UNIVERSIDAD POLITÉCNICA DE VALENCIA
Instituto Universitario de Ingeniería de Alimentos para el Desarrollo

EFFECT OF SOY PROTEIN-BASED EDIBLE COATINGS WITH ANTIOXIDANT ACTIVITY AND MODIFIED ATMOSPHERE PACKAGING ON THE QUALITY OF FRESH-CUT PRODUCES

EFECTO DE RECUBRIMIENTOS COMESTIBLES A BASE DE PROTEÍNA DE SOJA CON ACTIVIDAD ANTIOXIDANTE Y EL ENVASADO EN ATMÓSPFERAS MODIFICADAS EN LA CALIDAD DE PRODUCTOS FRESCOS CORTADOS

TESIS DOCTORAL
Presentada por:
Christian Ghidelli

DIRECTORA:
Dra. María Bernardita Pérez Gago

TUTOR:
Dr. Daniel Vidal Brotons

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Dra. María Bernardita Pérez Gago, Investigadora del Centro de Tecnología Poscosecha del Instituto Valenciano de Investigaciones Agrarias,

CERTIFICA: Que la memoria titulada "Efecto de recubrimientos comestibles a base de proteína de soja con actividad antioxidante y el envasado en atmósferas modificadas en la calidad de productos frescos cortados", que, para aspirar al grado de Doctor en Ciencia, Tecnología y Gestión Alimentaria presenta D. Christian Ghidelli, realizada bajo mi dirección en el Centro de Tecnología Poscosecha del Instituto Valenciano de Investigaciones Agrarias, cumple las condiciones adecuadas para su aceptación como Tesis Doctoral, por lo que

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Y para que conste a los efectos oportunos, presento La referida memoria, firmando el presente certificado en Valencia a 16 de Junio de 2014.

Fdo. Dra. María Bernardita Pérez Gago

“Wonder is the beginning of all science”

Aristotles

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El estilo moderno de vida y el interés de los consumidores por su salud, unido al aumento del poder adquisitivo, ha incrementado la demanda de fruta y verdura mínimamente procesada en el mercado. Sin embargo, los cambios en el metabolismo de producto cortado requieren el uso de distintas tecnologías para mantener la calidad y extender su vida útil.

El objetivo de este trabajo ha sido el desarrollo de recubrimientos comestibles a base de proteína de soja con actividad antioxidante y la evaluación de su efecto combinado con un envasado en atmósfera modificada (AM) para alargar la vida útil de productos altamente susceptibles al pardeamiento enzimático como la alcachofa 'Blanca de Tudela', berenjena 'Telma' y caqui 'Rojo Brillante' mínimamente procesados almacenados a 5 ºC.

En primer lugar, se estudió la efectividad de diferentes concentraciones de ácido ascórbico (AA), ácido cítrico (CA), ácido peracético (PA), cloruro cálcico ($CaCl_2$), ciclodextrina (CD), cisteina (Cys), hexametafosfato (HMF), 4-hexilresorcinol (Hexyl) a diferentes concentraciones para controlar el pardeamiento enzimático en extractos y precipitados (estudios *in vitro*). Seguidamente, se seleccionaron los antioxidantes a su concentración más efectiva para su aplicación en alcachofa, berenjena y caqui mínimamente procesados almacenados a 5 ºC (estudios *in vivo*).

Entre los diferentes antioxidantes aplicados, la Cys fue la más efectiva controlando el pardeamiento enzimático en alcachofas y berenjenas frescas cortadas. La vida útil alcanzó un máximo de 4 y 9 días de almacenamiento a 5 ºC para alcachofa y berenjena cuando se aplicó 0,5% y 1% Cys, respectivamente. La aplicación de 1,12% de AA y 0,21% de CA extendió la vida útil del caqui 'Rojo Brillante' cortado hasta 5-7 días de almacenamiento a 5 ºC; mientras, el $CaCl_2$ contribuyó a alargar su vida útil en menor medida.

Los recubrimientos comestibles a base de proteína de soja (SPI) fueron preparados en combinación con los agentes antioxidantes más efectivos en los estudios *in vivo* para la reducción del pardeamiento enzimático y extender la vida útil de los productos seleccionados. En alcachofa mínimamente procesada, la optimización del recubrimiento comestible SPI:cera de abeja (BW) se realizó en base al contenido de

BW y Cys. El incremento en BW del 20 al 40% (base seca) permitió reducir la concentración de Cys en las formulación del 0,5 al 0,3%, contribuyendo así a la disminución del color amarillento proporcionado por la aplicación de Cys al tejido de alcachofa. Este recubrimiento también resultó efectivo controlando el pardeamiento enzimático de la alcachofa 'Blanca de Tudela' mínimamente procesada, la cual alcanzó una vida útil de 4 días a 5 ºC sin inducir la aparición de malos olores. La aplicación de la Cys, sola o incorporada en el recubrimiento, ayudó a controlar el pardeamiento enzimático y alargar la vida útil comercial de la berenjena cortada en fresco. La evaluación visual de las muestras recubiertas con SPI-BW-1% Cys presentaron menos pardeamiento que el resto de tratamientos y alcanzaron un máximo de 9 días de vida útil comercial a 5 ºC. Para el caqui 'Rojo Brillante' mínimamente procesado se incorporó un 1% CA y 0,3% $CaCl_2$ al recubrimiento comestible a base de SPI. La aplicación de este recubrimiento no tuvo un efecto negativo en el sabor global del producto, resultando así un tratamiento efectivo para extender la vida útil del caqui 'Rojo Brillante' fresco cortado.

Finalmente, se evaluó la combinación de los recubrimientos comestibles seleccionados y el envasado en AM en la calidad y vida útil de los productos cortados. Las condiciones del envasado en AM, incluyendo una AM activa (5 kPa O_2 + 15 kPa CO_2), una AM pasiva y una AM alta en O_2 (>50 kPa), fueron comparadas con un control que mantuvo condiciones atmosféricas durante el almacenamiento. El envasado en AM activa o pasiva con baja concentración de O_2 y alta en CO_2, dañó el tejido de las berenjenas y las alcachofas cortadas, mientras que la AM con alta concentración de O_2 (>30-50 kPa) no fue efectiva para el almacenamiento del caqui fresco cortado. La combinación de los recubrimientos comestibles optimizados y las distintas AMs no extendió la vida útil de la alcachofa, pero ayudó a mantener la capacidad antioxidante del producto cuando se compara con el envasado en aire. Igualmente, el recubrimiento SPI-Cys envasado en aire resultó ser el mejor y el mas económico tratamiento para extender la vida útil de las berenjenas cortadas hasta 9 días de almacenamiento. Por lo contrario, la combinación del recubrimiento a base de SPI-CA-$CaCl_2$ y combinado con el envasado en AM activa (5 kPa O_2 + 15 kPa CO_2) mostró un efecto sinérgico en el control del pardeamiento enzimático de caqui 'Rojo

Brillante’, manteniendo la calidad visual por encima del limite de comercialización durante 8-10 días de almacenamiento a 5 ºC.

L'estil modern de vida i l'interès dels consumidors per la seua salut, unit a l'augment del poder adquisitiu, ha incrementat la demanada de fruita i verdura mínimament processada en el mercat. No obstant això, els canvis en el metabolisme del producte tallat requereix l'ús de distintes tecnologies per a mantenir la qualitat i estendre la vida útil.

L'objectiu d'aquest treball ha sigut el desenvolupament de recobriments comestibles a base de proteïna de soia amb activitat antioxidant i l'avaluació del seu efecte combinat amb un envasat en atmosfera modificada (AM) per allargar la vida útil de productes altament susceptibles al pardetjament enzimàtic com la carxofa 'Blanca de Tudela', albergínia 'Telma' i caqui 'Rojo Brillante' mínimament processat i emmagatzemats a 5 ºC.

En primer lloc, es va estudiar l'efectivitat de diferents concentracions d'àcid ascòrbic (AA), àcid cítric (CA), àcid peracètic (PA), clorur càlcic ($CaCl_2$), ciclodextrina (CD), cisteïna (Cys), hexametafosfat (HMF), 4-hexilresorcinol (Hexyl) a diferents concentracions per a controlar el pardetjament enzimàtic en extractes i precipitats (estudis *in vitro*). Seguidament, es van seleccionar els antioxidants a la seua concentració més efectiva per a la seua aplicació en carxofa, albergínia i caqui mínimament processat i emmagatzemat a 5 ºC (estudis *in vivo*).

Entre els diferents antioxidants aplicats, las Cys va ser la més efectiva controlant el pardetjament enzimàtic en carxofes i albergínies tallades en fresc. La vida útil va aconseguir un màxim de 4 i 9 dies d'emmagatzemament a 5 ºC per a carxofa i albergínia quan es va aplicar 0,5% i 1% Cys, respectivament. L'aplicació de 1,12% de AA i 0,21% de CA va allargar la vida útil del caqui 'Rojo Brillante' tallat fins a 5-7 dies d'emmagatzemament a 5 ºC; mentre que el $CaCl_2$, va contribuir allargant la vida útil en menor mesura.

Els recobriments comestibles a base de proteïna de soia (SPI) van ser preparats en combinació amb els agents antioxidants més efectius en els estudis *in vivo*, que van reduir el pardetjament enzimàtic i van allargar la vida útil dels productes seleccionats. En carxofa mínimament processada, l'optimització del recobriment comestible SPI:cera d'abella (BW) es va realitzar en base al contingut de BW i Cys. L'increment en BW del 20 al 40% (base seca) va permetre reduir la concentració en Cys en les

formulacions del 0,5 al 0,3%, contribuint així a la disminució del color groguenc proporcionat per l'aplicació de Cys al teixit de carxofa. Aquest recobriment també va ser efectiu al control del pardetjament enzimàtic de la carxofa 'Blanca de Tudela' mínimament processada, la qual va aconseguir una vida útil de 4 dies a 5 ºC sense induir l'aparició de mals olors. L'aplicació de la Cys, sola o incorporada amb el recobriment, va ajudar a controlar el pardetjament enzimàtic i a allargar la vida útil comercial de l'albergínia tallada en fresc. L'avaluació visual de les mostres recobertes amb SPI-BW-1% Cys presentaren menys pardetjament que la resta de tractaments i aconseguiren un màxim de 9 dies de vida útil comercial a 5 ºC. Per al caqui 'Rojo Brillante' mínimament processat es va incorporar un 1% CA i 0,3% $CaCl_2$ al recobriment comestible a base de SPI. L'aplicació d'aquest recobriment no va tenir un efecte negatiu en el sabor global del producte, resultant així un tractament efectiu per allargar la vida útil del caqui 'Rojo Brillante' tallat en fresc.

Finalment, es va avaluar la combinació dels recobriments comestibles seleccionats i el envasat en AM en la qualitat i la vida útil dels productes tallats. Les condicions del envasat en AM, incloent una AM activa (5 kPa O_2 + 15 kPa CO_2), una AM passiva i una AM alta en O_2 (>50 kPa), van ser comparades amb un control que va mantindré les condicions atmosfèriques durant l'emmagatzemament. L'envasat en AM activa o passiva amb baixa concentració de O_2 y alta en CO_2 va danyar el teixit de albergínies i carxofes tallades, mentre que la AM amb alta concentració d'O_2 (>30-50 kPa) no va ser efectiva per al emmagatzemament de caqui fresc tallat. La combinació dels recobriments comestibles optimitzats i les distintes AMs no va allargar la vida útil de la carxofa, però si va ajudar a mantenir la capacitat antioxidant del producte quan es compara amb l'envasat en aire. Igualment, el recobriment SPI-Cys envasat en aire va resultar ser el millor i el més econòmic tractament per allargar la vida útil de les albergínies tallades fins a 9 dies d'emmagatzemament. Per el contrari, la combinació del recobriment a base de SPI-CA-$CaCl_2$ i combinat amb l'envasat en AM activa (5 kPa O_2 + 15 kPa CO_2) va mostrar un efecte sinèrgic en el control del pardetjament enzimàtic de caqui 'Rojo Brillante', mantenint la qualitat visual per damunt del límit de comercialització durant 8-10 dies d'emmagatzemament a 5 ºC.

The market demand for fresh-cut fruits and vegetables has experienced a rapid expansion due to the increased health consciousness of consumers, busy lifestyles, and purchase power. However, the shelf life of fresh-cut products is greatly reduced because of the rapid metabolism of the wound tissue, requiring the use of different technologies to maintain their quality and extend the shelf life.

The objective of the present work was to develop a soy protein-based edible coating with antioxidant activity and to evaluate the combined effect of selected edible coatings and modified atmosphere packaging (MAP) conditions on the quality and shelf life of fresh-cut 'Blanca de Tudela' artichoke, 'Telma' eggplant, and 'Rojo Brillante' persimmon during storage at 5 ºC, which are characterized by a relatively short shelf life due to a rapid onset of enzymatic browning.

Firstly, the effect of ascorbic acid (AA), citric acid (CA), peracetic acid (PA), calcium chloride ($CaCl_2$), cyclodextrin (CD), cysteine (Cys), hexametaphosphate (HMP), and 4-hexylresorcinol (Hexyl) at different concentrations was studied as a pre-screening at controlling enzymatic browning in extracts and precipitates (*in vitro* studies). Then, the most effective antioxidants type and concentration were selected to be tested in fresh-cut tissue of artichoke, eggplant and persimmon stored at 5 ºC (*in vivo* studies).

Among the different antioxidants tested, Cys was the most effective to control enzymatic browning of fresh-cut artichokes and eggplants. The maximum commercial shelf-life was 4 and 9 days of storage at 5 ºC for fresh-cut artichokes and eggplants when Cys was applied at concentrations of 0.5% and 1%, respectively. In fresh-cut 'Rojo Brillante' persimmon, application of 1.12% AA and 0.21% CA extended the limit of marketability in the range of 5-7 days of storage at 5 ºC; whereas, $CaCl_2$ contributed in a lower extend.

Soy protein isolate (SPI)-based edible coatings were prepared with the most effective antioxidant agents from *in vivo* studies to reduce enzymatic browning and extend shelf of selected produces. In fresh-cut artichokes, the optimization of the SPI:Beeswax (BW) edible coating was based on BW and Cys content. An increase in the BW content from 20 to 40% (dry basis) allowed to reduce Cys concentration from 0.5% to 0.3%,

diminishing the yellow color provided by Cys application to artichoke tissue. This coating contributed to the control of enzymatic browning and improved the quality of fresh-cut 'Blanca de Tudela' artichokes, reaching 4 days of commercial shelf life at 5 ºC without off-odors. The application of Cys, either alone or incorporated to the SPI-BW coating, helped at controlling enzymatic browning and extending the commercial shelf life of fresh-cut eggplants. The visual assessment evaluated the samples coated with the SPI-BW-1% Cys coating as significantly less brown than the rest of the treatments, reaching the maximum commercial shelf life of 9 days at 5 ºC. For fresh-cut persimmon, the formulated SPI edible coating contained 1% CA and 0.3% $CaCl_2$. The application of this coating did not affect negatively the overall sensory quality of the product, which makes this coating a potential treatment to extend the commercial shelf life of minimally processed 'Rojo Brillante' persimmon.

Finally, the combination of selected SPI-based edible coatings with antioxidant capacity and MAP were evaluated on fresh-cut 'Blanca de Tudela' artichoke, 'Telma' eggplant, and 'Rojo Brillante' persimmon. MAP conditions included active conventional MAP (5 kPa O_2 + 15 kPa CO_2), passive MAP, and high O_2 MAP (>50 kPa) and they were compared to atmospheric conditions as control. 'Telma' eggplants and 'Blanca de Tudela' artichokes were susceptible to tissue damage when packaged under active or passive MAP with low O_2 and high CO_2 levels, whereas high O_2 MAP (>30-50 kPa) resulted detrimental for the storage of fresh-cut 'Rojo Brillante' persimmons. The combination of the optimized coating with the different MAP did not extend the shelf life of artichoke slices, but helped maintain the antioxidant capacity of the product as compared to control packaging conditions. Similarly, the SPI-Cys coating in atmospheric conditions packaging provided the best and cheapest approach for extending the shelf life of fresh-cut eggplants up to 9 days of storage. On the contrary, the combination of the SPI-based coating with the active MAP packaging (5 kPa O2 + 15 kPa CO2) showed a synergic effect in controlling tissue browning of fresh-cut 'Rojo Brillante' persimmon, maintaining the visual quality above the limit of marketability up to 8-10 days of storage at 5 ºC.

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INTRODUCTION

Coatings for minimally processed fruits and vegetables

SHARON DEA[1], CHRISTIAN GHIDELLI[2], MARÍA B. PÉREZ-GAGO[2,3], ANNE PLOTTO[1].

[1]Citrus and Subtropical Products Laboratory, U.S Department of Agriculture, Agricultural Research Service (USDA-ARS), Winter Haven, FL, USA.
[2] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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1.1 Introduction

Before dealing with coatings, this chapter first addresses the basic physiology and quality issues of fresh-cut fruit and vegetables, the better to appreciate the coatings devised to improve quality of these products.

First available to restaurants, hospitals and other food service operators, minimally processed fruits and vegetables, also known as fresh-cut fruit and vegetables, are now readily available in supermarkets and sold in numerous package sizes, including individual (i.e., single serving) portions. In the United Stase, the fresh-cut industry is the fastest growing sector in the fresh produce business (Premier et al., 2007)

Minimal processing is defined to include all operations such as washing, sorting, trimming, peeling, slicing, dicing, chopping, and shredding, that would not extensively affect the fresh-like quality of the produce (Shewfelt, 1987). The product remains biologically and physiologically active, in that the tissues are living and respiring, with a shifting of cellular processes and interactions in response to the tissue damage inflicted by the operations. Therefore, minimal processing increases the degree of perishability of the processed materials, thus challenging their marketability (Gorny et al., 1999).

Quality of fresh-cut items is determined by a consistent and fresh appearance, acceptable texture, characteristic flavor, and sufficient shelf-life to survive the distribution system. Those characteristics can be preserved to a certain extent by understanding the physiology of the fresh-cut fruit or vegetable and applying different techniques. Such techniques would be to choose the proper cultivar and ripeness stage, to use adequate sanitation procedures and processing techniques, to maintain low temperature from harvest to retail (cold chain), and to apply physical and chemical treatments such as modified atmosphere packaging (MAP) or dips and edible coatings.

1.2 Physiology of minimally processed fruit and vegetables

Fresh-cut products are wounded tissues that are prone to rapid deterioration; therefore, their physiology differs from that of intact fruits and vegetables (Brecht, 1995; Toivonen and Brummell, 2008). Within

seconds after removal of the protective skin (pericarp) or cutting the product into pieces, wound signals of different natures (i.e., electrical, chemical, and hormonal) are sent through the tissues and initiate defense responses that promote wound healing, guard against bacterial attack, and generally protect cells from further stress. Additionally, as a result of peeling and cutting, the subcellular compartmentalization at the cut surface is disrupted, mixing substrates and enzymes, and initiates reactions that do not normally occur in the intact fruit or vegetable. These physiological changes cause increased respiration rate and synthesis of wound–induced ethylene as well as increased ion leakage, loss of components, alteration in flux potential, and loss of turgor. These changes are also accompanied by loss of firmness and flavor, discoloration of cut surfaces, possible decrease in vitamins, and increase in water activity (a_w) at the cut surface which accelerates water loss (Beaulieu and Gorny, 2004; González-Aguilar et al., 2007b). In addition, cut fruit surfaces provide a favorable environment for microbial growth, due to increased moisture, sugars and analytes leaking from open cells.

1.2.1 Ethylene production as a response to stress

One of the most common responses to wounding in plant tissue is an increase in both respiration rate and ethylene production (Saltveit, 1997; Escalona et al., 2003). Wound-induced ethylene is caused by the increased formation of 1-aminocyclopropane-1-carboxylic acid (ACC) and the subsequent conversion of ACC to ethylene (Boller and Kende, 1980, Yu and Yang, 1980). An increased rate of wound-induced ethylene may cause physiological disorders and consequently affects the quality attributes of the product. Physiological changes induced by elevated ethylene concentration include increased cell permeability, loss of compartmentation, increased senescence and respiratory activity, and increased activity of enzymes (Hyodo et al., 1983). Some specific examples include accumulation of phenolic compounds in carrots, sprouting in potatoes, lignification in asparagus, brown spot (russet spotting) in lettuce, and general softening (Reid, 1992).

The increased rate of ethylene production, in response to cutting, have been shown in kiwifruit (Watada et al., 1990; Agar et al., 1999), apple (Hu et al., 2007b), papaya (Paull and Chen, 1997), strawberry

(Rosen and Kader, 1989), endive (Salman et al., 2009), tomato (Lee et al., 1970; Mencarelli et al., 1989; Abeles et al., 1992; Brecht, 1995; Artés et al., 1999), and squash (Abeles et al., 1992; Hu et al., 2007a). However, Gorny et al. (2000) reported no change in ethylene production following the slicing of pears. Also, preparation of fresh-cut mango cubes, resulted in ethylene production rates 1.5 times lower than that of the whole fruit (Chatanawarangoon, 2000). In fact, it was found that the peel was the major contributor to ethylene production for mango.

When ethylene is induced by wounding, the increased rate varies depending on the type of commodity, cultivar, ripeness stage, and storage temperature. The rate of ethylene production stimulated by stress typically occurs with an initial short lag period, followed by a progressive increase, reaching a peak before subsiding to a stable level. Hyodo et al. (1983) reported that cut winter squash showed an increased rate of ethylene production after a 3-hour lag, and the rate reached its peak in 30 hours, followed by a decline to a low level, 40 hours after incubation at 24 °C. Although the rate increase is usually in the range of 5-20-fold, more than 100-fold increase in response to wounding has been reported in some cases (Hyodo and Nishino, 1981; Hoffman and Yang, 1982; Hyodo et al., 1983). Moreover, the wound response is usually greater in fruit at the preclimacteric and climacteric stages than in postclimacteric fruit (Toivonen and DeEll, 2002).

Storage temperature has a significant effect on wound-induced ethylene production as well. It has been shown that storage of cantaloupe pieces at 0 to 2.5 ºC will almost completely suppress wound-induced ethylene as compared to higher storage temperatures (Toivonen and DeEll, 2002). Artés-Hernández et al. (2007) observed, in fresh-cut lemons, that the production rate of ethylene at 0 ºC was four times higher than that of whole fruit, while at 10 ºC, the production was up to 10 times higher.

1.2.2 Increased respiration as a response to stress

The effects of processing on tissue metabolism can be observed very rapidly, often within minutes to a few hours after cutting (Toivonen and Brummell, 2008). The initiation of respiration in response to wounding is delayed compared to that found for wound-induced ethylene (Brecht,

1995). In general, high respiration rates, measured by the CO_2 produced in fresh-cut products, are directly associated with a rapid increase in the tissue metabolism and consequently with accelerated loss of acids, sugars, and other components that determine flavor quality and nutritive value (Cantwell and Suslow, 2002). Biochemical changes triggered by cellular response include stimulation of degradation of carbohydrates, activation of glycolysis and the pentose phosphate pathway, activation in mitochondrial activity, and increase in the synthesis of proteins and enzyme activities (Uritani and Asahi, 1980). These activations and accelerations in cellular processes serve to provide energy and precursors for the biosynthesis of secondary metabolites that are important to wound healing. Wound-induced respiration has been associated with enhanced synthesis of enzymes involved in the respiratory pathway and to a transitory increase in aerobic respiration in fresh-cut carrots (Surjadinata and Cisneros-Zevallos, 2003).

Increase of respiration has been observed in many fresh-cut products including banana (Palmer and McGlasson, 1969), tomato (MacLeod et al., 1976), cantaloupe (McGlasson and Pratt, 1964), celery (Gómez and Artés, 2005), salad mixture (lettuce, celery, carrot, radish, onion, endive) (Priepke et al., 1976), pear and strawberry (Rosen and Kader, 1989) and lettuce (Cantwell and Suslow, 2002). In these studies, the typical increase in respiration is in the range of 2-10-fold. However, Watada et al. (1996) reported that, for some fresh-cut products, such as zucchini, muskmelons, honeydews and crenshaws, the respiration rate was found similar or lower than that of the intact product at 0, 5 and 10 ºC, but dramatically higher at 20 ºC, probably due to rapid physiological deterioration and microbial growth.

1.2.3 Enzyme activity

Mechanical injury from cutting of a fruit or vegetable destroys the integrity of cell tissues and the compartmentation of endogenous enzymes and substrates. Some of these enzyme-catalyzed changes are well known: enzymatic browning and cell wall degradation due to enzymatic hydrolysis of the cell wall pectin substances.

The amount of phenolic compounds is increased through wound-induction of phenylalanine ammonia lyase (PAL), the committed enzyme

in phenolic biosynthesis; these phenolics can be oxidized by polyphenol oxidase (PPO) and peroxidase (POD) to quinones which ultimately polymerize to produce the browning appearance common to wounded lettuce (Degl'Innocenti et al., 2005). Such increase in enzymatic activity as a result of the fresh-cutting process has been reported for fresh-cut potato strips (Cantos et al., 2002), broccoli florets (Gong and Mattheis, 2003), jicama cylinders (Aquino-Bolaños et al., 2000), carrots (Goldberg et al., 1985), and lettuce segments (Hisaminato et al., 2001; Mutara et al., 2004).

Generally, the activity of lipolytic enzymes, including phospholipase and lipoxygenase (LOX), increases during senescence, and have been implicated as one of the major causes of tissue breakdown in a number of vegetables (Wardale and Galliard, 1977; Galliard et al., 1976). The phospholipase activity releases unsaturated fatty acids from the membrane that can serve as substrates for LOX. In response to physical wounding, LOX may act positively, through its role in the production of defense related signaling molecules, or negatively, through participation in autocatalytic peroxidation reactions (Karakurt and Huber, 2003). LOX hydroperoxides can contribute to tissue damage through inactivation of protein synthesis and deterioration of cellular membranes (Karakurt and Huber, 2003). LOX catalyzes formation of fatty acid radicals which can react with cell components, leading to further breakdown. Bleaching of plant pigments, loss of β-carotene and chlorophyll a, has been shown to occur during LOX-mediated reactions (Schieberle et al., 1981; Klein et al., 1984).

In addition to the above examples, numerous less visible reactions occur unnoticed, but can cause drastic changes to the flavor, texture and palatability of the product. Enzyme reactions initiate and catalyze biological pathways which may otherwise be inactive, generating undesirable products. These reactions frequently catalyze and activate further enzyme reactions, resulting in a cascade of events (Schwimmer, 1983).

Stress due to wounding or infection often induce plant tissues to accumulate unusual metabolites, such as glycoalkaloids in damaged potato tubers, and polyphenolic compounds in injured sweet potatoes (Haard and Cody, 1978; Grisebach, 1987). Moreover, chlorogenic acid, isochlorogenic acid, caffeic acid, and methyl caffeate are among the

common products formed via the phenylpropanoid pathway. Increased production of polyphenols due to wounding was also accompanied by lignin synthesis. Moreover, suberization, common in injured tomato, potato and bean pod, involves the synthesis of suberin polymers consisting of ω-hydroxy and dicarboxylic acids of long chain fatty acids and alcohols as major components (Dean and Kolattukudy, 1976; Sukumaran et al., 1990).

1.2.4 Nutritional and flavor changes

The initial nutritional value of a fresh-cut product can only be as good as its whole counter-part. Thus, any preharvest or postharvest event that affects the quality of the whole fruit or vegetable can jeopardize the final flavor and nutritional value of the fresh-cut product.

Antioxidants, such as ascorbic acid (AA), lycopene, β-carotene and phenolics, are of great interest regarding the nutritional content of fruit and vegetables. If included in the diet, these compounds are known to prevent oxidation caused by reactive oxygen species that lead to damage the cells and DNA, and cause some degenerative diseases (Hu and Jiang, 2007).

The compound AA is a key marker for determining the extent of oxidation in fresh-cut fruits and vegetables and is easily oxidized during fresh-cut processing. Changes in AA content in fresh-cut produce are a result of both biosynthesis and degradation reactions during storage (Hu and Jiang, 2007). Increased ascorbate content with time was observed for fresh-cut slices of mangoes kept at 5 and 13 °C, even though the levels never reached that of whole fruit (Tovar et al., 2001). Gil et al. (2006) reported an increase in AA content in fresh-cut slices and whole strawberries stored at 5 °C, while a loss of AA was observed in pineapple pieces, kiwi fruit slices and in cantaloupe cubes. Increases in AA were attributed to de-novo synthesis from monosaccharides (e.g. D-glucose) (Liao and Seib, 1988; Loewus and Loewus, 1987; Tolbert and Ward, 1982). One study demonstrated initiation of AA-synthesis enzymes upon potato tuber injury, suggesting that the same metabolic process may also occur in other wounded tissue (Ôba et al., 1994). Furthermore, considering that the AA concentration is often reported on a fresh weight

basis, an increase in AA may be a result of water loss during storage rather than an actual increase in AA (Nunes et al., 1998).

Carotenoid degradation is accelerated with exposure to unfavorable conditions such as low pH, oxygen or light exposure (Wright and Kader, 1997a), conditions created during fresh-cut processing. Ethylene production induced by wounding hastens tissue senescence, including fatty acid oxidation by LOX, which in turn contributes to carotenoid co-oxidation (Wright and Kader, 1997a, 1997b; Brecht et al., 2004). A reduction of 25% of the initial total carotenoid content of fresh-cut mango was observed 9 days after slicing and storage at 5 °C (Gil et al., 2006). Odriozola-Serrano et al. (2008) reported that four of six cultivars of fresh-cut tomatoes retained their initial lycopene content for a period of 21 days at 4 ºC. For the remaining two cultivars, one kept the initial lycopene content for up to 14 days to then decrease for the rest of the storage period, while the other had its lycopene content depleted slightly and continuously throughout storage.

Reyes et al. (2007) demonstrated that the increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. They measured changes in antioxidant capacity, total soluble phenolics, AA, total carotenoids and total anthocyanins after wounding in zucchini, white and red cabbage, iceberg lettuce, celery, carrot, parsnips, red radish, sweet potato, and potato. The phenolic changes ranged from 26% decrease to an increase up to 191%, while antioxidant capacity changes ranged from a 51% decrease to an increase up to 442%. Reduced ascorbic acid decreased up to 82%, whereas the changes in anthocyanins and carotenoids were less evident (Reyes et al., 2007).

Nevertheless, even if some nutritional loss is expected during the shelf-life of a fresh-cut product, it has been shown that for several fresh-cut fruits (i.e., strawberry, persimmon, peach, papaya, mango, strawberry, pineapple, kiwi fruit, cantaloupe, and watermelon), the visual quality was appreciably reduced before any significant nutrient decrease has occurred (Wright and Kader, 1997a, 1997 b; Lamikanra and Richard, 2002; Rivera-López et al., 2005; Gil et al., 2006).

Cut fruit products rapidly lose their typical flavor, even when stored under refrigerated conditions. It is well-known that they can develop staleness or loss of freshness within a day of refrigerated storage (Lamikanra and Richard, 2002). Moreover, physical stress that inevitably

occurs during fresh-cut processing results in enzymes coming in contact with substrates, and contributes to changes in flavor. Such changes are mainly due to the loss of the principal flavor-related volatiles and the synthesis of stress related off-flavor volatiles such as ethanol (Lamikanra et al., 2002; Hodges and Toivonen, 2008).

During storage of fresh-cut cantaloupe, the breakdown of esters is an early and important reaction step that, by providing precursors for the synthesis of secondary aroma volatile compounds, leads to loss of freshness (Lamikanra et al., 2002; Lamikanra and Richard, 2002; Beaulieu, 2005). Sothornvit and Rodsamran (2008) showed that longer storage time and higher temperature significantly damaged fresh-cut mango flavor by favoring the development of off-flavor associated with fermentative metabolites such as ethanol and acetaldehyde, which sensory panelists identified as the main attribute affecting flavor.

