. Pear slices were covered with the buffered phenolic solutions for 30 s and color (CIE L*a*b*) change following 30 min at room temperature was assessed.

pH optima for PPO activity depended on the phenolic substrate. Activity was optimal at pH 5.0 for catechol and 4-methylcatechol; pH 6.0 for chlorogenic acid; pH 7.0 for dopamine, caffeic acid, and catechin; and pH 8.0 for pyrogallol. High PPO activity at every pH tested was observed when catechol was used as substrate. Discrepancies were observed between the pH dependency of PPO activity and browning. Significant correlations were obtained between activity and L* or metric-hue difference (ΔH*) over the pH range 3.0 to 8.0 except for chlorogenic acid and 4-methylcatechol. With chlorogenic acid, the main PPO substrate in 'Rocha' pear, tissue browning was higher at pH 3.0 (higher ΔH*), but PPO activity was very low at the same pH. Similarly with the other phenolic

substrates, browning at pH 3.0 was higher than the corresponding PPO activity. PPO inhibition by chemical compounds was affected by the pH of the buffer, and was more effective when ascorbic acid, N-acetyl-L-cysteine and Ca-ascorbate were used. Infer on tissue browning based on the results from PPO activity can be misleading. The pH of additives for cut pear should be corrected to reduce the browning potential.

1. Introduction

The industry is challenged to provide products for the growing segment of consumers who seek convenience but will not lower their high expectations regarding the intrinsic quality and food safety of fresh-cut fruit. Processing induces physiological responses that negatively impact the quality of the final fresh-cut product. Metabolic changes induced by cutting can result in excessive softening and color changes that strongly reduce the shelf-life of fresh-cut fruit (Toivonen and Brummell, 2008).

Enzymic browning occurs when *o*-diphenol substrates react with oxygen to generate *o*-quinones, which subsequently polymerize resulting in dark melanins (Yoruk and Marshall, 2003). The oxidative reaction is catalyzed by polyphenoloxidase (PPO; EC 1.14.18.1), although the co-involvement of peroxidases (POD; EC 1.11.1.7) in the presence of H_2O_2 has been proposed to explain browning in pear (Richard-Forget and Gauillard, 1997). Since enzyme and substrates are localized in different cellular compartments, plastids and vacuoles, respectively, browning occurs only when cellular compartmentation is compromised. The pH optimum for the activity of plant PPO is in the range pH 4.0–8.0 and the enzyme is nearly inactive at pH lower than 4.0 (Weemaes et al., 1998; Yoruk and Marshall, 2003). The reported values for pear are dependent on cultivar and phenolic substrate used to assay PPO activity in vitro, and ranges from 4.3 (Espín et al., 1998) to 7.2 (Ziyan and Pekayardimci, 2004). Acidifying additives (e.g., ascorbate, citrate) are

frequently used in the processing of cut fruits to limit microbial development and to reduce PPO action (Martinez and Whitaker, 1995; Ahvenainen, 1996; Yoruk and Marshall, 2003). The rate of browning is related with PPO activity, the type of phenolic substrate and its concentration (Coseteng and Lee, 1987; Amiot et al., 1992, 1995; Galviz-Sánchez et al., 2004). However, no significant correlation was found between browning and the activity of PPO or POD, or the concentrations of hydrogen peroxide, ascorbic acid, and total and individual phenolics during storage of fresh-cut potato (Cantos et al., 2002). Browning was correlated to phenylalanine ammonia lyase (PAL; EC 4.3.1.5) activity only in the first 4 days after wounding, suggesting that the rate-limiting factor for browning can be membrane integrity, rather than enzyme activity or substrate concentration (Cantos et al., 2002). pH influences *in vitro* activity of PPO and POD (Yoruk and Marshall, 2003; Fortea et al., 2009) and a significant effect of pH on the surface color of pear slices has also been reported (Gomes et al., 2010b). Therefore, it is anticipated that conditions that, intentionally or unintentionally, alter the surface pH of fresh-cut fruit will have an impact on enzymic browning.

The objective of this study was to assess the effect of pH, phenolic substrate, and food additives, on PPO activity *in vitro* and relate it to *in vivo* tissue browning in fresh-cut pear. This information will allow that pH effects on discolorations can be considered when food additives are being developed or used to prevent quality losses in fresh-cut pear.

2. Material and Methods

2.1 Plant material and processing

Pear (*Pyrus communis* L. 'Rocha') fruit were harvested at commercial maturity from orchards located in the Western Region of Portugal (39º14' N, 9º06' W). Fruits were

stored in commercial cold rooms, for 8 months, at -0.5 ºC, 90-92% relative humidity, 2.5 kPa O_2, and 0.7 kPa CO_2 (balance N_2). Fruits were processed with an average flesh firmness of 54±3 N, measured with a penetrometer mounted on a stand drill and equipped with an 8 mm probe. Whole fruit were sanitized with 150 µL L^{-1} NaOCl and rinsed with tap water before processing. Pears were cut by hand with a sharp knife previously sanitized with 90% ethanol, and one half of each fruit was used to measure tissue browning *in vivo* and the other half was frozen in liquid nitrogen and stored at -20 ºC before extraction of PPO for *in vitro* activity assays.

2.2 Color measurement in pear slices

Fruits for *in vivo* assays of color changes were cut in slices with 5-10 mm of thickness, without skin removal. Each one of seven slices was covered with 1 mL of each treatment solution, consisting of combinations of 6 pH values with 8 phenolic substrates. After 30 s, the treated surface was blot dried by hand with paper towels, and the color was measured immediately. The slices were then kept on a tray covered with aluminum foil to protect from light for 120 min at room temperature (24 ºC).

Tissue browning was evaluated by color, immediately after treatment and at 30 min intervals for a total period of 120 min. Color of the treated surface was measured in the CIE L*a*b* color space with a Konica-Minolta CR-400 chromameter (Osaka, Japan) equipped with a D_{65} illuminant and the observer at 2º. Chroma ($C^* = [(a^*)^2 + (b^*)^2]$ ½), hue angle [$h^o = \tan^{-1}(b^*)/(a^*)$], and the metric-hue ($\Delta H^* = [(\Delta a^*)^2 + (\Delta b^*)^2 - (\Delta C^*)^2]$ ½) and the total color ($\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]$ ½) differences between the initial and subsequent readings were computed and are reported.

2.3 Buffer and phenolic substrates

The effect of pH was evaluated in a single buffer solution prepared with 100 mM citric acid-200 mM sodium phosphate and pH adjusted to 3.0 to 8.0, with one pH unit increases. Phenolic compounds (Sigma-Aldrich, Madrid, Spain) used in *in vivo* color measurements and *in vitro* PPO activity assays were *o*-dihydroxyphenols [4-methyl catechol, pyrocatechol (catechol), chlorogenic acid, caffeic acid, dopamine hydrochloride], *o*-dihydroxy (bi)phenol [(+)-catechin hydrate)], trihydroxyphenol (pyrogallol), and monohydroxyphenol (L-tyrosine). In the *in vivo* experiment the concentration of phenolic compounds was 20 mM, while 50 mM were used in the *in vitro* experiment, except for tyrosine, caffeic acid and catechin that were used at 25 mM due to reduced solubility. In the *in vivo* experiment, buffer solutions at pH 3.0 to pH 8.0 without phenolics were used as controls. In the *in vitro* experiment an aqueous natural pear extract was used as control.

2.4 Natural substrate extraction

The effect of pH solution on PPO activity was also assayed *in vitro* against a natural extract containing the soluble phenolic compounds present on pear slices. Triplicate frozen fruit samples (20 g) were peeled and homogenized for 2 min (Ultra-Turrax T25, IKA, Staufen, Germany) in 100 mL of absolute ethanol at −20 ºC. The homogenate was boiled under reflux for 20 min to inactivate enzymes, and kept overnight at −20 ºC to assure complete precipitation of cell polymers. The suspension was filtered through Miracloth (Calbiochem Corporation, La Jolla, CA, USA), the supernatant was collected and completely evaporated (Laborota 4000 HB/G1, Heidolph, Kelheim, Germany) at 35 ºC for 40 min. The residue was suspended in 20 mL of ultra-pure water.

2.5 Crude PPO extract

Frozen pear tissue (10 g) were peeled and homogenized for 2 min with 20 mL of cold 0.2 M phosphate buffer containing 5 mM cystein hydrochloride (pH 7.2). The samples were centrifuged at 600 ×g for 20 min, the supernatant was collected and the pellet suspended in 20 mL of cold 1% potassium chloride and stirred for 30 min. Following centrifugation, the supernatant was collected, combined with the previous one, and the final volume of the crude extract adjusted to 45 mL. Total protein in the extract was 0.316 mg mL-1, as determined by the dye-binding method of Bradford (1976), using bovine serum albumin as the standard.

2.6 PPO *in vitro* activity assay

PPO activity was determined using the method described by Coseteng and Lee (1987), adapted to use citric acid-phosphate buffer at pH values ranging from 3.0 to 8.0. The buffer solution (2 mL) was combined with 0.5 mL of the phenolic solution and placed in a quartz cuvette with a 1 cm path length. Crude enzyme extract (0.5 mL) or a distilled water (blank) was added and the initial absorbance was measured at 420 nm. PPO activity was assayed by measuring the rate of increase in absorbance at 420 nm between time zero and after a period of incubation of 30 min at 25 ºC. One unit of enzyme activity was defined as a change in absorbance of 0.001 per min per mg of protein in the enzyme extract. All measurements were performed in triplicate.

2.7 Chemical inactivation of PPO

An independent trial was performed to study the effect of pH on the inactivation of pear PPO activity by additives that can be used in fresh-cut fruit. Four calcium salts - ascorbate (Ca-Asc), chloride (Ca-Chl), lactate (Ca-Lact) and propionate, all from Sigma-

Aldrich (Madrid, Spain) - were dissolved at a final concentration of 250 mM of Ca^{2+} (1%), 5.7 mM (1%) of ascorbic acid (AA), 6.1 mM (1%) of N-acetyl-L-cysteine (NAC) and 0.3 mM (0.05%) of 4-hexylresorcinol (4-HR) were dissolved separately in each buffer solution. PPO was assayed *in vitro* as described, using catechol as the phenolic substrate. Buffered substrate–enzyme mixtures without inhibitors (referred to as catechol) were used as controls.

2.8 Data analysis

Data from the *in vitro* PPO activity assays were analyzed by a two-factor ANOVA with phenolic substrate and pH as fixed factors. Mean separation was performed with the Duncan's multiple range test at $\alpha = 0.05$. The association between color measurement (*in vivo*) and PPO activity (*in vitro*) were estimated using Pearson's correlation coefficient. All ANOVA and correlation analyses were performed with the statistical software SPSS 17.0 for Windows (SPSS, Chicago, USA).

3. Results and discussion

3.1 Effect of substrate and pH on browning intensity

Endogenous enzymes were able to oxidize phenolic compounds in the presence of oxygen and the surface color of pear slices changed accordingly (Fig. 1). The kinetics of color changes revealed that most browning occurred within the first 30 min and subsequently remained relatively constant (not shown). Similarly, up to 75% of the browning occurring in buffer extracts of peach and nectarine skin tissue takes place in the initial 30 min, and was almost complete within the first 60 min (Cheng and Crisosto, 1995). Therefore, *in vivo* color changes are reported and discussed taking into account the

measurement performed at 30 min. Tissue browning during 30 min at room temperature affected all color coordinates, L*, a*, b*, C*, and hue angle (Tables 1 to 5). Lightness and hue angle (Tables 1 and 5) decreased after the treatments, indicating darkening and increased redness, respectively. The color coordinates a*, b*, and C* increased, which indicates more red, yellow, and saturated colors, respectively (Tables 2 to 4). The metric-hue difference (Table 6) adequately described the color changes occurring in the tissue. Since the other color coordinates are redundant for our analysis, the metric-hue difference will be used hereafter as a surrogate for browning.

Phenolic substrates significantly affected the surface color of pear slices ($P<0.001$). Metric-hue difference (Table 6) was higher when the pear mesocarp was in contact with 4-methylcatechol, irrespective of pH. After 4-methylcatechol, the higher browning intensity was induced by the substrates catechol and catechin. Color change was minor when tyrosine was used as substrate or in buffer alone (control). Browning (ΔH^*) of slices treated with catechol and, specially, with chlorogenic acid followed the same pattern as buffers without phenolics ($R^2>0.9$), in the pH range from 3 to 8 (Fig. 1). Based on these results, a quick *in vivo* test can be developed to assess the effects of additives in fresh-cut pear, by measuring surface color differences after 30-min incubation at room temperature in the presence of 20 mM of chlorogenic acid.

Flesh color changes were also affected by pH in a substrate-dependent manner (Fig. 1). Acidic buffers (pH 3.0 to 5.0) in the absence of phenolic substrates (control) or containing catechol, chlorogenic acid, and even dopamine and 4-methylcatechol, generally induced higher browning intensity (Table 6, Fig. 1) than neutral buffers. Caffeic acid, catechin and pyrogallol increased browning when dissolved in neutral to basic buffers (pH 6.0 to 8.0).

Visibly, browning development on the tissue surface is affected by pH and by the phenolic substrate. Substrates that induce rapid color changes can be used to develop quick tests of food additives, but interactions between pH and substrate must be considered. Since the reactions that lead to browning are initiated by PPO, it is important to examine the effect of pH and phenolic substrates on PPO from 'Rocha' pear.

3.2 Effect of substrate and pH on PPO activity

'Rocha' pear PPO activity was significantly affected by the phenolic substrate (P<0.001), pH (P<0.001), and a significant (P<0.001) interaction between both variables was also observed. 'Rocha' pear PPO exhibited activity towards di- and tri-hydroxyphenols (Table 7), but no activity was observed with the monohydroxyphenol L-tyrosine, in agreement with reports from other pear cultivars (Siddiq et al., 1994; Espín et al., 1998; Ziyan and Pekayardimci, 2004). Monophenolase activity has been documented in pear PPO (Espín et al., 1998) but no PPO activity was detected when L-tyrosine was used as substrate (Table 7). This can be explained by the absence of monophenolase activity in this cultivar or, alternatively, may be due to a low catalytic constant for the monophenolase activity of 'Rocha' pear PPO.

On average, enzyme activity was higher when catechin was used as substrate (74 U min^{-1} mg^{-1} protein), followed by catechol (58), dopamine (52), and 4-methylcatechol (50). Activities with pyrogallol, chlorogenic acid, and caffeic acid were lower than 30 U min^{-1} mg^{-1} protein (Table 7). High PPO activity at every pH tested was observed when catechol was used as a substrate. These results are consistent with previous reports considering catechol and 4-methylcatechol as the most reactive substrates for pear PPO (Siddiq et al., 1994; Espín et al., 1998; Ziyan and Pekayardimci, 2004), although enzyme activity was only determined at optimal pH. Lower concentrations of chlorogenic acid than catechol are

required for the maximum activity of ‘Conference’ PPO (Arias et al., 2007), justifying the use of the former phenolic as a standard substrate for enzyme assay.