In general, biochemical parameters associated with sugars and acids, such as pH, titratable acidity, soluble solids content, and organic and amino acids are important indicators of the overall flavor of fruit and vegetables. However, for fresh-cuts, these parameters are not recommended to be used as quality indicators since they are not significantly affected by storage (Lamikanra and Richard, 2002). For example, the pH, titratable acidity, Brix, organic acids, sugars and amino acids measured in cut cantaloupe after 2 weeks of storage at 4 °C were not significantly different from the amounts present in the freshly cut fruit (Lamikanra, et al., 2000). Similar observations have also been reported for fresh-cut mango (Ngarmsak et al., 2005; Gil et al., 2006; Donadon et al., 2004; Tovar et al., 2001), and lemons (Artés-Hernández et al., 2007).

Taste aroma quality are important attributes for consumers and therefore should be carefully evaluated when determining the shelf life of fresh-cut products, because an acceptable postcutting visual appraisal does not necessarily imply that a product has satisfactory flavor quality (Beaulieu and Gorny, 2004). It is difficult to establish overall shelf-life limits for fresh-cut fruit, taking flavor quality into consideration, because of the effects of initial fruit variability, the different post-cutting treatments and effect of packaging (Beaulieu and Gorny, 2004). Moreover, the optimum visual appearance of fresh-cut fruits accepted by the retailers and the consumers is often times attained when the fruit are

processed immature or unripe, thus compromising taste and aroma (Beaulieu and Gorny, 2004).

1.2.5 Color changes/browning

The visual quality of fresh-cut fruits and vegetables can be assessed using several attributes, including overall appearance, absence of defects, shape and size, glossiness, and most importantly, color. In fact, appearance is a primary quality attribute that greatly influences the consumer purchase decision. From initial maturity to wound-related effects and microbial colonization, many factors have major effects on the appearance of fresh-cut products (Beaulieu and Gorny, 2004; Toivonen and Brummell, 2008).

Enzymatic browning is one of the most limiting factors on the shelf-life of fresh-cut products and consequences of enzymatic browning are not restricted to discoloration; undesirable flavors and nutrients loss may result. Enzymatic browning is a complex process that can be subdivided in two parts. The first part is mediated by PPO resulting in the formation of *o*-quinones (slightly colored), which through non-enzymatic reactions, lead to the formation of complex brown pigments. *o*-Quinones are highly reactive and can rapidly undergo oxidation and polymerization. Usually brown pigments are formed, but, reddish-brown, blue-gray and even black discolorations can be synthesized (Garcia and Barrett, 2002).

In green vegetables, the senescence process usually leads to a yellow coloration of the tissues, normally considered the major consequence of chlorophyll degradation (Toivonen and Brummell, 2008). Also, in some minimally processed green vegetables, the synthesis of pheophytin, an olive-colored pigment, appears when the chlorophyll loses its bond with the magnesium atom and substitutes it with a hydrogen atom (Artés et al., 2007). The maintenance of a low temperature and a high relative humidity, combined with atmospheres lowered in O_2 and moderately rich in CO_2, are shown to be the main advisable techniques to delay this disorder (Artés et al., 2007).

Carrots may develop “white blush”, also known as “white bloom”, a discoloration defect that results in the formation of a white layer of material on the surface of peeled carrots, giving poor appearance to the product (Garcia and Barrett, 2002). This superficial whitish layer has

been associated with the synthesis of lignin (Lavelli et al., 2006), natural healing of the tissue, although it has also been related to dehydration of cells that are damaged or removed from the tissue (Tatsumi et al., 1993; Avena-Bustillos et al., 1994).

Besides, browning on the leaf edge of non-photosynthetic tissues has been associated with CO_2 injury when present in concentrations higher than 2 kPa during cold storage (Artés et al., 2007). The "russet spotting" characterized by the presence of pink to brown stains in the mid-rib of the leaf is quite frequent and has been related to an ethylene concentration levels higher to 0.1 ppm during cold storage (Artés et al., 2007). Onions, garlic and leeks can develop pink, red, green, blue-green or blue discolorations as a consequence of cell disruption (Toivonen and Brummell, 2008).

1.2.6 Texture and softening

Wounding hastens senescence and induces tissue softening, which is considered a major shelf-life limitation for fresh-cut produce (Soliva-Fortuny and Martin-Belloso, 2003; Beaulieu and Gorny, 2004). Many of the textural changes occurring on fresh-cut fruit are a continuation of the normal ripening events that lead to softening (Toivonen and Brummell, 2008). In whole fruit, cell walls undergo a natural degradation during fruit ripening, reducing cell wall firmness and intercellular adhesion. Softening is attributed to changes in turgor pressure and in the structure and composition of cell walls, such as disassembly of the pectin matrix, mediated at least in part, by the sequential action of pectin methyl esterase (PME) and polygalacturonase (PG) enzymes (Beaulieu and Gorny, 2004; Pinheiro and Almeida, 2008). In addition, softening may be attributable to the accumulation of osmotic solutes in the intercellular space and partly to postharvest water loss from ripening fruit (Toivonen and Brummell, 2008). In climacteric fruit, wound induced ethylene would have the same effect as treating tissue with exogenous ethylene, causing a hastening of ripening and softening.

Compared with fruit, vegetables generally have a much greater proportion of cells with thickened secondary walls and consequently are much firmer and less susceptible to softening. The loss of textural quality is related to aging processes and senescence, water loss, reduced turgor

and wounding effects, including the leakage of osmotic solutes. Wilting is the major cause of loss of visual appearance and texture in delicate leafy produce such as lettuce and spinach.

Flesh translucency, characterized by the alteration of flesh texture to become dark and glassy, and an overripe appearance, seriously limit the use of fruit by the fresh-cut fruit industries (Lana et al., 2006). Translucency is caused by the filling of cellular free space with liquid, giving to the tissue a transparent appearance. Fresh-cut cucumber melon, papaya, pears, tomatoes, and watermelon are susceptible to this disorder (Artés et al., 2007).

1.2.7 Surface microbial flora

Fresh-cut produce are very susceptible to microbial spoilage due to their high water activity (a_w), the presence of nutrients at the cut surface, and the absence of preservative processes known to delay undesirable biological and biochemical changes, such as bleaching, freezing or sterilization.

Raw fruit and vegetables have a naturally occurring microflora that is affected by several external factors, namely, product origin, agricultural production practices, harvesting and processing techniques, initial quality and maturity, transportation mode, storage temperature, and the use of controlled (CA) and modified (MA) atmosphere (Ngarmsak et al., 2006). The microbial load can further be enhanced by the different processing methods, such as handling, cutting, shredding, slicing and grating (Abadias et al., 2008). In addition, during distribution and storage, temperature fluctuations and the high humidity present in packages provide a favorable environment and incubation time for proliferation of spoilage organisms and microorganisms of public health significance (Heard, 2002; Fan and Song, 2008).

The major microbial concerns related to fresh-cut produce are mesophilic and psychrotrophic microorganisms affecting product shelf-life, and human pathogens. Most microbes on fresh fruits and vegetables are bacteria, and 80 to 90% of bacteria are Gram-negative rods, predominantly *Pseudomonas*, *Enterobacter* or *Erwinia* species (Zhang, 2007). Lactic acid bacteria such as *Leuconostoc mesenteroides* and *Lactobacilus* spp., and several species of yeast and molds are also

commonly found (Brackett, 1987; Fan and Song, 2008). Those microorganisms may degrade the sensory quality by affecting the appearance, cause off-odor/off-flavor, and to a lesser extend, may cause texture loss.

The detection of off-odors or obvious visual defects on fresh-cut vegetables is often accompanied by a bacterial count exceeding 8 log cfu/g or a yeast count exceeding 5 log cfu/g (Ragaert et al., 2007). For instance, unacceptable changes of appearance during storage of minimally processed artichoke at 4 ºC appeared at day 15, when the psychrotrophic microbial count reached 8.8 log cfu/g (Giménez et al., 2003). Li et al. (2001) also found that the visual quality of minimally processed iceberg lettuce became unacceptable at day 14 during storage at 5 ºC, which corresponded to aerobic psychrotrophic and yeast counts of 8.8 and 6.4 log cfu/g, respectively.

Although there has been a number of reports about microbiological contamination involving whole fresh produce and fresh-cut vegetables (Abadias et al., 2008), there is still little information about microbial contamination of fresh-cut fruits. For most fruit, due to their low pH, the natural microflora is restricted to acid-tolerant microorganisms, such as fungi and lactic acid bacteria. Fruits may also be a vehicle for non acid-tolerant microorganisms, although these may not grow (Brackett, 1987). Beaulieu and Gorny (2004) indicated that aerobic plate count, total plate count and, more significantly, yeast and mold count correlated closely with the shelf-life of fresh-cut fruits.

In fresh-cut fruit products, microorganism minimum detection levels based on visual observation of spoilage may vary depending on the microorganism and type of product. For example, in mango cubes, mesophilic and psychrotrophic aerobic and lactic bacterial counts detection level was reached at 2.4 log CFU/g, while for yeast the detection level was 3 log CFU/g (Poubol and Izumi; 2005a, 2005b). For fresh-cut melon, spoilage became detectable by consumers when yeast counts reached a level above 5 log CFU/g and aerobic psychrophilic counts reached 8 log (CFU/g) (Oms-Oliu et al., 2008a). In melon cubes, the cause of off-odor was associated with yeast and mold counts above 7 log CFU/g (Bai et al., 2003).

The incidence of food-borne outbreaks caused by contaminated fresh fruit and vegetables has increased in recent years. The pathogens

most frequently associated with produce-related outbreaks include bacteria (*Salmonella*, *Escherichia* coli), viruses (Norwalk-like, hepatitis A), and parasites (*Cryotosporidium*, *Cyclospora*), with *Salmonella* and *E.* coli O157:H7 being the leading causes of produce-related outbreaks in the United States (Abadias et al., 2008). *Listeria monocytogenes* is also considered a potential vehicle of foodborne outbreaks caused by the consumption of contaminated minimally processed fresh vegetables (Ryser and Marth, 1991). Previous works have shown that *Listeria monocytogenes* can grow or survive at refrigeration temperatures on many raw or processed vegetables, such as cabbage and shredded cabbage (Beuchat et al., 1986; Kallander et al., 1991), iceberg lettuce (Steinbruegge et al., 1988; Beuchat and Brackett, 1990), asparagus, broccoli and cauliflower (Berrang et al., 1989).

Moreover, while the acidic pH of most fruits prevents the development of most pathogens, they are not totally without risk (Brackett, 1987). Human pathogens may gain entry to fruit products when animal fertilizers or contaminated irrigation water or water used for rinsing come into contact with fruits during production and processing (Brackett, 1987; Bordini et al., 2007).

1.3 Techniques and approach to increase shelf-life of minimally processed products

Cultural practices, harvest maturity, postharvest handling, ripeness stage, storage conditions (i.e., temperature, humidity, atmosphere), and storage duration are all factors affecting the wound response in fresh-cut tissue (Portela and Cantwell, 2001; Cantwell and Suslow, 2002; Beaulieu and Gorny, 2004), and therefore affect the shelf-life quality of the minimally processed product. This section will review the intrinsic and external factors and techniques that affect the overall product quality and can help optimizing its shelf-life.

1.3.1 Quality of the raw product

The choice of the cultivar (genotype) is of prime importance to assure the optimal quality of a fresh-cut product. Cultivars often differ in organoleptic, compositional and nutritional qualities and consequently

behave differently when processed into fresh-cut products. For example, it was shown that the shelf-life of 14 cultivars of peaches and 8 cultivars of nectarines varied between 2 and 12 days at 0 ºC, and their positive response to CA and to an antibrowning treatment varied greatly (Gorny et al., 1999). Similarly, Beaulieu (2005) noticed differences in the volatile and quality attributes in six cantaloupe cultivars, and a range of quality attributes for different cultivars of fresh-cut mango cubes was reported by Rattanapanone et al. (2001), and Poubol and Izumi (2005 a, 2005b).

It is well known that fruit physiological and metabolic activities change with the ripeness stage (Allong et al., 2001). In general, when selecting less mature fruit for processing, a longer shelf-life is expected due to better firmness retention and decreased changes in appearance compared with processed ripe fruit. However, when using unripe fruit for processing, the amount and composition of volatiles present or released by the end product, and consequently the flavor, will not be satisfactory and the final fresh-cut product will lack good sensory quality (Gorny et al, 2000; Beaulieu and Lea, 2003; Beaulieu and Gorny, 2004). A mature fruit on the other hand will have superior eating quality but shorter shelf-life (Watada and Qi, 1999). Because some vegetables, which are the actual “fruit” of the plant (squash, bell pepper, cucumber) do not usually ripen once harvested, and that other vegetables, which comprise non-fruit parts of the plant such as roots, stems and flowers (potato, carrot, asparagus, broccoli) are better tasting when harvested immature, an optimal harvest maturity must be carefully targeted for each commodity. Thus, determining the optimal ripeness stage and harvest maturity that combines acceptable shelf-life and eating quality is the key to successful commercialization of fresh-cut fruits and vegetables.

Biotechnology can help change the characteristics of fresh-cut fruits and vegetables in order to improve their shelf-life quality (Zhang, 2007). For example, changes in flavor, starch, vitamin and anthocyanin contents, enzymatic activity, or simply characteristics for superior processing or improved visual quality could be engineered. The Flavr Savr tomato developed in the early 1990s by Calgene, Inc., a biotechnology company with headquarters in Davis, California, is a good example of genetically modified product that may benefit the fresh-cut industry. This tomato has a gene that slows the natural softening process

that accompanies ripening, thus the biotechnologically modified tomatoes can remain on the vine longer and soften more slowly, resulting in more flavor and color.

1.3.2 Sanitation treatments prior processing

Sanitizers are commonly used in fresh-cut processing operations to prevent contamination of food products by maintaining low levels of microorganisms in the environment. The utilization of decontamination methods to prolong the shelf-life of fresh-cut produce should reduce the risk of food-borne infections and intoxications, decrease the microbial spoilage, preserve the fresh attributes and the nutritional quality and leave the product free of unacceptable toxic residues or by-products (Gómez-López et al., 2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut products have been recently reviewed by Artés et al. (2009).

Washing with chlorinated water is a widely employed sanitation procedure accomplished by immersing the product in solutions containing between 50 and 200 μL/L free chlorine (HOCl) during less than 5 min (Ngarmsak et al., 2006; Rico et al., 2007). Even though the application of chlorine is not considered very effective in reducing microbial levels in contaminated tissue, chlorine reduces microbial loads in the water and prevents cross-contamination. In addition, chlorine rinse acts directly on the tissue by inhibiting browning reactions while it also helps remove cellular contents present on the cut surfaces of fruits and vegetables that may promote browning (Brecht et al., 1993). However, there is some controversy about using chlorine as an antimicrobial agent due to the possible formation of carcinogenic chlorinated compounds in the rinsing water, namely chloramines and trihalomethanes (Rico et al., 2007). Chlorine dioxide (ClO_2), a strong oxidizing and sanitizing agent, has a broad and high biocidal effectiveness and is also less affected than chlorine by pH and organic matter (Zhang, 2007). Hydrogen peroxide (H_2O_2), acidified sodium chloride, peroxyacetic acid, and organic acids have also been used to sanitize fresh-cut fruits and vegetables (Brecht et al., 2004; Narciso and Plotto, 2005; Ngarmsak et al., 2006; Rico et al., 2007). Peroxyacetic acid (PAA) is currently the most commonly used sanitizer in commercial fresh-cut processing facilities.

1.3.3 Processing practices

The sharpness of the cutting blade used for processing greatly affects the quality attributes of fresh-cut products (Hodges and Toivonen, 2008). That is, as a consequence of membrane rupture, a blunt blade will cause accumulation of liquid in the intercellular spaces; which can in turn reduce gas diffusion and induce anaerobic respiration, producing off odor due to ethanol synthesis, whereas a sharp blade minimizes tissue damage and associated wound stress responses such as increased respiration and ethylene production (Portela and Cantwell, 2001; González-Aguilar et al., 2007a; Oms-Oliu et al, 2008a).

The cutting shape may also influence the metabolism of fresh-cut tissue. For example, when stored at 5 °C or 10 °C, slices from fresh-cut papaya had better soluble solid content retention, lower weight loss, and better overall quality index than cubes from the same papaya fruit (Riviera-Lopez et al., 2005). Shredded root of radish had a higher respiration rate and lower content of soluble solids and AA than sliced radishes (Aguila et al., 2006). Moreover, trapezoidal cuts were shown to extend melon shelf life compared to slices or cylinder cuts (Aguyao et al., 2004). The surface area of the cut tissue is often the reason for such differences.

Washing after cutting may improve firmness retention of fresh-cut fruit by removing from the cut surfaces solutes and stress-related signaling compounds such as acetaldehyde and phenolics (Toivonen and Brummell, 2008). Moreover, washing increases the activities of catalase, peroxidase and superoxide dismutase enzymes, which are involved in scavenging oxygen free radicals that contribute to membrane injury.

1.3.4 Post-processing treatments

1.3.4.1 Temperature

Storage temperature has received the widest attention among the various postharvest environmental factors as it plays an important role in the postharvest physiology and quality of horticultural crops. In addition to providing effective action against fruit decay, low temperature reduces the RR (Q_{10}) which provides the energy to drive the reactions occurring

during ripening (Kays, 1991; Mohammed and Brecht, 2002). In addition, low temperature slows down the quantitative and qualitative changes in the normal complement of enzymes that bring about the characteristic synthetic and degradative changes associated with ripening, such as softening, color changes, and flavor and compositional changes, thus increasing fruit postharvest life (Kays, 1991; Ponce de León et al., 1997).

Proper temperature management during postharvest handling, processing and distribution is the most important external factor that must be controlled to preserve the quality and safety of fresh-cut fruits and vegetables (Zhang, 2007). Temperature has a direct relationship with the shelf-life of fresh-cut products. That is, the lower the temperature, the longer the shelf life of the fresh-cut fruit or vegetable. For instance, the marketable period of fresh-cut mango cubes was 3 to 5 days at 10 °C, but could be extended 5 to 8 days at 5 °C (Rattanapanone et al., 2001). Moreover, temperature was the main factor affecting postcutting life of fresh-cut pineapple, which ranged from 4 days at 10 ºC to over 14 days at 2.2 and 0 ºC (Marrero and Kader, 2006).

Because fresh-cut products are stored or displayed at the retail store for only a short period of time, and because they are extremely perishable compared with the whole fruits or vegetables, exposure to a temperature that causes a slight amount of chilling injury (CI) is preferred over a temperature that causes more rapid deterioration due to ripening and senescence (Watada and Qi, 1999). A significant number of fresh-cut fruits do not seem to be as chilling sensitive as the corresponding intact fruit. Furthermore, CI symptoms are often only manifested when fruit are transferred to nonchilling temperatures and may never become visible if the product is maintained exclusively at chilling temperatures. In fact, some authors have suggested that fresh-cut products may be subject to CI despite little visual manifestation of injury. For example, higher respiration rates of fresh-cut products compared with the corresponding whole fruit may in some cases, and to some extent be an indicator of CI (Brecht et al., 2004). Moreover, poor flavor retention in fresh-cut products, especially fruits, due to the inhibition of aroma volatile production, is a widely recognized problem and may be caused by CI (Beaulieu and Gorny, 2004).

1.3.4.2 Chemical treatments

Several chemical and physical treatments may be applied in synergy with proper temperature management and handling practices to extend the shelf-life and maintain the product quality. For example, dips in organic acids solutions (e.g. lactic acid, citric acid, acetic acid, tartaric acid) have been described as having a strong antimicrobial action against psychrophilic and mesophilic microorganisms in fresh-cut fruits and vegetables (Rico et al., 2007). The powerful antimicrobial action of organic acids is attributed to their capacity to reduce external pH, disrupt membrane transport or permeability, cause anion accumulation, and reduce the internal cellular pH by dissociation of hydrogen ions from the acid fraction (Rico et al., 2007).

Reducing agents, most commonly AA or its isomer erythorbic acid, isoascorbate or sodium erythorbate, are some of the most commonly used agents to reduce or eliminate cut surface discoloration, which is mainly attributed to enzymatic browning (Brecht et al., 2004; Beaulieu and Gorny, 2004). The use of isoascorbic acid has been shown to be more effective than AA or *N*-acetyl-L-cysteine, another reducing agent, in preventing tissue softening, surface browning, and decay on fresh-cut pineapple slices, extending shelf-life to 14 days compared to a shelf-life of 9 days for the non-treated slices (González-Aguilar et al., 2004).

Calcium and its salts have been used to decrease tissue softening of a great variety of fresh-cut fruits (Soliva-Fortuny and Martin-Belloso, 2003; Toivonen and Brummell, 2008; Aguayo et al., 2008). These compounds help maintain cell wall integrity by interacting with pectin to form calcium pectate, and help reduce tissue softening by cross-linking with cell wall and middle lamella pectins (Luna-Guzman et al., 1999; Rico et al., 2007). A combination of calcium chloride, AA, and citric acid significantly reduced color deterioration and loss of firmness, without affecting sensory characteristics, of fresh-cut mangoes stored at 5 °C (González-Aguilar et al., 2007a).

The use of 1-MCP was proven effective to slow the changes associated with loss of quality and extend the shelf-life of fresh-cut product (Vilas-Boas and Kader, 2007). The action of 1-MCP is mediated through the inhibition of ethylene perception of plant tissues by interacting with the receptor and competing with ethylene for binding

sites (Watkins, 2006). Therefore, the effectiveness of inhibition of ripening and/or senescence of fruit and vegetables is a function of the concentration of 1-MCP applied, up to saturation of the binding sites (Watkins, 2006). Response of fresh-cut products to 1-MCP treatment depends on the dose applied, the type of crop, the maturity or the ripeness stage, the exposure time, and the temperature (Blankenship and Dole, 2003). 1-MCP can be applied as a pre- or post-cutting treatment.

Slices made from 1-MCP-treated papayas had double the shelf-life compared to slices made with untreated papayas (Ergun et al., 2006). Arias et al. (2009) reported that slices made from 1-MCP-treated 'Blanquilla' pears (treated just after harvest) were firmer and had improved color compared to nontreated fruit. In pineapple, fresh-cut slices made from 1-MCP-treated fruit, had lower respiration rates, browning and hydrolysis of ascorbic acid compared to non-treated fruit (Budu and Joyce, 2003). Exposure of fresh-cut apples slices to 1-MCP (after processing) decreased ethylene production, respiration, softening, color change and synthesis of aroma compounds (Perera et al., 2003; Calderón-López et al., 2005).

1.3.4.3 Physical treatments

Along with good temperature management, the use of ozone, radiation, ultraviolet (UV) light, heat treatment, and modified atmosphere packaging (MAP), are physical treatments that can be applied to fresh-cut products in order to extend their shelf-life.

Ozone (O_3) is a strong antimicrobial agent with high reactivity and penetrability, and spontaneously decomposes to O_2 in air, or to $O_2 + H_2O$ in water (Rico et al., 2007). A low dose of gamma irradiation is also very effective in reducing bacterial, parasitic, and protozoan pathogens in raw food (Rico et al., 2007). A maximum irradiation level of 1.0 kGy was approved by the U.S. FDA for use on fruits and vegetables and could be used without damage to the cut product (Fan and Sokorai, 2008). Another effective antimicrobial agent is UV light, referred to as UV-A, UV-B or UV-C according to the wavelength from shortest to longest, respectively. UV light damages the DNA of microorganisms as well as acting indirectly against spoilage pathogens due to the induction of resistance mechanisms in different fruits and vegetables (Boynton, 2004). UV-C

irradiation was reported to improve the total antioxidant capacity of fresh-cut mango (González-Aguilar et al., 2007a), and improve the phenolic profile of honey pineapple, banana and guava (Alothman et al., 2009).

Mild heat treatments have been shown to potentially benefit the texture of fresh-cut fruit and vegetables. The treatment strengthens the cell wall due to the activation of the enzymes PME which promotes de-esterification of the pectin molecule, thus increasing the number of calcium binding sites. For example, in minimally processed celery, treatment by immersion in hot water allowed a better retention of the original color and the total chlorophyll content (Viña et al., 2007). In fresh-cut peach, heating the fruit at 50 ºC for 10 minutes, 4 hours before cutting, effectively controlled browning and retained firmness during storage (Koukounaras et al., 2008). A hot air treatment (48 ºC for 3 hours), applied to fresh-cut broccoli, was successful in delaying yellowing, maintaining chlorophyll content and retaining tissue integrity compared to controls (Lemoine et al., 2009). Similar results have been observed for fresh-cut apples (Kim et al., 1993; Barrancos et al., 2003). However, low heat (38 °C for 12 or 24 hours) of whole mangoes accelerated softening of fresh-cut slices (Plotto et al., 2003). Such treatments are difficult to take into commercial applications because of the multiple combinations of treatment time and temperature that need to be adjusted for each commodity, cultivar and ripeness stage.

A low O_2 or elevated CO_2 atmosphere, plus saturated or near-saturated humidity environments generated in MAP have been successfully used to extend fresh-cut produce shelf life. In fact, MAP systems in association with low temperature are extensively used commercially to extend shelf-life of fresh-cut products by reducing respiration rate, cell wall degradation, water loss, phenolic oxidation, microbial growth, and ethylene biosynthesis and action (Gorny 2003). Due to the active metabolism of fresh-cut fruits, MAP alters the atmosphere composition surrounding the product, affecting the concentrations of O_2, CO_2, water vapor, and other volatiles compounds that impact the physiology and overall quality of the product (Forney, 2007).

MAP systems are designed to maintain a respiring product in a favorable atmosphere, usually incorporating reduced O_2 and elevated

CO_2. The MA is created and maintained through the interplay of product respiration and gas permeation through the package (Yam and Lee, 1995; Mir and Beaudry, 2004). It can be created either passively or actively.

A passive MAP system is generated by allowing the desired atmosphere to develop naturally because of the product respiration and the diffusion of gases through the selected film or perforations (Moleyar and Narasiam 1994; Yam and Lee, 1995). In passive MAP systems, the development of a desirable atmosphere composition may take a considerable amount of time (up to several days), depending on the product respiration rate and the void volume within the package (Rodov et al., 2007). A similar situation can occur with coatings although with much less control. On the other hand, in an active MAP system, the atmosphere is created rapidly by flushing the headspace of the package with a desired gas mixture, usually consisting of N_2 mixed with O_2 and CO_2 concentrations that are near the anticipated equilibrium concentrations of those gases. In both cases, once the MA is established, the dynamic equilibrium of respiration and permeation maintain the appropriate atmosphere. Bai et al. (2003) reported that fresh-cut honeydew cubes packed in active MAP (5 kPa O_2 + 5 kPa CO_2) had better color retention, reduced respiration rate and microbial population and a longer shelf-life than those stored in passive MAP (Bai et al., 2003).

The tolerance to and physiological effects of elevated CO_2 are highly variable and depend on the commodity, maturity or ripeness stage, and storage temperature. In addition, elevated CO_2 may alter the response of the product to reduced O_2 concentrations, since with an increase in CO_2 concentration, the tolerance limits to reduced O_2 decrease (Watkins, 2000; Kader, 2002; Kader and Saltveit, 2003). In some cases, elevated CO_2 may cause discoloration and softening, and induce fermentative metabolism (Mir and Beaudry, 2004). Nevertheless, elevated CO_2 concentration (> 8 to 10 kPa) may inhibit ethylene action and can effectively inhibit microorganism growth.

Holding fresh-cut mango slices in 10 kPa O_2 and CO_2 at 5 °C retarded browning and softening (Limbanyen et al., 1998). MAP led to lower microbial infection, less translucency and better color retention in fresh-cut cantaloupe cubes stored in active MAP (4 kPa O_2 plus 10 kPa CO_2) (Bai et al., 2001). Moreover, MAP-treated fresh-cut kohlrabi,

stored in 6 kPa O_2 plus 13 kPa CO_2 and 13 kPa O_2 plus 8 to 9 kPa CO_2, had reduced microbial population growth and better color retention than air stored material (Escalona et al., 2003).

One hazard that may occur with the use of a nonoptimal MAP system for a given fresh-cut commodity is the generation of anaerobic conditions or accumulation of high CO_2 levels in the package. This can be caused by the utilization of inappropriate film or gas flushing protocols, variation in respiration rates from different cultivars or varieties, seasonal variation, and storage duration of the product prior to processing (Kim et al., 2005a, b; Hodges and Toivonen, 2008). Such conditions can cause the product to pass from aerobic to anaerobic respiration, which causes the production of ethanol and acetaldehyde leading to off-flavors and odors (Beaulieu, 2006; Saltveit, 2003).

1.4 Edible coatings for minimally processed products

Edible coatings improve the quality and extend shelf life of lightly processed fruit and vegetables by acting as a barrier to water loss and gas exchange, creating a micromodified atmosphere around the product. In addition, edible coatings can serve as carriers for other generally recognized as safe (GRAS) compounds, such as preservatives and other functional ingredients from natural sources (Baldwin et al., 1995; Olivas and Barbosa-Cánovas, 2005; Vargas et al., 2008). For example, the addition of a texture enhancer, such as calcium chloride, in an edible coating formulation may enhance fruit quality during storage by maintaining firmness (Olivas and Barbosa-Canovas, 2005). Furthermore, calcium in the form of calcium ascorbate provides a dual function of cross-linking (from Ca++) and preventing cut surface from browning (from ascorbate) (Wong et al., 1994). The incorporation of natural antioxidants, such as AA, citric acid, cysteine, and antimicrobials such as lactic acid, peracetic acid, can help reducing enzymatic browning and controlling microbial growth of fresh-cut products. Furthermore, edible coatings contribute to the reduction of synthetic packaging waste (Vargas et al., 2008).

Due to the particular physical and chemical properties of fresh-cut produce, edible coatings must be designed and formulated to suit their specific physiology. Some coatings may not adhere well to fresh-cut

surface, while others may offer good adherence but may be a poor barrier to moisture or not resist water vapor diffusion (Garcia and Barret, 2002). On a general basis, edible coatings used with fresh-cut products must be transparent, tasteless and odorless, in addition to containing safe and food-grade substances. They must have an adequate water vapor permeability (WVP), solute permeability and selective permeability to gases and volatile compounds. Further, the cost of technology and raw materials from which coatings are made has to be relatively low (Vargas et al., 2008).

The following sections will describe the nature and the properties of the main components found in edible coatings for fresh-cut fruit (i.e. proteins, polysaccharides, lipids, and resins), and how their combination can improve the shelf-life of minimally processed fruits and vegetables. Table 1 summarizes the use of coating materials and additives for fresh-cut fruits and vegetables, with corresponding references. More information on edible coatings for minimally processed fruits and vegetables can be found in the following reviews: Baldwin et al., 1995; Olivas and Barbosa-Cánovas, 2005; Lin and Zhao, 2007; Bourtoom, 2008; Vargas et al., 2008; Rojas-Graü et al., 2009.

Table 1. Edible coatings and minimally processed fruits.

Commodity	Coating material	Additives and plasticizer	References
Apple	Alginate, apple puree	N-acetylcysteine,$CaCl_2$, oregano, lemongrass, vanillin, Gly	Rojas-Graü et al., 2007
	Alginate, gellan, sunflower oil	N-acetylcysteine, $CaCl_2$	Rojas-Graü et al., 2008
	Alginate	N-acetylcysteine, glutathione, cinnamon, clove, lemongrass, citral, cynnamaldehyde, eugenol, calcium lactate; malic acid, Gly	Raybaudi-Massilia et al., 2008b
	Apple puree, BW, vegetal oils	AA, citric acid, Gly	McHugh and Senesi, 2000
	Alginate, AMG, linoleic acid	$CaCl_2$	Olivas et al., 2007
	Carragennan, CMC, WPC	CaCl2, Gly, PEG	Lee et al., 2003
	CMC, CC, WPI	$CaCl_2$, Gly	Le Tien et al., 2001
	Nature Seal™, CMC, SPC	AA, PS, SB, soy oil, CaCl2, Gly, PEG	Baldwin et al., 1996
	SWP, SPI, CC	Sorbitol	Shon and Haque, 2007
	WPI, BW	Gly	Pérez-Gago et al., 2003
	WPI; WPC, HPMC, BW, CW	Gly	Pérez-Gago et al., 2005a

	WPC; BW	AA, Cys, Gly	Pérez-Gago et al., 2006
	Candellilla wax	Aloe vera juice, ellagic acid, gallic acid	Saucedo et al., 2007
Avocado	Candellilla wax	Aloe vera juice, ellagic acid, gallic acid	Saucedo et al., 2007
Banana	Carragennan	AA, Cys, $CaCl_2$, Gly, PEG	Bico et al., 2009
	Candellilla wax	Aloe vera juice, ellagic acid, gallic acid	Saucedo et al., 2007
Carrot	Alginate	Citric acid, $CaCl_2$	Amanatidou et al., 2000
	Chitosan, yam starch	Gly	Durango et al., 2006
	Chitosan, yam starch	Gly	Simões et al., 2009
	Chitosan, MC, oleic acid	—	Vargas et al., 2009
	Xantam gum	Gluconal cal, Vit. E	Mei et al., 2002
	Cellulose-based	—	Peiyin and Barth, 1998
	Pectin, CMC, CC, WPI	Cynnamaldehyde, Gly	Caillet et al., 2006
	HPMC, sucrose ester	—	Villalobos-Carvajal et al., 2009
	CC, WPI	Gly	Lafortune et al., 2005
	SWP, SPI, CC	Sorbitol	Shon and Haque, 2007
Celery	CC, AMG	—	Avena-Bustillos et al., 1997
Eggplant	SPI, BW	AA, Cys, Gly	Ghidelli et al., 2010b
Grapefruit	Wax microemulsion	—	Baker and Hagenmaier, 1997
Lettuce	Alginate	$CaCl_2$	Tay et al., 2004
Lichi	Chtitosan	—	Dong et al., 2003

Commodity	Coating material	Additives and plasticizer	References
Mango	Chitosan	—	Chien et al., 2007
	CMC, chitosan, dextrin, stearic acid	AA, citric acid, calcium lactate	Ducamp-Collin et al., 2009
	CMC, maltodextrin	Calcium ascorbate, N-acetylcysteine	Plotto et al., 2004
Melon	Alginate, gellan, pectin, sunflower oil	$CaCl_2$, Gly,	Oms-Oliu et al., 2008a
	Alginate	Cinnamon, palmarosa, lemongrass, eugenol, geraniol, citral, malic acid, calcium lactate, Gly	Raybaudi-Massilia et al., 2008a
	SPI	malic acid, lactic acid, Gly	Eswaranandam et al., 2006
Mushroom	Chitosan	—	Eissa, 2007
Onion	SWP, SPI, CC	Sorbitol	Shon and Haque, 2007
Papaya	Alginate, gellan	AA, $CaCl_2$, Gly	Tapia et al., 2007
Pear	Alginate, gellan, pectin	N-acetylcysteine, glutathione, $Cacl_2$, Gly	Oms-Oliu et al., 2008b
	MC, stearic acid	AA, PS, $CaCl_2$, Gly, PEG	Olivas et al., 2003
Persimmon	WPI, BW	Sodium ascorbate, Gly	Pérez-Gago et al., 2005b
	SPI	citric acid, CaCl2, Gly	Ghidelli et al., 2010a
Potato	Nature Seal™, CMC, SPC	AA, PS, SB, soy oil, CaCl2, Gly, PEG	Baldwin et al., 1996
	CMC, CC, WP	$CaCl_2$, Gly	Le Tien et al., 2001

	SWP, SPI, CC	Sorbitol	Shon and Haque, 2007
Strawberry	Chitosan	—	Campaniello et al., 2008
Water chestnut	Chtitosan	—	Pen and Jiang, 2003

BW = beeswax, AMG = acetylated monoglyceride, CMC = Carboxymethyl cellulose, WPC = whey protein concetrate, CC = calcium caseinate, SPC = soy protein concentrate, SWP = sour whey powder, WPI = whey protein isolate, HPMC = Hydroxypropyl methylcellulose, CW = carnauba wax, MC = methylcellulose, SPI = soy protein isolate, CaCl2 = calcium chloride, Gly = glycerol, AA = ascorbic acid, PEG = polyethylene glycol, Cys = cystein, PS = potassium sorbate, SB = sodium benzoate.

1.4.1 Films versus emulsion coatings

Minimally processed products can be coated with stand-alone films preformed by casting or extrusion processes, or by formation of the film layer directly on the surface of the product by dipping or spraying the coating solution. Stand-alone edible films are usually used to cover, wrap or separate food components from each other and from the environment. Many of the films studied are actually dry films of layered structures, and most films are not suitable for foods with high surface water activity because they swell, dissolve and/or disintegrate on contact with water (Guilbert, 1986). However, adding a hydrophobic portion may contribute to decreasing water permeability. In this sense, McHugh et al. (1996) developed stand-alone edible films from fruit and vegetable purees that presented good mechanical and barrier properties to be used to wrap fresh-cut fruits. A film made from apple puree, beeswax, pectin, glycerol, ascorbic acid and citric acid was effective at reducing moisture loss and browning in fresh-cut apples (McHugh and Senesi, 2000). Another film made from pure mango puree reduced weight loss of whole mango fruit and extended shelf-life of fresh-cut mango slices by 2 to 3 days (Sothornvit and Rodsamran, 2008).

Coating formulations applied directly to food products usually, but not necessarily, consist of emulsions that contain immiscible components with one component dispersed as fine droplets (dispersed phase) in the other (continuous phase). This way, gas exchange, adherence, and moisture barrier properties of the composite coating are improved, with results very beneficial to fresh-cut fruits and vegetables (Baldwin et al., 1995). Emulsion coatings require the consideration of stability, which is related to a balance between attractive and repulsive forces-including van der Waals, electrostatic, steric, and hydration forces.

1.4.2 Polysaccharides

Polysaccharides (chitosan, alginate, carageenan, cellulose, starch, pectin, gums) are widely used as edible coatings for fresh-cut fruits (Krochta and de Mulder-Johnston, 1997). These coatings present generally good gas-mostly oxygen-barriers due to their hydrogen-bonded network structure (McHugh et al., 1994), and adhere well to the hydrophilic cut surfaces of

fruits and vegetables. However, they are poor water barriers (Baldwin et al., 1995), which may increase product desiccation and weight loss.

1.4.2.1 Chitosan

Chitosan [2-amino-2 deoxy- β –D-Glucan] is a cationic polymer obtained from the deacetylation of chitin originating from the exoskeleton of crustaceans (crab, shrimp and crayfishes), and from the cell wall constituents of fungi and insects (Andrady and Xu, 1997; Hirano, 1999). Chitosans are described in terms of degree of deacetylation and average molecular weight. Extraction methods make the differences in the degree of deacetylation, the distribution of acetyl groups, the chain length and the conformational structure of chitin and the chitosan molecule (Tsai et al., 2002). Chitosans have been widely studied because of their antimicrobial properties as well as their cationic character and their film-forming properties (Muzzarelli, 1996; Geraldine et al., 2008). They are also used by the food industry as clarifying agents, antioxidants, and as enzymatic browning inhibitors (Devlieghere et al., 2004). The amino groups in the chitosan molecule provide positive charges which make possible modifications to the molecule by covalent bonding with anions, such as those from fatty acids and other proteins under the right pH (Janjarasskul and Krochta, 2010). It is also believed that the cationic property of chitosan is partially responsible for its antimicrobial properties, by binding to the negatively charged microbial cell membranes (Papineau et al., 1991; Young et al., 1982). The positive charges of the chitosan molecule also provide some antioxidant activity, as well as the ability to carry and slow-release functional ingredients (Coma et al., 2002).

Chitosan edible coatings were shown to effectively maintain quality and extend the shelf-life of many fresh-cut products such as carrot (Vargas et al., 2009), Chinese water chestnut (Pen and Jiang, 2003), litchi (Dong et al., 2004), mango (Chien et al., 2007; Ducamp-Collin et al., 2009), mushrooms (Eissa, 2007), and strawberries (Campaniello, et al., 2008). Combination of chitosan with other polysaccharides has also shown to improve its functional properties. For example, an edible yam starch and chitosan coating was successful in controlling the microbial growth, preventing surface whitening and maintaining the sensory quality

of minimally processed carrots (Durango et al., 2006; Simões et al., 2009). In another study, chitosan and cellulose were made into a stand-alone film, with polyethylene glycol added as a plasticizer, which controlled microbial growth on fresh cut melon and pineapple as compared to uncoated fruit, or fruit wrapped in a commercial stretch film (Sangsuwan et al., 2008).

1.4.2.2 Alginates

Alginates are the salts of alginic acid, a linear copolymer of D-mannuronic and L-guluronic acid monomers, extracted from brown seaweeds of the *Phaeophyceae* class (Cha and Chinnan, 2004; Sime, 1990; Vargas et al., 2008). Alginates form films or gels by reacting with divalent and trivalent cations (e.g. calcium, magnesium, and ferric ions) which are added as texturing and gelling agents (Cha and Chinnan, 2004). Using calcium as the gelling agent with alginates has shown to provide additional properties, such as maintaining firmness of fresh-cut apples (Rojas-Graü et al., 2007, 2008; Raybaudi-Massilia et al., 2008b; Olivas et al., 2007), carrots (Amanatidou et al., 2000), lettuce (Tay and Perera, 2004), melon (Raybaudi-Massilia et al., 2008a; Oms-Oliu et al., 2008a), papaya (Tapia et al., 2008), and pears (Oms-Oliu et al., 2008b).

Alginates produce uniform, transparent and water soluble films, which give the fruit a bright, translucent, and fresh-like appearance (Olivas et al., 2007). These films are also effective gas barriers and were shown to decrease the respiration rate and the ethylene production by creating a modified atmosphere around the cut pieces of apple (Raybaudi- Massilia et al., 2008b; Rojas-Graü et al., 2007), melon (Raybaudi- Massilia et al., 2008a), and pears (Oms-Oliu et al., 2008b).

Like other hydrophilic polysaccharides, alginate coatings require the addition of plasticizers to increase coating flexibility and decrease brittleness by reducing the internal hydrogen bonds between the polymer chains and increasing molecular spaces. In addition, due to their hydrophilic nature, the incorporation of a lipidic substance to the alginate coating mix may be necessary to improve water vapor barrier properties. Tapia et al. (2008) studied the effect of the addition of glycerol, ascorbic acid and sunflower oil to alginate-based coatings in order to improve their moisture barrier on fresh-cut papaya. The coatings improved the

firmness of the fresh-cut product during the period studied, and the addition of 0.025% (w/w) sunflower oil resulted in a 16% increase in the water vapor resistance of the coated samples.

Several studies (Rojas-Graü et al., 2007, 2008; Raybaudi-Massilia et al., 2008a, b; Oms-Oliu et al., 2008b; Tapia et al., 2008) successfully used alginate edible coatings with anti-browning agents, such as ascorbic acid, *N*-acetyl-L-cysteine and glutathione, to control enzymatic browning and extend shelf-life of fresh-cut commodities. Lemongrass and cinnamon (0.7%), citral (0.5%) or cinnamaldehyde (0.5%) essential oils added to an alginate-based coating (alginate, malic acid, *N*-acetyl-L-cysteine, glutathione and calcium lactate) effectively prevented microbiological growth on fresh-cut apples (Raybaudi-Massilia et al., 2008b). The addition of palmarosa oil (0.3%) to a similar alginate based coating inhibited the native flora and reduced *Salmonella enteritidis* population while maintaining the fresh-cut melon quality parameters (Raybaudi-Massilia et al., 2008a).

1.4.2.3 Gellan

Gellan is a polysaccharide of microbial origin and is secreted by the bacterium *Sphingomonas elodea* (formerly referred to as *Pseudomonas elodea*). Like alginate, gellan coatings are made by adding calcium ions (calcium chloride) to cross-link the carbohydrate polymer (Oms-Oliu et al., 2008b).

In a comparative work between gellan and alginate, the gellan films showed better vapor barrier properties than alginate stand-alone films and water solubility values found for gellan films at 25 ºC were significantly lower (0.47 to 0.59 g soluble solids/g total solids) than the values found for the alginate film (0.74 to 0.79) (Tapia et al., 2007). This implies that gellan films, which are more difficult to dissolve in water, present a slightly higher hydrophobicity that could be desirable for their use on fresh-cut fruits. Also in this study, fresh-cut apples and papaya cylinders were successfully coated with these films and the addition of sunflower oil (0.025 mL/ 100mL film forming solution) to the gellan coating applied to fresh-cut apples improved its water barrier property, thus reduced moisture loss and maintained texture.

Gellan-based edible films and coatings are good carriers for antioxidant agents such as glutathione, *N*-acetyl-L-cysteine, ascorbic and citric acids (Rojas-Graü et al., 2008). For example, incorporation of *N*-acetyl-L-cysteine and glutathione to a gellan-based coating effectively controlled enzymatic browning, reduced ethylene production, and maintained the desirable quality characteristics of fresh-cut apples (Rojas-Graü et al., 2008) and pears (Oms-Oliu et al., 2008b). Moreover, this coating maintained the vitamin C content and antioxidant potential in pears (Oms-Oliu et al., 2008b) and in fresh-cut melon (Oms-Oliu et al., 2008a).

1.4.2.4 Carrageenan

Extracted from several red seaweeds, mainly *Chondrus crispus*, carrageenan is a complex mixture of at least five different water soluble galactose polymers (Karbowiak et al., 2007; Vargas et al., 2008). Carrageenans form gels in presence of monovalent or divalent cations during moderate drying, leading to a three-dimensional network formed by polysaccharide double helices which becomes a solid film after solvent evaporation (Karbowiak et al., 2007). Among the different types of polymers (κ; ι, and λ carrageenan), ι-carrageenan is preferred for coatings as it makes clear and elastic gels (Karbowiak et al., 2007)

Application of carrageenan edible coatings with an antibrowning agent was a good method to prevent weight and firmness losses, to reduce respiration rate, to maintain eating quality, and to maintain microbial growth within acceptable limits during storage of fresh-cut banana (Bico et al., 2009), and fresh-cut apples (Lee et al., 2003).

1.4.2.5 Xanthan gum

Xanthan gum is the product of glucose fermentation by the *Xanthomonas campestri* bacterium. It is an anionic polymer with cellulosic backbone substituted on alternate glucose residues with a trisaccharide side chain (Mei et al., 2002). A xanthan gum coating was used as carrier of calcium and vitamin E on fresh-cut baby carrots (Mei et al., 2002). These formulations were efficient at delaying the white surface discoloration of carrots, probably by acting as surface moisturizer, and they did not affect

the aroma, flavor, sweetness, crispness, and carotene levels of the baby carrots.

1.4.2.6 Cellulose and cellulose derivatives

Cellulose, the structural material of plant cell walls, is composed of linear chains of (1->4)-β-D-glucopyranosyl units (Nisperos, 1994). Because it is naturally insoluble in water, chemical modification of cellulose by etherification is necessary to make it usable. Water soluble cellulose derivatives are methylcellulose (MC), hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC) and ionic carboxymethylcellulose (or sodium carboxymethylcellulose CMC). These cellulose derivatives tend to have excellent film forming property, and films are generally odorless, tasteless, transparent, flexible and of moderate strength. Regarding their physical properties, cellulose derivative films provide some barrier to oil and fats and have moderate barrier properties to moisture and oxygen (Krochta and Mulder-Johnson, 1997; Gennnadios et al., 1997; Turhan and Sahbaz, 2004; Maftoonazad and Ramaswamy, 2005). MC is the most hydrophobic of the cellulose derivatives (Kester and Fennema, 1986), even though the WVP of cellulose stand-alone films is still relatively high. MC-based coatings were used on fresh-cut apples (Vargas et al., 2009) and pear wedges (Olivas et al., 2003). In both studies, the application of MC edible coating did not reduced weight loss, probably due to the high relative humidity conditions. However, the incorporation of stearic acid, a fatty acid (hydrophobic molecule), to MC coatings reduced the weight loss of pear wedges compared to MC alone.

Scarce reports of application of HPMC-based coatings to fresh-cut fruit are available. Villalobos-Carvajal et al. (2009) studied the barrier properties of HPMC edible coatings containing surfactant mixtures prepared in aqueous or hydroalcoholic solution, applied to fresh-cut carrots. HPMC edible coating prepared in a hydroalcoholic solution provided greater resistance to water vapor migration than those prepared in an aqueous solution. In addition, coatings prepared in an alcoholic media induced lesser color changes (whitening) than those prepared in an aqueous solvent, probably because the film formed was thinner (Villalobos-Carvajal et al., 2009). In fresh cut apples coated with HPMC

containing beeswax or carnauba wax formulations, there was no significant reduction in either weight loss or browning (Pérez-Gago et al., 2005b).

CMC, or cellulose gum, is available for food applications in a variety of types based on degree of substitution, particle size, viscosity and hydration characteristics (Nisperos-Carriedo, 1994). On fresh-cut fruit, the use of CMC-based edible coatings with an antioxidant agent such as *N*-acetyl-L-cysteine, ascorbic and/or citric acid, gave positive results in controlling enzymatic browning, improving visual appearance and maintaining fruit aroma and flavor in fresh-cut mangoes (Plotto et al., 2004; Ducamp et al., 2009). Combinations of CMC with other hydrocolloids, such as whey or milk protein, showed good synergy, and was useful in reducing respiration rate, delaying browning, maintaining firmness and increasing the antioxidative power of coated fresh-cut apples (Lee et al., 2003), and potatoes (Le Tien et al., 2001). It was hypothesized that the carboxyl groups in CMC may effectively trap peroxide radicals (Le Tien et al., 2001). Similar effectiveness was demonstrated by Baldwin et al. (1996) with minimally processed potato and apple, where the antibrowning activity of CMC-based edible coating with ascorbic acid was more effective than an ascorbic acid aqueous solution alone. The latter results suggest that ascorbic acid undergoes less degradation within the cellulose matrix, thus enhancing its antibrowning activity. Moreover, application of CMC retarded water loss and the addition of potassium sorbate and citric acid showed a synergistic effect for microbial control (Baldwin et al., 1996). Finally, two commercial cellulose based edible coatings of varying pH (2.7 and 4.6) significantly retarded surface whitening, preserved carotene content, retarded peroxidase activity and maintained fresh appearance of lightly-processed carrots (Li and Barth, 1998).

1.4.2.7 Pectin

Pectin is a soluble plant fiber derived from plant cell walls, and the major commercial source for food applications is from citrus peel and apple pomace. Pectin is divided into types according to the degree of esterification (DE) that is an indication of the content and branching of methyl esters on the polymer chain composed of (1->4)-α-D-

galactopyranosyluronic acid units. The DE imparts solubility and gellation properties. Pectins with a DE of 50% are divided into low- and high-methoxyl pectins. Low-methoxyl pectin can form gels under a narrow pH range and with the presence of soluble solids, while high-methoxy pectin can form gels in the presence of calcium ion (Nisperos-Carriedo, 1994). Pectin-based edible coatings with added sunflower oil contributed to reduced moisture and firmness loss, and inhibition of ethylene synthesis in fresh-cut melon and pears (Oms-Oliu et al., 2008a, b). Further addition of *N*-acetyl-L-cysteine and glutathione as antioxidants to pectin was effective in avoiding browning in fresh-cut pears. The addition of these antioxidants preserved vitamin C and phenolic content in fresh-cut pears, which was attributed to a reduction of O_2 diffusion. Also, fresh-cut melon coated with pectin maintained their quality attributes better compared to samples coated with gellan or alginate after one week storage, without affecting their taste (Oms-Oliu et al., 2008a,b).

1.4.2.8 Starch and starch derivatives

Starch is a polymeric carbohydrate composed of anhydroglucose units [(1->4)-α-D-glucopyranosyl monomers]. Most starches contain two types of glucose polymers: a linear chain molecule termed *amylose* and a branched polymer of glucose termed *amylopectin* (Nisperos-Carriedo, 1994). Starch edible coatings exhibit different properties than other polysaccharides, which is attributed to the amylose content (Lawton, 1996).

Among the polysaccharides available commercially for production of edible coatings, starch is the natural biopolymer most commonly used because it is abundant, relatively low cost and has a wide range of functionalities (Mali et al., 2002). Mali et al. (2002) showed that yam (*Dioscorea* sp.*)* starch, with about 30% amylase, presented good film forming properties; therefore, it was a good source for the production of edible coatings. Yam starch was used on minimally processed carrots (Durango et al., 2006). When combined with chitosan, it effectively controlled microbiological growth and enhanced visual quality and phenolic content of carrot sticks (Simões et al., 2009).

Dextrin, derived from starch with smaller molecular size, could be used in edible coating providing a better water vapor resistance than starch coatings (Lin and Zhao, 2007). Ducamp et al. (2009) reported the effectiveness of dextrin potato starch with calcium lactate and ascorbic acid to reduce respiration rate in fresh cut mangoes. Meanwhile, combination of maltodextrin with CMC did not improve mango flavor or texture in minimally processed mangoes (Plotto et al., 2004).

1.4.3 Proteins

Proteins used in edible coating destined for fresh-cut fruits and vegetables are usually whey protein and casein extracted from milk, or soy proteins (Baldwin and Baker, 2002). Due to their chemical nature, proteins can impart a range of physical and chemical properties to coatings. The type, sequence and amount of amino acids will determine their molecular size, shape (globular, random coil or helix conformation), and charges depending on the pH. Therefore, coatings and films made with proteins will vary in their flexibility (rigid versus flexible), thermal stability, and barrier properties (Vargas et al., 2008). Proteins, like polysaccharides, are highly polar polymers, and are capable of forming strong films, with low permeability to O_2 and CO_2, but poor water barrier properties (Kester and Fennema, 1986; McHugh and Krochta, 1994, Baldwin and Baker, 2002). Plasticizers can improve the flexibility and strength of those films (Brault et al., 1997; Sothorvith and Krochta, 2001), however their application implies a decrease in film and coating moisture barrier. In spite of the inherent hydrophilicity of proteins, protein-based coatings made with high levels of hydrophobic amino acids, like those found in soy protein and casein, present greater moisture barrier properties than proteins with less hydrophobic amino acids, especially if they are combined with lipids.

1.4.3.1 Whey protein and casein

Milk proteins include approximately 80% of casein and 20% of whey protein (Gennadios et al., 1994) and are attractive for coating manufacturers due to their numerous functional properties (Chen, 2002; Krochta, 1997, 2002; Vargas et al., 2008). During manufacturing, casein

is separated from whey protein by adjusting milk to the isoelectric pH of casein, then centrifugating the casein precipitate (Gennadios et al., 1994). The acid casein can be converted to soluble caseinates through neutralization with an alkali, obtaining sodium, calcium, magnesium, potassium, and ammonium caseinates (Chen, 2002). Caseins have low levels of cystein, and as a result, have an open random coil shape (Gennadios et al., 1994). Because of that nature, they can easily form films from aqueous solutions without further treatment. Casein-based edible coatings are transparent, flavorless, flexible, and have high nutritional qualities, excellent sensory and gas barrier properties (Lin and Zhao, 2007; Vargas et al., 2008). Different casein products may result in films of different permeabilities and mechanical properties, for example films made with calcium caseinate were stronger and more flexible and presented lower WVP than films made with sodium caseinate (Brault et al., 1997).

Liquid whey is a by-product of cheese processing and is commercially purified to produce whey protein concentrate (WPC) with 25% to 80% protein content, or whey protein isolate (WPI) with protein content above 80%, prepared from WPC by adding an ion-exchange step (Gennadios et al., 1994; Krochta, 2002). Unlike caseins, whey proteins require heat denaturation for film formation (Gennadios et al., 1994; McHugh et al., 1994). Whey protein produces transparent, flavorless, and flexible water based edible coatings.

Casein and WPI coatings efficiently delayed browning of apple and potato slices by acting as oxygen barriers. Moreover, they were shown to have a high antioxidant activity due to the presence of amino acids and the simple sugar lactose known for its free radical quenching effect (Le Tien et al., 2001).

The same effectiveness in controlling enzymatic browning was reported in the application of both WPI and WPC edible coatings with the addition of lipid compounds (beeswax or carnauba wax) on fresh-cut apples. This was attributed to a possible antioxidant effect of cysteine, presented in high levels in whey proteins, or the oxygen barrier provided by the coatings (Pérez-Gago et al., 2003, 2005a). The addition of lipids to these whey protein coatings did not significantly reduce moisture loss. However, combination of casein-coating with a hydrophobic compound that acted as a plasticizer (acetylated monoglyceride) contributed to

significantly increase water vapor resistance, thus reducing moisture loss, and respiration rate of celery sticks (Avena-Bustillos et al., 1997). Browning of fresh-cut apples and persimmon was further retarded when whey protein coating was combined with anti-browning agents (Pérez-Gago et al., 2005b, 2006). Ascorbic acid (Pérez-Gago et al., 2006) or cysteine (Pérez-Gago et al., 2005b) reduced enzymatic browning more effectively when incorporated into the whey protein coatings than when applied alone. The polymer coating might offer a protective effect to degradation of the antioxidants, such as observed earlier in a CMC coating (Baldwin et al., 1996).

Application of WPI and casein edible coating with irradiation treatment (1 kGy), and packed under air was used to prevent the whitening of baby carrots, maintaining firmness and quality during a 21-day storage period (Lafortune et al., 2005; Caillet et al., 2006).

1.4.3.2 Soy protein

Soy protein (SP) is the major plant origin protein studied as coating material for minimally processed products. SP coatings can be prepared from soy protein isolates (SPI) or concentrates (SPC), containing about 70% and 90% protein, respectively, with a plasticizer, typically glycerol or sorbitol, to improve flexibility (Gennadios et al., 1994). Like with other carbohydrate and protein coatings, SP coatings exhibit poor moisture resistance and water vapor barrier properties due to the inherent hydrophilicity of protein and plasticizers added (Rhim et al., 2000).

Few studies reported the application of soy protein on fresh-cut fruits and vegetables. SPI coatings effectiveness was shown to control browning in potato slices, and reduce moisture loss in carrots and apple slices (Shon and Haque, 2007). A SPI-cysteine-based edible coating was successful at maintaining the quality of fresh-cut eggplants and minimally processed persimmon (Ghidelli et al., 2010a,b). The incorporation of cysteine as an antibrowning agent further delayed the enzymatic browning and prevented softening of fresh-cut eggplant tissue (Ghidelli et al., 2010b).

In addition, SPI coating combined with low O_2 and high CO_2 modified atmosphere showed a positive synergic effect in controlling

tissue browning and maintained the general visual quality of fresh-cut persimmon up to 8-10 d at 5 ºC (Ghidelli et al., 2010a).

The influence of the SPI application alone or with additives (malic or lactic acid) on the sensory quality of fresh-cut cantaloupe was studies by Eswaranandam et al. (2006). SPI alone or with lactic acid improved the sweetness of fresh-cut cantaloupe, but did not have any added effect on the taste, overall appearance, enzymatic browning or moisture loss compared to controls.

1.4.4 Lipids

Lipids used in edible coatings for fresh-cut fruits include beeswax, candelilla and carnauba wax, tryglicerides, acetylated monoglycerides, fatty acids, fatty alcohols and surfactants such as sucrose esters. Lipids have long been used to protect horticultural crops from dehydration, to slow down senescence, and to improve surface appearance (Kester and Fennema, 1986; Hagenmaier and Baker, 1994; Hernandez, 1994; Morillon et al., 2002). However, because of their hydrophobic properties, lipids tend to form thicker and brittle coatings, and they do not adhere well to the moist surface of fresh-cut tissue. Consequently, for fresh-cut produce, the best use of lipids in a coating is to combine them with hydrophilic film forming agents such as polysaccharides or proteins.

In stand-alone films, many studies describe the effect of lipid type on WVP, showing an effect of lipid polarity created by chemical groups (e.g. carboxylic, alcohol), aliphatic chain length and degree of unsaturation (Morillon et al., 2002). The study by McHugh and Senesi (2000), where different types of lipids were added to an apple puree-based edible film, is a good example of property modification due to the lipidic phase. Waxes, high molecular weight fatty acids and fatty alcohols showed good adhesion to the casting surface. Vegetable oil significantly reduced WVP and the puree film with vegetable oil did not need additional plasticizers. Among the fatty acid tested, oleic acid exhibited the best water barrier properties. Moreover, increasing concentrations of lipids resulted in significant decrease in WVP for coatings with oleic acid, palmitic acid and beeswax (McHugh and Senesi, 2000). These apple-based wraps significantly reduced moisture loss and browning in fresh-cut apples and color was preserved for 12 days at 5ºC.

The use of candelilla wax by itself improved the shelf-life of fresh-cut avocados, bananas and apples (Saucedo-Pompa et al., 2007). Moreover, the addition of *Aloe vera* juice or ellagic acid, an antioxidant compound, reduced weight loss, retarded browning and retained firmness of fresh-cut fruits (Saucedo-Pompa et al., 2007).

The addition of lipids to protein or polysaccharide coatings helped improve coating moisture barrier. Stearic acid added to a MC coating significantly reduced fresh-cut pear weight loss (Olivas et al., 2003). However, incorporation of beeswax and carnauba wax in coatings composed of whey or soy proteins, or HPMC did not significantly reduce moisture loss of fresh-cut apples and eggplants (Pérez-Gago et al., 2003, 2005a; Ghidelli et al., 2010b). Nevertheless, these combinations improved visual appearance by reducing enzymatic browning of these fresh-cut produces.

Due to their hydrophobic nature, lipid compounds do not adhere well to the high moisture surface of fresh-cut products. Since wax coatings can withstand high a_w with little loss of integrity, Baker and Hagenmaier (1997) developed wax microemulsion coatings to inhibit fluid leakage from grapefruit segments. Generally, wax microemulsion coatings provided an acceptable mean which to control fluid leakage from grapefruit segments; coatings made with either polyethylene wax or carnauba wax with C12-C18 fatty acids the most effective coatings, without compromising appearance or general acceptability.

Acetylated monoglyceride is a vegetable oil derivative which is solid at room temperature. Therefore, its application as a coating for fruits or vegetables requires an emulsifier such as calcium or sodium caseinate. Optimization of caseinate-acetylated monoglyceride coating produced a 75% reduction in moisture loss and minimized wound response in celery sticks by reducing respiration (Avena-Bustillos et al., 1997).

1.4.5 Composite and bilayer coatings

As reviewed in the preceding section, each coating material has its own physico-chemical properties: hydrocolloids (proteins and carbohydrates) tend to form hydrophilic networks with good barriers to oxygen and carbon dioxide, but poor water permeability; while lipids form

hydrophobic coatings with good water barrier properties but that do not adhere well to fresh cut tissue. Thus, when combined in proper proportions, they complement each other and form successful edible coatings for fresh-cut fruits.

An edible composite coatings can be formed as a bilayer or as an emulsion. In bilayer edible coatings, the polysaccharide or protein solution is applied on the fruit surface and after drying of the coating, a second layer with the lipid is applied. In emulsion composite edible coatings, the lipid is dispersed and entrapped in the hydrophilic phase forming a homogeneous emulsion, which is directly applied on the fruit surface (Krochta, 1997). Therefore, emulsion composite coatings are more convenient to apply than bilayer coatings because they only required one application and drying step. They also have a better adherence to a larger number of surfaces due to the presence of both polar and nonpolar components, and exhibit good mechanical resistance provided by the continuous polymer matrix (Quezada-Gallo et al., 2000; Pérez-Gago and Krochta, 2001). Therefore, only very few studies can be found in the literature where fresh-cut fruits and vegetables are coated with a bilayer composite coating. Wong et al. (1994) reported a reduction of water loss and of internal oxygen concentration of fresh-cut apple cylinders coated with a bilayer coating composed of a first layer of polysaccharides (pectin, carrageenan, alginate, and microcrystalline cellulose), followed by a layer containing acetylated monoglyceride. Recently, application of composite emulsion coatings on fresh-cut products have been reported by several authors and were discussed in previous sections (Avena-Bustillos et al., 1997, Baker and Hagenmaier, 1997; Olivas et al., 2003; Pérez-Gago et al., 2003, 2005a, 2005b, 2006; Villalobos-Carvajal et al., 2009; Ghidelli et al., 2010b).