PPO activity increased with pH from 3.0 to 7.0, decreasing slightly at pH 8.0, but this general pattern was affected by the substrate. The optimum pH was found to be 8.0 for pyrogallol, 7.0 for catechin, dopamine, and caffeic acid, 6.0 for chlorogenic acid, and 5.0 for catechol and 4-methylcatechol (Table 7). PPO activity against a natural extract contain the native soluble phenolics was very low, with optimum at pH 3.0 (Table 7). pH optima for PPO activity also depends on the pear cultivar (Siddiq et al., 1994). The optimum pH, when 4-methylcatechol is used as substrate, was found to be 4.3 for PPO from ‘Blanquilla’ (Espín et al., 1998), 5.0 and 5.5 for Bosc’ and ‘Red’ (Siddiq et al., 1994), and pH 7.2 for ‘Ankara’ pear PPO (Ziyan and Pekayardimci, 2004). Maximum activity of ‘Conference’ PPO against catechol or chlorogenic acid is pH 4.5 (Arias et al., 2007), a pH optimum lower than that obtained here for ‘Rocha’ pear PPO (Table 7). No PPO activity against 4-methylcatechol was detected at pH 7.0 for ‘Bosc’ and ‘Red’ (Siddiq et al., 1994), but the relative activity of PPO from ‘Rocha’ pear at the same pH was 45% (Table 7). Differences in the reported pH optima for pear PPO may be due to the methodologies used to extract, purify, and assay the enzyme activity (Yoruk and Marshall, 2003). Relative ‘Rocha’ PPO activity at pH 3.0 was low (10–20% of maximum) for 4-methylcatechol, and most other others phenolic substrates, except catechol and the natural pear extract (Table 7). This is consistent with the results reported for ‘Red’ (≈ 10%), but not for ‘Bosc’ (≈ 60%) pear PPO (Siddiq et al., 1994). There are several causes for PPO inhibition induced by pH (Yoruk and Marshall, 2003), such as protonation of catalytic groups essential for catalysis, conformational changes in the active site of the enzyme, denaturation of the protein, and/or instability of the substrate.

Since pH and the phenolic substrate have a strong effect on both browning and PPO activity of 'Rocha' pear, it is warranted to evaluate if the relationship between PPO activity and color changes is also affected by these factors.

3.3 Relationship between PPO activity and tissue browning

Pearson's correlation coefficients were calculated to examine the relationship between enzyme activity and color parameters. Significant correlations were obtained between PPO activity and L* or ΔH* (Table 8) over the pH range 3.0 to 8.0 except for the substrates chlorogenic acid, 4-methylcatechol, and control buffers without added phenolic substrates. Similar results with catechol were reported by Soliva-Fortuny et al. (2002), who correlated L* and ΔE* values of packed pear cubes with PPO activity throughout storage, while Galviz-Sánchez et al. (2004) only obtained a poor relationship between PPO activity and the a* or b* color parameters of flesh of whole 'Rocha' pear after long-term storage. In extracts of peach and nectarine fruit skin, browning (determined by absorbance at 420 nm) was not correlated with PPO activity, even if changes in L* in ground peaches were directly related to PPO activity (Lee et al., 1990). The correlation between browning and the content of chlorogenic acid and epicatechin was significant after a 1-h incubation at 30 ºC, but not significant in the subsequent 4 h, indicating a rapid oxidation of phenolic substrates (Lee et al., 1990). In 'Charentais' melon, a higher contribution of POD (pH optimum 4.5) than PPO (pH optimum 7.5) to browning was established on the basis of the correlation between enzyme activities and color parameters (Chisari et al., 2008). POD activity was highly correlated with parameters a*, b*, C*, and hue angle, whereas PPO was only slightly correlated with L*.

Discrepancies were observed between the pH dependency of PPO activity and browning (Tables 6 and 7). With chlorogenic acid, the main PPO substrate in 'Rocha' pear

(0.08 mg kg^{-1}), tissue browning was higher at pH ≤4.0 (higher ΔH*), although PPO activity against the same substrate was low in this pH range. Similarly with the other phenolic substrates, browning at pH 3.0 was higher than the corresponding PPO activity, which may be due to the acid inactivation of pear PPO at pH < 3.5-4.0 (Rivas and Whitaker, 1973; Weemaes et al., 1998), and to the ability of the citric acid used in the buffer solution in chelating the copper of the prosthetic group of PPO. In contrast, an activation of PPO and POD enzymes by low pH (acid shock) has been observed (Yoruk and Marshall, 2003; Fortea et al., 2009). Discrepancies were also observed at higher pH values, which can be attributed to the increased instability of *o*-quinones at high pH (Valero and García-Carmona, 1992). Differences between browning patterns and PPO activity can also relate to the fact that PPO extracts were only partially purified and the presence of isoforms or even other enzymes with diverse pH optima cannot be excluded. However, only one peak was observed when activity was assayed in the pH range 3.0 to 8.0 suggesting that PPO activity is predominant.

Clearly, there is not a simple and direct relationship between PPO activity and color changes in fresh-cut pear, as pH and substrate affected these two variables in different ways.

3.4 Effect of inhibitory additives on enzyme activity and on browning intensity

Food additives are used in susceptible fresh-cut fruit to prevent or minimize enzymic browning and extend storage life. The additives most commonly used by the industry for this purpose were assessed their effect on PPO activity at various pH values.

The spectrophotometrical PPO assay was not possible when calcium propionate was used at all pH values, and when the calcium salts of ascorbate, chloride, and lactate were used at pH above 7.0 due to precipitation of calcium in the phosphate buffer. Ascorbic acid

(AA), and N-acetyl-L-cysteine (NAC) concentrations were in the range proposed by Oms-Oliu et al. (2006), but concentrations of 4-hexylresorcinol (4-HR) lower than commonly reported were used to avoid darkish pigments on the surface of the pear wedges (Oms-Oliu et al., 2006) or impart flavors (Dong et al., 2000). Since this *in vitro* experiment was not coupled with an *in vivo* experiment, the relationship between the effect of the additives on PPO and on browning is discussed taking into account the published literature and the data reported on Chapter 3.

All chemicals tested were able to decrease 'Rocha' pear PPO activity to some extent (Table 9). Ascorbic acid, N-acetyl-L-cysteine, and calcium ascorbate were found to be the most effective compounds (Table 9). L-cysteine and ascorbic acid are reported to be very good inhibitors of PPO from pear (Siddiq et al., 1994) and lychee (Sun et al., 2008). Ascorbate delays the polymerization of oxidized substrates molecules to brown pigments and can reduce them back to their original state, but does not interact directly with pear PPO (Arias et al., 2007). Ascorbic acid alone in the range 0.75% to 3% could not prevent browning reactions in fresh-cut pears (Gorny et al. 2002; Oms-Oliu et al., 2006). AA acted differently on PPO activity and on color as a function of its concentration (Oms-Oliu et al., 2006). Ascorbate in combination with calcium chloride was a more efficient antibrowning treatment for fresh-cut pear under neutral (pH 7.7) than under acidic (pH 3.3) conditions (Sapers and Miller, 1998). Application of calcium ascorbate (0.8% ascorbate) at pH 3.0 and pH 7.0 was equally successful in reducing discolorations in pear slices during 6 days at 4.5 ºC (Gomes et al., 2010b). Some activity of 'Rocha' pear PPO is present at pH 3.0 but it decreased to negligible values at pH 4.0-6.0 (Table 7). Wedges of 'Flor de Invierno' pear treated with 9.2 mM (1.5%) NAC maintained the initial color for 28 days at 4 ºC due to the inhibition of PPO (Oms-Oliu et al., 2006). Differences between concentration of inhibitors capable of inactivate enzyme activity (*in vivo*) and reduce color changes (*in vivo*) were

reported. N-acetyl-L-cysteine at concentrations higher than 0.3 mM was very effective in inactivating PPO of loquat fruit, but concentrations up to 1 mM are needed to prevent browning in the fresh loquat juice (Ding et al., 2002).

PPO from ‘Conference’ pear is inhibited by 4-hexylresorcinol in pH-dependent manner (Arias et al., 2007). The stronger inhibition of PPO by 4-hexylresorcinol at low pH was also observed in ‘Rocha’ pear (Table 9). In fresh-cut pear, 4-HR did not inhibit browning at 0.01% (personal observation) or at 0.03% without ascorbic acid (Dong et al., 2000), and high concentrations (2%) promote dark pigmentation of surface (Oms-Oliu et al., 2006).

Chloride as sodium salt or calcium salt (de Poix et al., 1980) and sodium chlorite (Lu et al., 2006) were reported to reduce activity of PPO in apples. Apple PPO inhibition increase with the acidification of a sodium chlorite solution from pH 5.5 to values below pH 4.0, probably because chloride dioxide generated under acidic conditions inhibits the enzyme (Lu et al., 2006). Similarly, 'Rocha' pear PPO activity can be suppressed in a solution of calcium chloride at pH 3.0-5.0, but not at pH 6.0 (Table 9). However color changes in stored fresh-cut pear did not follow same trend observed for PPO; the application of acidic or neutral dips of calcium chloride reduced browning in the same degree (Gomes et al., 2010b).

Among the calcium salts, calcium lactate had the least inhibitory effect on PPO, and the same PPO activity pattern (U min^{-1} mg^{-1} of protein) was followed by buffer-lactate-catechol and buffer-catechol mixtures (Table 9). This behavior can explain why calcium lactate alone has no antibrowning effect on cut pear (Dong et al., 2000; Gorny et al., 2002; Gomes et al., 2010b).

In general, PPO inactivation cannot be easily correlated with antibrowning capacity of an inhibitor, although differences in methodologies adopted in different studies make

direct comparisons unreliable. Combinations of different additives has been proposed to improve effectiveness in browning control of fruit pieces, since it is possible to supply the 'active' compounds in moderate concentrations, avoiding detrimental effect such as surface damage due to elevated ionic strength, or off-flavors.

4. Conclusions

Significant positive correlations between PPO activity *in vitro* and color changes *in vivo* were observed for caffeic acid, catechin, catechol, dopamine, and pyrogallol. In general, browning at pH 3.0 was higher than the corresponding PPO activity. PPO activity against a water-soluble pear extract did not correlate with tissue color changes. In addition, lack of a clear correlation between PPO activity and browning, and differences among inhibitors in their effectiveness to inhibit browning and PPO make it difficult to predict the response of fruits based on *in vitro* outcomes. The results reported herein suggest that PPO activity is not a good indicator of tissue browning in fresh-cut 'Rocha' pear, either in the presence or in the absence of inhibitors. Direct assessment of color changes after application of catechol or chlorogenic acid to the cut surface can provide an expedite and more accurate method to anticipate color changes induced by dipping solutions on fresh-cut pear storage.

Tables

Table 1. Changes in lightness (L^*) observed on the surface of pear slices after 30 min at 24 ºC. Mean values (n=7) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Time	Buffer pH					
	(min)	3.0	4.0	5.0	6.0	7.0	8.0
Caffeic acid	0	78.40 ^{a}	78.52 ^{a}	78.08 ^{a}	76.48 ^{a}	77.07 ^{a}	76.96 ^{a}
	30	72.61^{a,b}	73.16 ^{a}	72.91^{a,b}	70.64 ^{b}	67.33 ^{c}	66.29 ^{c}
Catechin	0	77.89 ^{a}	77.37 ^{a}	76.85 ^{a}	78.24 ^{a}	78.24 ^{a}	78.24 ^{a}
	30	70.94 ^{a}	70.15^{a,b}	68.42^{a,b}	69.30^{a,b}	62.22 ^{c}	67.49 ^{b}
Catechol	0	77.24 ^{a}	77.39 ^{a}	77.61 ^{a}	77.86 ^{a}	76.79 ^{a}	75.84 ^{a}
	30	44.80 ^{b}	44.88 ^{b}	43.66 ^{b}	65.32 ^{a}	67.00 ^{a}	68.42 ^{a}
Chlorogenic	0	77.12 ^{a}	76.37 ^{a}	74.68 ^{a}	77.00 ^{a}	75.07 ^{a}	76.65 ^{a}
	30	69.47 ^{a}	67.47 ^{a}	67.62 ^{a}	68.17 ^{a}	66.06 ^{a}	66.85 ^{a}
Dopamine	0	80.26 ^{a}	79.59 ^{a}	77.05 ^{b}	76.51 ^{b}	77.04 ^{b}	77.04 ^{b}
	30	75.37 ^{a}	64.17^{b,c}	64.57 ^{b}	60.03 ^{c}	61.88^{b,c}	59.94 ^{c}
4-Methylcatechol	0	76.76 ^{a}	75.74^{a,b}	77.47 ^{a}	77.23 ^{a}	73.95 ^{b}	76.26 ^{a}
	30	52.72 ^{b}	50.63 ^{b}	53.59 ^{b}	53.09 ^{b}	54.83 ^{b}	59.65 ^{a}
Pyrogallol	0	75.85 ^{a}	75.64 ^{a}	77.15 ^{a}	77.83 ^{a}	76.81 ^{a}	76.46 ^{a}
	30	72.23^{b,c}	72.31^{b,c}	73.84^{a,b}	74.95 ^{a}	71.47 ^{c}	68.51 ^{d}
Tyrosine	0	75.86 ^{c}	77.83^{a,b}	75.27 ^{c}	76.99^{b,c}	77.98^{a,b}	79.05 ^{a}
	30	78.56 ^{a}	77.69^{a,b}	76.01 ^{b}	76.30 ^{b}	76.80 ^{b}	77.57^{a,b}
Control	0	79.15 ^{a}	79.32 ^{a}	79.77 ^{a}	78.72 ^{a}	79.45 ^{a}	78.65 ^{a}
	30	77.87 ^{a}	77.82 ^{a}	78.81 ^{a}	77.73 ^{a}	78.16 ^{a}	78.29 ^{a}

Table 2. Changes in color coordinate *a** observed on the surface of pear slices after 30 min at 24 ºC. Mean values (*n*=7) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Time	Buffer pH					
	(min)	3.0	4.0	5.0	6.0	7.0	8.0
Caffeic acid	0	-2.63 ^{b}	-2.30^{a,b}	-1.98 ^{a}	-1.93 ^{a}	-1.97 ^{a}	-1.99 ^{a}
	30	1.26 ^{c}	0.84 ^{c}	0.48 ^{c}	3.25 ^{b}	6.19 ^{a}	6.54 ^{a}
Catechin	0	-2.80 ^{b}	-2.16 ^{a}	-2.38^{a,b}	-2.02 ^{a}	-2.02 ^{a}	-2.02 ^{a}
	30	0.63 ^{d}	-0.26 ^{d}	2.99 ^{d}	7.99 ^{c}	22.89 ^{a}	13.42 ^{b}
Catechol	0	-2.41^{a,b}	-2.05 ^{a}	-2.44^{a,b}	-2.35^{a,b}	-2.66 ^{b,c}	-3.03 ^{c}
	30	15.71 ^{a}	16.70 ^{a}	16.69 ^{a}	3.80 ^{b}	1.50 ^{c}	2.15^{b,c}
Chlorogenic	0	-2.10 ^{b}	-2.81 ^{c}	-2.27 ^{b}	-1.93^{a,b}	-1.59 ^{a}	-1.81^{a,b}
	30	3.44 ^{a}	2.76^{a,b}	2.09^{b,c}	1.37 ^{c}	2.49^{a,b,c}	1.40 ^{c}
Dopamine	0	-2.35 ^{b}	-1.96 ^{b}	-2.11 ^{b}	-0.76 ^{a}	-0.44 ^{a}	-0.44 ^{a}
	30	-0.16 ^{d}	10.17 ^{a}	10.24 ^{a}	8.27 ^{b}	3.39 ^{c}	2.66 ^{c}
4-Methylcatechol	0	-2.70 ^{c}	-2.33^{b,c}	-2.31^{b,c}	-1.95 ^{b}	-0.64 ^{a}	-0.02 ^{a}
	30	21.29 ^{a}	22.40 ^{a}	20.42 ^{a}	21.40 ^{a}	19.81 ^{a,b}	16.70 ^{b}
Pyrogallol	0	-0.53 ^{a}	-1.48 ^{b}	-2.25 ^{c}	-2.11 ^{c}	-2.41 ^{c}	-1.47 ^{b}
	30	-0.01^{b,c}	-0.56 ^{c}	-1.85 ^{d}	-2.50 ^{e}	0.16 ^{b}	2.54 ^{a}
Tyrosine	0	-2.30 ^{a}	-2.70 ^{a}	-2.15 ^{a}	-1.94 ^{a}	-2.22 ^{a}	-2.02 ^{a}
	30	-2.03 ^{a}	-1.91 ^{a}	-1.57 ^{a}	-1.63 ^{a}	-1.79 ^{a}	-1.72 ^{a}
Control	0	-2.03 ^{b}	-1.63 ^{a}	-1.72^{a,b}	-1.51 ^{a}	-1.35 ^{a}	-1.41 ^{a}
	30	-1.59 ^{a}	-1.15 ^{a}	-1.33 ^{a}	-1.37 ^{a}	-1.23 ^{a}	-1.47 ^{a}

Table 3. Changes in color coordinate *b** observed on the surface of pear slices after 30 min at 24 ºC. Mean values (n=7) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Time	Buffer pH					
	(min)	3.0	4.0	5.0	6.0	7.0	8.0
Caffeic acid	0	12.85^{b,c}	14.14^{a,b,c}	12.22 ^{c}	14.88^{a,b}	15.53 ^{a}	15.38^{a,b}
	30	21.36 ^{b}	20.66 ^{b}	20.34 ^{b}	23.24^{a,b}	24.75 ^{a}	23.13^{a,b}
Catechin	0	14.46 ^{a}	14.36 ^{a}	12.24 ^{a}	14.38 ^{a}	14.38 ^{a}	14.38 ^{a}
	30	32.24 ^{d}	48.74 ^{c}	53.40 ^{c}	51.39 ^{c}	64.87 ^{a}	58.93 ^{b}
Catechol	0	14.70^{a,b}	15.40 ^{a}	15.64 ^{a}	13.32 ^{b}	13.03 ^{b}	12.90 ^{b}
	30	22.63 ^{a}	21.36 ^{a}	22.08 ^{a}	15.62 ^{b}	14.88 ^{b}	15.81 ^{b}
Chlorogenic	0	16.09 ^{a}	18.54 ^{a}	16.71 ^{a}	16.38 ^{a}	15.98 ^{a}	15.83 ^{a}
	30	28.12^{a,b}	29.57 ^{a}	29.83 ^{a}	29.03 ^{a}	26.39^{b,c}	24.38 ^{c}
Dopamine	0	13.82 ^{a}	14.00 ^{a}	13.67 ^{a}	14.38 ^{a}	14.77 ^{a}	14.77 ^{a}
	30	22.20^{b,c}	26.44 ^{a}	25.56 ^{a}	24.82 ^{b}	20.05 ^{c}	21.77 ^{c}
4-Methylcatechol	0	16.51 ^{b}	17.90 ^{a,b}	16.81 ^{b}	19.54^{a,b}	21.01 ^{a}	17.24 ^{b}
	30	36.41 ^{a}	36.43 ^{a}	36.69 ^{a}	33.90^{a,b}	31.54^{b,c}	29.52 ^{c}
Pyrogallol	0	15.68 ^{c}	16.99 ^{b,c}	17.00^{b,c}	15.99 ^{c}	19.02^{a,b}	20.01 ^{a}
	30	27.43^{b,c}	29.71 ^{a,b}	32.00 ^{a}	28.47 ^{b}	25.38 ^{c}	27.46^{b,c}
Tyrosine	0	13.50 ^{a}	14.84 ^{a}	12.58 ^{a}	14.62 ^{a}	13.07 ^{a}	14.87 ^{a}
	30	13.14 ^{a}	14.52 ^{a}	15.10 ^{a}	15.15 ^{a}	14.27 ^{a}	15.36 ^{a}
Control	0	12.84 ^{a}	11.87 ^{a}	12.23 ^{a}	12.25 ^{a}	11.53 ^{a}	12.39 ^{a}
	30	14.62 ^{a}	13.20 ^{a,b}	13.42^{a,b}	12.83 ^{b}	11.98 ^{b}	13.59^{a,b}

Table 4. Changes in chroma (C^*) observed on the surface of pear slices after 30 min at 24 ºC. Mean values (n=7) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Time	Buffer pH					
	(min)	3.0	4.0	5.0	6.0	7.0	8.0
Caffeic acid	0	13.12 ^{a,b}	14.34^{a,b}	12.39 ^{b}	15.02 ^{a}	15.66 ^{a}	15.52 ^{a}
	30	21.41 ^{b,c}	20.69 ^{c}	20.36 ^{c}	23.47^{a,b,c}	25.53 ^{a}	24.05^{a,b}
Catechin	0	14.74 ^{a}	14.52 ^{a}	12.47 ^{a}	14.53 ^{a}	14.52 ^{a}	14.52 ^{a}
	30	32.26 ^{d}	48.75 ^{c}	53.50 ^{c}	52.06 ^{c}	68.86 ^{a}	60.53 ^{b}
Catechol	0	14.90^{a,b,c}	15.54^{a,b}	15.84 ^{a}	13.53 ^{b,c}	13.31 ^{c}	13.26 ^{c}
	30	27.64 ^{a}	27.15 ^{a}	27.70 ^{a}	16.15 ^{b}	14.97 ^{b}	16.04 ^{b}
Chlorogenic	0	16.23 ^{a}	18.75 ^{a}	16.87 ^{a}	16.49 ^{a}	16.06 ^{a}	15.94 ^{a}
	30	28.34 ^{a,b}	29.71 ^{a}	29.91 ^{a}	29.09 ^{a,b}	26.51^{b,c}	24.44 ^{c}
Dopamine	0	14.03 ^{a}	14.15 ^{a}	13.85 ^{a}	14.43 ^{a}	14.78 ^{a}	14.78 ^{a}
	30	22.29 ^{b}	28.35 ^{a}	27.58 ^{a}	26.18 ^{a}	20.34 ^{b}	21.94 ^{b}
4-Methylcatechol	0	16.74 ^{b}	18.05^{a,b}	16.97 ^{b}	19.65 ^{a,b}	21.03 ^{a}	17.26 ^{b}
	30	42.25 ^{a}	42.81 ^{a}	42.01 ^{a}	40.27 ^{a,b}	37.28^{b,c}	34.02 ^{c}
Pyrogallol	0	15.70 ^{c}	17.07^{b,c}	17.16^{b,c}	16.13 ^{c}	19.17^{a,b}	20.07 ^{a}
	30	27.43 ^{b,c}	29.72^{a,b}	32.06 ^{a}	28.59 ^{b}	25.38 ^{c}	27.59^{b,c}
Tyrosine	0	13.70 ^{a}	15.09 ^{a}	12.77 ^{a}	14.75 ^{a}	13.26 ^{a}	15.02 ^{a}
	30	13.30 ^{a}	14.65 ^{a}	15.19 ^{a}	15.25 ^{a}	14.39 ^{a}	15.47 ^{a}
Control	0	13.00 ^{a}	11.99 ^{a}	12.36 ^{a}	12.35 ^{a}	11.61 ^{a}	12.47 ^{a}
	30	14.71 ^{a}	13.26^{a,b}	13.49^{a,b}	12.91 ^{b}	12.05 ^{b}	13.68^{a,b}

Table 5. Changes in hue angle (h^o) observed on the surface of pear slices after 30 min at 24 ºC. Mean values (n=7) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Time	Buffer pH					
	(min)	3.0	4.0	5.0	6.0	7.0	8.0
Caffeic acid	0	101.47 ^{a}	99.25^{a,b}	99.48^{a,b}	97.89 ^{b}	97.35 ^{b}	97.49 ^{b}
	30	86.62 ^{a}	87.95 ^{a}	88.68 ^{a}	82.10 ^{b}	75.94 ^{c}	74.36 ^{c}
Catechin	0	101.10 ^{a}	98.57 ^{b}	100.99 ^{a}	97.92 ^{b}	98.00 ^{b}	98.00 ^{b}
	30	88.95 ^{a,b}	90.33 ^{a}	86.91 ^{b}	81.47 ^{c}	70.73 ^{e}	77.48 ^{d}
Catechol	0	99.26 ^{c,d}	97.69 ^{d}	98.82^{c,d}	100.01^{b,c}	101.66^{a,b}	103.31 ^{a}
	30	55.22 ^{c}	52.07 ^{c}	52.94 ^{c}	76.76 ^{b}	84.25 ^{a}	82.36 ^{a}
Chlorogenic	0	97.46^{a,b,c}	98.65 ^{a}	97.87^{a,b}	96.73^{b,c}	95.76 ^{c}	96.64^{b,c}
	30	83.09 ^{c}	84.67^{b,c}	86.08^{a,b}	87.46 ^{a}	84.69^{b,c}	86.96 ^{a}
Dopamine	0	99.84 ^{a}	97.99 ^{a}	98.82 ^{a}	93.35 ^{b}	91.77 ^{b}	91.72 ^{b}
	30	90.48 ^{a}	68.97 ^{c}	68.52 ^{c}	71.66 ^{c}	80.48 ^{b}	82.99 ^{b}
4-Methylcatechol	0	99.22 ^{a}	97.45^{a,b}	97.78^{a,b}	95.97 ^{b}	92.02 ^{c}	90.21 ^{c}
	30	59.63 ^{a}	58.41 ^{a}	60.93 ^{a}	57.81 ^{a}	58.05 ^{a}	60.65 ^{a}
Pyrogallol	0	92.04 ^{c}	95.01 ^{b}	97.46 ^{a}	97.63 ^{a}	97.26 ^{a}	94.23 ^{b}
	30	90.05 ^{c,d}	91.10 ^{c}	93.31 ^{b}	95.10 ^{a}	89.66 ^{d}	84.81 ^{e}
Tyrosine	0	99.55 ^{a,b}	100.33 ^{a}	99.89 ^{a}	97.49 ^{c}	99.74^{a,b}	97.84^{b,c}
	30	98.86 ^{a}	97.43 ^{a}	96.02 ^{a}	96.33 ^{a}	97.05 ^{a}	96.38 ^{a}
Control	0	99.09 ^{a}	97.88^{a,b}	97.90^{a,b}	97.17 ^{b}	96.67 ^{b}	96.49 ^{b}
	30	96.26 ^{a}	95.17 ^{a}	95.64 ^{a}	96.17 ^{a}	95.93 ^{a}	96.18 ^{a}

Table 6. Metric-hue difference of the surface of pear slices after 30 min at 24 ºC. Mean values (n=7) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Buffer pH					
	3.0	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
Caffeic acid	4.34 ^{b}	3.42 ^{c}	2.97 ^{c}	5.06 ^{b}	7.33 ^{a}	7.72 ^{a}
Catechin	4.60^{d,e}	3.82 ^{e}	6.35^{c,d}	8.04 ^{c}	14.94 ^{a}	10.62 ^{b}
Catechol	15.22 ^{a}	15.93 ^{a}	16.32 ^{a}	6.01 ^{b}	4.29 ^{b}	5.26 ^{b}
Chlorogenic acid	5.35^{a,b}	5.71 ^{a}	4.59^{b,c}	3.56 ^{d}	3.96^{c,d}	3.35 ^{d}
Dopamine	2.89 ^{c}	10.04 ^{a}	10.23 ^{a}	7.26 ^{b}	3.41 ^{c}	2.73 ^{c}
4-Methylcatechol	18.00 ^{a}	18.57 ^{a}	16.87 ^{a}	18.36 ^{a}	16.27 ^{a}	12.28 ^{b}
Pyrogallol	0.71 ^{e}	1.59^{c,d}	1.70 ^{c}	0.93^{d,e}	2.92 ^{b}	3.89 ^{a}
Tyrosine	0.45 ^{a}	0.74 ^{a}	0.94 ^{a}	0.54 ^{a}	0.70 ^{a}	0.65 ^{a}
Control ^{x}	0.70 ^{a}	0.57 ^{a}	0.51^{a,b}	0.29^{b,c}	0.31^{b,c}	0.25 ^{c}

^{x} Controls were buffer solutions at different pH values without phenolics

Table 7. Effect of pH on PPO activity (U min^{-1} mg^{-1} of protein) against various phenolic substrates. Mean values ± S.E (*n*=3) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

PPO substrate	Buffer pH					
	3.0	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
Caffeic acid	5.7 ± 0.2 [b]	12.2 ± 0.3 [b]	12.2 ± 0.2 [b]	24.0 ± 0.8 [a]	27.8 ± 0.1 [a]	24.4 ± 6.2 [a]
Catechin	2.9 ± 0.1 [f]	8.7 ± 0.1 [e]	42.9 ± 0.6 [d]	97.1 ± 0.6 [c]	151.3 ± 1.4 [a]	143.7 ±1.3 [b]
Catechol	53.0 ± 0.8 [d]	69.3 ± 1.0 [b]	76.6 ± 0.9 [a]	58.8 ± 0.9 [c]	47.2 ± 0.2 [e]	46.0 ± 1.0 [e]
Chlorogenic acid	3.4 ± 0.1 [e]	20.0 ± 0.2 [c]	33.3 ± 0.0 [b]	37.3 ± 0.5 [a]	32.7 ± 0.6 [b]	12.7 ± 0.1 [d]
Dopamine	5.6 ± 0.2 [e]	31.5 ± 0.1 [d]	54.5 ± 0.5 [c]	71.8 ± 0.2 [b]	79.0 ± 0.1 [a]	69.8 ± 2.0 [b]
4-Methylcatechol	6.6 ± 0.3 [e]	70.3 ± 1.0 [c]	92.2 ± 1.6 [a]	79.9 ± 0.7 [b]	41.4 ± 1.2 [d]	9.6 ± 0.8 [e]
Pyrogallol	6.1 ± 0.1 [f]	10.0 ± 0.1 [e]	11.2 ± 0.1 [d]	17.5 ± 0.3 [c]	52.2 ± 0.8 [b]	82.4 ± 0.2 [a]
Tyrosine	NA	NA	NA	NA	NA	NA
Pear extract	2.4 ± 0.1 [a,b]	2.7 ± 0.1 [a]	1.0 ± 0.3 [d]	0.8 ± 0.0 [d]	1.4 ± 0.1 [c,d]	1.9 ± 0.4 [b,c]

NA, no activity

Table 8. Pearson's correlation coefficient between PPO activity and slice color coordinates, as affected by pH (*n*=6).