1.4.6 Coating optimization

The success of an edible coating is based on the physicochemical and barrier properties of its components (proteins, polysaccharides, lipids). Thus, determining the proper composition and proportions of the components is of prime importance in order to extend the shelf-life and enhance the quality of fresh-cut fruit and vegetables. Considerable work can be found in the literature regarding the effect of the different

components on the barrier and mechanical properties of stand-alone films (Guilbert, 1986; Kester and Fennema, 1986; Krochta and De Mulder-Johnston, 1997). For example, the ratio of polymer to plasticizer have phenomenal effects on stand-alone film strength and elasticity. Brault et al. (1997) showed that glycerol had a double function by acting as a plasticizer in a calcium caseinate film, thereby improving film viscoelasticity, and also enhanced formation of cross-links within the caseinate chains resulting in a stronger film.

Similarly, many factors, such as coating composition and formulation solid content, proven to affect the performance of edible coatings on the postharvest life of whole fruit, have not been studied in details on fresh-cut products. The investigation of these factors prior to the incorporation of additives such as antioxidants and/or antimicrobials is very important to optimize the coating performance on fresh-cut produces. In this sense, Pérez-Gago et al. (2003) found that the solid content of the formulation and lipid content of WPI-beeswax edible coatings without incorporation of antioxidants also had an effect in the degree of browning of fresh-cut apples. As beeswax and solid content increased the browning index of cut apples decreased (Figure 1a, 2a). However, high beeswax or solid content imparted a whitish appearance to the coated apples that were considered as unacceptable by the sensory panel. The optimum solid content of the emulsion and beeswax content in order to reduce browning were 16% and 20% (dry basis), respectively (Figure 1b, 2b).

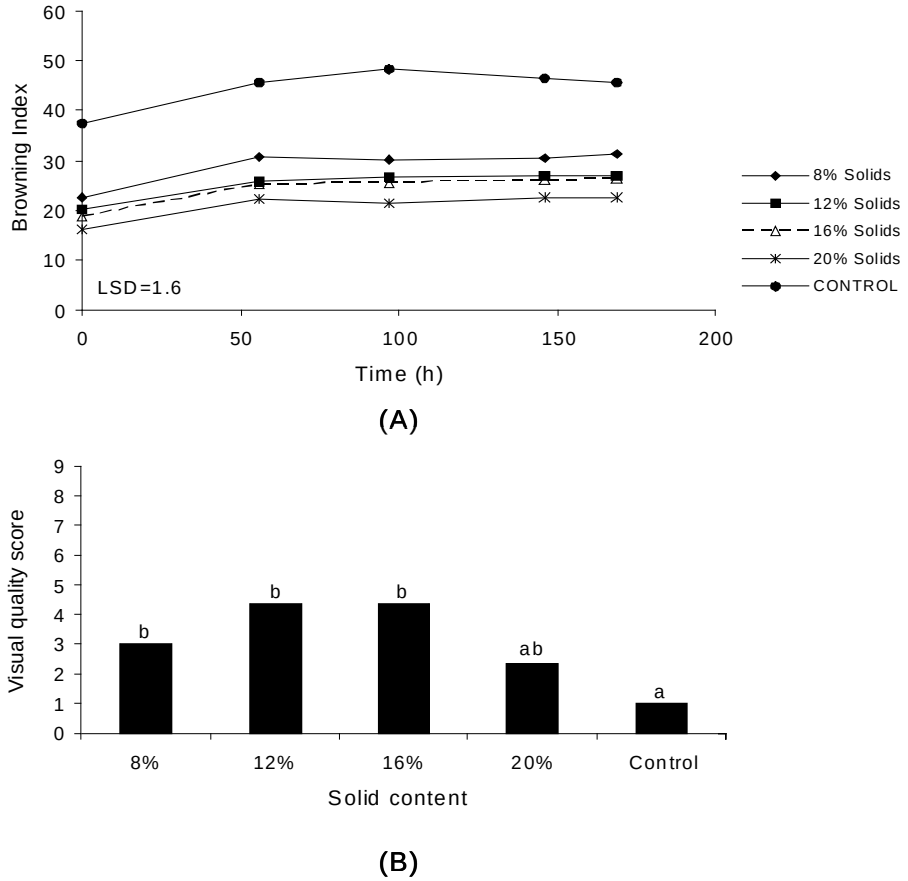

Figure 1. Effects of solid content in whey protein isolate-beeswax coating on (A) browning index and (B) visual quality (1 = poor quality, 9 = excellent) of fresh-cut apples during storage at 5 ºC. (Adapted from Pérez-Gago et al., 2003).

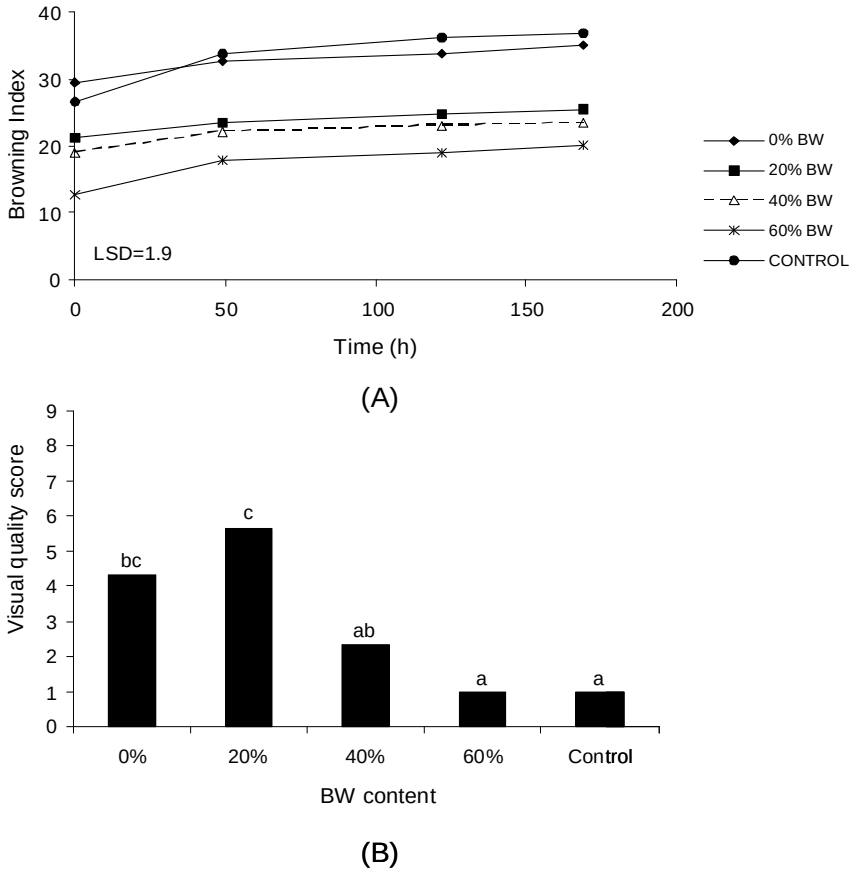

Figure 2. Effects of beeswax content in whey isolate-beeswax coating on (A) browning index and (B) visual quality (1 = poor quality, 9 = excellent) of fresh-cut apples during storage at 5 ºC. (Adapted from Pérez-Gago et al., 2003).

In a more recent study, Pérez-Gago et al. (2005a) showed that the selection of the hydrophilic component is important in the formulation of coatings for fresh-cut products. Results showed that whey protein-based coatings without incorporation of antioxidants were more effective in reducing enzymatic browning of 'Golden Delicious' apples than HPMC-based coatings, probably due to the antioxidant effect of some amino

acids such as cysteine, or the higher oxygen barrier that the protein exerts. However, no differences on visual appearance were found between the uses of WPI having 98% protein or WPC with 65% protein content. When different lipids (carnauba wax or beeswax) were incorporated into the formulations, results indicated that beeswax was more effective at reducing browning as measured with the colorimeter, but visual differences between waxes were less evident at the end of the storage time by the sensory panel (Figure 3a, b).

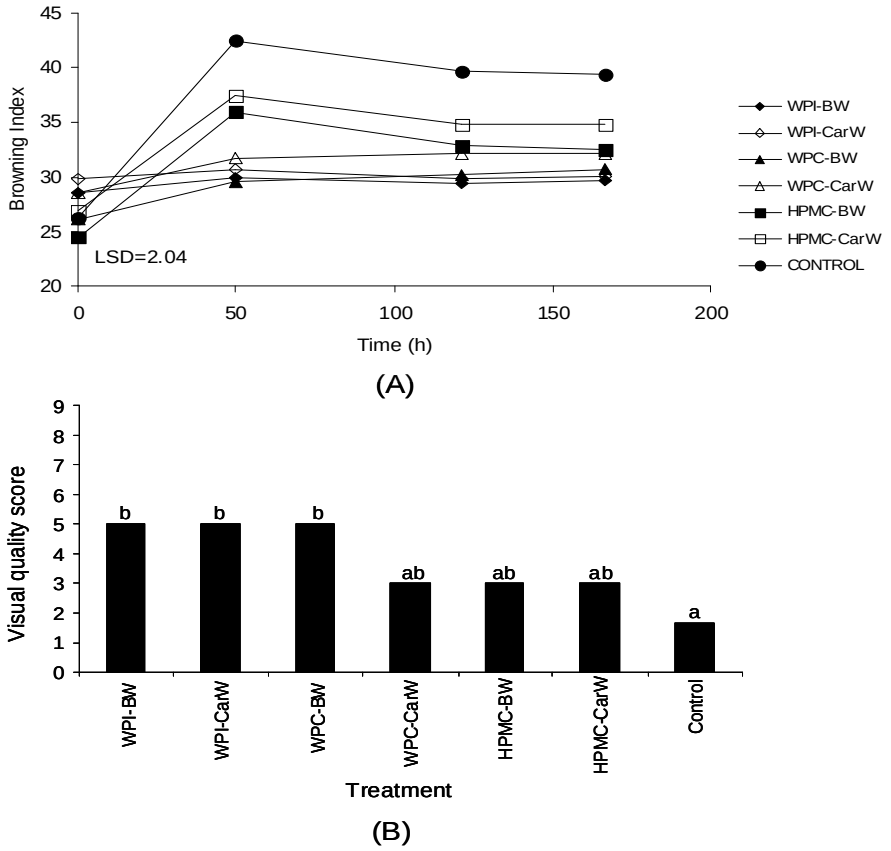

Figure 3. (A) Browning index and (B) visual quality (1 = poor quality, 9 = excellent) of fresh-cut apples coated with whey protein isolate (WPI), whey protein concentrate (WPC), hydroxypropyl methylcellulose (HPMC), beeswax (BW), or carnauba wax (CarW), during storage at 5 ºC. (Adapted from Pérez-Gago et al., 2005a).

1.4.7 Future needs and trends of minimally processed fruits and vegetables

It is widely known that edible coatings provide significant benefits in extending shelf-life and enhancing quality and microbial safety of fresh-cut fruits and vegetables. Nevertheless, the use of edible coatings on a wide range of fresh-cut products and on a commercial scale is still limited by several factors. Many available edible coating formulations are characterized by high hydrophilicity, which does not provide a satisfactory moisture barrier to the fresh-cut product with their high water activity. Higher moisture on the cut side of the flesh, or presence of natural hydrophobic waxy layer on the peel side of the fruit can also create problems with coating adhesion and durability. Therefore, more studies are required to develop new formulations with higher moisture barrier and surface adhesion, as well as to understand the functionality and interactions among the different components.

It is important to investigate sensory quality of coating materials and coated products, including appearance, color, aroma, taste, and texture, since they are important factors that influence commercial success of fresh-cut products. Application of edible coatings, alone or with additives such as as antibrowning or antimicrobial agents, could give the product an unattractive surface appearance, or develop exogenous flavors affecting consumer repeat purchase.

The development of new technologies that allow more control of coating properties and functionalities are being investigated. Among them, a new technique, called micro- and nanoencapsulation – as opposed to macroencapsulation, consists in incorporating functional ingredients and antimicrobial compounds into edible coatings. This technology could pack solid, liquid or gaseous substances in miniature (micro or nanoscale) sealed capsules that can release their content at controlled rates under specific conditions (e.g. changes of pH, temperature, irradiation, osmotic shock). This technology shows the important advantage to protect encapsulated ingredients from moisture, heat or other extreme storage conditions, as they are very perishables to oxygen, light or lipid oxidation. Most of the research so far has focused on nanoencapsulation of silver or zinc particles for microbial control on fruit surfaces (An et al., 2008; Fayaz et al., 2009; Jin et al., 2008; Rhim et

al., 2006). In addition to increased functionality, incorporation of nanoparticles from clay derivatives advantageously modified physical properties (tensile strength and WVP) of a chitosan-based film (Rhim et al., 2006). One aspect that will need to be addressed with the development of nanotechnology in foods is their regulatory status. There is currently no regulation concerning nanoparticles, and it has been speculated that some harmless ingredients in their natural (i.e. "macro") form may become harmful under nanoparticle form (Sozer and Kokini, 2008).

Finally but not lastly, another trend in the development of new coatings is incorporation of healthful additives, including probiotics (Rojas-Graü et al., 2009). Whether adding simple vitamins – ascorbic acid (Tapia et al., 2008), calcium or vitamin E (Han et al., 2004; Mei et al., 2002), or live probiotics, Bifidobacterium lactis (Tapia et al., 2007), the possibilities are immense and each food developer can exert his/her creativity.

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Recent advances in modified atmosphere packaging and edible coatings to maintain quality of fresh-cut fruits and vegetables

CHRISTIAN GHIDELLI [1] AND MARÍA B. PÉREZ-GAGO [1,2*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

Abstract

Processing of fruits and vegetables generates physiological stresses in the still living cut tissue, leading to quality deterioration and shorter shelf-life as compared with fresh intact produces. Several strategies can be implemented with the aim to reduce the rate of deterioration of fresh-cut commodities. Such strategies include low temperature maintenance from harvest to retail and the application of physical and chemical treatments such as modified atmosphere packaging (MAP) with low O_2 and high CO_2 levels and antioxidant dips. Other technologies such as edible coatings with natural additives, new generation of coatings using nanotechnological solutions such as nanoparticles, nanoencapsulation, and multilayered systems, and non-conventional atmospheres such as the use of pressurized inert/noble gases and high levels of O_2 have gained a lot of interest as a possibility to extend the shelf life of minimally processed fruits and vegetables. However, the high perishability of these products challenges in many cases their marketability by not achieving sufficient shelf life to survive the distribution system, requiring the combination of treatments to assure safety and quality. This review reports the recent advances in the use of MAP, edible coatings, and the combined effect of both technologies to extend the shelf life of fresh-cut fruits and vegetables.

Keywords: Edible coating, minimally processed fruits and vegetables, modified atmosphere packaging.

Introduction

Minimally processed or fresh-cut produces are ready-to-eat or ready-to-use fresh fruits and vegetables that have been washed, chopped and packaged in sealed polymeric films or trays. This trading form was developed in the 1980s to respond to the emerging consumer demand for convenient, high quality and preservative-free products that maintain fresh appearance, while being less severely processed than canned or frozen products. The demand for fresh-cut fruit and vegetables has continuously increased during the last years, being the convenience factor the main reason for the growth, although now the healthy eating trend is helping to make this sector even stronger.

Contrary to other food processing techniques such as drying, freezing or canning, fresh-cut processing does not extend the shelf-life of the produce. In fact, processing and packaging of fresh-cut fruits and vegetables generates physiological stresses in the still living cut tissues leading to quality deterioration and a shorter shelf-life as compared with intact fruits and vegetables (Gil et al., 2006; Rojas-Graü et al., 2009a). The greatest hurdle to the commercial marketing of fresh-cut produces is related to their higher susceptibility to enzymatic browning, tissue softening, increasing of respiration, microbial growth, and environmental factors. Among them, temperature, humidity, atmospheric composition and ethylene concentration directly influence the deterioration process. Mechanical damages caused by harvesting, handling, storage and transportation before processing are also responsible of the shelf-life of the fresh-cut produces (Al Ati and Hotchkiss, 2002).

There is no single technology that limits the overall quality deterioration. Several strategies can be implemented with the aim to reduce the rate of deterioration of fresh-cut commodities. These include the use of high quality raw produces at the optimum harvest maturity, sanitation during handling and processing, good processing practices reducing mechanical damage, and postprocessing treatments such as low temperature and low oxygen atmosphere packaging to reduce respiration rate and ethylene production. Other technologies such as edible coatings with natural additives and non-conventional atmospheres have gained a lot of interest as a possibility to extend the shelf life of minimally processed fruits and vegetables.

Successful applications of modified atmosphere packaging (MAP) with low O_2 and high CO_2 for minimally processed fruits and vegetables have been extensively reported in the literature (Oms-Oliu et al., 2009a; Rojas-Graü et al., 2009a; Sandhya, 2010). The effect of low O_2 and high CO_2 MAP to reduce quality deterioration of fresh-cut produces during storage is related to a reduction in produce respiration rate, ethylene biosynthesis and action, water loss, phenolic oxidation, and aerobic microbial count (Toivonen and DeEll, 2002). However, the beneficial quality effects of MAP on the packaged fresh-cut fruits and vegetables depend upon a number of uncontrollable factors, such as the species, cultivar, cultural practices, stage of development, postharvest handling, as well as controllable factors, including packaging material gas

permeability, respiration rate, and storage conditions (Zhuang et al., 2014). Exposure of fresh-cut produces to too low O_2 and excessive CO_2 levels may lead to anaerobic respiration and fermentation with the production of undesirable metabolites and other physiological disorders (Soliva-Fortuny and Martín-Belloso, 2003). Therefore, the range of O_2 and CO_2 in the package must be defined for each product and handling/processing characteristic (e.g. processed form, package format, storage conditions, etc.).

Innovative MAP such as the use of pressurized inert/noble gases and high levels of O_2 have also been reported as effective in extending shelf life of minimally processed fruits and vegetables. The main benefits of superatmospheric O_2 and noble gases atmospheres are related to the prevention of microbiological spoilage and anaerobic fermentation, as observed in fresh-cut melon, cabbage, baby spinach leaves, green peppers, broccoli, and lettuce (Jamie and Salveit 2002; Allende et al., 2004; Oms-Oliu et al., 2008a; Lee et al., 2011; Meng et al., 2012). Moreover, inert gases and high O_2 atmospheres have been found to be particularly effective at inhibiting enzymatic reactions and maintaining firmness of fresh-cut products as reported in iceberg lettuce, mushrooms, potatoes, apples, green pepper, and melons (Amanatidou et al., 2000; Day, 2001; Jacxsens et al., 2001; Jamie and Salveit, 2002; Limbo and Piergiovanni, 2006; Oms-Oliu et al., 2008a; Meng et al., 2012). Nevertheless, the effect of innovative MAP is dependent on similar factors as with conventional MAP (i.e. type of commodity, temperature, storage duration, etc.) (Kader and Ben-Yehoshua, 2000).

Another approach to extend the shelf life of fresh-cut fruits and vegetables that has gained a lot of attention in the last decade is the use of edible coatings alone or combined with MAP. Edible coatings can provide a semipermeable barrier to gases and water vapor, which might translate in a reduction in respiration rate, enzymatic browning and water loss (Pérez-Gago et al., 2005), and their protective function may be also enhanced with the addition of ingredients such as antioxidants, antimicrobials, flavors, etc. Several reviews present the beneficial effect of edible coatings to maintain the quality properties of fresh-cut fruits and vegetables (González-Aguilar et al., 2010; Dea et al., 2012; Dhall, 2013). Considering the importance of these technologies in horticultural products, this paper provides a critical review about the most recent

works in the literature regarding MAP and edible coating application, alone or in combination, to extend the shelf life of fresh-cut fruits and vegetables. The most recent studies on these technologies are summarized in Table 1.

Modified atmosphere packaging

MAP of fresh-cut produces consists of enclosing the commodity in polymeric films in which the gas composition is modified from normal air to provide an atmosphere for increasing shelf life and maintaining the quality. Because of the respiration process, the fresh-cut product consumes O_2 and produces CO_2, therefore the O_2 and the CO_2 concentration within the package is reduced and increased, respectively. The steady state of equilibrium is reached when the amount of O_2 consumed and CO_2 produced inside the package equals the O_2 and CO_2 amount permeating through the film. Therefore, the specific gas composition at equilibrium is determined by the product weight and physiology (e.g. respiration rate, maturity state, etc), environmental conditions (e.g. temperature, relative humidity), and properties of the packaging material (e.g. film thickness, permeability, perforation density and surface area) (Al Ati and Hotchkiss, 2002; Sandhya, 2010; Caleb et al., 2013). The modified atmospheres (MA) can be achieved passively or actively. The passive MAP relies on the natural process of produce respiration and film permeability. While, active MAP is achieved by displacing the air within the package with a known mixture of gases to create an initial atmosphere that evolves during storage according to the produce's respiration rate, the storage conditions and film permeability (Al Ati and Hotchkiss, 2002).

Table 1. Modified atmosphere packaging (MAP) and/or edible coating application, alone or in combination for fresh-cut fruits and vegetable.

Commodity	Atmosphere conditions	Coating material	Additives and plasticizer	Benefits	References
Apple	0-10 kPa O_2 + 0-30 kPa CO_2	—	AA, CA, $CaCl_2$	Reduce respiration rates Reduce ethylene production Control browning Preserve visual appearance	Gunes et al., 2001
	Passive	—	CaAsc	Control browning Preserve visual appearance Maintain antioxidants and vit C content	Aguayo et al., 2010
	—	Alginate, apple puree	N-acetylcysteine, $CaCl_2$, oregano, lemongrass, vanillin, Gly	Inhibit psychrotrophic bacteria, yeasts and molds growth Reduce CO_2 production Reduce ethylene production Control browning Maintain texture	Rojas-Graü et al., 2007a
	—	Alginate, gellan, sunflower oil, fatty acids	N-acetylcysteine, glutathione, $CaCl_2$, Gly	Maintain texture Reduce water loss	Rojas–Graü et al., 2007b
	—	Alginate, gellan, sunflower oil	N-acetylcysteine, $CaCl_2$, Gly	Inhibit browning Maintain texture	Rojas-Graü et al., 2008
	—	Alginate	N-acetylcysteine, glutathione, cinnamon, clove, lemongrass, citral, cynnamaldehyde, eugenol, calcium lactate, malic acid, Gly	Prevent microbiological growth Reduce CO_2 production Inhibit browning	Raybaudi-Massilia et al., 2008a
	—	Carragennan, WPC	CA, OA	Reduce CO_2 production Inhibit browning Reduce water loss	Lee et al., 2003

Commodity	Atmosphere conditions	Coating material	Additives and plasticizer	Benefits	References
Apple	—	Alginate, gellan, sunflower oil	N-acetylcysteine, AA, CA, Gly	Reduce weight loss	Tapia et al., 2007
	—	Chitosan	—	Inhibition PPO activity Inhibit browning	Qi et al., 2011
	—	WPC, BW	AA, Cys, 4-HR, Gly	Inhibit browning	Pérez-Gago et al., 2006
Artichoke	Passive	—	—	Reduce weight loss Maintain vit C and phenolic compounds content	Gil Izquierdo et al., 2002
	80 kPa O_2	—	Lemon juice	Inhibit browning	Gómez di Marco et al., 2011
	—	Alginate	CA, $CaCl_2$	Inhibit browning	Del Nobile et al., 2009
Banana	2-4 kPa O_2 + 5-10 kPa CO_2	—	AA, Cys, $CaCl_2$	Control browning	Vilas-Boas et al., 2006
	—	Carragennan	AA, Cys, $CaCl_2$, Gly, PEG	Inhibit browning Reduce water loss	Bico et al., 2009
Broccoli	—	Chitosan	—	Reduce total mesophilic and psychrotrophic bacteria growth Inhibition of total coliform growth Decrease of *E.coli* growth	Moreira et al., 2011
	—	Chitosan	Propolis, resveratrol, tea tree essential oil	Inhibit mesophilic and phsychrotrophic bateria growth Control *E. coli* and *L monocytogenes* growth	Alvarez et al., 2012
Cabbage	70 kPa O_2 + 15 kPa CO_2	—	—	Inhibit microbial growth Inhibit *E. coli* and *S. aureus* growth	Lee et al., 2011
Carrot	50 kPa O_2 +	Alginate	CA, $CaCl_2$	Inhibit browning	Amanatidou et al., 2000

	30 kPa CO_2			Inhibit *Enterobacteriacea* spp, *Pseudomonas*, lactic acid bacteria grrowth Reduce firmness loss	
	—	Chitosan, yam starch	Gly	Control microbial growth Prevent surface whitening Maintain sensory quality	Durango et al., 2006
	—	Chitosan, yam starch	Gly	Control microbial growth Prevent surface whitening Maintain sensory quality	Simões et al., 2009
	—	Chitosan	—	Reduce weight loss Preserve phenolic content	Pushkala et al., 2012
	60 kPa O_2 + 30 kPa CO_2	CC	Cynnamaldehyde, Gly	Inhibit *L. innocua* growth	Caillet et al., 2006
	60 kPa O_2 + 30 kPa CO_2	CC, WPI	Gly	Reduce microbial growth Retard whitening	Lafortune et al., 2005
Eggplant	2-5 kPa O_2 + 2-5 kPa CO_2	—	—	Control browning Preserve visual appearance	Catalano et al., 2007
Galega kale	1-2 kPa O_2 + 15-20 kPa CO_2	—	—	Control browning Preserve visual appearance	Fonseca et al., 2005
Kiwi	10 kPa O_2 + 10 kPa CO_2	Alginate	Hydro-alcoholic solution, grape seed extract	Control dehydration Reduce respiration rate Maintain sensory quality	Mastromatteo et al., 2011
Kohlrabi	Passive	—	—	Maintain sensory quality	Escalona et al. 2007
Lettuce	4.6-6.2 kPa O_2 + 2.1-4.3 kPa CO_2	—	—	Inhibit microbial growth Retard the *L. monocytogenes* growth	Carrasco et al., 2008
	70-80 kPa O_2 + 10-20 kPa CO_2	—	—	Reduce respiration rate Prevent anaerobic fermentation	Escalona et al., 2006
	80 kPa O_2	—	—	Reduce firmness loss Maintain vit C content	Day, 2001

Commodity	Atmosphere conditions	Coating material	Additives and plasticizer	Benefits	References
Litchi	—	Chitosan	—	Inhibition PPO activity Reduce weigh loss Maintain vit C content	Dong et al., 2003
Mango	Passive	—	AA, 4-HR, PS	Inhibit browning Maintain sensory quality	González-Aguilar et al., 2000
	60 kPa O_2	—	—	Inhibit *Rhodotorula mucilaginosa* yeast growth Reduce respiration rate	Poubol and Izumi, 2005
	10 kPa O_2 + 0 kPa CO_2	Mango film	—	Reduce off flavor	Sothornvit and Rodsamran., 2010
	—	Chitosan	—	Inhibition PPO activity	Djioua et al., 2010
	—	Alginate, sunflower oil	AA, CA, $CaCl_2$, Gly	Inhibit browning	Robles-Sánchez et al., 2013
Melon	2.5 kPa O_2 + 7 kPa CO_2	—	AA, $CaCl_2$	Control browning Preserve visual appearance	Oms-Oliu et al., 2007a
	2.5 kPa O_2 + 7 kPa CO_2	—	$CaCl_2$	Inhibit ethylene syntesis Decrease CO_2 emission	Oms-Oliu et al., 2008a
	70 kPa O_2	—	$CaCl_2$	Reduce psychrotrophic growth Inhibit *Rhodotorula mucilaginosa* yeast growth Reduce CO_2 production Prevent anaerobic fermentation Inhibit browning Reduce firmness loss	
	70 kPa O_2	—	$CaCl_2$	Reduce CO_2 production Prevent anaerobic fermentation Reduce firmness loss	Oms-Oliu et al., 2008d
	—	Alginate	Cinnamon, palmarosa,	Inhibit microbial growth	Raybaudi-Massilia et al.,

			lemongrass, eugenol, geraniol, citral, malic acid, calcium lactate, Gly	Inhibition native flora growth Reduce *S. enteritidis* growth	2008b
	—	Chitosan, MC	Vanillin, PEG	Control microbial growth	Sangsuwan et al., 2008
Mixed salad	95 kPa O_2	—	—	Inhibit lactic acid bacteria, *Enterobacteriaceae* spp growth	Allende et al., 2002
Mushroom	< 0.1 kPa O_2 + 15 kPa CO_2	—	—	Inhibition of microbial growth	Simon et al., 2005
	95 kPa O_2	—	—	Inhibit browning	Jacxsen et al., 2001
	—	Chitosan	—	Inhibit PPO activity Maintain texture	Eissa, 2007
Papaya	5 kPa O_2 + 10 CO_2		CA, $CaCl_2$	Reduce microbial growth Control browning Maintain texture	Whaghmare and Annapure, 2013
	—	Alginate, gellan, sunflower oil	N-acetylcysteine, AA, CA, Gly	Reduce water loss	Tapia et al., 2007
	—	Alginate, gellan, sunflower oil	AA, Gly	Reduce weight loss	Tapia et al., 2008
	—	Chitosan	—	Inhibit microbial growth	González-Aguilar et al.,2009
Pear	0.25-0.5 kPa O_2 + 5-10-20 kPa CO_2	—	AA, Cys, calcium lactate	Control browning	Gorny et al., 2002
	2.5 kPa O_2 + 7 kPa CO_2	—	N-acetylcysteine, glutathione	Inhibit microbial growth Inhibit ethylene syntesis Decrease CO_2 production Control browning Preserve visual appearance	Oms Oliu et al., 2008c
	2.5 kPa O_2 + 7 kPa CO_2	—	—	Inhibit ethylene syntesis Decrease CO_2 production Control browning Preserve visual appearance	Oms-Oliu et al., 2009b

Commodity	Atmosphere conditions	Coating material	Additives and plasticizer	Benefits	References
Pear	2.5 kPa O_2 + 7 kPa CO_2	—	AA, $CaCl_2$	Inhibit ethylene syntesis Decrease CO_2 emission Control browning Preserve visual appearance	Soliva-Fortuny et al., 2007
	70 kPa O_2	—	N-acetylcysteine, glutathione	Reduce psychrotrophic growth Inhibit *Rhodotorula mucilaginosa* yeast growth	Oms Oliu et al., 2008c
	80 kPa O_2	—	—	Increase production of phenolics compounds and anthocyanin	Li et al., 2012b
	—	Alginate, gellan, pectin	N-acetylcysteine, glutathione, $CaCl_2$, Gly	Reduce CO_2 production Inhibit browning	Oms-Oliu et al., 2008f
	—	MC, stearic acid	AA, PS, $CaCl_2$, Gly, PEG	Inhibit browning Reduce weigh loss	Olivas et al., 2003
Pepper	50-80 kPa O_2 + 15 kPa CO_2	—	—	Control mesophilic and psychrotrophic bacteria, *Enterobacteriaceae* spp. growth	Conesa et al., 2007
Pineapple	< 8 kPa O_2 + 10 kPa CO_2	—	—	Control browning Preserve visual appearance	Marrero and Kader, 2006
	—	Chitosan, MC	Vanillin, PEG	Control microbial growth	Sangsuwan et al., 2008
	—	Alginate, sunflower oil	Lemongrass, Gly	Reduce microbial growth	Azarakhsh et al., 2014
	—	Alginate, sunflower oil	AA, CA, $CaCl_2$, Gly	Reduce juice leakage	Montero-Caldéron et al., 2008
Pomelo	3 kPa O_2 + 5 kPa CO_2	—	—	Inhibit microbial growth	Li et al., 2012a
	75 kPa O_2	—	—	Inhibit *Rhodotorula mucilaginosa* yeast growth	

Potato	55-100 kPa O_2	—	AA, CA	Prevent anaerobic fermentation	Limbo and Piergiovanni, 2006
Red Chard	> 85 kPa O_2	—	—	Inhibit psychrotrophic growth	Tómas Calleja et al., 2011
Spinach	Passive	—	—	Reduction of aerobic mesophilic bacteria growth	Allende et al., 2004
	Passive	—	—	Reduce psychrotrophic bacteria growth, and *Pseudomonas* growth Control browning Preserve visual appearance	Tudela et al., 2013
	100 kPa O_2	—	—	Reduce mesophilic bacteria growth	Allende et al., 2004
Strawberry	80 kPa O_2 + 20 kPa CO_2	Chitosan	—	Inhibit mesophilic and psychrotropic bacteria, yeast growth	Campaniello et al., 2008
	5 kPa O_2 + 30 kPa CO_2	Chitosan	—	Inhibit mesophilic and psychrotropic bacteria, yeast growth Maintain color	
Tomato	2.5 kPa O_2 + 5 kPa CO_2	—	—	Maintain vit C Increase production of phenolics compounds, flavonoids and carotenoids	Odriozola-Serrano et al., 2009

AA = ascorbic acid, CA = citric acid, $CaCl_2$ = calcium chloride, CaAsc = calcium ascorbate, Gly = glycerol, WPC = whey protein concetrate, OA = oxalic acid, 4-HR = hexylresorcinol, PPO = polyphenol oxidase, BW = beeswax, Cys = cystein, PEG = polyethylene glycol, CC = calcium caseinate, WPI = whey protein isolate, PS = potassium sorbate, MC = methylcellulose.