PPO substrate	L*	a*	b*	C*	h°	ΔH*	ΔE*
Caffeic acid	-0.846*	0.868*	0.880*	0.892**	-0.858*	0.794*	0.780*
Catechin	-0.793*	0.935**	0.832*	0.856*	-0.959**	0.938**	0.856*
Catechol	-0.733*	0.751*	0.652	0.702	-0.773*	0.753*	0.741*
Chlorogenic acid	-0.449	-0.516	0.340	0.320	0.552	-0.401	0.367
Dopamine	-0.904**	0.203	-0.262	-0.197	-0.307	-0.062	0.459
4-Methylcatechol	-0.525	0.515	0.469	0.502	-0.210	0.513	0.487
Pyrogallol	-0.818*	0.787*	-0.540	-0.526	-0.788*	0.945**	-0.573
Controls [x]	-0.403	-0.026	0.470	0.466	-0.312	0.569	0.430

[x] Controls for color determination in slices were buffer solutions at different pH values without phenolics, while control for PPO activity was an aqueous natural pear extract.

Significant correlation (1-tailed) at the 0.05 and 0.01 level are indicated by * and **, respectively.

Table 9. Effect of food additives on the PPO activity (relative to catechol at pH 5.0 as 100%); within parenthesis, activity is indicated as percentage of inhibition in relation to the activity measured in catechol at each pH. Mean values ± S.E (n=3) within lines followed by the same letter are not significantly different at α=0.05 by the Duncan's multiple range test.

Compound	**Buffer pH**					
	3.0	**4.0**	**5.0**	**6.0**	**7.0**	**8.0**
Catechol	48.4 ±0.6 ^{d} (0)	90.9 ±0.2 ^{b} (0)	100.0 ±1.0 ^{a} (0)	93.2 ± 1.7 ^{b} (0)	54.7 ±1.6 ^{c} (0)	36.3 ±0.5 ^{e} (0)
Ca-Asc	7.7 ±0.3 ^{a} (84)	1.4 ±0.5 ^{c} (98)	2.8 ±0.3 ^{b} (97)	3.7 ± 0.2 ^{b} (96)	ND -	ND -
Ca-Chl	0.0 ±0.0 ^{b} (100)	1.1 ±0.3 ^{b} (99)	8.5 ±3.0 ^{b} (92)	32.6 ±11.1 ^{a} (65)	ND -	ND -
Ca-Lact	40.4 ±3.5 ^{d} (17)	68.7 ±0.8 ^{b} (24)	76.1 ±2.1 ^{a} (24)	50.5 ± 1.1 ^{c} (46)	ND -	ND -
Ca-Prop	ND	ND	ND	ND	ND	ND
AA	1.9 ±0.1 ^{a} (96)	1.4 ±0.2 ^{b} (98)	1.4 ±0.1 ^{b} (99)	0.4 ± 0.1 ^{c} (100)	0.0 ±0.0 ^{d} (100)	0.0 ±0.0 ^{d} (100)
4-HR	1.9 ±0.3 ^{b} (96)	7.2 ±0.3 ^{b} (92)	8.4 ±0.4 ^{b} (92)	10.1 ± 0.9 ^{b} (89)	15.1 ±0.3 ^{a} (72)	13.8 ±1.8 ^{a,b} (62)
NAC	0.8 ±0.1 ^{b} (98)	0.6 ±0.3 ^{b} (99)	3.4 ±1.0 ^{a} (97)	4.6 ± 0.4 ^{a} (95)	0.0 ±0.0 ^{b} (100)	0.0 ±0.0 ^{b} (100)

ND – PPO activity not determined due to precipitation.

Figures

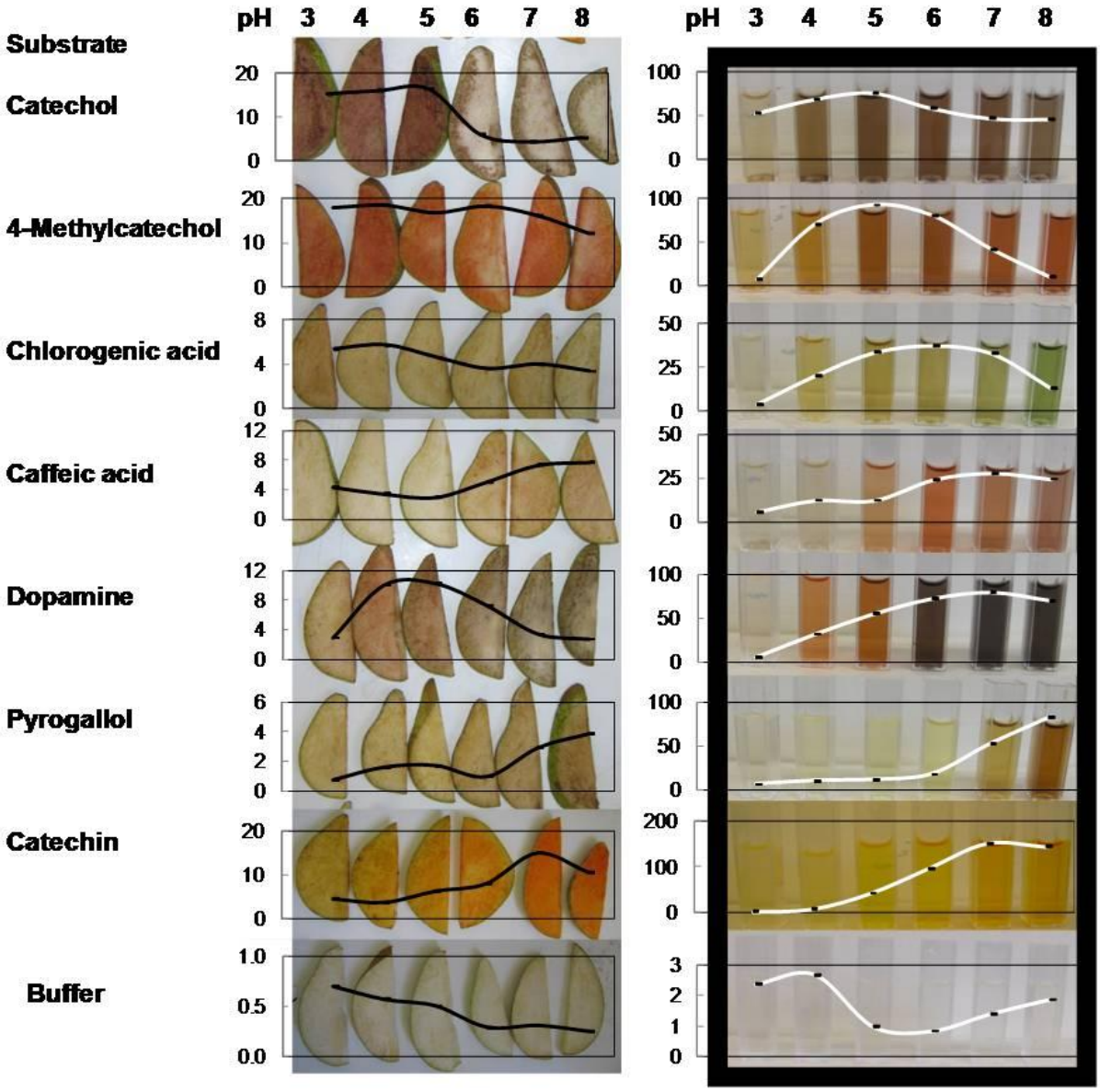

Figure 1. Color development on pear slices, reported as metric-hue difference (ΔH*) (left), and on cuvette during *in vitro* PPO activity assay (U min^{-1} mg^{-1} of protein) (right).

CHAPTER 5

EFFECTS OF PACKAGE OXYGEN CONCENTRATION AND CALCIUM ASCORBATE PH ON THE QUALITY RETENTION OF FRESH-CUT 'ROCHA' PEAR

With:

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Abstract

Theoretical estimations suggest that optimizing oxygen concentration inside modified atmosphere packages is of limited benefit for fresh-cut 'Rocha' pear. Packages were designed to achieve three equilibrium levels of oxygen partial pressures, high (HO) with 18.2 kPa O_2 + 1.0 kPa CO_2, low (LO) with 2.0 kPa O_2 + 5.1 kPa CO_2 and very-low (VLO) with 0.5 kPa O_2 + 5.1 kPa CO_2. Fresh-cut 'Rocha' pear were treated with 250 mM calcium ascorbate solutions buffered at pH 3.0 and pH 7.0, packaged under the three MAP conditions, and stored at 5 ºC for 20 days. Levels (mean ± confidence interval at 95%) of oxygen during the experiment were 16.7±0.2, 1.8±0.2 and 0.25±0.04 kPa with corresponding CO_2 levels of 1.3±0.1, 4.3±0.2 and 6.5±0.4 kPa. Changes in quality attributes related to fruit metabolism, namely firmness, titratable acidity, pH, and soluble solids content were not affected by oxygen levels. Ascorbate levels decreased exponentially during storage with a rate independent on oxygen levels. Water activity was also unaffected by oxygen concentration. Oxygen partial pressure inside the packages affected browning and microbial growth, which were reduced at VLO. Sensory analyses performed after 8 days in storage confirmed that panelists perceived the differences in color but did not notice differences in firmness or taste among the samples under different oxygen levels. pH affected browning and microbial growth. Browning was more intense at pH 3.0 and microbial growth faster at pH 7.0. Results show that no significant improvements of quality attributes dependent on the physiology of respiration of fresh-cut 'Rocha' pear can be obtained by reducing oxygen partial pressure inside the packages, confirming the theoretical estimation.

1. Introduction

Ready-to-eat fresh-cut fruit are a convenient means to foster fruit consumption by modern consumers. Packaging is required to prevent post-processing contamination and reduce water loss by fresh-cut fruit. A modification in the composition of the atmosphere surrounding the fruit occurs when rigid plastic containers or plastic films with various permeabilities to gases wrap respiring fruit pieces. It has long been recognized that modified atmospheres with O_2 partial pressures lower and CO_2 partial pressures higher than those in the normal atmosphere are beneficial to extend the shelf-life (Gorny, 1998; Rocculi et al., 2004; Fonseca et al., 2005).

The effects of modified atmosphere packaging (MAP) have been largely documented in fresh-cut produce. The effects of different packaging films, and oxygen and carbon dioxide levels inside packages have been studied in pear (Sapers and Miller, 1998; Soliva-Fortuny et al., 2002a, 2002b, 2007; Oms-Oliu et al., 2008b). Methods for designing MA packages to achieve target O_2 and CO_2 levels have also been developed and tested (Talasila and Cameron, 1997; Jacxsens et al., 2000; Del Nobile et al., 2007). The recognition of low oxygen atmospheres as beneficial for shelf-life extension of fresh-cut fruit, including pear, is prevalent in the literature (Sapers and Miller, 1998; Soliva-Fortuny et al., 2002a,b). Beneficial modified atmosphere can have initial oxygen levels around 2.5 kPa (Soliva-Fortuny et al., 2007; Oms-Oliu et al., 2008b), or even 0 kPa oxygen (Gil et al., 1998; Soliva-Fortuny et al., 2002b; Soliva-Fortuny et al., 2007), or be balanced with nitrous oxide or noble gases (Rocculi et al., 2004). However, the benefits of MAP in particular commodities are often generalizations that warrant detailed examination of the effects of packaging on individual quality attributes.

Quality of fresh-cut produce is a multivariable attribute. Quality changes that determine the shelf-life of fresh-cut fruit include microbial growth, softening, browning,

and water loss (Ahvenainen, 1996). These quality changes are caused by different sets of factors and are likely to have different kinetics. Some quality attributes depend, to a great extent, on the metabolic activity of the fresh-cut tissue, e.g., firmness, electrolytes efflux, soluble solids content, juice pH and titratable acidity. Browning and ascorbate content (related to the antibrowning additive) are partially dependent on the metabolic activity but are largely biochemical relations occurring in the cell-free environment of the cut surface. Water loss is regulated by film characteristics and physical factors; microbial growth has its own metabolic regulation, largely independent on the metabolic rate of fresh-cut fruit.

The systematic approach to MAP design involves the knowledge of respiration rate and film permeability to gases at the operating temperature. Respiration rate is inversely correlated with shelf-life (Rolle and Chism, 1987; Fonseca et al., 2005), and can be slowed down by low temperatures or, in some cases, by modified atmosphere. The kinetics of respiration as a function of oxygen partial pressure has been established for fresh-cut 'Rocha' pear (Chapter 2.1; Gomes et al., 2010a). At 5 ºC, the shift from aerobic to anaerobic metabolism occurs at 0.25 kPa of O_2 and the apparent K_m is 0.24 kPa; therefore, the maximum reduction in respiration rate that can be achieved at 5 ºC via reducing the O_2 partial pressure is 49%. We hypothesize that such a reduction in respiration rate is not sufficient for significant extension of quality attributes related to the metabolic activity of the tissue and the physiological behavior of fresh-cut fruit will be similar at any oxygen concentration above the fermentation threshold. If this hypothesis is confirmed, no significant benefits on quality attributes dependant on the metabolic rate can be achieved via MAP in fresh-cut 'Rocha' pear. MAP, however, is likely to affect quality attributes unrelated to the metabolic activity of the tissue, such as microbial contamination and water loss.