Passive modified atmosphere for fresh-cut fruits and vegetables

There is scarce information in literature about the effect of passive MAP on fresh-cut products compared to active MAP. Gil-Izquierdo et al. (2002) and Giménez et al. (2003) studied the effect of packaging films of different permeability (polyvinylchloride (PVC), low density polyethylene (LDPE) and polypropylene (PP)) on the quality (weight losses, color, texture and sensory acceptability) and microbial growth (mesophiles, psychrotrophs, anaerobic micro-organisms, sporeformers, faecal coliforms, *Salmonella* and *Escherichia coli*) of minimally processed artichoke. In these works, the films with the highest permeability reached equilibrium rapidly and the highest atmosphere modification was detected with the PP film. The MA reached in the package affected the vitamin C and phenolic content (Gil-Izquierdo et al., 2002) and the microbial quality (Giménez et al., 2003) of minimally processed artichokes. Microbial counts in those batches where the equilibrium atmosphere was anaerobic were below the legal limit, whereas some batches with an acceptable sensory quality had microbial counts higher than those allowed by the legislation.

Escalona et al. (2007) studied the effect of passive MAP on quality attributes and shelf life of kohlrabi sticks during 14 days of storage at 0 ºC. Two commercial films were tested (oriented polypropylene (OPP) and amide-polyethylene (amide-PE)) and compared to microperforated OPP film as control. On day 14 only sticks stored in MAP conditions scored above the limit of marketability; meanwhile, a poor appearance and slight tissue dehydration in control sticks shorten their shelf life. The use of MAP helped maintaining firmness and induced a good fresh quality of sticks, especially when those were placed in amide-PE bags. Sticks packed in this polymeric material reached an equilibrium atmosphere of 7 kPa O_2 and 9 kPa CO_2 after 6 days of storage and under these conditions the product presented an acceptable sensorial quality for 14 days.

Allende et al. (2004) studied the effect of polyethylene film bags with two different O_2 permeabilities on plant metabolism, sensory quality and microbial growth of minimally processed baby spinach. Spinaches packed in the higher barrier film exhibited a more rapid accumulation of CO_2 than those in the permeable film, with a CO_2 level of 16.2 in the barrier film package, versus 6.1 kPa in the permeable film package at the

end of 12 days of storage at 5 ºC. Fresh-cut spinach packed with the barrier film exhibited a significant reduction in the growth of aerobic mesophilic bacteria compared to control conditions, but induced a strong off-odor and loss of tissue integrity due to the combination of extremely low O_2 and high CO_2 concentrations inside the packaging.

The application of antibrowning agents such as hexylresorcinol, potassium sorbate, and ascorbic acid in combination with passive MAP obtained with Cryovac LDX-5406 film reduced enzymatic browning and maintained the sensory quality of fresh-cut mangoes stored 14 days at 10 °C (González-Aguilar et al., 2000). The in-package atmosphere (O_2 and CO_2) changes of fresh-cut mango were affected by the different treatments. The different pattern in the atmospheric changes was associated with the variation in tissue deterioration. However, because the ripening process had already been induced before slicing, the in-package modified atmosphere created in most of the antioxidant combinations tested did not affect senescence and deterioration of tissue to any meaningful extent. In any of the antioxidant treatments the O_2 and CO_2 concentrations reached at the end of the 14 days of storage were below 5 kPa or higher than 10 kPa, respectively. On the other hand, Beltran et al. (2005) reported that the respiratory activity of the potato strips packaged in LDPE in response to different sanitizers (traditional and non-traditional sanitizers) was similar for all treatments. After 5 days of storage, the steady state within packages was reached with O_2 and CO_2 levels of about 0.3–1.4 and 6.3–8.3 kPa, respectively. The atmospheres created in the packages were in the range recommended for fresh-cut potatoes with O_2 and CO_2 levels 1–3 and 6–9 kPa, respectively (Gorny, 2003). In this work, the MAP was compared to vacuum packaging. The sanitizing treatments in vacuum packaging increased the lag phase of mesophilic bacteria up to 11 days and were the best packaging method to preserve the sensory quality of fresh-cut ‘Monalisa’ potatoes up to 14 days at 4 ºC.

Packaging fresh-cut cantaloupe in passive MAP by sealing with Cryovac LDX-5406 film maintained a commercial shelf life of 9 days at 5 ºC, whereas in perforated films shelf life was only 5 to 7 days, mainly due to tissue translucency and/or off-odor development (Bai et al., 2001). Similar results were found in fresh-cut honeydew melon harvested in two different seasons and packed in a similar packaging system. Passive

MAP extended the shelf life of honeydew melon by 1.3-3.7 days, depending on storage temperature (the higher the temperature the shorter the period), compared to those packaged in perforated films. Although quality attributes differed between cubes of fruit available in winter and summer, the shelf-life was similar for both winter and summer cubes stored under passive MAP (Bai et al., 2003).

Low oxygen atmosphere-active MAP for fresh-cut fruits and vegetables (LOA)

MAs requirements and recommendations for fresh-cut fruits and vegetables report low O_2 (1–5 kPa) and⁄or elevated CO_2 (5–10 kPa) levels to maintain quality and consequently extend shelf life of many produces (Gorny, 2003). In some cases, displacing the air within the package with a known mixture of gases close to the recommended atmospheres helps to extend the shelf life of the cut product compared to the use of passive MAP by reducing the metabolism, delaying browning reactions, and inhibiting microbial growth as soon as the product is cut. Thus for example, fresh-cut honeydew and cantaloupe packaged in active MAP with 5 kPa O_2 + 5 kPa CO_2 and 4 kPa O_2 + 10 kPa CO_2, respectively, had better color retention, reduced respiration rate and microbial population, and longer shelf-life than those in passive MAP (Bai et al., 2002; 2003). In fresh-cut pears and melon, packaging under 2.5 kPa O_2 + 7 kPa CO_2 inhibited ethylene synthesis (Soliva-Fortuny et al., 2007; Oms-Oliu et al., 2008a,c; 2009b). However, O_2 consumption and CO_2 production rates of just-packaged fresh-cut pears were stimulated more significantly under 2.5 kPa O_2 and 7 kPa CO_2 atmospheres than under initial 21 kPa O_2. This phenomenon was attributed to a possible effect of the vacuum created in packages before flushing the gas mixture, promoting changes in the pear tissue structure, as well as a dramatic modification of the internal atmosphere. On the contrary, packages of fresh-cut pears stored under 21 kPa O_2 did not suffer such stress due to vacuum. This effect was only observed at early storage and decreased with storage. This atmosphere also reduced enzymatic browning of fresh-cut pears and melons.

Effectiveness of LOA active MAP controlling enzymatic browning, preserving visual appearance and extending the shelf life has also been reported in other fresh-cut products such as eggplants (Catalano et al.,

2007), Galega kale (Fonseca et al., 2005), pineapple (Marrero and Kader, 2005), papaya (Waghmare and Annapure, 2013), among others. However, in products rich in antioxidant compounds such as artichokes, pears, banana, persimmons, mangos, papaya, etc. the only use of LOA may be insufficient to prevent browning and provide sufficient shelf life to the cut product. Therefore, most of the works in the literature report the combine effect of antioxidants such as citric and ascorbic acid, cystene, hexylresorcinol and MAP to control enzymatic browning and extend the shelf life of various fresh-cut produces as mango (González-Aguilar et al., 2000), pear (Gorny et al., 2002), and banana (Vilas-Boas et al., 2006). In a recent work with fresh-cut papaya, whereas LOA passive MAP alone (5 kPa O_2 + 10 kPa CO_2) or the antioxidant dip ($CaCl_2$ and citric acid) were not effective in preserving quality, the combination improved color, maintained firmness and reduced microbial count, extending the shelf life up to 25 days at 5 ºC (Waghmare and Annapure, 2013). In this work, the combination of antioxidants and passive MAP showed a significantly lower decrease in O_2 concentration in the package and correspondingly lower increase in CO_2, avoiding off-flavor development.

Excessive low O_2 concentrations surrounding the fresh-cut product obtained by either passive or active MAP may accelerate the decay and the accumulation of fermentative metabolites leading to off-flavor and odors. Tudela et al. (2013) described severe off-odor development caused by ammonia accumulation in baby spinach after 10 days at 7 ºC when exposed to low O_2 and high CO_2 (stabilizing near 1 kPa O_2 and 11 kPa CO_2). Nevertheless, senescence of baby spinach occurred more rapidly in samples stored under higher O_2 (stabilizing near 10 kPa O_2 + 9 kPa CO_2) concentrations. Soliva-Fortuny et al. (2007) observed that storage of fresh-cut pears under low O_2 and high CO_2 concentrations was detrimental to flavor perception and even harmful to the fruit tissue after 3 weeks at 5 ºC. Increase of tissue softening and fermentative metabolites related to excessively low O_2 and high CO_2, and ethanol accumulation in packages has also been observed in fresh-cut pears and melon (Oms-Oliu et al., 2008c,d). However, Gunes et al. (2001) reported that elevated CO_2 might provide a mechanism to reduce accumulation of fermentation products in fresh-cut apples stored under low-O_2 atmospheres, resulting

in about a 50% reduction in acetaldehyde, ethanol and ethyl acetate concentrations.

The effect of low O_2 and high CO_2 concentrations on the growth of Gram negative bacteria, moulds, and aerobic microorganisms, as *Pseudomonas* is well known. For example, packaging under active and passive LOA significantly inhibited the growth of spoilage microorganism in fresh-cut pears, melon, honey pomelo, shredded lettuce, and mushroom slices, among others (Simon et al., 2005; Carrasco et al., 2008; Oms-Oliu, 2008c; Oliveira et al., 2010; Li et al., 2012a) and reduced the development of aerobic psycrhrotrophic bacteria and *Pseudomonas* in leaf spinaches (Tudela et al., 2013). However, restrictive O_2 atmospheres have also been shown to stimulate the development of yeasts, Gram-positive bacteria as *Lactobacillus* or facultative anaerobes pathogens as *E. coli*, *Listeria monocytogenes*, and *Clostridium botulinum* (Farber et al., 2003). The extend of the MAP activity depends on the type and concentration of the microorganism, as well as the O_2 and CO_2 concentrations, pH and ripeness stage of the commodity at processing, and the storage temperature (Al Ati and Hotchkiss, 2002). Oms-Oliu et al. (2009b) observed that active MAP (2.5 kPa O_2 + 7 kPa CO_2) inhibited bacterial growth, yeast and mould proliferation in mature-green pears, but did not control microbial growth in partially ripe and ripe pears.

Postharvest losses in nutritional quality, particularly vitamin C content, can be substantial and might be enhanced by physical damage, extended storage duration, high temperatures, low relative humidity, and chilling injury of chilling-sensitive commodities (Lee and Kader, 2000). Although the influences of processing and storage on the nutritional content of fresh-cut fruits seemed to depend on the commodity, storage under MAP might reduce the rate of nutritional losses (Gil et al., 2006). In general, the range of O_2 availability is the main factor affecting antioxidant properties of fresh-cut products, whereas high CO_2 concentrations appear to have a significant effect on ascorbic acid, phenolic compounds and carotenoids. Fonseca et al. (2005) reported higher ascorbic acid degradation of shredded Galega kale in air than under 1–3 kPa O_2 atmospheres. Packaging under 2.5 kPa of O_2 and 5 kPa of CO_2 reduced the formation of carotenoids and also maintained vitamin C in fresh-cut tomatoes (Odriozola-Serrano et al., 2009). Aguayo et al. (2010) reported that passive MAP significantly reduced the loss of

antioxidant activity and ascorbic acid concentrations of fresh-cut apples during storage at 4 ºC, specially when apple slices were treated with calcium ascorbate. Others authors have reported that high concentrations of CO_2 (more than 5 kPa) had a negative effect on ascorbic acid maintenance of apple slices (Cocci et al., 2006) and fresh-cut pear (Oms-Oliu et al., 2007b), especially when hypoxic conditions were reached in the packages. According to Tudela et al. (2002), high CO_2 levels increased ascorbic acid loss of fresh-cut potatoes by accelerating ascorbate peroxidase-catalysed oxidation processes. In agreement with the latter result, an important increase in peroxidase activity was shown during storage of fresh-cut melon packed under 2.5 kPa O_2 and 7 kPa CO_2 MA conditions (Oms-Oliu et al., 2008b).

High oxygen atmosphere for fresh-cut fruits and vegetables (HOA)

The application of O_2 concentrations higher than 70 kPa has been found to be particularly effective at controlling aerobic and anaerobic microorganisms, preventing anaerobic fermentation and controlling enzymatic browning (Kader and Ben-Yehoshua, 2000). However, the effect greatly depends on the commodity, cultivar, physiological stage, and storage conditions, as well as the type and concentration of the antimicrobial compound. The effect of high O_2 concentrations reducing microbial growth has been related to the accumulation of reactive oxygen species that damage vital cell components, affecting cellular antioxidant protection systems of cell metabolism (Kader and Ben-Yehoshua, 2000).

In vitro tests proved that the exposure to high O_2 levels (>70 kPa) causes a delay in aerobic and anaerobic microbial growth and foodborne pathogenic microorganisms associated with refrigerated fresh-cut vegetables such as *S. enteritidis*, *S. typhymurium*, *L. monocytogenes*, and *E. coli* (Amanatidou et al., 1999; Jacxsens et al., 2001). In agreement with this findings, O_2-enriched MAP reduced the growth of psychrotrophic microorganisms in fresh-cut Red Chard stored up to 8 days at 5 °C with 100 kPa O_2 (Tómas-Calleja et al., 2011) and fresh-cut pear and melon packed under 70 kPa O_2 during storage at 4 ºC (Oms-Oliu et al., 2008a,c). Allende et al. (2004) also reported a reduced growth of mesophilic aerobes in fresh-cut baby spinach packed in 80-100 kPa O_2 for 12 days at 5 ºC. However, reports on the effect of high O_2 concentrations on the growth of the aerobic microbiota on fresh-cut

mixed salads were inconclusive (Allende et al., 2002). Lactic acid bacteria and *Enterobacteriaceae* spp. appeared to be inhibited under high O_2 concentrations but, on the other hand, the growth of yeasts and *Aeromonas caviae* was stimulated under high O_2 levels, whereas psychrotrophic bacteria and *L. monocytogenes* were not affected. An inhibitory effect on some spoilage microorganisms such as *Rhodotorula mucilaginosa* yeast has been observed in fresh-cut pears, 'Piel de Sapo' melons, mango cubes and honey pomelo when exposed to high O_2 concentrations (Poubol and Izumi, 2005; Oms-Oliu et al., 2008a; Li et al., 2012a). Whereas, *Candida parapsilosis* survived on inoculated cut pears stored under 70 kPa O_2 (Oms-Oliu et al., 2008c). In strawberries, 80–100 kPa O_2 atmospheres inhibited the growth of *Botrytis cinerea* (Wszelaki and Mitcham, 2000) and an initial atmosphere of 70 kPa O_2 retarded the growth of molds and yeasts on strawberries and raspberries (Van der Steen et al., 2002).

The inhibitory effect on bacterial growth has been noticed to be greater when the high O_2 is combined with high CO_2 concentrations (10 to 20 kPa) rather than when the individual gas is used alone (Amanatidou et al., 1999). Lee et al. (2011) suggested that high O_2 combined with CO_2 could give a bacteriostatic and bactericidal effect through suppression of aerobes by high CO_2 and anaerobes by high O_2. Particularly, the antibacterial effect was evidently shown on *E. coli* and *S. aureus* among other inoculated strains (*P. fluorescence, S. Typhimurioum, L. monocytogenes*) in fresh-cut cabbage. Amanatidou et al. (2000) also reported that packaging carrots slices under 50 kPa O_2 and 30 kPa CO_2 significantly inhibited the growth of *Enterobacteriacea* spp., *Pseudomonas* and lactic acid bacteria. In fresh-cut bell peppers, MAP with 50 or 80 kPa O_2 and 15 kPa CO_2 controlled the growth of mesophilic, psycrhrotrophic bacteria and *Enterobacteriaceae* spp. up to 8-9 days at 5ºC; however, high O_2 alone did not prevent microbial growth (Conesa et al., 2007). Zhang et al. (2013) evaluated on honeydew melon agar at 7 °C the effect of modified atmospheres with high O_2 and high CO_2 concentrations on the growth and volatile organic metabolite production of *Candida sake*, *Leuconostoc mesenteroides* and *Leuconostoc gelidum*, which had all been previously isolated from spoiled commercial fresh-cut honeydew melon. These atmospheres greatly retarded the growth, CO_2 and volatile metabolite production of *C.*

sake, especially MAs consisting of 50 kPa O_2 + 50 CO_2 and 70 kPa O_2 + 30 kPa CO_2, and had minor effect on the rest of microorganisms. Overall, fresh-cut honeydew melon cubes packaged in MA of 50 kPa O_2 + 50 kPa CO_2 had appreciably lower populations of yeasts and lactic acid bacteria, and lower quantities of volatile organic compounds after 5 days of storage at 7 ºC.

Beside the effect on microbial growth control, the application of superatmospheric O_2 may stimulate, have no effect, or lower the respiration rate of fresh-cut produces, depending on the commodity, cultivar, physiological stage, storage conditions, etc. (Kader and Ben-Yehoshua, 2000). Pouboul and Izumi (2005) studied the physiological behavior of two fresh-cut mango cultivars held in high O_2 atmospheres (60 kPa) for 42 h at 5 ºC. The authors observed that the 60 kPa O_2 atmosphere reduced the respiration rate of 'Carabao' mango cubes slightly during storage at 5 °C. Nevertheless, the same high O_2 concentrations did not affect the respiration rate of 'Nam Dokmai' mango cubes, which affected the shelf life and overall quality of this mango cultivar. Escalona et al. (2006) observed that the application of 70-80 kPa O_2 concentrations combined with 10-20 kPa CO_2 successfully reduced respiration rate and prevented anaerobic fermentation of fresh-cut butter lettuce. In fresh-cut melon, active MAP with initial low O_2 levels reduced in-package ethylene concentration, whereas superatmospheric O_2 levels (70 kPa) avoided anaerobic metabolism by reducing CO_2 production rate and preventing ethanol production during 3 weeks of storage (Oms-Oliu et al., 2008a,d). Similarly, Limbo and Piergiovanni (2006) observed that high oxygen partial pressures (55 and 100 kPa) had an inhibitory effect on the anaerobic volatiles production in potato slices. However, other works have shown contradictory results regarding the effect of high O_2 levels on volatile production. In fresh-cut pear, superatmospheric O_2 did not prevent the production of acetaldehyde and ethanol during storage due to a stress response related at the same time with the highly oxidative environment and the accumulation of CO_2 within the package (Oms-Oliu et al., 2008c).

The application of high O_2 atmospheres has also been suggested as an alternative to low O_2 and moderate CO_2 concentrations to inhibit enzymatic browning. It has been hypothesized that high O_2 levels may cause substrate inhibition of the enzyme polyphenol oxidase (PPO), or

alternatively, that high levels of colorless quinones formed in the oxidation reaction may cause a feedback inhibition of the PPO (Day, 2001). In sliced mushrooms and fresh-cut melon, high oxygen atmospheres (70-95 kPa O_2) were particularly effective at inhibiting enzymatic browning as compared with low-oxygen atmosphere MAP (Jacxsens et al., 2001; Oms-Oliu et al., 2008a). Allende et al. (2004) reported lower tissue damage of spinach leaves in high O_2 packages (80 and 100 kPa) stored during 12 days at 5 ºC. In some commodities, high O_2 concentrations alone cannot effectively prevent browning of fresh-cut produce. Limbo and Piergiovanni (2006) showed the positive effect of high-oxygen partial pressures combined with dipping in acid solutions to control enzymatic browning of fresh-cut potato for 10 days at 5 ºC. Gómez di Marco et al. (2011) reported that the best combination to reduce artichoke heads browning was the application of 80 kPa O_2 and lemon juice as antioxidant. However, Poubol and Izumi (2005) reported that the application of superatmospheric O_2 concentrations (60 kPa) to fresh-cut mango showed a similar or higher degree of browning than the use of ambient air conditions at 5 ºC. Similarly, Gorny et al. (2002) reported that high O_2 (40, 60, 80 kPa) did not effectively prevent surface browning of fresh-cut pears.

The use of high oxygen concentrations has been shown to reduce firmness loss in several fresh-cut products such as sliced carrot (Amanatidou et al., 2000), shredded iceberg lettuce (Day et al., 2001), and fresh-cut melon (Oms-Oliu et al., 2008a,d). According to Amanatidou et al. (2000), the effect of high O_2 atmospheres on firmness may be related to an inhibition on the proliferation of pectolytic *Pseudomonas*. Whereas, Deng et al. (2005) suggested that firmness retention in high O_2 levels could be related with the lower activity of cell wall hydrolytic enzymes as observed in table grapes.

Few works has been published on the effect of high O_2 concentrations on the nutritional content of fresh-cut fruits and vegetables. Day (2001) described that high O_2 atmospheres had a beneficial effect on ascorbic acid retention on prepared lettuce. However, vitamin C content of fresh-cut pears stored under 70 kPa O_2 was rapidly lost in comparison with those stored in low O_2 atmosphere (Oms-Oliu et al., 2007b). Similarly, ascorbic acid content and antioxidant capacity underwent a significant depletion in ready-to-eat honey pomelo slices packed under high O_2

atmosphere in comparison with low O_2 active and passive MAP (Li et al., 2012a). Li et al. (2012b) also reported the highest decrease in vitamin C in fresh-cut pears stored in 80 kPa O_2 packaging with more than 50% loss in vitamin C content after 12 days of storage at 4 ºC. Whereas, phenolics and anthocyanin contents of the samples packed in 80 kPa O_2 were 2.5 and 12 times higher, respectively, than those in the passive package, and 3 and 2 times higher than those in low O_2 package. Higher production of phenolic compounds was also observed by Odriozola-Serrano et al. (2009) in tomato slices packed under high O_2 and passive atmosphere. According to these authors, the increase on phenolic compounds in fresh-cut products under both passive MAP and high O_2 concentrations could be directly associated with a physiological response to stress conditions. The fresh-cut tomatoes stored under 80 kPa of O_2 atmospheres also scored higher on flavonols, lycopene, β-carotene, chlorogenic acid, and total antioxidant capacity than those packaged under lower O_2 concentrations. These results indicate that the effect of high O_2 on the nutritional content of fresh-cut products may vary depending on the commodity, O_2 concentration, storage time and temperature.

Noble gases for fresh-cut fruits and vegetables

The use of noble gases such as Helium (He), Argon (Ar), and Xenon (Xe) to replace N_2 as the balancing gas in MAP is considered one of the major advances to preserve and extend the shelf life of fresh and minimally processed fruits and vegetables. He, Ar and Xe have been successfully applied in MAP and controlled atmosphere storage to reduce microbial growth and maintain the quality of fresh products (Jamie and Saltveit, 2002; Meng et al., 2012; Wu et al., 2012 a,b). The beneficial effect of noble gases Ar and He have been related to their solubility and diffusivity characteristics (Day 2001; Jamie and Salveit 2012). The similar atomic size to molecular O_2 and higher solubility in water than N_2 and O_2 make probably inner gases more effective at displacing O_2 from cellular sites and enzymatic O_2 receptors with the consequence that oxidative deterioration reactions are likely to be inhibited (Day et al., 2001). Furthermore, replacing the N_2 with He/Ar may modify the diffusion of O_2, CO_2, and C_2H_4 in fresh commodities. Burg and Burg (1965) showed that replacing N_2 with He enhanced gas diffusion and reduced the concentration gradient of O_2 between the inside and outside

of a commodity. These changes allow fresh commodities that experience internal low O_2 deficiencies at lower O_2 storage to tolerate the low O_2 environment better than they could tolerate in the presence of N_2 atmospheres (Jamie and Saltveit, 2002).

Various studies have investigated the effect of the noble gases on the quality of fresh-cut produces. Ar used as major component in MAP has been reported to reduce microbial growth and improve the quality retention of fresh products like broccoli, lettuce and sliced mushroom (Day 2001; Jamie and Salveit 2002). Jamie and Salveit (2002) reported that the content of *o*-quinones in fresh-cut crisphead lettuce was higher in tissue stored in 2 kPa controlled atmosphere made up in Ar than in that in He or N_2, whereas no differences were observed in green leaf lettuces. In both cultivars, the *o*-quinone content was reduced by controlled atmosphere storage compared to storage in air. The inhibition of the activity of apple and mushroom PPO was also observed in low O_2 MAP enriched with argon (O'bernie et al., 2002). Rocculli et al. (2005) also reported that the use of 90 kPa Ar on fresh-cut kiwifruit was significantly effective at maintaining firmness values and slowing down respiration, but not at controlling color browning. Better results were obtained by packaging of kiwifruits under 90 kPa N_2O, limiting the firmness loss and maintaining color values during 12 days at 4 ºC.

Tomas-Callejas et al. (2011) investigated the antimicrobial and quality effect of 100 kPa O2-, He-, N_2-, or N_2O-enriched active MAP compared to a passive MAP on fresh-cut red chard baby leaves at 5 ºC during 8 days of storage. No differences in microbial growth were observed between He-, N_2-, and N_2O-enriched MAPs and the passive MAP. The active MAP helped to retain better the vitamin C content compared with the passive MAP control; whereas, total phenolics content drastically increase during storage in samples under O_2-, He-, and N_2-enriched MA packages. Among the treatments tested in active MAP, He preserved the total chlorophyll content throughout the shelf life. In fresh broccoli, however, atmospheres containing 90 kPa Ar and 2 kPa O_2 did not delay the loss of chlorophyll (Jamie and Salveit; 2002).

Under appropriate temperature and pressure conditions inner gases can form ice-like crystals called clathrate hydrates, in which molecules are trapped within cage-like structure of water molecules, lowering water activity in fresh and fresh-cut produce, thereby reducing the leaching of

organic material from fresh-cuts and movement of microbes into deeper tissues in comparison to other pretreatments (Caleb et al., 2013). Meng et al. (2012) reported that the application of pressurized Ar (2, 4 and 6 MPa) on fresh-cut green pepper packed with 5 kPa O_2 and 8 kPa CO_2 were able to maintain the cell integrity of the produce by inhibiting the production of malondialdehyde, as well as the activities of catalase and peroxidase. The treatments were also reported to reduce the proliferation of spoilage microorganisms such as coliforms, yeast and moulds. Wu et al. (2012a) studied the effect of high pressure Ar (150 MPa) on preserving fresh-cut apples at 4 ºC. The pressurized Ar reduced the respiration rate and ethylene production of fresh-cut apples. However, it caused some negative effects on color and firmness, which were overwhelmed by combining the pressurized Ar and antioxidant treatments with calcium chloride, citric and ascorbic acid. This combination reduced color change and firmness loss and maintained the sensory quality of fresh-cut apples for 12 days at 4 ºC. Similarly, fresh-cut pineapple treated with pressurized Ar (1.8 Mpa) effectively maintained the quality and delayed the microbial growth of the product, reaching 6 days of shelf life at 4 ºC (Wu et al., 2012b).

The use of noble gas mixtures has also been studied in order to preserve foods. Under such treatments, the metabolism of fruits and vegetables is restrained, which might result in extended shelf life (Zhan et al., 2005; Zhang et al., 2008). Raymond et al. (2013) studied the changes in some physical and biochemical properties of fresh-cut green peppers under a compressed mixed noble gas (Ar and Krypton) compared to compressed Ar treatment alone at 5 °C for 15 days. Both treatments allowed maintenance of better quality compared to the untreated cut peppers. The noble gases reduced the respiration rate and vitamin C loss of fresh-cut peppers compared to the untreated samples, but did not inhibit the PPO activity. Mixed Argon-Krypton samples presented lower mass loss and cell membrane permeability than the Ar treatment alone.

Wu et al. (2013) studied the effects of high pressure argon and xenon (HP Ar + Xe) mixed treatment on wound healing and microbial growth in fresh-cut apples and pineapples inoculated with *E. coli* and *Saccharomyces cerevisiae* and stored at 4 ºC. Samples under HP Ar + Xe mixed treatment exhibited a positive wound healing response due

probably to the increase in H_2O_2 production and the accumulation of phenolics and lignin during storage. The enhanced wound healing ability provided by the HP Ar + Xe mixed treatment was also found to contribute at the inhibition of the growth of *E. coli* or *S. cerevisiae* in tested apple and pineapple samples.

Although noble gases applied as mixed or alone treatment have been proven to ensure safety, maintain the overall quality and extend the shelf life of several fresh-cut products, the high cost of some noble gases may limit their application.

Edible coatings for fresh-cut fruits and vegetables

Traditionally, edible coatings have been used as a tool to reduce the deleterious effects of processing on fruit and vegetable tissues. An edible coating is a thin layer of edible material formed on the surface of a fruit and vegetable that provides a semipermeable barrier to gases, water vapor, and volatile compounds between the product and the surrounding atmosphere (González-Aguilar et al., 2010; Oms-Oliu et al., 2009a). Therefore, edible coatings can contribute to extend the shelf life of fresh-cut fruits and vegetables by reducing respiration rate as they create a MA within the produce, enzymatic browning, and water loss (Pérez-Gago et al., 2005).

The effectiveness of edible coatings to preserve the quality of fresh-cut products may vary depending on the composition and thickness of the coating, the type of commodity, variety and maturity, the degree of surface coverage, and the storage conditions (González-Aguilar et al., 2010).

Compounds most commonly used to form edible coatings include polysaccharides as chitosan, alginate, cellulose, carrageenan, pectin, starch; proteins as whey, casein, soy protein; and lipids as carnauba, beeswax and fatty acids (Dea et al., 2012).

Several works have described the beneficial effect of polysaccharide and protein-based edible coatings on reducing the respiration rate of fresh-cut produces, which has been attributed to their good oxygen barrier (Olivas and Barbosa-Cánovas, 2005). Wong et al. (1994) observed a lower CO_2 production rate in apple pieces coated with pectin, alginate or microcrystalline cellulose-lipid bilayer edible coatings. Lower CO_2 production has also been observed in fresh-cut apples, melons, and

pears coated with an alginate-based edible (Rojas-Graü et al., 2007a; Oms-Oliu et al., 2008e,f; Raybaudi-Massilia et al., 2008a,b). Lee et al. (2003) showed that the respiration rate of apple slices could be decreased by 20% by applying a whey protein-based coating.

The hydrophilic nature of proteins and polysaccharides usually requires the addition of lipids to improve the moisture barrier of the edible coatings in the fresh-cut products. Lipids such as sunflower oil added to alginate, gellan or pectin-based coatings and stearic acid incorporated to methyl cellulose-based edible coatings have been proven to significantly reduce weight loss of fresh-cut apple, papaya and pear (Tapia et al., 2007, 2008; Olivas et al., 2003). However, some works have also reported that edible coating based on gellan and carragennan with no lipid incorporated reduced water loss of fresh-cut apples, papaya and banana (Lee et al., 2003; Tapia et al., 2007; Bico et al., 2009).

The use of edible coatings with antimicrobial properties has been considered a potential tool to improve the safety of fresh-cut produces. Chitosan edible coatings have been widely used in fresh-cut fruits and vegetables for their antimicrobial properties. González-Aguilar et al. (2009) reported that dipping fresh-cut papaya in medium molecular weight edible chitosan resulted in antimicrobial activity suppressing mesophilic plate count, and the growth of molds and yeast. The antimicrobial effects of chitosan on the native microflora and on the survival of *E. coli* O157:H7 inoculated in fresh-cut broccoli were evaluated by Moreira et al. (2011). The authors reported a significant reduction in total mesophilic and psychrotrophic bacteria counts. Moreover, chitosan coating inhibited the growth of total coliform and significantly decreased *E. coli* counts throughout the storage.