Processing of fresh-cut pear requires the use of antibrowning additives (Gorny et al., 2002; Oms-Oliu et al., 2008b). Calcium ascorbate is a commonly used antioxidant for fresh-cut fruit. The effect of calcium ascorbate on color and firmness depends on the pH (Gomes et al., 2010b). Since both MAP and calcium ascorbate are expected to affect the quality of fresh-cut pear it is warranted to examine their combined effects.

The aim of this work was to test the hypothesis that quality attributes dependent on metabolic activity cannot be modulated via MAP in fresh-cut 'Rocha' pear in the presence of the antibrowning additive calcium ascorbate. Kinetic data on the effect of O_2 partial pressure on respiratory activity were used to design packages to achieve three levels of reduction in respiration rate. The effect of O_2 partial pressure on the kinetics of changes was evaluated for several quality attributes that limit shelf-life of the product: physiological (firmness, electrolytes efflux, soluble solids content, juice pH and titratable acidity), physical (water activity), microbiological (aerobic mesophilic and psychrophilic bacteria), and biochemical (color and ascorbate content). The effect of the pH of the antibrowning additive used to reduce undesirable discolorations was also assessed.

2. Materials and methods

2.1 Plant material and minimal processing

Pear (*Pyrus communis* L. 'Rocha') fruit were harvested at commercial maturity and stored for 6 months at -0.5 ºC, 90-95% RH under controlled atmosphere with 2.0 kPa O_2 + 0.5 kPa CO_2 (balance N_2). Fruits were removed from cold storage and allowed to soften at 20 ºC to a flesh firmness of 50 ± 1 N before processing. Whole fruits were cooled to a pulp temperature of 5 ºC, surface sanitized with 2 mM sodium hypochlorite (pH 6.5), and rinsed with tap water before processing. Pears were cut by hand into longitudinal slices (*ca.* 10

mm thick) and slices were dipped for 1 min in cold solutions, drained, and placed inside plastic bags. Solutions contained calcium ascorbate (250 mM Ca^{2+}) were buffered at pH 3.0 or pH 7.0. To assure the complete solubility of calcium ascorbate, buffer solution at pH 3.0 was 100 mM citric acid-200 mM sodium phosphate and at pH 7.0 was 100 mM Tris-100 mM HCl (Gomes et al., 2010b). All processing operations were performed in a cold room at 10 ºC sanitized with sodium hypochlorite and fruit manipulations were made with gloved hands.

2.2 Active modified atmosphere packaging

Data on the kinetics of respiration of fresh-cut 'Rocha' pear as affected by O_2 partial pressure (Eq. 1) were used to design packages in which the predicted O_2 partial pressures would stabilize at *ca.* 18.2, 2.0, and 0.5 kPa, corresponding to 99, 89, and 68% of the maximal respiration rates of fresh-cut 'Rocha' pear at 5 ºC, respectively (Gomes et al., 2010a). The change in gas composition was mathematically simulated and adjustment of packaging dimensions (A) and fruit weight (*M*), for given film permeability (P_{O_2}) and thickness (*l*), were manipulated to control the desirable levels of gases under steady-state conditions, according to the Eq. 2 (Talasila and Cameron, 1997).

$$R_{O_2} = \frac{R_{O_2}^{\max,T} \times pO_{2\,pkg}}{K_{m,O_2} + pO_{2\,pkg}} \qquad (1)$$

Estimates at 5 ºC (278.15 K) given by the Michaelis-Menten model were $R_{O_2}^{max,T}$ = 0.139 mmol kg^{-1} h^{-1}, and K_{m,O_2} = 0.240 kPa. Expected respiration rates at each oxygen level are displayed in Table 1.

$$pO_{2pkg} = pO_{2atm} - \frac{\left(R_{O_2} \times \mathrm{M}\right) \times l}{P_{O_2} \times A} \quad (2)$$

Fruit slices were placed inside open plastic bags with the characteristics summarized in Table 1. The bags were then flushed with a known gas mixture and sealed (Multivac A300/41/42, Germany). Gas mixtures with 18.2 kPa O_2 + 1.0 kPa CO_2, 2.0 kPa O_2 + 5.1 kPa CO_2, and 0.5 kPa O_2 + 5.1 kPa CO_2 (balance N_2) were obtained from Gasin (Barcelona, Spain). These active modified atmospheres are hereafter referred to as high oxygen (HO), low oxygen (LO) and very-low oxygen (VLO), respectively.

Levels of O_2 ($pO_{2\ pkg}$) and CO_2 inside the packages were monitored throughout storage with a gas analyzer (CheckMate II, PBI Dansensor, Ringsted, Denmark).

2.3 Storage conditions and sample analyses

Packaged fresh-cut pear was stored at 5.0 ± 0.5 ºC for 20 days. Samples were analysed immediately after processing (day 0) and after 2, 5, 7, 9, 12, 15, and 20 days in storage for flesh color and firmness, water activity, electrolyte efflux, juice soluble solids content, titratable acidity and pH. Microbial counts were obtained at 0, 3, 7, 10, 14, and 20 days in storage. Ethanol was measured 5 times throughout storage period in 2 packages of each oxygen level and pH buffer. Ascorbate content was determined at days 0, 2, 5, 8, 15, and 20 following the European standard EN14130:2003.

2.4 Color measurement

Color of the cut surface was measured on 45 slices sampled from sufficient bags (6 at minimum using heavier bags) per treatment. Color was measured in the CIE L*a*b* color space with a Konica-Minolta CR-400 chromameter (Osaka, Japan) equipped with a

D_{65} illuminant and the observer at 2º. Lightness (L*), chroma ($C^* = [(a^*)^2 + (b^*)^2]$ ½), hue angle [$h^o = \tan^{-1}$ (b*)/(a*)], and the metric hue difference ($\Delta H^* = [(\Delta a^*)^2 + (\Delta b^*)^2 - (\Delta C^*)^2]$ ½) between the initial reading and the observation date are reported.

2.5 Firmness assessment

Firmness was measured with a TA-XT2 Plus texture analyzer (Stable Micro Systems, Surrey, UK) in 45 slices per treatment and the maximum peak force required to force to drive a 3 mm flat head probe 5 mm into de slice with a travel speed of 1.5 mm s^{-1} was registered. All measurements were made with the fruit slices at 5 ºC.

2.6 Water activity

Disks with 20 mm of diameter were excised with a cork borer from slices. Water activity was measured in 8 pear disks (20-25 g) with a HygroLab3 meter (Rotronic, Hauppauge, NY, USA).

2.7 Electrolyte efflux

Four disks (*ca.* 2.5 g) were excised from pear slices with a cork-borer, in triplicate, rinsed and immersed in 25 mL of an isotonic mannitol solution (700 mM). Electrical conductivity of the incubation solution was measured with a Con 510 meter (Eutech Instruments, Nijkerk, The Netherlands) immediately after immersion of disks (EC_0) and at the end of a 2 h incubation period at 20 ºC (EC_f). The tissue and bathing solution was then frozen overnight at -20 ºC, thawed, and boiled under reflux for 15 min. Total tissue electrolytes (EC_t) were measured in this solution after cooling to ambient temperature. Electrolyte leakage (EL) was expressed as percentage of total tissue electrolytes $EL = \left(EC_f - EC_0\right) / \left(EC_t - EC_0\right) \times 100$] (Gomes et al., 2010b).

2.8 Measurement of soluble solid content, pH, and titratable acidity

Three composite samples per treatment and storage time were homogenized, filtered and the juices obtained were used to measure soluble solids content (SSC), juice pH, and titratable acidity (TA) (Gomes et al., 2010b).

2.9 Microbial counts

Random samples of *ca*. 10 g of pear slices, in triplicate, were taken from the packages and prepared plates on PCA were incubated at 30 ºC for 3 days for mesophilic aerobic bacteria, and at 5 ºC for 10 days for psychrophilic bacteria count (Gomes et al., 2010b). Microbial counts are reported as $\log_{10}$ colony forming units per gram of fresh sample weight [log (CFU g^{-1})].

2.10 Sensory evaluation

Pear slices were sampled after 8 days of storage at 5 ºC. Samples were prepared in enough quantities to allow that slices from the same pear subjected to different treatments were tasted by each panelist. A 28-member trained panel assessed the fresh-cut pears for browning intensity, firmness, and taste. Slices from each sample were presented to the panelists on coded plates. The analyses were performed in a sensory testing room equipped with individual booths, white tables, and controlled cool white fluorescent light according to the standard ISO 8589:1988.

A paired comparison test was used to address the effect of pH of the dipping solution. Samples were served to the panelists in three pairs with the same oxygen level but treated at different pH and the panelists were asked to answer to the questions: “which sample is firmer?” and “which sample is browner?”.

Ranking tests were used to assess the sensory quality of the combined MAP and pH of the additive treatments. Panelists were asked to rank all six samples (combinations of three O_2 levels and two pH treatments) by firmness and by brown color, using the number 1 (minimum) to the softer or clear sample and 6 (maximum) to the firmer or browner sample (ISO 8587:2006). Additionally, the panelists were asked to classify the taste of the six samples using the 9-point hedonic categorical scale, in which 1 = dislike extremely, 3 = dislike, 5 = neither like nor dislike, 7 = like, and 9 = like extremely.

2.11 Data analyses

Exploratory data analysis was done with two-factor analysis of variance (ANOVA) with packaging oxygen level and pH as fixed factors. Mean separation for factors was performed with Duncan's multiple range test at α=0.05. Experimental data were subsequently fitted to linear, exponential, and fractional conversion models to describe the kinetics of quality attributes. The 95% confidence interval on the parameter estimate was used to evaluate the significance of differences between parameters. The differences between predicted and observed values were quantified by the root mean square error (RMSE). Zero-order (Eq. 3), and first-order exponential (Eq. 4) and fractional conversion (Eq. 5) kinetics models were used to describe the changes in quality attributes.

$$P_t = P_0 + k \times t \quad (3)$$

$$P_t = P_0 \times e^{k \times t} \quad (4)$$

$$P_t = (P_0 - P_\infty) \times e^{-k \times t} + P_\infty \quad (5)$$

where P_t is the measured physical parameter at time t; P_0 is the initial physical parameter at time zero; k is the reaction rate coefficient; t is the time of storage (days); P_∞ is the final nonzero equilibrium value.

Sensory data were analyzed by the binomial test and the Friedman test, for the paired test comparison and the ranking test, respectively. When consistent differences among the rank orders of the samples resulted from the Friedman test, the Least Significant Difference (LSD) at α=0.05 was used for mean separation.

All ANOVA and regression analyses were performed with the statistical software SPSS 17.0 for Windows (SPSS, Chicago, USA).

3. Results

3.1 Packaging O_2 and CO_2 and respiration rates

The evolution of O_2 and CO_2 inside the packages is shown in Fig. 1. The actual levels (mean ± 95% confidence interval) of oxygen were 16.7±0.2, 1.8±0.2, and 0.25±0.04 kPa with corresponding CO_2 levels of 1.3±0.1, 4.3±0.2, and 6.5±0.4 kPa for HO, LO, and VLO treatments, respectively. The composition of the atmosphere was not affected by the pH of the dipping solution (not shown). Average respiration rates during 20 days of storage, calculated based on the oxygen partial pressures inside the packages and Eq. 2 solved in order at R_{O_2}, and package characteristic displayed in Table 1, were 0.298±0.027, 0.184±0.001, and 0.106±0.000 mmol kg^{-1} h^{-1} for HO, LO, and VLO, respectively.

3.2 Effect of packaging O_2 on the quality of fresh-cut pear

The changes in firmness of fresh-cut pear during storage were best described by fractional conversion kinetics (Table 2), although a zero-order model also fit experimental data accurately (not shown). The pear slices softened from initial experimental values of 10.2 N to 8.6-8.8 N by day 20 (Fig. 2A), with no significant effect of O_2 levels on the rate of firmness loss or final equilibrium value, F_∞ (Table 2).

Soluble solids content decreased slightly (1.5 ºBrix) during storage, a variation described by fractional conversion kinetics (Table 2, Fig. 2B). Juice pH increased during storage from initial values of 4.47 to a maximum of 4.79 (data not shown). The adjustment of a linear model was poor (Table 2) but suggests a rate close to zero. Consistently, experimental data on titratable acidity (TA) were very poorly adjusted by the linear model and had a null rate of variation (Table 2). The TA experimental values for all treatments during 20 days at 5 ºC were inside the confidence interval of [21.34, 22.40].

Experimental data on electrolyte efflux (not shown) and the respective adjusted models (Table 2) were not significantly affected by O_2 levels, although the linear models show a tendency of increase during storage, perceptible after day 2 (data not shown).

Significant color changes occurred during storage. These could be adequately described by a decrease in hue angle according to a first-order fractional conversion model (Table 2, Fig. 3A). After day 7 the hue angle was significantly lower in HO and very similar to the initial value in slices stored in VLO (Fig. 3A), although no statistical differences were observed in the model estimates of parameters (Table 2).

Ascorbate content decreased at similar rates for all oxygen treatments (Fig. 3B, Table 2). Ascorbate levels increased from 0.08 g kg^{-1} in freshly sliced pear to 3.71 g kg^{-1} following immersion in the antibrowning treatment solution, and declined exponentially during subsequent storage to 0.16 g kg^{-1} by day 20 (average of all treatments). Fractional

conversion models were able to explain more than 95% of the variation observed in the experimental data (Table 2).

Water activity remained constant [0.96, 0.97] throughout storage period as indicated by the reaction rate coefficient equal to zero for all treatments (Table 2).

Microbial counts, reported as log (CFU g^{-1}), increased linearly during the storage period (Table 2, Fig. 4). Although confidence intervals of the model parameters overlap (Table 2), means at each individual sampling date were significantly (P<0.05) higher at greater oxygen levels. Aerobic mesophilic counts of 6 log (CFU g^{-1}) are considered satisfactory for fresh-cut produce (Gilbert et al., 2000) and were reached in after 10 days in HO and LO, but later under VLO. Mean separation between LO and VLO treatment was possible after day 10 for psychrophiles, while for mesophiles the means were statistically different only on day 20.

The sensory panel was unable to detected differences in firmness or taste among MAP treatments (Table 3). However, the panel classified the browning intensity of the slices according to the oxygen levels inside the packages (Table 3).

3.3 Effect of pH on the quality of fresh-cut pear

The effect of pH on the kinetics of change in each quality attribute is indicated in Fig. 5 to Fig. 7 and Table 4. pH of the calcium ascorbate dipping solution had no statistical effect on the parameters of adjusted models for any of the quality attributes (Table 4), but differences in general trends were observed. The softening rate of fresh-cut pear slices during storage was slightly higher in slices treated at pH 3.0 than in those treated at pH 7.0 (Table 4, Fig. 5A) and can be described by fractional conversion models (Table 4).

The fractional conversion model provided a good adjustment of soluble solids content data, showing a faster decrease at pH 7.0 than at pH 3.0 but equal equilibrium

values (Table 4, Fig. 5B). No clear differences between treatments were observed in juice pH (data not shown). Linear models fitted to juice pH data (Table 4) indicate that initial values around 4.5 increased during storage at a slow rate of 0.001 and 0.004 units day^{-1}, at pH 7.0 and pH 3.0, respectively. Experimental values of titratable acidity of pear slices treated at pH 3.0 were inside the confidence interval of [22.72, 24.27], and were significantly higher ($P<0.05$) throughout storage than that of pears treated at pH 7.0 [19.77, 20.77]. Linear regressions show distinct treatments intercepts (Table 4), suggesting a direct effect of the exogenously applied solution.

Electrolyte efflux was higher in slices treated at pH 3.0 until day 12, but toward the end of the storage period slices treated at pH 7.0 had higher electrolyte leakage (data not shown), as can be assessed by the linear models (Table 4).

pH strongly affected the color of pear slices. Changes in hue angle followed a fractional conversion model (Table 4). Hue angles were significantly lower in slices treated at pH 3.0 (Fig. 6A).

Ascorbate content decreased according to a fractional conversion model (Table 4) although the final equilibrium value for pH 7.0 (0.34 g kg^{-1}) is twice the experimental value obtained after 20 days in storage (0.17 g kg^{-1}). pH did not affect the kinetics of ascorbate depletion (Table 4, Fig. 6B).

Water activity was not affected by pH and remained constant at 0.97 throughout storage (Table 4).

Microbial load [log (CFU g^{-1})] followed a linear trend (Table 4), with higher bacterial counts at pH 7.0 than at pH 3.0 (Table 4, Fig. 7).

The sensory panel considered that slices treated at pH 3.0 were darker than those dipped in a solution at pH 7.0 (Table 5). Slices treated at pH 3.0 were also considered

softer, but not in all oxygen treatments (Table 3 and Table 5). The panel was unable to detect differences in the taste of slices treated at different pH values (Table 3).

4. Discussion

4.1 Kinetics of quality changes

MAP is an almost universal complement to refrigeration in the technology of fresh-cut fruit. In addition to MAP and refrigeration, fresh-cut fruit susceptible to enzymic browning require additives to help retain the fresh-like quality. The quality of fresh-cut fruit is a multidimensional attribute, whose changes involve metabolic, microbial and physical factors and follow different kinetic patterns.

Fractional conversion and linear kinetic models adequately described almost every quality attribute. The model that best fit experimental data, based on the lowest RMSE, was adopted to describe the kinetics of change. No curve adjustment was possible with enough accuracy for pH, TA, electrolyte leakage and a_W, due to the wandering of data around a fixed value, without any trend during the storage period considered. Naturally occurring random variation among samples is likely responsible for most of the unexplained variation (Lesage and Destain, 1996).

A fractional conversion model was used to describe the changes in firmness, SSC, hue angle, and ascorbate content. This model has been used to describe firmness loss in whole kiwifruit (Hertog et al., 2004), fresh-cut pear (Soliva-Fortuny et al., 2002b), cut tomato (Lana et al., 2005), and heated vegetables (Rizvi and Tong, 1997), and also lightness decrease in cut pear (Soliva-Fortuny et al., 2002a). Since firmness is not an extinguishable variable given the fact that cell wall structure do not allow for a final value of zero, the fractional conversion model was preferred over a zero-order kinetics, which

also fitted experimental firmness data with good accuracy. The limited storage period of samples can contribute to zero-order kinetics description of variable behavior as well as first-order kinetic models (*cit*. Rizvi and Tong, 1997). Ascorbate loss of intact pear, 40 days after harvest or during storage for 100 days, was described by first-order kinetics (Zerbini et al., 2002), similar to the performed in this trial. Linear regressions were a simple and accurate (R^2>0.96) way to modelled the microbial growth [log (CFU g^{-1})] under diverse oxygen and pH treatments, while Raybaudi-Massilia et al. (2009) preferred the Gompertz equation, using non-inoculated fresh-cut pears.

4.2 Effect of MAP on quality changes

The average respiration rates registered in fresh-cut pear stored under LO and VLO were very similar to those measured in packed cut pear obtained from whole fruit stored for 6 months (Table 1), but were 1.1 (VLO), 1.5 (LO) and 2.2 (HO) times higher than the respiration rates found at 5 ºC for packed fresh-cut pear obtained from intact pears stored for only 5 weeks (Gomes et al., 2010a; Table 1). Measured oxygen concentration in the VLO treatment was close to the fermentation threshold of 0.25 kPa (i.e. the lower limit of oxygen partial pressure needed for aerobic respiration) detected to fresh-cut pear stored at 5 ºC (Gomes et al., 2010a); in these packages, ethanol concentration was more than three times higher than in HO or LO packages (data not shown) denoting anaerobic metabolism to some extent.

The benefits of MAP have been attributed to the ability to reduce metabolic rate (Beaudry, 1999). However, MAP *per se* is ineffective in preventing enzymic browning (Gorny et al., 2002; Oms-Oliu et al., 2008b), and for that reason is considered of little benefit in fresh-cut pear (Gorny, 2001). Therefore, antibrowning additives are required in the processing technology for fresh-cut pear.

In ‘Rocha’ pear, the kinetics of respiration rate as affected by O_2 partial pressure at 5 ºC anticipate that MAP would be of little benefit in reducing the changes in quality attributes dependent on the metabolic rate of the tissue (Gomes et al., 2010a). In fact, for quality attributes whose changes are largely dependent on the metabolic activity of the tissue – firmness, SSC, TA, pH – no significant differences were observed among the MAP treatments (Table 2). Consistently, no differences on firmness loss were observed on fresh-cut pear subjected to 0.25, 0.50, or 21 kPa O_2 (plus 0, 5, 100 or 20 kPa CO_2) for 10 days at 5 ºC (Gorny et al., 2002) or to 2.5 or 21 kPa oxygen at least for 21 days at 5 ºC (Oms-Oliu et al., 2007b, 2008b). However, a metabolic linkage between gas exchange and softening may exist in intact apple and kiwifruit stored at different MA conditions (Hertog et al., 2001; 2004), since lowering oxygen levels decreased respiration rate and fruit firmness was better retained (assuming no influence of ethylene).

The similar rates of decrease in SSC during the first days under the three O_2 levels (Figs. 2B) and the steady pH values (Table 2) are also consistent with the residual effect of MAP on metabolic changes. Although higher respiration rate was observed in air atmospheres, no differences in SSC and pH were detected in apple slices packed in air or 5 kPa O_2 plus 5 kPa CO_2 for 12 days at 4 ºC (Rocculi et al., 2004). Organic acids, and not sugars, the major respiratory substrates of pear slices at 5 ºC, as indicated by the respiratory quotient of 1.38 (Gomes et al., 2010a), and the differences in respiration rate were not enough to impart a significant differences in pH or TA of pear slices during storage at 5 ºC under the three O_2 levels (Table 2). SSC of fresh-cut pear have been reported to remain relatively unchanged during storage under 0 and 2.5 kPa O_2 (Soliva-Fortuny et al., 2007) or to be better retained in low oxygen atmospheres than in air after 7 days at 5 ºC (Oms-Oliu et al., 2007b). Oms-Oliu et al. (2007b) reported an increase of juice pH of pear wedges stored more than 14 days in non-steady 2.5 kPa O_2 and in air and related it with anaerobic

processes and microbial contamination. No differences were detected between TA of cut pear stored under diverse oxygen levels (Table 2), while initial anoxic (0 kPa O_2) atmospheres could cause higher decrease in TA of pear cubes than hypoxic (2.5 kPa O_2) MAP (Soliva-Fortuny et al., 2007). Electrolyte leakage remained constant during storage and unaffected by MAP treatments (Table 2), indicating that membrane competence was not differentially affected by the O_2 levels used in this study.

Enzymic browning in cut surface of fresh-cut pear cannot be considered a metabolic change and is better envisioned as a biochemical reaction in a cell-free extract. In fact, polyphenoloxidase (PPO) previously located in plastids and phenolic compounds previously sequestered in the vacuole come in contact as a result of tissue wounding and react on the cut surface. PPO catalyses the oxidation of *o*-diphenols into *o*-quinones that polymerize subsequently to form dark melanins (Yoruk and Marshall, 2003). PPO has a relatively high affinity for O_2, with apparent K_m values of 0.11 mM in 'Bartlett' pear (Rivas and Whitaker, 1973). Saturation of the O_2 substrate for 'Conference' pear PPO activity is achieved at a concentration of 0.26 mM (Espín et al., 2000). Therefore, in the context of fresh-cut fruit, the effect of O_2 levels on enzymic browning is exerted directly and not via the metabolic activity. Oxygen concentration induced differences in color (Fig. 3A), possibly by the inhibition of polyphenoloxidase (Heimdal et al., 1997) and subsequent browning reactions, or by a more stable antioxidant activity of ascorbate at low O_2 levels (Aguayo et al., 2010), since Espín et al. (2000) suggested that PPO from 'Conference' pear has high affinity to ascorbic acid (at pH 7.0 apparent K_m of 0.55 mM is 30 times lower for AA than for chlorogenic acid) and can oxidize ascorbic acid.

Slices stored under higher oxygen levels became browner than those stored under lower levels (Fig. 3A), an effect that was clearly perceived by the sensory panel (Table 3). Other authors have reported an effect of O_2 on browning of pear and apple slices (Soliva-

Fortuny et al., 2007; Aguayo et al., 2010), but under very low O_2 levels (0-2.5 kPa) no differences in hue angle were observed (Soliva-Fortuny et al., 2002a). In the VLO treatment, the infinite value for hue obtained with fractional conversion model was slightly higher than the initial value, following an increase between day 0 and day 2 (Fig 3A). A similar effect was also detected in fresh-cut apple treated with calcium ascorbate (Aguayo et al., 2010).

Ascorbic acid content can be negatively related with CO_2 levels inside packages greater than 5 kPa (Cocci et al., 2006) or with CO_2 production (Oms-Oliu et al., 2007b; Techavuthiporn et al., 2008). However, despite the different respiration rates observed in cut 'Rocha' pear stored under three oxygen levels, no kinetic differences in the loss of exogenous ascorbate were observed during storage (Fig. 3B). Initial content of AA (371 g kg^{-1} fresh weight), achieved after immersion in dipping solutions, was reduced by 65 to 75% during the first 5 days of storage. Similarly, 1 day at 4 ºC was enough to lose 60-80% of AA in apple slices dipped in 1% AA and packed in air or in 5% O_2 plus 5% CO_2 (Cocci et al., 2006). Endogenous AA content of fresh-cut 'Flor de Invierno' pear stored at 5 ºC are better preserved under 2.5 kPa O_2 than in 21 kPa O_2 (Oms-Oliu et al., 2007b). Therefore, O_2 levels may have differential effects on endogenous or exogenously applied ascorbic acid. Dehydration of the cells at the cut surface is observed in fresh-cut pear and can limit consumer acceptability. Oxygen level (and subsequently water vapor production by respiration) did not affect water activity (Table 2). Plastic films used to make packages were from the same material, so diffusion rates were similar in all treatments.

Microbial counts were significantly affected by MAP oxygen levels; microbial contamination was higher under HO or LO than under VLO treatments. Similar results were obtained by Oms-Oliu et al. (2008b) with higher growth rate for aerobic psychrophilic bacteria in cut pears stored under passive MAP than under MAP with oxygen levels below

2.5 kPa. Low oxygen atmospheres were also beneficial to control yeast and mold proliferation on fresh-cut pear (Oms-Oliu et al., 2008b). Although there were statistically significant differences ($P<0.05$) among means, the limit of acceptability of 6 log CFU g^{-1} (Gilbert et al., 2000) was reached after 10-14 days in all treatments and no practical distinction can be made on shelf-life based on microbial load.

The sensory evaluation of firmness and color was consistent with the results from instrumental analysis. No preference distinctions were made among the three MAP conditions (Table 3). In oxygen-free atmosphere the high acetaldehyde and ethanol content was not detected by an informal organoleptic evaluation of apple slices (Gil et al., 1998), whereas in pear cubes the perception of a lack of flavor or sweetness was detrimental in the sensory evaluation (Soliva-Fortuny et al., 2007).

4.3 Effect of antibrowning additive

Additives are often required to help retain the overall quality of fresh-cut produce. In particular, in fruit prone to enzymic browning, e.g., pear, antibrowning additives are crucial to minimize quality deterioration. The pH of the formulation interferes with the efficacy of additives and pH, *per se*, is an important explanatory variable for the effects of additives (Gomes et al., 2010b). Calcium ascorbate is very effective in reducing browning because ascorbate reduces the *o*-quinones formed by polyphenoloxidase enzyme back to their precursor diphenols (Toivonen and Brummell, 2008). On fresh-cut pear treated with calcium ascorbate at pH 3.0 and pH 7.0 little changes in hue angle were observed after six days at 5 ºC (Gomes et al., 2010b). In contrast, in this experiment significant decreases in hue angle occurred by day 5 in slices treated at pH 3.0, and changes intensified until the end of storage period (Fig. 6A). This trend in color changes is related to the exponential decrease in ascorbate content during storage (Figs. 3B and 6B); by day 5, the initial

ascorbate content decreased by 75% and only weak antibrowning effect can be obtained thereafter. Low levels of ascorbic acid (1%) on apple slices lose its reducing power 20 min after the treatment (Son et al., 2001) and are less effective in preventing browning than high levels (6-20%) (Aguayo et al., 2010). Even in the absence of added ascorbate, pH, *per se*, can affect browning: browning of pear slices is faster and more intense after a dip in a solution at pH 3.0 than in the same solution at pH 7.0 (Gomes et al., 2010b). The pH effect was perceptible by the sensory panel (Tables 3 and 5).

Pear slices dipped in solutions at pH 3.0 become softer than those treated at pH 7.0 (Gomes et al., 2010b), and ascorbate solutions adjusted to pH 7.0 are more effective in reducing softening than acidic solutions (Gorny et al., 2002; Gomes et al., 2010b). Consistently, the sensory panel perceived the pear slices treated at pH 3.0 as softer than those treated with pH 7.0 in LO and VLO MAP, but not in HO (Tables 3 and 5), even though the differences in instrumental firmness were not significant (data not shown).