In fresh-cut products such as litchi, mushrooms, mango and apples, chitosan coatings have been effective to inhibit the increase of PPO activity and avoid the color change during storage (Dong et al., 2004; Eissa et al., 2007; Djioua et al., 2010; Qi et al., 2011). Moreover, application of chitosan was beneficial in retarding weight loss and maintaining ascorbic acid content in fresh-cut litchi (Dong et al., 2004), delaying texture changes in fresh-cut mushrooms (Eissa et al., 2007), and reducing weight loss and preserving total phenolic content in carrots shreds (Pushkala et al., 2012).

Combination of chitosan with other polysaccharides has also shown to improve its functional properties. For example, an edible yam starch-chitosan coating was successful at controlling the microbial growth, preventing surface whitening and maintaining the sensory quality of minimally processed carrots (Durango et al., 2006; Simões et al., 2009). Similarly, a chitosan-methyl cellulose film applied as food wrapper inhibited the growth of inoculated *E. coli* and *S. cerevisiae* on fresh-cut melon and pineapple as compared to un-wrapped fruit, or fruit wrapped in a commercial stretch film (Sangsuwan et al., 2008).

The functional properties of edible coatings can be enhanced by the incorporation of active ingredients such as antioxidant, flavors, texture enhancer, nutrients, and antimicrobials to reduce enzymatic browning, texture loss, the risk of pathogen growth on food surfaces and/or to improve nutritional and sensory quality (Olivas and Barbosa-Cánovas, 2005). Incorporating antimicrobial compounds into edible films and coatings provides a novel way to enhance the safety and extend the shelf life of ready-to eat products (Cagri et al., 2004). Several compounds have been investigated for incorporation into edible coatings, including organic acids (acetic, benzoic, lactic, propionic, sorbic), fatty acid esters (glyceryl monolaurate), polypeptides (lysozyme, peroxidase, lactoferrin, nisin) and plant essential oil (cinnamon, oregano, lemongrass) (Franssen et al., 2004). Raybaudi-Massilia et al. (2008a) reported that lemongrass and cinnamon (0.7%), citral (0.5%) or cinnamaldehyde (0.5%) essential oils added to an alginate-based coating (alginate, malic acid, *N*-acetyl-L-cysteine, glutathione and calcium lactate) effectively prevented microbiological growth on fresh-cut apples. Combination of lemongrass at 0.3 and 0.5% with an alginate-based edible coating (sodium alginate and sunflower oil) reduced significantly yeast, molds and total plate counts in fresh-cut pineapple (Azarakhsh et al., 2014). In other study, the addition of palmarosa oil (0.3%) to a similar alginate-based coating inhibited the native flora and reduced *S. enteritidis* population while maintaining the fresh-cut melon quality parameters (Raybaudi-Massilia et al., 2008b).

Alvarez et al. (2012) studied the effect of chitosan alone or enriched with bioactive compounds and essential oil (propolis, resveratrol and tea tree essential oil) in the microbiological load of fresh-cut broccoli. Chitosan alone or combined with essential oils or bioactive compounds

significantly inhibited the growth of mesophilic and psychrotrophic bacteria, and also controlled *E. coli* and *L. monocytogenes* survival, avoiding deleterious effects on the sensory attributes of fresh-cut broccoli.

Calcium chloride is generally used as firming agent. For example, adding calcium as a gelling agent to alginate, gellan and pectin-based edible coatings showed to be effective at maintaining firmness in several fresh-cut products (Dea et al., 2012). Montero-Calderón et al. (2008) reported that the use of an alginate coating significantly improved shelf life of cut pineapple, by reducing juice leakage. In alginate or gellan-based coatings, the use of $CaCl_2$ showed a beneficial effect on firmness retention of apple wedges during the storage (Rojas-Graü et al., 2007b; 2008).

Enzymatic browning of fresh-cut produces can also be reduced by the use of antioxidant compounds incorporated into the edible coating. Fresh-cut pears were preserved from surface browning by coating with a methyl cellulose coating containing ascorbic acid (Olivas et al., 2003) and alginate and gellan coatings containing N-acetylcysteine and glutathione (Oms-Oliu et al., 2008f).

Browning of fresh-cut apples has been controlled or reduced by dipping in carrageenan and whey protein edible coatings containing ascorbic, citric and oxalic acid (Lee et al., 2003); whey protein-beeswax edible coatings containing ascorbic acid and cysteine (Pérez-Gago et al., 2006); alginate and gellan coatings containing N-acetylcysteine (Rojas-Graü et al., 2008; Raybaudi-Massilia et al., 2008a); chitosan edible coating containing ascorbic acid (Qi et al., 2011). Browning of banana slices was reduced by a carrageenan edible coating containing ascorbic acid and cysteine (Bico et al., 2009); in carrot slices, artichoke heads and fresh-cut mangoes alginate coatings with ascorbic and citric acid were also effective to control browning (Amanatidou et al., 2000; Del Nobile et al., 2009; Robles-Sánchez et al., 2013).

A new generation of edible coatings is under development, which aims to incorporate and/or control release of active compounds using nanotechnological solutions such as nanoparticles, nanoencapsulation, and multilayered systems (Dhall, 2013). By using nanoscience, new forms of nanocomposites dispersed with nanoparticles, nanofibers, or nanoemulsions can be developed to improve the moisture barrier and

mechanical properties of the edible films and coatings (Avena-Bustillos and McHugh, 2012). The most commonly used metal and metal oxide nanomaterials used in edible films and coatings are silver (Ag) and zinc oxide (ZnO), which are known by their antimicrobial properties (Kim et al., 2007; Lok et al., 2007; Jin et al., 2009).

Although nanoparticles have been successfully incorporated to edible coating such as chitosan, alginate, carrageenan and gellan to improve mechanical and barrier properties, few works can been found about the use of edible coatings containing nanoparticles for fresh-cut fruits and vegetables (Chaudhry and Castle, 2011; Chen and Yada, 2011; Costa et al., 2012). Costa et al. (2012) studied the effects of an active alginate based-coating loaded with silver-montmorillonite (Ag-MMT) on the shelf life and microbiological quality of fresh-cut carrots. *Enterobacteriaceae* spp. and mesophilic bacteria in the active-coat sample were found below 10^4 and 5×10^7 CFU/g, respectively, that were set as the threshold for these microbial groups. Reduced cell loads by one or two log cycles of psychrotrophic bacteria, *Pseudomonas* spp. and yeasts were also observed in fresh-cut carrots packaged with the active coating. This coating also resulted effective at controlling dehydration and extended the shelf life of the carrots packed in OPP films to more than 45 days.

Meng et al. (2014) evaluated the efficacy of ultrasound treatment and nano-zinc oxide (ZnO) incorporated to a chitosan-based edible coating in preserving the quality of fresh-cut kiwifruit stored at 4 ºC. At the end of storage, the combined application of ultrasound and nano-ZnO coating slowed down ethylene and carbon dioxide production, reduced the water loss and softening, delayed the senescence and significantly extend the storage life of fresh-cut kiwifruit.

Luo et al. (2013) studied the effect of chitosan coating, alone or in combination with nano-chitosan (40-50 nm) on browning and lignification of fresh-cut *Zizania latifolia* stored at 1 ºC for 12 days. The results showed that nano-chitosan coating was more effective than chitosan to retard browning and lignification of fresh-cut *Z. latifolia* by inhibiting browning and lignification-related enzyme activity.

Micro- and nano-encapsulation consists in the incorporation of active compounds into edible coatings with the aim to control their release under specific conditions (e.g., changes of pH, temperature, irradiation,

osmotic shock). This technology protects the encapsulated active compounds from moisture, heat or other extreme conditions and enhances their stability and viability (Dea et al., 2012). Among the different biopolymers, alginate is the most widely used material for encapsulation. Enzymes, probiotic, prebiotic, essential and marine oils are among the most active compounds to be encapsulated (Dhall, 2013).

In some cases, the dipping of fresh-cut fruits and vegetables into an edible coating is limited by the difficult adhesion to the hydrophilic surface of the cut product. The multilayer coating technique has been used as a good alternative to overcome these problems. The preparation of multilayer structures consists of consecutive dipping of the cut product into two or more coating solutions containing oppositely charged polyelectrolytes. Between each dipping step, it may be necessary to have a drying step to remove the excess of coating solution attached to the cut surface (McClements et al., 2009). Coatings manufactured with this technique have been applied in fresh-cut produces as papaya (Brasil et al., 2012), pear (Medeiros et al., 2012), pineapple (Mantilla, 2013), and watermelon (Sipahi et al., 2013). Brasil et al. (2013) studied the effect of a microencapsulated beta-cyclodextrin and trans-cinnamaldehyde complex incorporated into a multilayered edible coating made of chitosan and pectin on the microbiological and physicochemical quality of fresh-cut papaya. The multilayered edible coating was very effective at inhibiting aerobic and psychrotrophic bacteria, yeast and mold growth. Application of the edible coating helped to extend the shelf life of fresh-cut papaya up to 15 days at 4 ºC and maintained firmness, color, vitamin c and b-carotene content. In addition, encapsulated trans-cinnamaldehyde had no negative impact on flavor of coated papaya, being more accepted by the panelists than control samples. Similarly, the application of a multilayered edible coating composed of 2% trans-cinnamaldehyde, 2% chitosan and 1% pectin helped extend the shelf life of fresh-cut cantaloupe up to 9 days at 4 ºC (Martiñon et al., 2014).

Similar systems have been prepared with sodium alginate for fresh-cut watermelon (Sipahi et al., 2013) and pineapple (Mantilla et al., 2013). The multiplayer edible coatings were prepared by layer by layer deposition of sodium alginate coating with pectin and calcium ion (calcium chloride or calcium lactate). The order was chosen based on the polyetrolyte interaction among opposite charges to obtain a stable and

uniform coatings and consisted of five steps (Calcium-antimicrobial coating -Calcium-Pectin-Calcium). The multilayered edible coating with microencapsulated beta-cyclodextrin and trans-cinnamaldehyde extended the shelf-life of fresh-cut pineapple and watermelon stored at 4 ºC for 15 days (Mantilla et al., 2013; Sipahi et al., 2013). Microbiological analyses demonstrated the effect of the coating reducing growth of psychrotrophic bacteria, yeasts and molds. The alginate and pectin-based multilayered antimicrobial coating also helped to maintain color, pH, ºBrix and firmness values without affecting odor and flavor attributes. Medeiros et al. (2013) also optimized nanolayered coatings based on carrageenan and lysozyme. The coating was successfully applied to fresh-cut 'Rocha' pear and controlled weight loss and maintained high and low values of the total soluble solids and the tritable acidity, respectively, after 7 days of storage at 4 ºC.

Combination MAP and edible coating for fresh-cut fruits and vegetables

The use of MAP or edible coatings in association with low temperature maintenance represent two of the main approaches to preserve the quality of minimally processed fruits and vegetables. However, the high perishability of these products challenges in many cases their marketability by not achieving sufficient shelf life to survive the distribution system. An approach to extend the shelf life of fresh-cut fruits and vegetables is the combination of these technologies (i.e. edible coatings and MAP).

Relatively few works can be found in the literature that study the synergic effect of MAP and edible coatings on fresh-cut fruits and vegetables shelf life. Mastromatteo et al. (2011) studied the effect of a sodium alginate edible coating enriched with active compounds (a hydro-alcoholic solution and grape seed extract) and two MAP conditions (passive and LOA with 10 kPa O_2 + 10 kPa CO_2) on the quality of minimally processed kiwifruits. The combination of active compounds with alginate-based coating was more effective at delaying the microbial growth than the active compounds alone. In particular, a viability loss of the mesophilic and psychrotrophic bacteria of about 2 log cycle for the coated samples with respect to control and dipped samples was found. Moreover, the combination of the active edible coating delayed the

presence of visible moulds and reduced weight loss and respiration rate of the product. Overall, the alginate-based coating increased the shelf life of the samples up to 14 and 12 days when packed in LOA and passive MAP, respectively, compared to 8 days for the control samples.

Sothornvit and Rodsamran (2010) studied the effect of a mango-based film to wrap fresh-cut mango in combination with LOA packaging (10 kPa O_2). The MAP, with or without the mango film wrap, extended the shelf life of fresh-cut mango to 6 days when stored at 5 ºC. Whereas, at room conditions the combination of the LOA and the edible mango wrap significantly reduced off-flavor and extended the shelf life from 2-3 days to 4 days.

Campaniello et al. (2008) studied the possible use of chitosan coating to improve the safety and quality on fresh-cut strawberries packaged in high (80 kPa) or low (5 kPa) oxygen MAP at different temperature (4, 8, 12, 15 °C) of storage. Chitosan showed a high antimicrobial activity at inhibiting the growth of psychrotrophic and mesophilic bacteria, yeast and molds, and extending the lag phase, particularly at the highest temperatures (12 and 15 °C) tested. The use of MAP as second hurdle technology resulted in a sensible benefit on the microbiological quality of fresh-cut strawberries. Conventional MAP contributed to better control psychrotrophic bacteria; whereas high oxygen concentrations had a beneficial effect on sensorial characteristics, color and inhibiting mesophilic bacteria.

The application of gamma irradiation and high O_2 - high CO_2 (60 kPa O_2 + 30 kPa CO_2) MAP significantly reduced the growth of aerobic microorganism on minimally processed carrots. The combination of these treatments with a calcium caseinate and whey protein isolated-based edible coating did not affect the microbial growth of the samples; however, the coating was necessary to protect carrots against dehydration and whitening. The high oxygen packaging in combination with the coating had a detrimental effect on firmness and appearance of minimally processed carrots, being the best combination for maintaining texture irradiation at 1 kGy of coated carrots packed under air (Lafortune et al., 2005). Caillet et al. (2006) also evaluated the effect of an antimicrobial edible coating based on calcium caseinate containing trans-cinnamaldehyde, combined with high oxygen (60 kPa O_2 + 30 kPa CO_2) or air and gamma irradiation on peeled carrots inoculated with *L innocua.*

The use of HOA contributed to completely inhibit the growth of *L. innocua* and lowered the radiation dose required for that purpose. The antimicrobial edible coating was needed to reduce the *L. innocua* growth when minimally processed carrots were packed under air.

Final remarks

This review presents several studies about MAP and edible coatings with promising results to improve the safety and extend the shelf life of fresh-cut fruits and vegetables. The higher susceptibility of these products to enzymatic browning, tissue softening, microbial growth, and loss of nutrients makes in many cases necessary to continue looking for alternatives to reach sufficient shelf life for commercial distribution of the product. Depending of the MAP conditions, much research is still required to avoid or retard fermentative reactions, off-flavor and loss of nutritional compounds. Innovative MAP such as the use of pressurized inert/noble gases and high levels of O_2 have resulted effective in extending shelf life of different fresh-cut fruits and vegetables, being the main benefits the prevention of microbiological spoilage and anaerobic fermentation. Nevertheless, the effect of innovative MAP is dependent on similar factors as with conventional MAP (i.e. type of commodity, temperature, storage duration, packaging material, etc.). Some of the promising area for development to improve quality of fresh-cut commodities could be the use of active MAP able to deliver active compounds and new packaging material to better match the respiration of fresh-cut fruits and vegetables.

On the other hand, the application of edible coatings with active compounds and the new trend using nanotechnological solutions, such as nanoparticles, nanoencapsulation, and multilayered systems, to improve the quality of fresh-cut products requires more research to understand the interactions among active compounds and coating materials to avoid sensory and physiological disorders in the produce.

New technology combinations of MAP with new edible coatings can be a feasible way at improving microbial stability and quality of fresh-cut fruits and vegetables, thus extending their shelf-life. Exploration of these technologies in highly perishable fruits and vegetables (i.e. high susceptibility to enzymatic browning, loss of integrity, microbial spoilage) that have received little or no attention is also required to

increase the offer of fresh-cut fruits and vegetables, providing producers and consumers products with sufficient shelf life and the maximal safety and quality.

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GENERAL OBJECTIVES

GENERAL OBJECTIVES

To study the effect of new edible coatings with antioxidant activity and modified atmosphere packaging on the quality and shelf life of fresh-cut 'Blanca de Tudela' artichoke, 'Telma' eggplant, and 'Rojo Brillante' persimmon, characterized by a relatively short shelf life due to a rapid onset of enzymatic browning.

Specific objectives

1. To study the effect of a wide range of antibrowning agents with different mechanisms of action at different concentrations to control browning in extracts and precipitates of artichoke, eggplant, and persimmon (*in vitro* studies).

2. To evaluate the effect of the selected antioxidant agents in *in vitro* studies on the shelf life of fresh-cut artichoke, eggplant, and persimmon (*in vivo* studies).

3. To develop soy protein-based edible coatings amended with antioxidant agents from *in vivo* studies to reduce enzymatic browning and extend shelf life of fresh-cut artichoke, eggplant, and persimmon during storage at 5 ºC.

4. To evaluate the combined effect of selected soy protein-based edible coatings with antioxidant capacity and conventional or high oxygen modified atmosphere packaging on the quality and shelf life of fresh-cut artichoke, eggplant, and persimmon during storage at 5 ºC.

Chapter 1

Antibrowning effect of antioxidants on extract, precipitate, and fresh-cut tissue of artichokes

CHRISTIAN GHIDELLI [1], MILAGROS MATEOS [2], CRISTINA ROJAS-ARGUDO [1] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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ABSTRACT
The effect of antioxidants controlling enzymatic browning of artichokes cv. 'Blanca de Tudela' was studied in extracts and fresh-cut tissue. Initially, the effect of ascorbic acid (AA), citric acid (CA), peracetic acid (PA), calcium chloride ($CaCl_2$), cyclodextrin (CD), cysteine (Cys), hexametaphosphate (HMP), and 4-hexylresorcinol (Hexyl) at different concentrations was studied in extracts and precipitates. Absorbance at 450nm of artichoke extract and color of the pellets were measured, as a preliminary screening of antioxidant effectiveness controlling browning. AA at 10 mol/m^3 was the most effective controlling browning in both the extract and the pellet; whereas, Cys and 4-Hexyl were effective at a higher concentration (50 mol/m^3) and CA was only effective in the extract. Application of PA, $CaCl_2$, CD, and HMP was not effective controlling browning in the extracts and precipitates, even at a concentration of 50 mol/m^3. Next, application of AA, CA, Cys, and Hexyl at different concentrations was studied on fresh-cut artichokes during storage at 5ºC. Samples treated with Cys (0.1, 0.3, 0.5, 1%) showed the highest L* and lowest a* values. An increase in Cys concentration decreased a* and increased b* values, which correlated with a decrease in browning and an increase in yellowness of the tissue. Application of CA (1, 2.7, 5.3%), AA (0.5, 1, 1.5, 2%) and Hexyl (0.002, 0.005%) did not inhibit enzymatic browning. Visual evaluation confirmed these results.

KEYWORDS: Artichoke extracts; fresh-cut artichokes; enzymatic browning; antioxidants.

1. Introduction

Artichoke (*Cynara scolymus* L.) is a typical vegetable of the Mediterranean area widely known for its high dietary fiber, and healthy protective actions as diuretic, hypocholesterolemic, hepatoprotective, anti-inflammatory and anti-microbial properties (Gebhardt, 1997). Due to the nutritional benefits and gastronomic properties artichoke demand is increasing among consumers. Minimally processing steps such as washing, removal of external leaves, slicing and packaging can bring important advantages for artichoke commercialization, reducing transport costs, storage space, and preparation time for consumers. However, these

steps induce enzymatic browning, in addition to a quality deterioration associated with water loss, softening, microbial contamination, increase of respiration and ethylene production, that result in a reduced shelf life (Ahvenainen, 1996). Among all the factors reducing shelf-life of fresh-cut artichoke, enzymatic browning caused by oxidation of phenolic compounds by polyphenol oxidase enzyme (PPO) is the major problem. Browning might be prevented by chemical and physical methods, including reduction of temperature and/or oxygen, use of modified atmosphere (MA) packaging, and application of antioxidant agents that act to inhibit enzyme, remove its substrates or function as preferred substrate (Garcia & Barret, 2002).

Antioxidant treatments used in several fresh-cut fruits and vegetables include acidulant and reducing agents such as citric and ascorbic acid (CA and AA) (Lattanzio, Linsalata, Palmieri, & Van Sumere, 1989; Son, Moon, & Lee, 2001), thiol containing compounds such as cysteine (Cys) (Pérez-Gago, Serra, & del Río, 2006; Oms-Oliu, Aguiló-Aguayo, & Martín-Belloso, 2006; Amodio, Cabezas-Serrano, Peri, & Colelli, 2011), chelating and complexing agents such as hexametaphosphate (HMP) and cyclodextrin (CD) (Pilizota & Sapers, 2004), or compounds that directly inhibit PPO such as 4-hexylresorcinol (Hexyl) and calcium chloride ($CaCl_2$) (Rosen & Kader, 1989; Monsalve-Gonzalez, Barbosa-Cánovas, Cavalieri, McEvily, & Iyengar, 1993; Luo & Barbosa-Cánovas, 1997; Arias, González, Peiró, Oria, & López-Buesa, 2007).

Effectiveness of an antibrowning agent depends on many factors such as cultivar, concentration, synergy with other antioxidants, pH, application system, etc. In artichoke, the degree of browning has been correlated with the nature and amount of its phenolic compounds that may vary according to the genotype (Doğan, Turan, Ertürk, & Oktay, 2005; Cabezas-Serrano, Amodio, Cornacchia, Rinaldi, & Colelli, 2009; Todaro, Peluso, Catalano, Mauromicale, & Spagna, 2010). Artichoke cv. Blanca de Tudela is one of the main cultivars grown in Spain. A few works have studied the effect of packaging and washing disinfection on spoilage and microbiological quality of minimally processed artichokes ‘Blanca de Tudela’ (Gimenez, Olarte, Sanz, Lomas, Echavarri, & Ayala, 2003; Sanz, Gimenez, Olarte, Lomas, & Portu, 2002; Sanz, Gimenez, & Olarte, 2003). However, enzymatic browning is still the main limitation for the development as a minimally processed produce, and no works

have been published with the aim of finding an effective antibrowning treatment. Few approaches have been conducted to inhibit browning in fresh-cut artichokes, mainly using CA and AA (Lattanzio et al., 1989; Giménez et al., 2003). In a recent work, Amodio et al. (2011) reported that only Cys had some effect controlling browning of fresh-cut 'Catanese' artichokes, whereas AA, CA, $CaCl_2$, and Hexyl had little or not effect.

In the bibliography, browning evaluation is based on reflectance measurement (L*, a*, b*) on fresh-cut surface of fruits and vegetables during storage (*in vivo* studies). Nevertheless *in vitro* studies, involving extraction of soluble browning products and measurement of absorbance at particular wavelengths, can be performed as pre-screening to determine the potential effect of antioxidant agents controlling enzymatic browning of fruit and vegetable tissues (Garcia & Barrett, 2002). Considering that not all PPO products are water soluble, Amiot, Tacchini, Aubert, & Nicolas (1992) suggested that the degree of browning of a tissue can be estimated by measuring the maximum optical absorbance of the supernatant and the reflectance of the pellet (L*, a* and b* values), obtained after centrifugation during the preparation of the soluble pigment extract, as values of soluble and insoluble brown pigments, respectively. Therefore, the aim of this work was to study the effectiveness of a wide range of antibrowning agents at different concentrations controlling browning in extracts and precipitates of artichoke (*Cynara scolymus* L., cv. Blanca de Tudela) (*in vitro* studies). Then the most effective antioxidant agents were studied on fresh-cut 'Blanca de Tudela' artichokes during storage at 5 ºC (*in vivo* studies).

2. Materials and methods

The study was divided in two steps. In the first part enzymatic browning was determined in artichoke extracts and precipitates, meanwhile the second part was carried out in fresh-cut artichoke cv. Blanca de Tudela.

2.1. Plant material and antioxidants

Artichoke (*Cynara scolymus* L., cv. Blanca de Tudela) were purchased in a local market (Valencia, Spain) and stored at 5 ºC for 24 hours until processing. Antibrowning agents tested included ascorbic acid

(AA) and citric acid (CA) from Quimivita (Barcelona, Spain), peracetic acid (PA) from Fluka (Sigma Co., Barcelona, Spain), calcium chloride ($CaCl_2$), hexametaphosphate (HMP) , cyclodextrin (CD), cysteine (Cys), and 4-hexylresorcinol (Hexyl) from Sigma-Aldrich (St. Louise, MO; USA).

2.2. Determination of enzymatic browning in artichoke extracts and precipitates

Samples were washed to eliminate soil and dirt. External bracts, leaves and stalk were removed and artichoke heads were cut in small sections, frozen with liquid nitrogen and crushed with a blender (Braun, Model MR350, Kronberg im Taunus, Germany). Ground samples were stored at –20 ºC till analysis to avoid browning of the tissue.

For the analysis, 3 g of frozen samples were introduced in a centrifuge tube containing 30 ml of the antibrowning agent. An initial concentration of 10 mol/m^3 was tested for all the antioxidants. Concentrations were either increased or decreased depending on absorbance and reflectance measurements obtained for each antioxidant. Table 1 shows the antioxidant concentrations tested. A reference sample or 'blank' was prepared with 113 mol/m^3 AA. This concentration of AA provided a complete inhibition of soluble and insoluble brown pigments. Water was used as untreated control. Samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland), left 1 hour at 20 ºC and then centrifuged for 10 min at 12.000 rpm at 5 ºC. Absorbance of extracts was determined at 450 nm with a UV spectrophotometer (Thermo Electron Corporation, Auchtermuchty, Fife, UK). This absorbance corresponded to the maximum difference observed among samples in the range 360-500 nm. The precipitate was poured into a petri dish and L* (lightness), a* (red to green), and b* (yellow to blue) values were measured with a Minolta (Model CR-300, Ramsey, N.Y., USA) on the bottom of the dish. A standard white calibration plate was employed to calibrate the equipment. Data were reported as the total color difference with the control sample (c) with no antioxidant as:

$$\Delta E = ((L^*-L_c^*)^2 + (a^*-a_c^*)^2 + (b^*-b_c^*)^2)^{1/2}$$

Both extracts and precipitate were evaluated visually by three judges using an enzymatic browning scale, where: 0 = totally browned, 1 =

Table 1. Effect of antioxidant type and concentration on browning of 'Blanca de Tudela' artichoke extract nd precipitate.

Treatment [z]	Concentration mol/m^3	Concentration g/100 ml	pH	Extract effectiveness		Precipitate effectiveness		Global effectiveness [w]
				Abs_{450}	Visual [y]	ΔE [x]	Visual [y]	
AA	1	0.02	6.08	1.752 b	0	1.33 a	0	NO
	10	0.18	4.53	0.083 a	3	22.93 b	3	YES
CA	10	0.21	3.64	0.169 b	3	13.30 a	1	NO
	20	0.42	3.12	0.052 a	3	13.60 a	1	NO
	50	1.07	2.70	0.049 a	3	15.66 b	2	NO
PA	10	0.08	4.93	1.319 c	0	3.67 a	0	NO
	25	0.20	4.60	1.141 b	1	5.05 b	0	NO
	50	0.40	4.35	0.971 a	1	5.93 c	0	NO
$CaCl_2$	10	0.12	4.89	1.245 ab	0	3.86 b	0	NO
	25	0.31	5.42	1.334 c	0	3.12 b	0	NO
	50	0.62	5.42	0.945 a	1	2.06 a	0	NO
CD	10	1.14	5.65	1.579 a	0	5.63 a	0	NO
	50	5.69	5.98	1.607 a	0	8.57 b	0	NO
Cys	10	0.12	5.49	0.707 b	2	15.27 a	3	NO
	20	0.25	5.86	0.750 b	2	15.41 a	3	NO
	50	0.62	5.73	0.463 a	3	17.81 b	3	YES
HMP	10	0.10	5.69	1.759 a	0	2.17 a	0	NO
	50	0.52	6.16	2.607 b	0	7.97 a	0	NO
Hexyl	10	0.20	5.98	2.913 b	0	14.63 a	0	NO
	50	0.99	6.50	1.326 a	3	23.55 b	3	YES
CONTROL	0	---	5.88	2.187	0	----	0	NO
Blank	113	2.00	3.20	0.057	3	28.47	3	YES

[z]AA=Ascorbic acid; CA=Citric acid; PA=Peracetic acid; CD=Cyclodextrin; Cys=Cysteine: HMP=Hexametaphosphate; Hexyl=4-hexylresorcinol; Blank=reference sample prepared with AA at 113 mol/m^3, which provided complete inhibition of browning in extract and precipitate.

[y] Visual evaluation: 0=totally browned, 3= no browned.

[x] Color difference with control sample $\Delta E = ((L^*-L_c^*)^2 + (a^*-a_c^*)^2 + (b^*-b_c^*)^2)^{½}$.

[w] Treatments were considered effective when visual values of extracts and precipitates were 3.

For each antioxidant, means values with the same letter are not different ($p \leq 0.05$) according to LSD.

partially browned, 2 = slightly browned, 3 = no browned. Treatment was considered effective when both extracts and precipitate were visually scored as 3.

2.3. Determination of enzymatic browning in fresh-cut artichokes

After washing, artichoke external bracts, leaves and stalk were removed. Artichoke hearts were cut in slices (5mm approximately) using a sharp stainless-steel knife. Pieces were dipped in the antioxidant solutions for 3 minutes, drained and dried under cold conditions. In the first experiment with fresh-cut artichokes, antioxidants were AA, CA, Cys, and Hexyl at concentrations reported in Table 2. A second experiment was conducted with AA (0.5 g/100 g, 1 g/100 g, and 1.5 g/100 g), Cys (0.1 g/100 g, 0.3 g/100 g, 0.5 g/100 g, and 1 g/100 g) and Hexyl (0.002 g/100 g, and 0.005 g/100 g) at different concentrations from those studied in experiment 1 to conclude the effect of the antioxidants in fresh-cut artichokes. Once dried, 4 pieces were placed in polypropylene trays that were heat-sealed with microperforated films (35 µm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure no modification of the atmosphere in the tray and study only the effect of the antibrowning agents, the polypropylene films were additionally perforated with a needle. Finally, samples were stored 7 days at 5ºC. A maximum of 15 artichokes were processed at the same time to minimize their exposure to oxygen and the whole process was carried out in a temperature-controlled room at 10±1 ºC under suitable hygienic conditions.

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, N.Y., USA) on 12 artichoke pieces per treatment using the CIELAB color parameters, L*, a*, and b*. Each measurement was taken randomly at three different locations of each sample piece. A standard white calibration plate was employed to calibrate the equipment. The results were expressed as the means of the 12 measured samples per each sampling day.

During storage, artichoke pieces were also evaluated visually by 10 judges. Each treatment was coded, presented in random order and the judges had to rank each sample from lowest to highest degree of browning. The visual quality in each treatment based on general visual appearance was also determined using a visual scale, where: 9 =

excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny, Hess-Pierce, & Kader, 1999). A color photograph of samples rated with this scale was provided to the judges.

Table 2. Effect of antioxidants on color attributes of fresh-cut artichokes 'Blanca de Tudela' stored up to 4 days at 5 ºC.

Treatment	Concentration mol/m^3	Concentration g/100 ml	L*	a*	b*
AA	10	0.18	54.2 e	2.4 b	27.1 df
	25	0.44	54.0 de	2.2 b	25.5 d
	50	0.88	51.3 cd	2.3 b	22.8 bc
CA	50	1.07	52.0 cde	2.8 b	25.7 de
	125	2.67	50.8 c	5.7 ce	24.8 cd
	250	5.33	52.7 cde	5.4 c	25.6 de
Cys	10	0.12	58.3 f	-1.6 a	30.3 g
	25	0.31	59.9 f	-1.2 a	32.9 h
	50	0.62	60.7 f	-1.2 a	36.0 i
Hexyl	10	0.20	38.5 b	6.6 e	21.6 a
	25	0.49	33.6 a	8.5 f	21.7 a
	50	0.99	35.3 a	9.1 f	22.3 ab
CONTROL	---	---	58.1 f	-0.8 a	27.9 f

Values at day 0: $L_0^* = 68.0$; $a_0^* = -6.7$; $b_0^* = 34.8$ (n=12).
Data are mean values of the entire storage duration (n=36).
Within each column, values followed by the same letter are significantly different (P≤0.05) according to LSD.

2.4. Statistical analysis

Statistical analysis was performed using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences between means were determined by least significant difference (LSD). Specific differences for the degree of browning obtained by sensory evaluation were determined by Friedman test, which is recommended with ranking (UNE 87 023, 1995). Significance of differences was defined at $p \leq 0.05$.

3. Results and discussion

3.1. Effect of antioxidants on color and visual quality of artichoke extracts and precipitates

Table 1 shows the effect of the antibrowning agents at different concentrations for extracts and precipitates based on absorbance at 450 nm and color difference compared to the control (ΔE), respectively. The global effectiveness in the extract and precipitate was also assessed by visual evaluation, where the judges evaluated the samples according to browning.

The extract and precipitate of the control presented an intense brown color, which translated in high absorbance (Table 1) and a* values (data not shown); whereas, the reference sample or 'blank' prepared with 113 mol/m^3 AA provided a complete inhibition of soluble and insoluble brown pigments. In general for all the samples tested, an increase in the antioxidant concentration showed a decrease in Abs_{450} and an increase in ΔE. AA at 10 mol/m^3 was the best treatment controlling enzymatic browning on artichoke extracts and pellets at that concentration, showing no significant differences with the 'blank' on absorbance and ΔE. Cys was effective controlling enzymatic browning of the extract and precipitate at a higher concentration of 50 mol/m^3. Lower concentrations of Cys were effective controlling browning of the precipitate as evaluated by the visual panel; however, the extracts were scored as slightly brown. The use of Hexyl, although prevented the formation of brown pigments in the precipitate and extract, provoked a sharp pinkish color in the artichoke precipitate and a translucent appearance in the extract, which translated in high absorbance values. Nevertheless, a concentration of 50 mol/m^3 was considered effective by the judges controlling browning of the extract and precipitate. CA at 50 mol/m^3 prevented browning on the artichoke extract showing absorbance values slightly lower than the 'blank'. However, it was less effective preventing the formation of insoluble brown pigments as shown by the low ΔE of the precipitate. This was confirmed in the visual evaluation, where the extract was evaluated as not brown and the precipitate as slightly brown. Application of lower concentrations (≤20 mol/m^3) of CA had the same effect than 50 mol/m^3 in the extract, but were less effective in the precipitate. Application of PA, $CaCl_2$, CD, and HMP was not effective controlling

browning in the extracts and precipitates, even at a concentration of 50 mol/m^3. Since an increase in the concentration of these antioxidants from 10 mol/m^3 to 50 mol/m^3 did not show a significant improvement controlling brown pigments in the extract and precipitate, higher concentrations were not studied.

The optimum pH range of artichoke PPOs was reported between 4.3 and 5.0 (Espin, Tudela, & Garcia-Cánovas, 1997) or with a broader range between 4.0 and 7.0, that depended on artichoke cultivar (Todaro et al., 2010). In the present work, the effect observed in the extract by CA at concentrations above 10 mol/m^3could be related to its inhibitory effect by lowering the pH. However, this effect was not observed in the precipitate, which even at low pHs it did not show a complete inhibition in the formation of insoluble pigments. The results might suggest a higher stability of the PPO insoluble fraction (membrane-bound PPO) than the soluble fraction in low pH conditions. With AA, Cys, and Hexyl, browning inhibition could be attributed to the specific mechanisms of action of each antioxidant. In the case of AA and Cys, their activity resides in its reducing character reacting with *o*-quinones to yield colorless products (Richard-Forget, Goupy, Nicolas, Lacombe, & Pavia, 1991); whereas Hexyl has been described as an inhibitor of the enzyme PPO (Monsalve-Gonzalez et al., 1993).

3.2. Color changes on artichoke fresh-cut tissue

Two experiments were conducted on fresh-cut tissue. In a first experiment, antioxidant type and concentrations were selected from the best results obtained in artichoke extracts (10 mol/m^3 AA, 50 mol/m^3 Cys, and 50 mol/m^3 Hexyl). CA was also included in the study due to its inhibitory effect in the extract, although it did not show a complete inhibition in the precipitate (i.e. at 50 mol/m^3 visual quality of the precipitate was evaluated as slightly brown). These concentrations were either increased or decreased depending on the antioxidant to values close to those reported in the bibliography for other fresh-cut commodities.

Table 2 shows the effect of antioxidant type and concentration, expressed in molar and weight basis, on color L*, a*, and b* values, as mean values for 4 days of storage at 5 ºC. Among the different antioxidants, samples treated with Cys showed the highest L* and lowest

a* values, although no differences were found with untreated samples after 2 days of storage. On the opposite side, Hexyl was the less effective antioxidant inducing damage in the tissue that translated in samples with the lowest L* and b* and the highest a* values. Furthermore, an increase in Hexyl concentration translated in a decrease in L* and an increase in a*, indicating that Hexyl caused damage of the tissue at the concentrations tested. In the bibliography, Hexyl is usually applied at much lower concentrations which a range between 0.01 and 0.02 g/100 g (Monsalve-González et al., 1993; Monsalve-Gonzalez, Barbosa-Cánovas, McEvily, & Iyengar, 1995; Luo & Barbosa-Cánovas, 1997; Arias et al., 2007). Therefore, it would be interesting to study the effect of lower concentrations in artichoke tissue.

Samples treated with AA and CA also had lower L* and higher a* values than control samples. In these treatments, an increase in antioxidant concentration increased tissue browning, indicating that those concentrations could be also creating some damage in the tissue. An increase in AA showed a decrease in L* and b* values; meanwhile, for CA a sharp increase in a* was observed as antioxidant concentration increased. In this experiment, the range of CA and AA concentrations applied were approximately 1-5 g/100 g and 0.2-0.9 g/100 g, respectively. Amodio et al. (2011) also observed a decrease in L* and an increase in a* values of fresh-cut artichokes cv. Catanese as CA concentration increased, presenting the highest a* value at 2 g/100 g CA. In the case of AA, these authors also observed higher a* values than in control samples. This was attributed to a possible stimulation of PPO activity or by an induction of important oxidative damage, as observed by other authors in Chinese water chestnuts (Jiang, Pen, & Li, 2004) and fresh-cut 'Fuji' apple (Larrigaudière, Ubach, Soria, Rojas-Graü, & Martín-Belloso, 2008). Palma, D'Aquino, D'Hallewin, & Agabbio (2004) also described an increase in browning of artichoke heads dipped in 1 g/100 g AA solution as pH was decreased from 5.8 to 2.8, which was attributed to the minimum PPO activity found in artichoke at pH 5.5 (Espin et al., 1997). Todaro et al. (2010) observed that depending on artichoke cultivar, PPO activity could be stimulated at high AA concentrations.

Considering these results, a second experiment was conducted aimed to test the effect of AA, Cys, and Hexyl at different concentration ranges.

Fig. 1, 2 and 3 show the effect of these antibrowning agents on L*, a*, and b* values of fresh-cut artichokes 'Blanca de Tudela' during 7 days of storage at 5 ºC. Increased enzymatic browning in artichoke pieces during storage was accompanied by an increase in a*, and a decrease in b* and L* values.

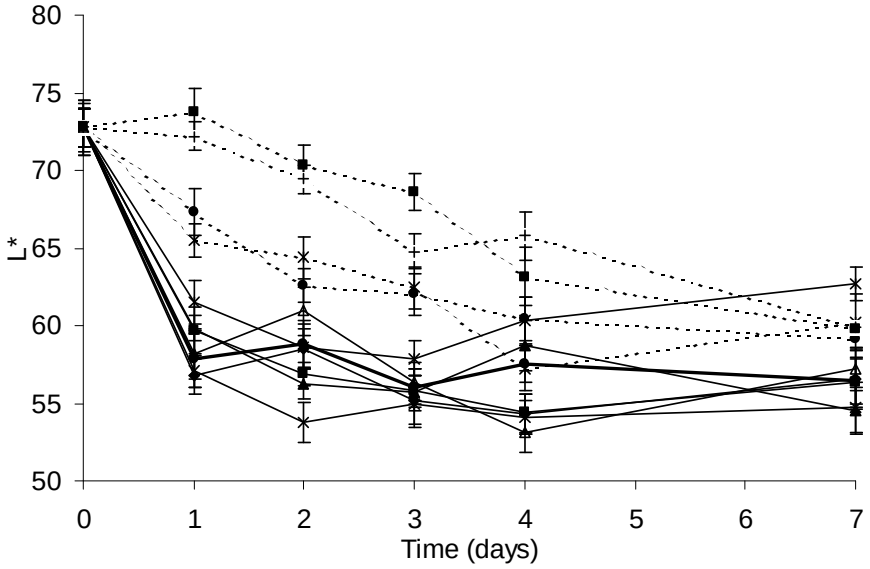

Fig. 1. Effect of antioxidant type and amount on lightness (L*) of fresh-cut artichokes 'Blanca de Tudela' stored 7 days at 5 ºC. Vertical bars represent standard error. (◆, 0.5 g/100 ml AA; ■, 1 g/100 ml AA; ▲, 1.5 g/100 ml AA; ✕ 2 g/100 ml AA; ✕, 0.1 g/100 ml Cys; ●, 0.3 g/100 ml Cys; +, 0.5 g/100 ml Cys; ■, 1 g/100 ml Cys; △, 0.002 g/100 ml Hexyl; ✳, 0.005 g/100 ml Hexyl; ●, Control).

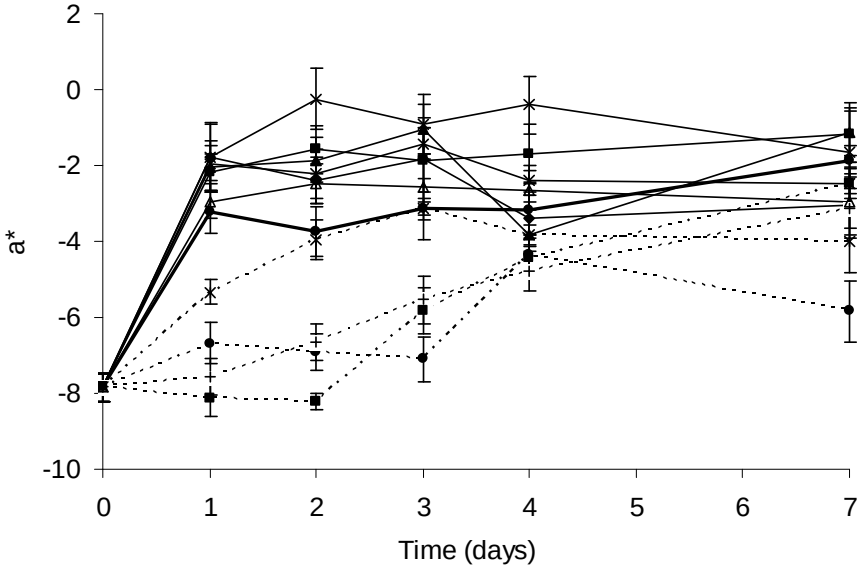

Fig. 2. Effect of antioxidant type and amount on a* (red-green) of fresh-cut artichokes 'Blanca de Tudela' stored 7 days at 5 ºC. Vertical bars represent standard error. (—◆—, 0.5 g/100 ml AA; —■—, 1 g/100 ml AA; —▲—, 1.5 g/100 ml AA; —✕— 2 g/100 ml AA; ···✕···, 0.1 g/100 ml Cys; ···●···, 0.3 g/100 ml Cys; ···+···, 0.5 g/100 ml Cys; -■- , 1 g/100 ml Cys; —△—, 0.002 g/100 ml Hexyl; —✳—, 0.005 g/100 ml Hexyl; —●—, Control).

L* increased as Cys concentration increased, observing significant differences during the first 4 days of storage between samples treated at concentrations below 0.3 g/100 g and those above 0.5 g/100 ml whereas, these differences disappeared at the end of storage. Samples treated with Cys at concentrations above 0.3 g/100 g showed a* values significantly lower than control samples during storage. Cys treatments also resulted in an increase in b* values (yellowness), that increased as Cys concentration increased. Thiol-containing compounds, such as Cys, have been described as reducing agents of o-quinones to their phenol precursors producing colorless stable products (Garcia & Barrett, 2002). However, a direct inhibition of PPO by Cys through the formation of

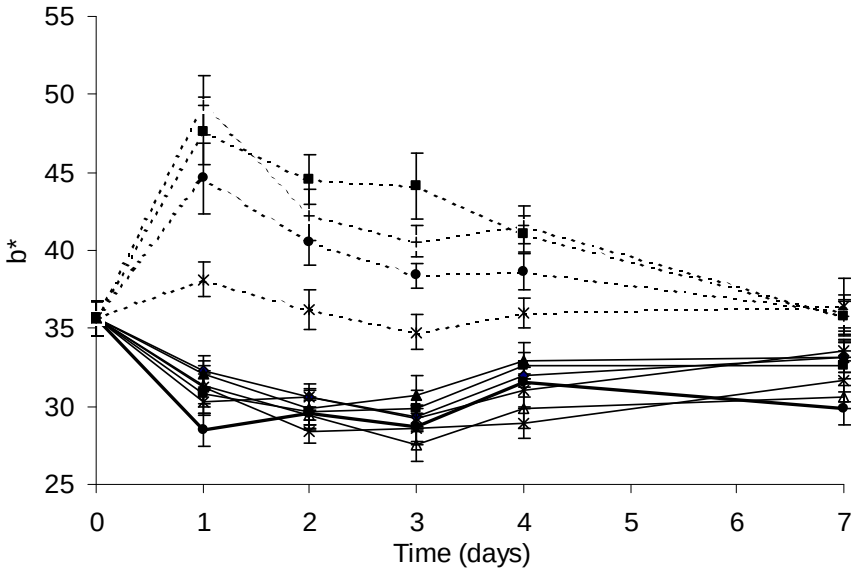

Fig. 3. Effect of antioxidant type and amount on b* (yellow-blue) of fresh-cut artichokes 'Blanca de Tudela' stored 7 days at 5ºC. Vertical bars represent standard error. (—◆—, 0.5 g/100 ml AA; —■—, 1 g/100 ml AA; —▲—, 1.5 g/100 ml AA; —✕— 2 g/100 ml AA; ··✕··, 0.1 g/100 ml Cys; ··●··, 0.3 g/100 ml Cys; ··+··, 0.5 g/100 ml Cys; -■- , 1 g/100 ml Cys; —△—, 0.002 g/100 ml Hexyl; —✳—, 0.005 g/100 ml Hexyl; —●—, Control).

stable complexes with the copper of active PPO sites has also been proposed (Kahn, 1985). Richard-Forget et al. (1991) isolated cysteine-quinone addition compounds from different phenols that almost block the browning reaction, showing high affinity with PPO enzymes and subtracting substrates for the further oxidation of o-quinone. A Cys-phenol ratio above 1 was needed to fully degrade phenols in colorless addition compounds; whereas when Cys was applied at low concentration, the appearance of pinkish-red off-colored compounds have been reported and attributed to phenol regeneration with deep color formation (Richard-Forget et al., 1991; Pérez-Gago et al., 2006). On the other hand, Cavallini, De Marco, Duprè, & Rotilo (1969) described that

the addition of excess Cys to an alkaline Cu^{II} solution resulted in the appearance of a yellow compound identified with a Cys-copper complex. Considering that artichoke is a vegetable with a significant copper content (United States Department of Agriculture, 2011), the addition of high Cys concentrations could be inducing the formation of these yellow compounds. Amodio et al. (2011) also confirmed the effectiveness of Cys controlling enzymatic browning of 'Catania' fresh-cut artichokes. Similarly to our results, an increase in yellowness was observed as Cys content increased, indicating the formation of some color compounds at high Cys concentration that would be interesting to elucidate in future research. Amodio el al. (2011) also observed that in Cys-treated samples an increase in pH from the natural value of 2.1 to 3 induced a better color retention of the artichoke quarters. This was attributed to the fact that at higher pH values, the nucleophilic attack of quinones by cysteine is more effective due to the pKa of thiol group (Richard-Forget et al., 1991).

Application of AA in a concentration range of 0.5-2 g/100 ml increased browning as antioxidant concentration increased, as it was reflected by a decrease in L* and an increase in a*, confirming the results observed in the previous experiment at lower AA concentrations (Table 2). An increase in a* was also observed in fresh-cut Fuji apples dipped in 1 g/100 g AA, indicating tissue browning (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2008). Larrigaudière et al. (2008) showed that AA may cause important oxidative damage in fresh-cut 'Fuji' apples at the concentrations used in commercial applications to prevent browning.

Application of Hexyl at lower concentrations than those tested in the previous experiment was not effective reducing browning. Several researchers have studied its use individually or combined with other antibrowning agents in fresh-cut products. Amodio et al. (2011) reported that fresh-cut artichokes treated with 0.01 g/100 g Hexyl had the lowest L* value and scored worse than the control. Similar results were observed by Pérez-Gago et al. (2006), where application of Hexyl at 0.005 and 0.02 g/100 g was less effective than AA and Cys reducing browning of fresh-cut apples. However, Son et al., 2001 reported that 0.1 g/100 g Hexyl was the most effective antioxidant preventing browning of 'Liberty' apple slices. Rojas-Graü, Sobrino-López, Tapia, & Martín-Belloso (2006) reported that increasing the concentration of Hexyl up to 0.5 g/100 g lead to a decrease of a* in fresh-cut apples. Nevertheless,

higher concentration than 0.5 g/100 g provoked an increase in a*. On the other hand, several authors suggested that the effectiveness of Hexyl controlling browning is improved by the combination with other antioxidant agents (Monsalve-Gonzalez et al., 1993; Luo & Barbosa-Canovas,1997; Arias et al., 2007). Monsalve-Gonzalez et al. (1995) observed that combination of 0.025 g/100 g Hexyl and 0.25 g/100 g AA was an effective anti-browning agent in fresh-cut apple during 32 days of storage at 25 ºC. Arias et al. (2007) also described a synergic effect in the combination of 0.01 g/100 g Hexyl and 2 g/100 g AA with a reduction in PPO activity of fresh-cut pears.

The results in fresh-cut artichokes contrast with those found in the extract, where AA, and Hexyl were effective reducing soluble and insoluble browning products at different concentrations. Application of antioxidants has an effect not only in browning reactions, but also in the metabolic activity and cell wall changes during wound-induced reactions. Bolwell, Butt, Davies, & Zimmerlin (1995) indicated that Cys and AA may influence wound-induced cell wall structural changes by stimulating H_2O_2 production. Application of CA, AA and L-Cys to fresh-cut potatoes increased their metabolic activity and influenced their sugar composition, especially after treatment with L-Cys (Rocculi et al., 2007) and AA induced oxidative damage in fresh-cut apples (Larrigaudière et al., 2008).

3.3. Visual quality on artichoke tissues

Degree of browning of fresh-cut artichokes treated with different antioxidants was also assessed by a sensory panel with the aim of determining whether the color differences observed instrumentally could be observed visually. Samples treated with Cys were evaluated as the best during storage (Fig. 4). After 1 day of storage control and samples dipped in AA and Hexyl were judged as inedible or in the limit of usability; whereas samples dipped in Cys were evaluated above the limit of marketability. During storage, quality evaluation of fresh-cut artichokes dipped in Cys decreased, reaching the limit of marketability after 4 days of storage at 5 ºC with concentrations above 0.5 g/100 g Cys. Although this period is not enough for product commercialization, application of Cys significantly prolonged the shelf life of fresh-cut artichoke compared to control samples and opens the possibility for

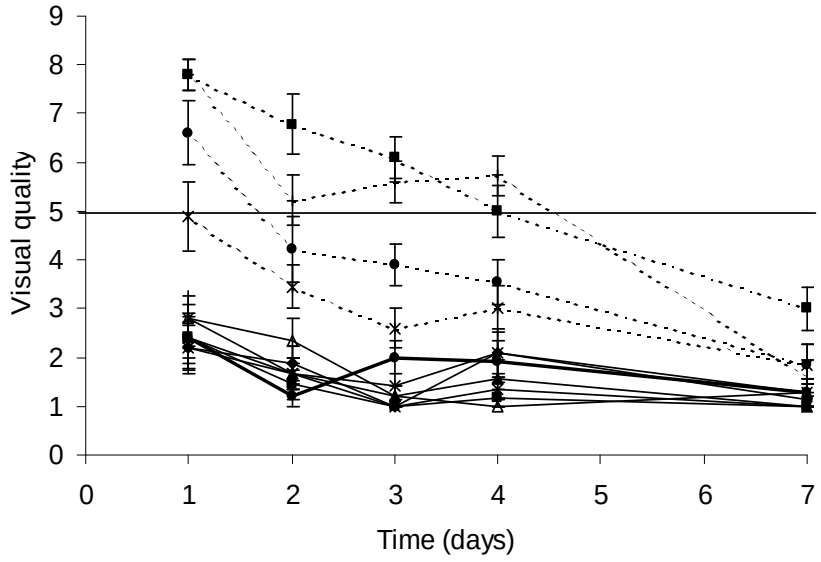

Fig. 4. Effect of antioxidant type and amount on visual appearance of fresh-cut artichokes 'Blanca de Tudela' stored 7 days at 5ºC. Visual appearance was based on a visual scale (9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible). Vertical bars represent standard error. (—◆—, 0.5 g/100 ml AA; —■—, 1 g/100 ml AA; —▲—, 1.5 g/100 ml AA; —✕— 2 g/100 ml AA; ··✕··, 0.1 g/100 ml Cys; ··●··, 0.3 g/100 ml Cys; ··+··, 0.5 g/100 ml Cys; -■-, 1 g/100 ml Cys; —△—, 0.002 g/100 ml Hexyl; —✳—, 0.005 g/100 ml Hexyl; —●—, Control).

future studies in combination with other technologies, such as modified atmosphere, edible coatings, etc.

The visual ranking confirmed the results obtained instrumentally in the color analysis. Samples dipped in 0.5 and 1 g/100 g Cys were ranked as the less browned during the entire storage period. Whereas, AA and Hexyl showed no significant differences compared to control samples during storage.

4 Conclusion

The use of antioxidants had a limited action controlling enzymatic browning of fresh-cut artichokes. In extracts and precipitates, AA, Cys and Hexyl were the most effective antioxidants preventing browning. However, in fresh-cut tissue only Cys at concentrations above 0.5 g/100 g significantly extended shelf life till 4 days of storage at 5 ºC. However, it provided an increase of yellowness on the cut surface of the artichokes. Application of AA in a concentration range of 0.5-2 g/100 g and Hexyl at 0.002 and 0.005 g/100 g induced browning, probably due to an increase in metabolic activity or induced oxidative damage.

Future work will require a study of the effect of Cys on the sensory quality of artichokes and its combination with other technologies, such as modified atmosphere packaging and edible coatings, in order to further extend the produce shelf life.

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Chapter 2

Effect of antioxidants on enzymatic browning of eggplant extract and fresh-cut tissue

CHRISTIAN GHIDELLI [1], MILAGROS MATEOS [2], CRISTINA ROJAS-ARGUDO [1] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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ABSTRACT
The effect of antioxidants controlling enzymatic browning of eggplants was studied in extract and fresh-cut tissue. Initially, the effect of ascorbic acid (AA), citric acid (CA), peracetic acid (PA), calcium chloride ($CaCl_2$), ciclodextrin (CD), cysteine (Cys), hexametaphosphate (HMP), and 4-hexylresorcinol (Hexyl) at different concentrations was studied in extracts and precipitates of eggplant. Cys and Hexyl were effective at 10 mM in both the extract and pellet. Higher concentrations were needed to have an effective control of enzymatic browning in samples treated with AA (20 mM), CA (50 mM), and PA (50 mM). Next, the application of AA, CA, PA, Cys, and Hexyl at different concentrations was studied on fresh-cut eggplant tissues during storage at 5C. Cys was effective as antioxidant, extending the shelf-life till 9 days of storage at 5C when applied at a concentration of 1%. Tissue browning increased as AA and Hexyl concentrations increased.

PRACTICAL APPLICATION
Commercialization of eggplants as a fresh-cut product is limited by its relatively short shelf life due to a rapid onset of enzymatic browning. The main approach to reduce enzymatic browning is the use of antioxidant solutions. However, little information can be found about effective technologies to control browning of fresh-cut eggplants. This work studies the effect of a wide range of antibrowning agents at different concentrations, combining *in vitro* (in extracts and precipitates) with *in vivo* (in tissue) studies and provides relevant information for further development of minimally processed eggplants during storage at 5C.

Keywords: extract; precipitate; fresh-cut eggplant; enzymatic browning; antibrowning agent.

INTRODUCTION
Eggplants (*Solanum melongena*) are ranked amongst the top ten vegetables in terms of antioxidant capacity due to its phenolic components, which makes this vegetable to be very appreciated by consumers (Cao *et al*. 1996). Commercialization of this vegetable as a fresh-cut product is limited by its relatively short shelf life due to a rapid onset of enzymatic browning, tissue softening and water loss. Among all

the factors reducing shelf life of fresh-cut eggplants, surface browning is one of the main cause of quality loss due to the reaction of polyphenol oxidase (PPO) with phenolic compounds that brings to the formation of o-quinones and subsequent brown pigments (Mishra *et al*. 2012).

Most of the works found in the literature related to enzymatic browning of eggplants are focused on the study of the effect of substrate specificity, temperature, pH, and antioxidants on the PPO activity obtained from eggplants (Almeida and Nogueira 1995; Dogân *et al*. 2002*;* Cheriot *et al*. 2006; Todaro *et al*. 2011). However, little information can be found about effective technologies to control browning of fresh-cut tissue. The application of ethanol vapors or the use of a sharp blade followed by immediate dipping in water have been effective in reducing enzymatic browning of minimally processed eggplants (Cao *et al*. 1996; Hu *et al*. 2010). Common technologies used to prevent browning include reduction of temperature, use of modified atmosphere (MA) packaging, and application of compounds that act to inhibit enzyme, remove its substrates or function as preferred substrate (Garcia and Barrett 2002). The antioxidants used in fresh-cut fruits and vegetables include acidulants, such as citric, ascorbic, and peracetic acid (CA, AA, and PA) (Lattanzio *et al*. 1989; Sapers and Miller 1992; Castañer *et al*. 1996; Dong *et al*. 2000; Son *et al*. 2001; Oms-Oliu *et al*. 2006; Pérez-Gago *et al*. 2006; Amodio *et al*. 2011), reducing agents such as cysteine (Cys) and AA, chelating and complexing agents such as hexametaphosphate (HMP) and ciclodextrin (CD) (Sapers *et al*. 1989; Pilizota and Sapers 2004), and enzymatic inhibitors such as 4-hexylresorcinol (Hexyl) (Monsalve-Gonzalez *et al*. 1993; Luo and Barbosa-Cánovas 1997; Arias *et al*. 2007a). Effectiveness of these antibrowning agents depends on many factors, such as commodity, cultivar, concentration and synergy with other antioxidants, pH, application system, etc.

In the bibliography, browning evaluation is generally based on reflectance measurement (L*, a*, b*) on fresh-cut surface of fruits and vegetables during storage (*in vivo* studies). Nevertheless *in vitro* studies, involving extraction of soluble browning products and measurement of absorbance at particular wavelengths, can be performed as pre-screening to determine the potential effect of antioxidant agents controlling enzymatic browning of fruit and vegetable tissues (Garcia and Barrett

2002). Considering that not all PPO products are water soluble, Amiot *et al*. (1992) suggested that the degree of browning of a tissue can be estimated by measuring the maximum optical absorbance of the supernatant and the reflectance of the pellet (L*, a* and b* values) as a value of soluble and insoluble brown pigments, respectively. Therefore, the aim of this work was to study the effect of a wide range of antibrowning agents at different concentrations in extracts and precipitates of eggplants (*in vitro* studies) and to evaluate the most effective antioxidant agents on fresh-cut eggplants during storage at 5C (*in vivo* studies).

MATERIALS AND METHODS

The study was divided in two steps. In the first part enzymatic browning was determined in eggplant extracts and precipitates, meanwhile the second part was carried out in fresh-cut eggplants.

Plant material and antioxidants

Eggplants (*Solanum melongena* L., cv. Telma) were purchased in a local market (Valencia, Spain) and stored at 5C for 24 h until processing. The antibrowning agents tested included AA and CA from Quimivita (Barcelona, Spain), PA from Fluka (Sigma Co., Barcelona, Spain), calcium chloride ($CaCl_2$), HMP, CD, Cys, and Hexyl from Sigma-Aldrich (St. Louise, MO, USA).

Determination of enzymatic browning in eggplant extracts and precipitates *(in vitro studies)*

Samples were washed, peeled, cut into rectangular pieces, frozen with liquid nitrogen, and crushed with a blender (Braun, Model MR350, Kronberg im Taunus, Germany). Ground samples were stored at -20C till analysis to avoid browning of the tissue.

For the analysis, 3 g of frozen samples were introduced in a centrifuge tube containing 30 mL of the antibrowning agent. An initial concentration of 10 mM was tested for all the antioxidants. Concentrations were either increased or decreased depending on absorbance and reflectance measurements obtained for each antioxidant. Table 1 shows the antioxidant concentrations tested expressed in mM and %. A reference sample or ‘blank’ was prepared with 113 mM AA. This

concentration of AA provided a complete inhibition of soluble and insoluble brown pigments. Water was used as untreated control. Samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland), left 1 h at 20C and then centrifuged at 17,390 x *g* and 5C for 10 min. The absorbance of the extracts was determined at 450 nm with a UV spectrophotometer (Thermo Electron Corporation, Auchtermuchty, Fife, UK). This absorbance corresponds to the maximum difference observed among samples in the range 360-500 nm. The precipitate was poured into a petri dish and L* (lightness), a* (red to green), and b* (yellow to blue) values were measured with a Minolta chromameter (Model CR-300, Ramsey, N.Y., USA) on the bottom of the dish. A standard white calibration plate. was employed to calibrate the equipment. Data were reported as the total color difference with the control sample (c) as:

$$\Delta E = ((L^*-L_c^*)^2 + (a^*-a_c^*)^2 + (b^*-b_c^*)^2)^{1/2}$$

Extracts and precipitates were also evaluated visually by three judges using an enzymatic browning scale: 0 = totally browned, 1 = partially browned, 2 = slightly browned, 3 = no presence of browning. A treatment was considered effective when both extract and precipitate were visually scored as 3.

Table 1. Effect of antioxidant type and concentrations on browning of eggplant extract and precipitate.

Antioxidant [z]	Concentration mM	Concentration (%)	pH	Extract effectiveness		Precipitate effectiveness		Global effectiveness
				Abs_{450}	Visual [y]	ΔE [x]	Visual [x]	
AA	10	0.17	4.78	0.144 a	3	5.67 b	2	NO
	20	0.35	3.90	0.153 a	3	22.03 c	3	YES
CA	10	0.21	3.77	0.288 c	2	14.42 b	1	NO
	20	0.42	3.11	0.228 b	3	14.71 b	2	NO
	50	1.07	2.57	0.095 a	3	20.87 c	3	YES
PA	10	0.08	5.13	0.487 c	1	11.26 b	0	NO
	25	0.20	4.48	0.408 b	2	7.64 a	0	NO
	50	0.40	4.23	0.217 a	3	13.69 c	3	YES
$CaCl_2$	10	0.12	5.74	0.317 a	1	4.71 a	0	NO
	20	0.24	4.64	0.341 a	2	6.21 b	0	NO
	50	0.62	4.44	0.425 b	1	6.27 b	0	NO
CD	10	1.14	6.14	0.826 a	0	3.74 a	0	NO
	50	5.69	5.58	0.826 a	0	17.56 b	0	NO
Cys	1	0.01	5.52	0.296 b	2	6.41 b	2	NO
	10	0.12	5.85	0.182 a	3	22.46 c	3	YES
HMP	10	0.10	6.19	0.830 a	0	2.36 a	1	NO
	50	0.52	6.00	0.804 a	0	3.39 a	2	NO
Hexyl	1	0.02	5.55	0.840 b	0	4.98 a	0	NO
	10	0.20	6.36	0.702 a	3	22.91 b	3	YES
CONTROL	0		5.72	0.641	0	----	0	NO
BLANK	113	1.9	3.18	0.095	3	25.28	3	YES

[z]AA=Ascorbic acid; CA=Citric acid; PA=Peracetic acid; CD=Ciclodextrin; Cys=Cysteine: HMP=Hexametaphosphate; Hexyl=4-hexylresorcinol; Blank=reference sample prepared with AA at 113 mM (1.9%), which provided complete inhibition of browning in extract and precipitate.