Titratable acidity was affected by the dipping solution during storage ($P<0.05$), and linear regressions show higher intercept to slices treated at pH 3.0 than at pH 7.0 (Table 4). Titratable acidity is a measure of the total acid concentration, even the amount of hydrogen ions released from undissociated acid during titration, and the chemical nature of buffer solution at pH 3.0 (citrate, $pK_a>3.0$) interferes with this measure, as also reported by Cocci et al. (2006).

Electrolyte efflux in pears treated with calcium ascorbate at pH 3.0 remained relatively constant and tended to increase after treatment at pH 7.0 (Table 4). Electrolyte leakage from both treatments are close to the ones obtained previously with slices treated with calcium ascorbate at pH 7.0 (Gomes et al., 2010b) and indicate that no tissue deterioration occurred during storage.

The antimicrobial effect of acidic solutions observed in this experiment are consistent with those reported elsewhere (Karaibrahimoglu et al., 2004; Raybaudi-Massilia et al., 2009; Gomes et al., 2010b).

5. Conclusions

MAP with aerobic low oxygen concentrations did not retard the changes in the quality attributes of fresh-cut pear that depend directly on metabolism, such as firmness, SSC, juice pH, TA, and electrolytes efflux. However, low levels of oxygen inside packages clearly benefit color and, eventually, microbial safety, although the practical significance of MAP for these purposes is questionable. pH of dipping solution had significant effects on browning, on TA and on microbial growth, and should be considered in the use of additives. Sensory evaluation did not find taste differences in fresh-cut pear stored under the three MAP conditions.

Tables

Table 1. Combinations of packaging dimensions, film permeability and thickness, and fruit weight needed to obtain the desired level of oxygen at 5 ºC.

O_2 level	**Respiratory rate** [a] (mmol h^{-1} kg^{-1})		**Product Weight**	**Films**			**Package surface** [d]
	5 weeks storage	6 months storage	(kg)	**Type** [b]	**Thickness** (cm)	**OTR** [c]**, at 5 ºC** (mmol cm cm^{-2} h^{-1} kPa^{-1})	(cm^2)
HO	0.138	0.215	0.027	OPP	0.0040	9.048×10^{-09}	800
LO	0.125	0.173	0.110	OPP	0.0025	3.257×10^{-09}	800
VLO	0.095	0.104	0.116	OPP	0.0025	3.257×10^{-09}	450

[a] Respiratory parameters estimates for pears stored for 5 weeks were published in Gomes et al. (2010a), while values for pears stored for 6 months are based on unpublished data using the same methodology (Gomes et al., 2010a) in a different set of data [$R_{O_2} = (1.56\times10^{-18} \times e^{0,1421*T} \times pO_{2pkg}) / (1.33\times10^{-11} \times e^{0,088*T} + pO_{2pkg})$]

[b] OPP – oriented polypropylene (Amcor Flexibles, Ledbury, UK);

[c] OTR – oxygen transmission rate; CO_2 permeability can be obtained multiplying OTR by 4.

[d] Package surface = length × width × 2 sides

Table 2. Estimated value (± 95% confidence interval) of the linear and the fractional conversion models parameters used to describe quality evolution of fresh-cut pear under MAP with three oxygen levels during 20 day at 5 ºC.

Parameter	O_2 level	P_0	k (day^{-1})	P_∞	R^2
Firmness	HO	10.40±1.23	0.133±0.425	8.76±2.03	0.575
(N)	LO	10.23±0.66	0.046±0.222	8.00±7.11	0.767
	VLO	10.16±0.51	0.043±0.170	7.78±6.45	0.841
SSC	HO	13.36±0.66	0.234±0.320	11.91±0.54	0.830
(%)	LO	13.26±0.40	0.294±0.265	11.99±0.26	0.915
	VLO	13.27±0.42	0.403±0.393	12.03±0.23	0.904
pH	HO	4.43±0.32	0.006±0.030		0.035
	LO	4.49±0.16	0.002±0.015		0.018
	VLO	4.53±0.15	0.000±0.014		0.001
TA	HO	21.75±2.35	0.071±0.218		0.095
(mmol H^+L^{-1})	LO	22.12±2.57	-0.082±0.238		0.106
	VLO	21.13±2.87	0.081±0.266		0.085
Electrolyte efflux	HO	18.74±1.99	0.089±0.185		0.188
(%)	LO	18.09±3.59	0.121±0.333		0.116
	VLO	18.57±1.95	0.051±0.181		0.073
hº	HO	101.5±4.2	0.040±0.300	90.0±59.3	0.720
	LO	101.0±1.8	0.024±0.314	94.0±74.3	0.613
	VLO	100.9±1.2	-0.239±0.849	101.0± 1.4	0.442
Ascorbate	HO	3.65±0.48	0.289±0.120	0.19±0.36	0.992
(g kg^{-1})	LO	3.65±0.60	0.367±0.191	0.26±0.40	0.988
	VLO	3.56±1.15	0.301±0.311	0.26±0.85	0.953
aW	HO	0.97±0.02	0.000±0.003		0.014
	LO	0.96±0.02	0.000±0.002		0.008
	VLO	0.97±0.02	0.000±0.002		0.211
Mesophiles	HO	2.63±0.34	0.326±0.030		0.996
[log (CFU g^{-1})]	LO	2.46±0.64	0.320±0.057		0.984
	VLO	2.40±0.46	0.313±0.041		0.991
Psychrophiles	HO	2.92±0.62	0.255±0.055		0.976
[log (CFU g^{-1})]	LO	2.62±0.58	0.262±0.052		0.980
	VLO	2.59±0.62	0.258±0.055		0.977

Table 3. Sensory assessment of quality attributes of pear slices stored for 8 days under three oxygen levels and treated with calcium ascorbate buffered at pH 3.0 and pH 7.0.

Oxygen level	pH	Rank[A] color	Rank[A] firmness	Taste[B]
HO	**3**	161[a]	105[a]	5.5 ± 0.3[a]
	7	113[b]	90[a]	5.8 ± 0.4[a]
LO	**3**	85[c]	79[a]	5.8 ± 0.3[a]
	7	91[b,c]	99[a]	6.0 ± 0.3[a]
VLO	**3**	65[c,d]	91[a]	6.1 ± 0.3[a]
	7	52[d]	103[a]	5.9 ± 0.3[a]

Values within columns followed by a common letter are not significantly different ($P > 0.05$).

[A] Values are sums of attributed number that varied between 1 (minimum, to the softer or pale sample) and 6 (maximum, to the firmer or browner sample), $n = 27$.

[B] Values are means (±SE) of classification using the 9-point hedonic category scale, in which 1 = dislike extremely and 9 = like extremely, n=27.

Table 5. Sensory assessment of quality attributes by paired comparison test (PCT) between samples subjected to the same oxygen level and different solution pH.

Oxygen level	Color		Firmness	
	pH 3.0	pH 7.0	pH 3.0	pH 7.0
HO	26[a]	2[b]	14[a]	14[a]
LO	24[a]	4[b]	10[a]	18[a]
VLO	19[a]	9[a]	8[b]	20[a]

Values within lines followed by a common letter are not significantly different ($P > 0.05$); values are responses, $n = 28$.

Table 4. Estimated value (± 95% confidence interval) of the linear and the fractional conversion model parameters used to describe quality evolution of fresh-cut pear treated with calcium ascorbate at pH 3.0 and at pH 7.0 and stored under MAP at 5 ºC for 20 days.

Parameter	**pH**	P_0	k (day^{-1})	P_∞	R^2
Firmness	3.0	10.55±0.60	0.132±0.198	8.85±0.99	0.861
(N)	7.0	10.04±0.79	0.047±0.047	8.00±8.00	0.660
SSC	3.0	12.99±0.33	0.261±0.265	12.03±0.24	0.896
(%)	7.0	13.59±0.47	0.313±0.252	11.93±0.30	0.931
pH	3.0	4.47±0.17	0.004±0.016		0.068
	7.0	4.50±0.23	0.001±0.022		0.001
TA	3.0	23.38±4.15	0.010±0.386		0.001
(mmol H^+L^{-1})	7.0	20.17±2.08	0.014±0.193		0.005
Electrolyte efflux	3.0	19.60±2.66	0.004±0.247		0.000
(%)	7.0	17.34±2.92	0.169±0.271		0.279
hº	3.0	101.5±2.2	0.030±0.189	90.0±53.9	0.842
	7.0	100.9±1.7	-0.124±0.405	101.2± 3.7	0.565
Ascorbate	3.0	3.33±0.56	0.259±0.138	0.14±0.45	0.987
(g kg^{-1})	7.0	3.94±0.98	0.396±0.319	0.34±0.64	0.972
aW	3.0	0.97±0.02	0.000±0.002		0.078
	7.0	0.97±0.02	0.000±0.002		0.009
Mesophiles	3.0	2.49±0.54	0.314±0.049		0.988
[log (CFU g^{-1})]	7.0	2.51±0.31	0.325±0.027		0.996
Psychrophiles	3.0	2.70±0.59	0.254±0.053		0.978
[log (CFU g^{-1})]	7.0	2.72±0.57	0.263±0.051		0.981

Figures

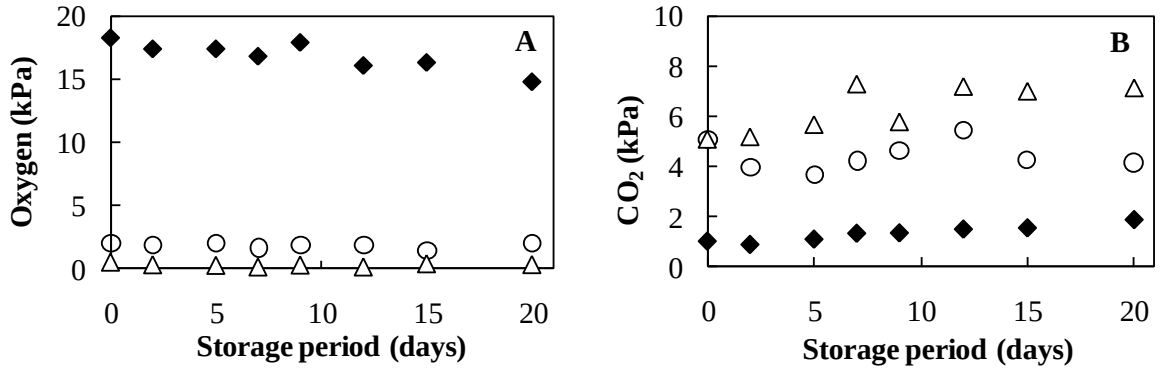

Figure 1. Oxygen (A) and carbon dioxide (B) inside the modified atmosphere packages during 20 day of storage at 5 ºC. Symbol treatments: ♦ – high oxygen, ○ – low oxygen, and Δ – very low oxygen.

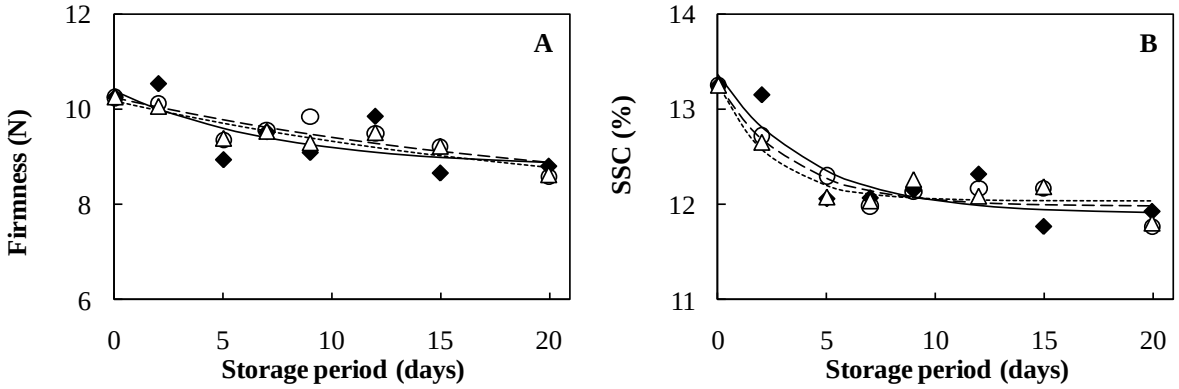

Figure 2. Firmness (A) and soluble solids content, SCC (B) evolution during 20 day of storage at 5 ºC. Symbol treatments: ♦ – high oxygen (HO), ○ – low oxygen (LO), and Δ – very low oxygen (VLO). Lines for curve adjustment: — HO, – – LO, and --- VLO.

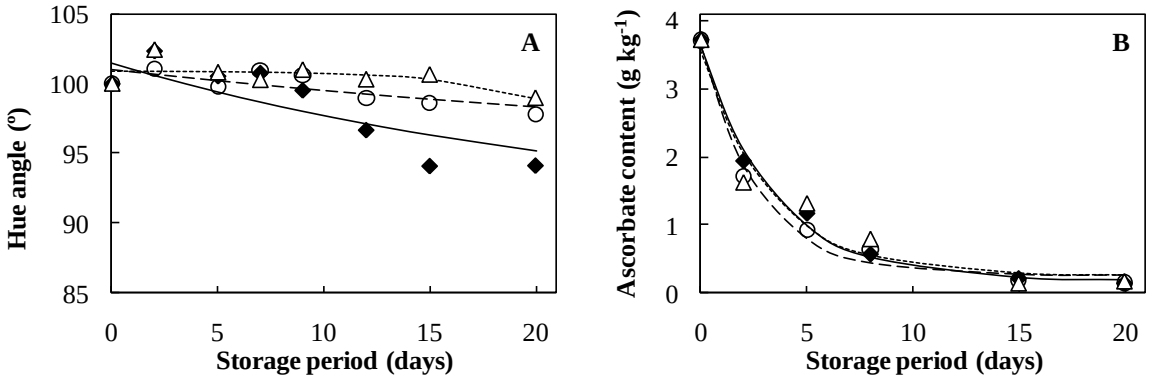

Figure 3. Hue angle (A) and ascorbate content (B) evolution during 20 day of storage at 5 ºC. Symbol treatments: ♦ – high oxygen (HO), ○ – low oxygen (LO), and Δ – very low oxygen (VLO). Lines for curve adjustment: — HO, – – LO, and --- VLO.

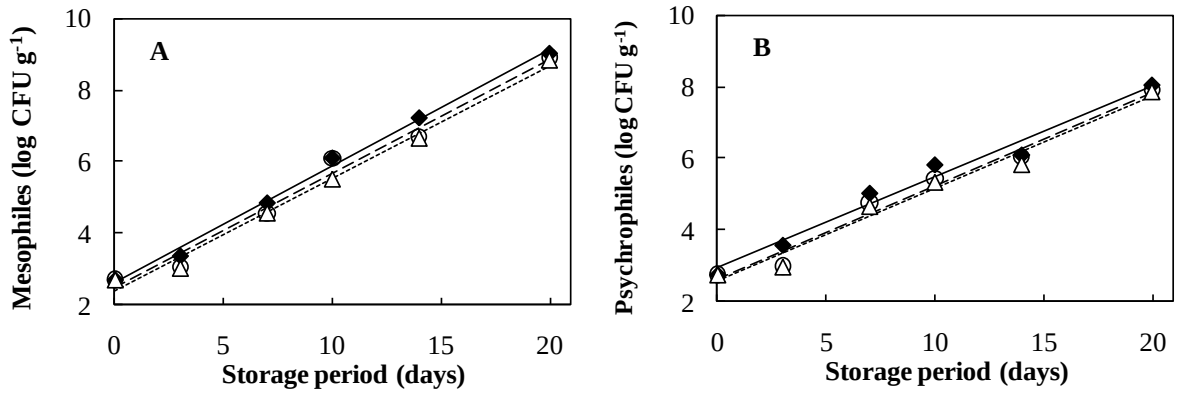

Figure 4. Aerobic mesophiles (A) and psychrophiles (B) evolution during 20 day of storage at 5 ºC. Symbol treatments: ♦ – high oxygen (HO), ○ – low oxygen (LO), and Δ – very low oxygen (VLO). Lines for curve adjustment: — HO, – – LO, and --- VLO.

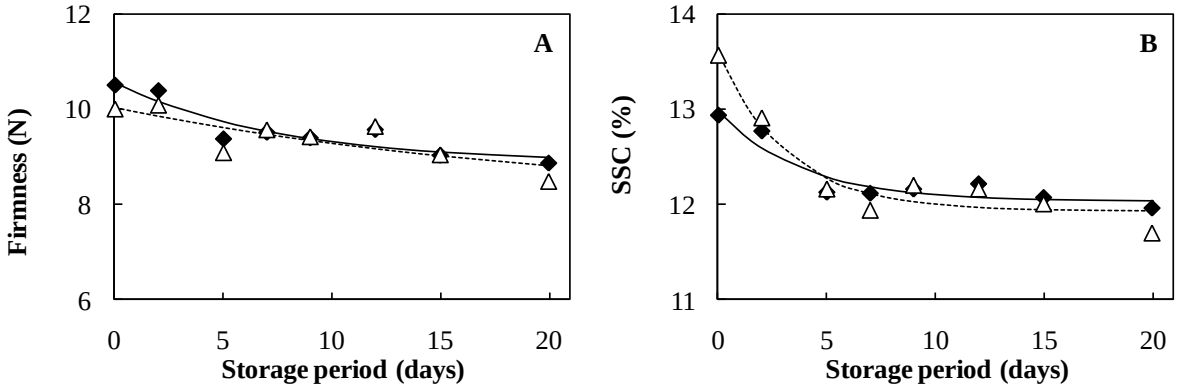

Figure 5. Firmness (A) and soluble solids content, SCC (B) evolution during 20 day of storage at 5 ºC. Symbol treatments: ♦ – pH 3.0, and Δ – pH 7.0. Lines for curve adjustment: — pH 3.0, and --- pH 7.0.

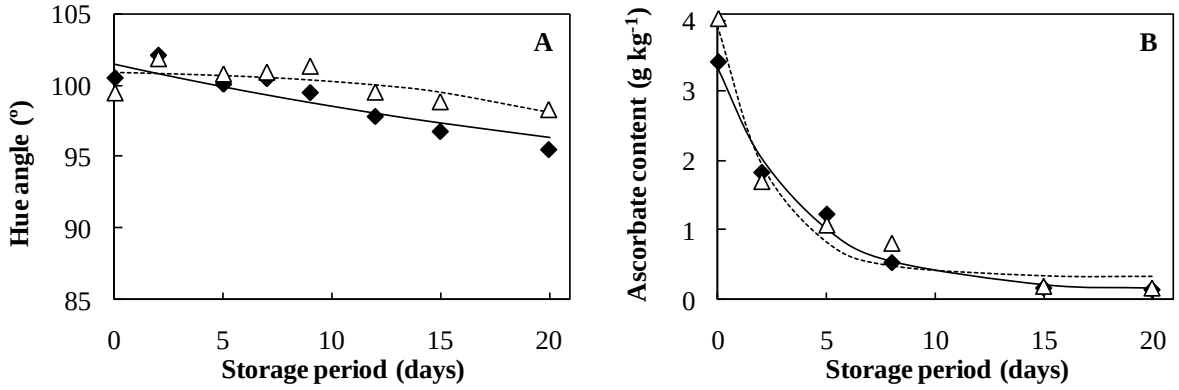

Figure 6. Hue angle (A) and ascorbate content (B) evolution during 20 day of storage at 5 ºC. Symbol treatments: ♦ – pH 3.0, and Δ – pH 7.0. Lines for curve adjustment: — pH 3.0, and --- pH 7.0.

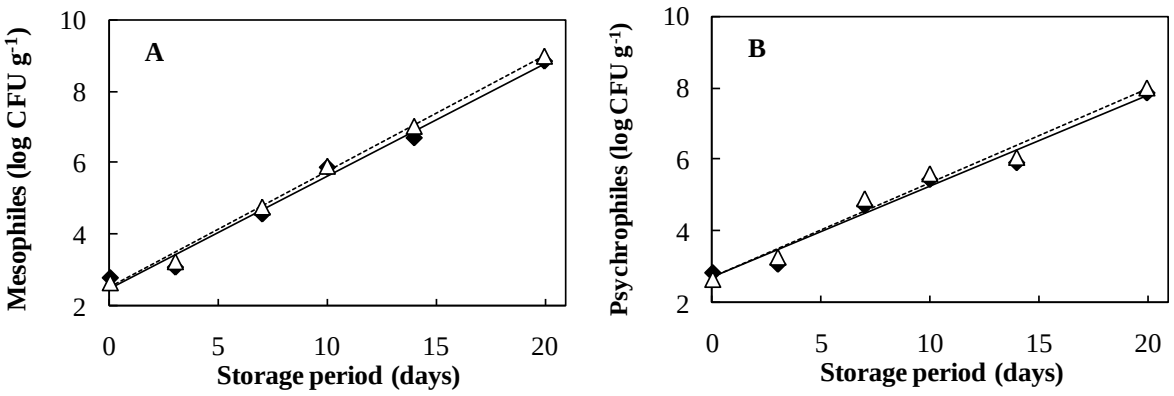

Figure 7. Aerobic mesophiles (A) and psychrophiles (B) evolution during 20 day of storage at 5 ºC. Symbol treatments: ♦ – pH 3.0, and Δ – pH 7.0. Lines for curve adjustment: — pH 3.0, and --- pH 7.0.

CHAPTER 6

GENERAL DISCUSSION

1. The need for a systematic approach

The goal of postharvest research is to understand the mechanisms of quality building and depreciation in living horticultural commodities and to develop and apply technologies aimed at preventing quality depreciation. In the literature pertaining fresh-cut fruit there are abundant reports on experiments characterizing quality, but systematic information about the physiological and biochemical bases underlying the quality changes is often limited. Differences in the methodologies applied, different levels of integration of independent variables, and the multiple interactions at play in these systems, pose challenges in the optimization of processes and in conjectures about their efficacy. In addition, genotypic variability among cultivars in the kinetics of quality changes in response to wounding recommends a cultivar-specific approach. Differences in storage periods between cut and whole fruit, and the consequent weight of metabolic processes involved, also play a role in limiting technology transfer to the industry.

Appearance and texture are two major attributes affecting quality of fresh-cut fruit (Toivonen and Brummell, 2008). Current technologies to keep quality of fresh-cut fruit resort on an efficient cold-chain, to minimize metabolic and microbial activities, and on packaging, to prevent post-processing contamination and reduce water loss. MAP *per se* is ineffective in preventing enzymic browning in fresh-cut pear, and antibrowning additives are required during processing. It has long been assumed that modified atmospheres, with O_2 partial pressures lower and CO_2 partial pressures higher than those in the normal atmosphere, extend the shelf-life of minimally processed produce (Gorny, 1998). However, MAP is considered of little benefit to fresh-cut pear (Gorny, 2001), but recent studies evaluating color, texture and/or microbial spoilage (Sapers and Miller, 1998; Soliva-Fortuny et al., 2002a,b; Oms-Oliu et al., 2008b) seem to suggest otherwise. As stated by Gorny (1998), many of modified atmosphere requirements and recommendations derived

from generalizations that warrant detailed examination, namely on the product's respiration rate to design proper MAP. The effects of packaging on individual quality attributes derived from physiological, biochemical, and microbiological changes, under a range of gases concentrations, needs to be study in order to judge the potential benefits of MAP and find the optimal environmental conditions for each commodity. The research reported in this thesis aimed at evaluating i) the potential MAP utilization for fresh-cut 'Rocha' pear and cantaloupe melon by using a physiological-based model of respiratory behavior (**Chapter 2**), and the effect of MAP on the kinetics of individual quality attributes by using packages designed to achieve target atmospheres to test hypothesis (**Chapter 5**), and ii) the efficacy of commonly used antibrowning and firming food additives, trying to isolate explanatory variables for the observed quality changes (**Chapters 3 and 4**). The results reported herein can be used by the fresh-cut industry to support innovation or improvement of processes and technologies.

2. Conclusions

A single process or technology can influence multiple quality attributes, some in a favorable way but detrimentally affecting others. These 'pleiotropic' effects were found in the use of additives as a preservation method using fresh-cut 'Rocha' pear as a model system (**Chapter 3**). pH of the dipping solution can affect, *per se*, color, texture, and microbial growth of fresh-cut pears. Acidification of dipping solutions to pH 3.0 to prevent microbial growth will result in enhancement of browning, softening, and disassembly of the cell wall. The choice of calcium formulation additives to prevent undesirable change on visual and sensorial quality of cut produce should consider the pH ranges that provide the expected benefits. The use of acidic solutions of calcium ascorbate, lactate, or propionate is not recommended to prevent tissue damage, while the effects of calcium chloride are less

affected by pH than those of other calcium salts. The results suggest that pH is a very important explanatory variable for the effects of surface treatments in fresh-cut fruit that must be considered in research and in industrial practice.

Results of *in vitro* PPO activity as affected by pH, phenolic substrates and food additives did not correlate with browning of pear slices affected by the same factors (**Chapter 4**). pH and the phenolic substrate have a strong effect on both browning and PPO activity of 'Rocha' pear, but interactions between pH and substrate must be considered. PPO activity was not found to be a good indicator of tissue browning in fresh-cut 'Rocha' pear, either in the presence or in the absence of inhibitors. Substrates that induce rapid color changes can be used to develop quick tests for antibrowning additives.

Respiration-based models (**Chapter 2**) revealed that a safe working atmosphere does not exist in fresh-cut 'Rocha' pear and cantaloupe melon; therefore, the optimization of low oxygen levels under modified atmosphere packaging will bring no significant benefit in slowing down metabolism without inducing fermentation. Packages were designed to establish an equilibrium modified atmosphere by matching package permeation to oxygen with the O_2 consumption by fresh-cut fruit. Oxygen partial pressures obtained inside packages were very close to those predicted by the models (**Chapter 5**) validating the proposed Michaelis-Menten kinetic for O_2 uptake and the Arrhenius-type equation for temperature effect in fresh-cut 'Rocha' pear (**Chapter 2.1**). Low oxygen under modified atmosphere packaging, in combination with calcium ascorbate at pH 7.0 as antibrowning additive, allowed marginal improvements in fresh-cut pear quality based on color and microbial growth, and no disadvantages were found in the use of MAP (**Chapter 5**). MAP with aerobic low oxygen concentrations did not retard the changes in the quality attributes of fresh-cut pear that depend directly on metabolism, such as firmness, SSC, juice pH, TA and electrolytes efflux, compared to high oxygen concentrations. Based on these results,

MAP would not extend shelf-life of fresh-cut pear, in agreement with the theoretical estimations proposed.

In synthesis, industry efforts in optimizing quality and safety of fresh-cut produce benefit from a systematic scientific-technological approach, starting with the physiological basis for quality changes, and integrating the interactions at play in the complex system that a package of fresh-cut fruit actually is. Without this systematic approach, the constraints of the 'pack-and-pray' way will remain.

3. Future research directions

In the research process, finishing lines often become starting points for new inquiries. The understanding of fresh-cut systems will certainly benefit from developments in fundamental areas of the physiology, biochemistry, and molecular biology of wounded fruit tissues. The effect of independent variable must be understood *per se* to identify the most critical steps in the rate and intensity of degradation reactions in minimally processed produce. However, integration and a thorough understanding of the interactions at play among the factores that affect quality of fresh-cut fruit are essential. Predictive models are a very useful tool in identifying the key factors and would allow the design of best strategy and the search of alternative technologies for keeping quality. If there are physiological limitations in some quality attributes, the efforts of optimizing existing technological solutions are misplaced; instead the focus (paradigm) should change and quality improvement need to be searched in new technologies or in areas such as breeding programs to find cultivars well adapted to minimally processing. In addition, the physiological-based models presented herein can be a useful tool in the postharvest area, namely supporting decisions in the long-term storage of intact 'Rocha' pear, where data and validation experiments are still missing.

In particular, the research results presented in this thesis suggest the following lines of future inquiry:

- The physiological response to CO_2, not addressed in the research reported in this thesis, remains to be clarified. A systematic study of the effect of CO_2 on respiration and metabolically related quality attributes, similar to the study reported herein for O_2, is necessary. Subsequent work should address the interaction between O_2 and CO_2, at play in MAP, and incorporate these refinements in the development of more efficient modified atmospheres packages for fresh-cut fruit.
- Enzymic browning is the earliest practical consequence of cutting in fresh-cut pear, and the use of antibrowning additives in combination with MAP can prevent color changes and increase shelf-life to 2-3 weeks. However, cut surface dehydration is visible few days after processing and constitutes a visual and sensorial problem in fresh-cut pear. Water loss and water mass transfer in fresh-cut systems (produce + microflora + additives + package) should also be subjected to systematic analyses based on the physical and physiological processes involved.
- Discrepancies observed between the pH dependency of PPO activity and browning need clarification and antibrowning potential of food additives should be assess in living tissues, in addition to *in vitro* tests.
- A consumer orientation to quality would search for good flavored fresh-cut fruits that MAP should preserve. The effect of MAP on aroma volatiles has been rarely characterized and calls for more research in order to design MAP that account for consumer flavor preferences.

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