[y] Visual evaluation: 0=totally browned, 3= not presence of browning.

[x] Color difference with control sample $\Delta E = ((L^*-L_c^*)^2 + (a^*-a_c^*)^2 + (b^*-b_c^*)^2)^{1/2}$.

For each antioxidant, means values with the same letter are not different ($P \leq 0.05$).

Determination of enzymatic browning in fresh-cut eggplant *(in vivo studies)*

After washing, eggplants were peeled and cut into rectangular pieces (approximately 5 cm x 3.5 cm x 1.5 cm) using a sharp stainless-steel knife. Pieces were dipped in the antioxidant solutions for 3 min, drained and dried under cold conditions. In a first experiment with fresh-cut tissue, the antioxidants tested were AA, CA, PA, Cys, and Hexyl. A second experiment was conducted in fresh-cut eggplant tissue with AA and Cys to corroborate the effect of these antioxidants. Antioxidant concentrations for both experiments are reported in Table 2. Once dried, 4 cut pieces were placed in polypropylene trays and were heat-sealed with microperforated films (35 µm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure no modification of the atmosphere in the tray and to study only the effect of the antibrowning agents, the polypropylene film was additionally perforated with a needle. Finally, samples were stored 7 and 9 days at 5C for the first and second experiments, respectively. A maximum of 15 eggplants were processed at the same time to minimize their exposure to oxygen and the whole process was carried out in a temperature-controlled room at 10±1C under suitable hygienic conditions.

Table 2. Antioxidant type and concentrations tested in eggplant tissue.

Antioxidant	Concentration (%)	
	Experiment 1	Experiment 2
AA	0.35, 0.88, 1.75	0.35, 0.88, 1.75, 3.50
CA	0.40, 1.00, 2.00	-
PA	0.40, 1.00, 2.00	-
Cys	0.01, 0.03, 0.06	0.10, 0.50, 1.00
Hexyl	0.20, 0.50, 1.00	-

Color measurements were made periodically with a Minolta chromameter (Model CR-300, Ramsey, N.Y., USA) on 12 eggplant pieces per treatment using the CIELAB color parameters, L*, a*, and b*. Each measurement was taken randomly at three different locations of each sample piece. A standard white calibration plate was employed to

calibrate the equipment. The results were expressed as the means of the 12 measured samples per each sampling day.

During storage, eggplant pieces were also evaluated visually by 10 judges. Each treatment was coded, presented in random order and the judges had to rank each sample from lowest to highest degree of browning. The visual quality in each treatment based on general visual appearance was also determined using a visual scale, where: 9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny *et al*. 1999). A color photograph of samples rated with this scale was provided to the judges. Results for ranking based on color and visual quality, performed by the same panel members, were expressed as an average of the scores.

Statistical analysis

Statistical analysis was performed using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences between means were determined by least significant difference (LSD). Specific differences for color obtained by sensory evaluation were determined by Friedman test, which is recommended with ranking (UNE 87 023 1995). Significant difference was defined at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Effect of antioxidants on color and visual quality of eggplant extracts and precipitates (in vitro studies)

Enzymatic browning of eggplant extracts containing the soluble pigments was determined at 450 nm; whereas, insoluble brown pigments were determined by L*, a*, and b* values of the pellets obtained after centrifugation. Table 1 shows the effect of the antioxidants at different concentrations for the extracts and precipitates based on absorbance at 450 nm and color difference compared to the control (ΔE), respectively. The global effectiveness in the extract and precipitate was also assessed by visual evaluation, where the judges evaluated the samples according to browning.

The extract and precipitate of the control presented an intense brown color, which translated in high absorbance and a* values (data not shown); whereas, the reference sample or ‘blank’ prepared with 113 mM AA provided a complete inhibition of soluble and insoluble brown

pigments. Generally, a decrease in Abs_{450} and an increase in ΔE were observed as antioxidant concentrations increased. The best treatments controlling enzymatic browning in eggplant extracts and precipitates were Cys and Hexyl, which inhibited soluble and insoluble brown pigments at 10 mM. No significant differences in absorbance were found between samples treated with 10 mM Cys and the blank. The application of lower concentration of Cys (1mM) also reduced the absorbance and ΔE of the extract and precipitate, being evaluated by the judges as slightly browned which indicates the potential of this antioxidant to control browning of eggplants. The application of 10 mM Hexyl, however, provoked a translucent appearance in the extract, that translated in high absorbance values; nevertheless, the judges evaluated the extract and precipitate of these samples as not browned. Knapp (1965) reported the competitive inhibition of eggplant PPO by resorcinol using chlorogenic acid as substrate. The presence of a hydrophobic substituent in the 4-position (4-Hexylresorcinol) increased the inhibitory potential of the PPO using tert-Butylcatechol as substrate (Pérez-Gilabert and García Carmona 2000). However, Cheriot *et al*. (2006) observed a small effect of Hexyl (pH 5) and Cys (0.25 M, pH 2) on eggplant PPO activity using 4-methylcatechol as substrate.

pH is a key factor affecting enzyme activity. The optimum pH values for PPO activity varies depending on the commodity. For example, optimum pH values reported in the literature are 5.5 for strawberries (Wesche-Ebeling and Montogomery 1990), 7 for 'Amasya' apples (Oktay *et al.* 1995) and 6 for wild pear (Yerlitürk *et al.* 2008). In eggplants, PPO activity has been found in a broad pH range between 4 and 9 (Pérez-Gilabert and García Carmona 2000). Depending on the cultivar and substrate, the pH optimum for PPO activity has been narrowed to a range of 4.8-6 (Concellón *et al*. 2004) or to a value of 6 or 7 (Dogân *et al*. 2002; Todaro *et al*. 2011). Therefore, a reduction of pH may help reducing browning in the eggplant extract and precipitate. In our work, high concentrations of CA and PA (50 mM) were required to control browning of the extract and precipitate, with a pH of the solutions below 4. Application of AA offered a complete inhibition of soluble and insoluble brown pigments at a concentration of 20 mM; whereas a concentration of 10 mM was only effective in the extract but not in the precipitate. Todaro *et al*. (2011) studied the inhibition of eggplant PPO

by several organic acids with catechol as substrate, obtaining a strong inhibition of PPO activity by CA (about 70% reduction at 0.11 M and 95% at 0.42 M) and a lower inhibition by tartaric and acetic acid (about 40% and 20% at 0.11 M, respectively). Almeida and Nogueira (1995) studied the interaction between the use of AA, CA, ethylenediaminetetraacetic acid (EDTA), sodium metabisulphite and a heat treatment (70C for 2 min) in the control of PPO activity of several fruits and vegetables, showing that eggplant PPO was the most resistant to the different combinations tested. Only the combination of AA, CA, sodium metabisulphite and heat reached a 98.2% enzyme inhibition, while, the best alternative to the use of SO_2 was the combination of AA, CA and heat treatment, resulting in 84.3% inhibition of eggplant PPO activity.

Application of CD, $CaCl_2$, and HMP did not inhibit browning either in the extract, or in the precipitate, even at a concentration of 50 mM. Since an increase in the concentration of these antioxidants from 10 mM to 50 mM did not show a significant improvement in controlling brown pigments, higher concentrations were not studied. In the case of CD, higher concentrations could not be tested because of the limited solubility of this compound (Sapers *et al*. 1989).

Color changes on eggplant fresh-cut tissue (in vivo studies)

Two experiments were conducted on fresh-cut eggplant tissue. In a first experiment, antioxidant type and concentrations were selected from the best results obtained in eggplant extracts for AA (20 mM), CA (50 mM), PA (50 mM), and Hexyl (10 mM); whereas the lower concentration was selected for Cys (1 mM), which was evaluated with slight browning. These concentrations were increased to values close to those reported in the bibliography for other fresh-cut commodities. Table 2 shows the concentrations studied in %.

Fig. 1 shows the effect of antioxidant type and amount on L* and a* values of fresh-cut eggplants during storage at 5C. Browning was accompanied by a decrease in L* and an increase in a*. Cys was the most effective antioxidant controlling browning of fresh-cut eggplants with lower a* and higher L* values than control samples, even though this antioxidant was tested at the lowest concentrations (0.01-0.06%). Eggplant pieces dipped in 0.35 and 0.88% AA showed higher L* and

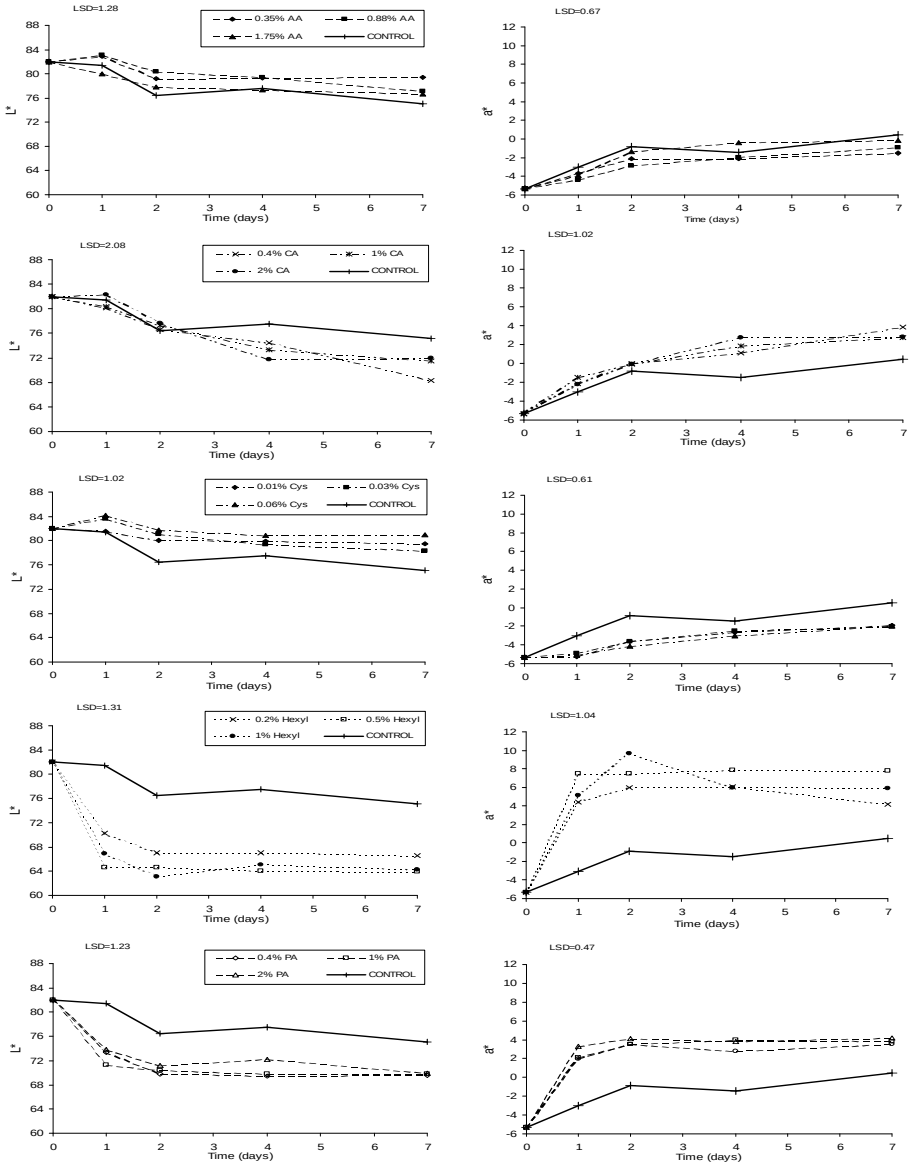

Fig. 1. Effect of antioxidant type and concentrations on L* and a* of fresh-cut eggplants stored at 5C. LSDs at the 5% level.

lower a* values than the untreated samples. Whereas, an increase in tissue browning was observed when AA concentration increased to 1.75%. A similar behavior has been described in fresh-cut artichokes when AA concentration was increased, which might indicate a possible stimulation of PPO activity (Amodio et al. 2011; Ghidelli et al. 2013). An oxidative damage of the tissue with cell disruption and subsequent decompartmentalization of enzymes was also reported by the application of high concentrations of AA in Chinese water chestnuts (Jiang et al. 2004), fresh-cut 'Fuji' apples (Larrigaudière et al. 2008), and pears (Oms-Oliu et al. 2006).

Among all the antibrowning agents tested, Hexyl was the antioxidant that caused the highest degree of browning in cut eggplants, showing the lowest L* and the highest a* values. Rojas-Graü *et al*. (2006) reported that increasing the concentration of Hexyl up to 0.5% lead to a decrease of a* in fresh-cut apples, but higher concentrations provoked an increase in a*. Son *et al*. (2001) observed that the application of 0.1% Hexyl was one of the most effective antioxidant tested preventing browning of 'Liberty' apple slices. In the bibliography, Hexyl has been applied successfully at much lower concentrations, in a range between 0.01 and 0.02% (Monsalve-Gonzalez *et al*. 1993, 1995; Luo and Barbosa-Cánovas 1997; Dong *et al*. 2000; Arias *et al*. 2007b). Therefore, the concentrations used in the present experiment might be harmful for eggplant tissue. On the other hand, several authors have suggested that the effectiveness of Hexyl controlling browning is improved by the combination with other antioxidant agents (Monsalve-Gonzalez *et al*. 1993; Arias *et al*. 2007a). Monsalve-Gonzalez *et al*. (1995) observed that combination of 0.02% Hexyl and 0.25% AA was an effective anti-browning agent for fresh-cut apples during 32 days of storage at 25C. Arias *et al*. (2007a) also described a synergic effect by combining 0.01% Hexyl and 0.2% AA with a reduction in PPO activity of fresh-cut pears.

Todaro *et al*. (2011) found a strong inhibition of eggplant PPO by CA at low concentration. Other organic acids, such as acetic acid and tartaric acids also showed some enzyme inhibition, although with a lower effectiveness, suggesting that CA could be useful to increase the quality and shelf life of minimally processed eggplants. In our work, the concentrations tested for CA and PA increased browning of fresh-cut eggplants, showing lower L* and higher a* values than control samples.

As observed with AA, dipping eggplants pieces in low pH solutions could be harmful for the tissue, inducing an oxidative damage or a possible stimulation of PPO activity (Jiang *et al*. 2004; Larrigaudière *et al*. 2008).

Considering the above results, a second experiment was carried out on fresh-cut eggplants with AA and Cys. The experiment was designed to corroborate the behavior observed with AA (i.e. tissue browning is increased when AA concentration increased) and to improve the effect of Cys concentration controlling enzymatic browning of fresh-cut eggplants (Table 2). Fig. 2 shows the effect of AA and Cys on L* and a* values of fresh-cut eggplants stored 9 days at 5C. As in the previous experiment, Cys was more effective than AA controlling enzymatic browning of fresh-cut eggplants. Fresh-cut eggplants treated with 1% Cys had the highest L* values during storage, whereas, little or no differences were observed in L* values between 0.1 and 0.5 % Cys. However, eggplants dipped in 0.1% Cys showed higher a* values than those dipped in 0.5 and 1% Cys. Therefore, these results confirm that an increase in Cys content reduces enzymatic browning of fresh-cut eggplants. Several works have reported that the application of Cys at low concentrations to apples or pears slices induced the appearance of pinkish-red off-colored compounds due to phenol regeneration with deep color formation (Richard- Forget *et al*. 1991; Pérez-Gago *et al*. 2006). In our study, this effect was not observed even at concentrations as low as the one studied in the first experiment (0.01–0.06%). In the case of AA, the results also confirmed the trend observed in the first experiment. Eggplants treated with 0.35% AA had the lowest L* and the highest a* values, and the application of higher concentrations of AA only inhibited browning after 1 day of storage.

Comparing the results in cut tissue with those obtained in extract and precipitate, only Cys resulted effective controlling browning *in vitro* and *in vivo*; whereas, Hexyl was only effective *in vitro*, inducing browning in fresh-cut tissue. Similarly, the application of AA, CA, and PA at the concentrations tested also resulted in an increase in tissue browning. The differences found between *in vitro* and *in vivo* studies indicate that the application of antioxidants has an effect not only in the browning reactions, but also in the metabolic activity and cell wall damage during wound-induced reactions.

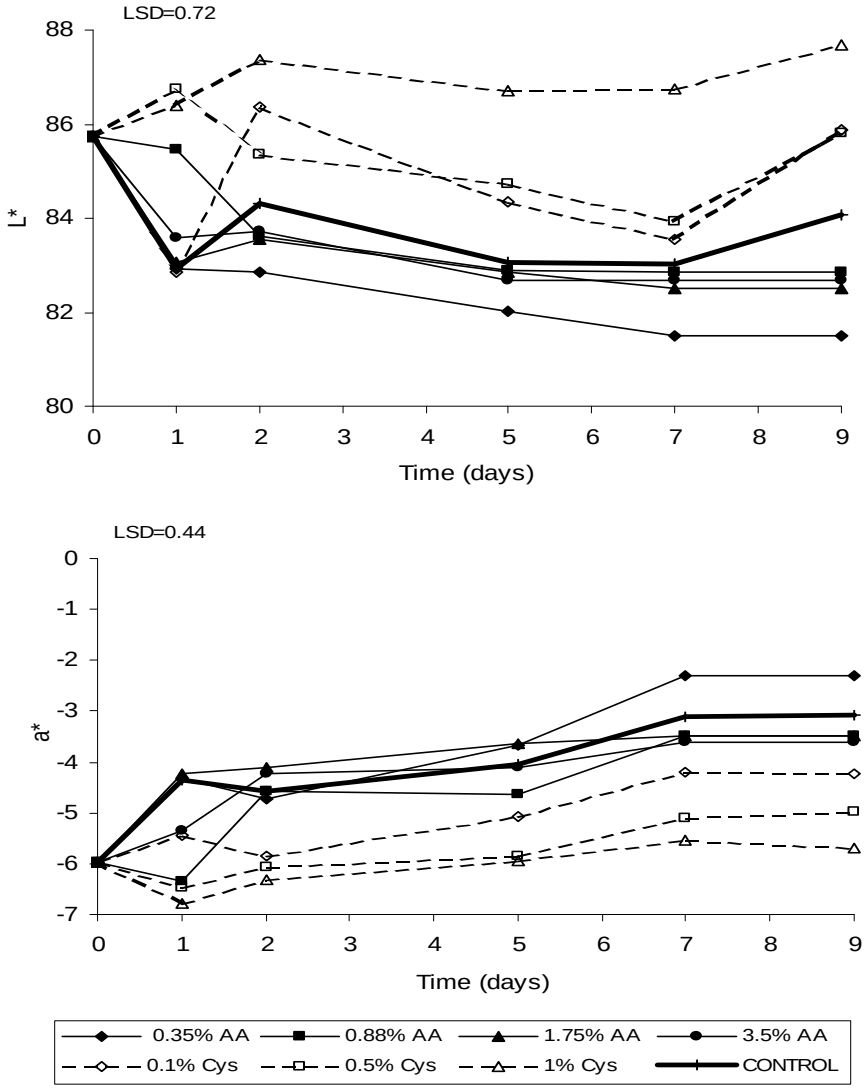

Fig. 2. Effect of antioxidant type and concentrations on L* and a* of fresh-cut eggplants stored at 5C. LSDs at the 5% level.

Visual quality of fresh-cut eggplants

Fig. 3 shows the visual quality of fresh-cut samples dipped in the different antioxidants tested in the first experiment during storage at 5C. All the samples, except those treated with Cys, were evaluated below the limit of marketability even after 1 day of storage. Eggplant pieces treated with Cys reached the limit of marketability after 4 days of storage, with no differences among concentrations (0.01-0.06%). In general, samples treated with 0.88% AA were evaluated with higher visual quality than samples treated with 1.75% AA throughout storage, which correlated with the color instrumental analysis (Fig. 1).

The browning rank of the samples followed the same trend observed in color measurements. The judges ranked the samples treated with Cys as the less brown and the samples treated with Hexyl as the most brown (Table 3). Eggplant pieces treated with CA and PA were evaluated with higher browning than the control; whereas, those treated with AA were evaluated as the control.

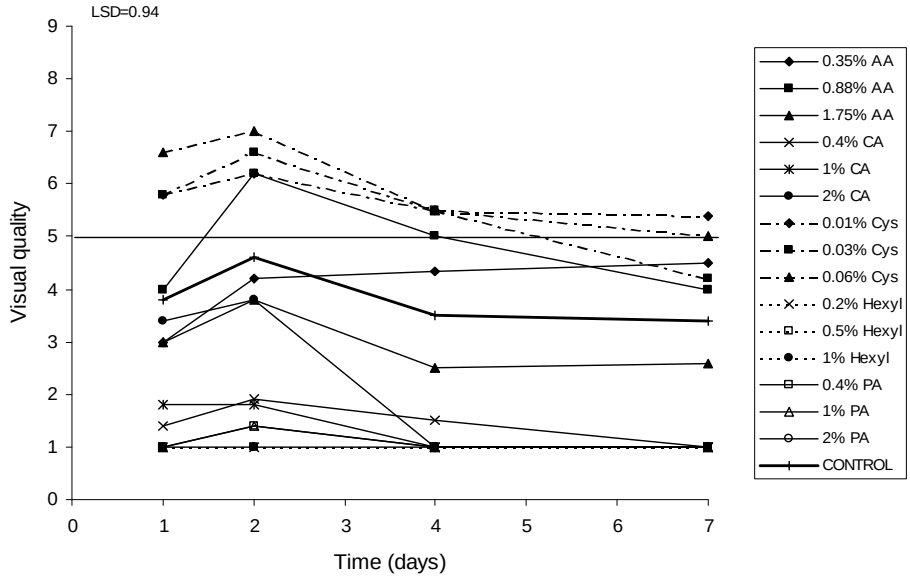

Fig. 3. Effect of antioxidant type and concentrations on visual quality of fresh-cut eggplants stored at 5C. LSDs at the 5% level.

Table 3. Effect of antioxidant type and concentrations on browning ranking of fresh-cut eggplants stored at 5C.

	Time (days)					
	1		4		7	
Lowest browning	0.06% Cys	a	0.01% Cys	a	0.01% Cys	a
	0.01% Cys	ab	0.03% Cys	a	0.06% Cys	a
	0.03% Cys	ab	0.88% AA	ab	0.88% AA	a
	0.88% AA	abc	0.06% Cys	ab	0.03% Cys	a
	CONTROL	abc	CONTROL	abc	0.35% AA	a
	2% CA	abc	0.35% AA	abc	CONTROL	ab
	1.75% AA	abc	1.75% AA	abc	1.75% AA	ab
	0.35% AA	abc	0.4% CA	abc	2% CA	ab
	1% CA	abc	1% CA	abc	1% CA	ab
	0.4% CA	abc	2% PA	abc	2% PA	ab
	0.4% PA	abc	1% PA	abc	0.4% CA	ab
	2% PA	bc	0.4% PA	abc	0.2% Hexyl	ab
	1% PA	bc	2% CA	abc	0.4% PA	ab
	0.2% Hexyl	c	0.2% Hexyl	bc	1% PA	ab
	0.5% Hexyl	c	0.5% Hexyl	bc	0.5% Hexyl	ab
Highest browning	1% Hexyl	c	1% Hexyl	c	1% Hexyl	b

Judges ranked eggplants from the lowest browning to the highest browning and were allowed to group those treatments that were considered similar. Means with the same letter are not significantly different ($P<0.05$).

In the second experiment, the limit of marketability of fresh-cut eggplants increased as Cys concentration increased (Fig. 4). Samples dipped in 0.1% Cys were evaluated above the limit of marketability up to 5 days of storage at 5C, and those treated with 1% Cys were still evaluated as very good after 9 days of storage at 5C. During the first 2 days of storage, samples dipped in 1.75 and 3.5% AA were evaluated as bad, whereas samples dipped in lower AA concentrations were evaluated close to the limit of marketability. This was due to tissue damage at high AA concentrations. On increasing the storage time, the differences among AA-treated samples were reduced due to an increase in browning. When comparing AA with Cys treatments, eggplant slices dipped in Cys were ranked with the lowest degree of browning, whereas those dipped in AA at 1.75 and 3.5% were evaluated with the highest browning (Table 4).

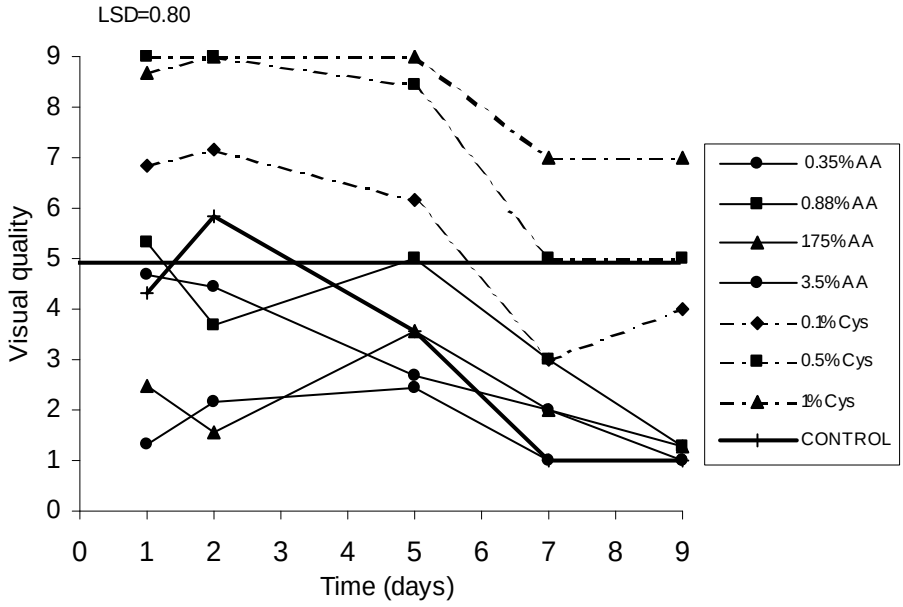

Fig. 4. Effect of antioxidant type and concentrations on visual quality of fresh-cut eggplants stored at 5C. LSDs at the 5% level.

Table 4. Effect of antioxidant type and concentrations on browning ranking of fresh-cut eggplants stored at 5C.

	Time (days)					
	1		5		9	
Lowest browning	1% Cys	a	1% Cys	a	1% Cys	a
	0.5% Cys	a	0.5% Cys	ab	0.5% Cys	ab
	0.1% Cys	ab	0.1% Cys	abc	0.1% Cys	abc
	0.88% AA	abc	0.88% AA	abcd	CONTROL	abcd
	0.35% AA	abc	CONTROL	bcd	0.35% AA	bcd
	CONTROL	abc	1.75% AA	cd	0.88% AA	bcd
	1.75% AA	bc	0.35% AA	cd	1.75% AA	cd
Highest browning	3.5% AA	c	3.5% AA	d	3.5% AA	d

Judges ranked eggplants from the lowest browning to the highest browning and were allowed to group those treatments that were considered similar. Means with the same letter are not significantly different ($P<0.05$).

CONCLUSION

This study demonstrates the potential of Cys, even at low concentrations, to retard browning of the fresh-cut eggplants. In *in vitro* studies, Cys and Hexyl were the most effective antioxidants controlling browning of eggplant extracts and precipitates. In fresh-cut tissue (*in vivo* studies), only Cys was effective as antioxidant, extending the shelf-life till 9 days of storage at 5C when applied at a concentration of 1%. Future work will require a study of the effect of this antioxidant on the sensory quality of eggplants and the possible combination with other technologies, such as modified atmosphere packaging and edible coatings.

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Chapter 3

Effect of antioxidants controlling enzymatic browning of minimally processed persimmon 'Rojo Brillante'

CHRISTIAN GHIDELLI [1], CRISTINA ROJAS-ARGUDO [1], MILAGROS MATEOS [2] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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Abstract

'Rojo Brillante' is an important variety of persimmon that after removal of the astringency with high levels of CO_2 maintains the firmness and sweetness, which makes possible its commercialization as a fresh-cut commodity. However, the commercial success of the product is limited mainly by enzymatic browning. This work presents the effect of a wide range of antioxidants on enzymatic browning of 'Rojo Brillante' persimmon combining *in vitro* (extracts and precipitates) and *in vivo* (cut tissue) studies. Preliminary screening of the antioxidants, determined by absorbance and color measurements of persimmon extracts and pellets, showed that 4-hexylresorcinol (Hexyl), citric acid (CA) and calcium chloride ($CaCl_2$) were effective at controlling browning at 10 mM; whereas, ascorbic acid (AA) required a higher concentration (25 mM). Peracetic acid, cyclodextrin, cysteine, and hexametaphosphate were not effective at controlling browning, even at a concentration of 50 mM. In *in vivo* studies, AA (1.12%) and CA (0.21%) were the most effective treatments to control enzymatic browning of fresh-cut 'Rojo Brillante' persimmon, reaching the limit of marketability in in 5-7 days; whereas, Hexyl and $CaCl_2$ did not reach 1 day of storage. The results showed that optimum concentrations in cut tissue did not always correlate with the *in vitro* studies, indicating that antioxidants have an effect not only in browning reactions, but also in the metabolic activity and cell wall changes during wound-induced reactions. The results provide relevant information for further development of minimally processed 'Rojo Brillante' persimmon during storage at 5 ºC.

Keywords: persimmon extract; fresh-cut; enzymatic browning; antioxidants.

1. Introduction

'Rojo Brillante' is an important persimmon cultivar in the zone Ribera Xuquer (Valencia, Spain). In the last decade, its production has significantly increased and the fruit is now considered as an important alternative to other crops, with an important presence in European markets. At harvest, this cultivar has excellent sensory quality and firmness; however, the presence of high concentrations of soluble tannins makes the fruit inedible for its astringency. Exposure to high levels of

carbon dioxide (95 % for 24 h at 20 ºC) has proven to be the most effective way to remove astringency while maintaining fruit firmness (Arnal and del Río, 2003). The effectiveness of this technology to remove astringency makes possible the commercialization of 'Rojo Brillante' persimmon as fresh-cut fruit. However, the high phenolic content of persimmons increases the susceptibility of oxidation by the enzyme polyphenol oxidase (PPO) in the presence of O_2, leading to the formation of brown pigments in the cut surface.

Common technologies used to prevent browning include reduction of temperature, use of modified atmosphere (MA) packaging, and application of antioxidants (Garcia and Barrett, 2002). In persimmon, very few studies have been carried out to control browning and extend the quality of the fresh-cut fruit. Wright and Kader (1997) described that controlled atmosphere storage with 12% of CO_2 slightly increased the shelf life of sliced persimmon fruit, delaying the appearance of black areas on the surface. The application of honey solution dips extended the shelf life of fresh-cut persimmon fruit by delaying off-aroma development, firmness loss and jelling (Ergun and Ergun, 2010); whereas, persimmon cubes subjected to vacuum impregnation with sucrose did not avoid browning, suggesting the need of antioxidants (Igual et al., 2008).

The antioxidants used in fresh-cut fruits and vegetables include acidulants such as citric, ascorbic, and peracetic acid (CA, AA, and PA), reducing agents such as cysteine (Cys) and AA, chelating and complexing agents such as hexametaphosphate (HMP) and cyclodextrin (CD), or enzymatic inhibitors such as 4-hexylresorcinol (Hexyl) and calcium chloride ($CaCl_2$) (Garcia and Barrett, 2002). In general, the effect of these antioxidants controlling enzymatic browning of fresh-cut fruits depends on many factors such as commodity, cultivar, concentration, synergy with other antioxidants, pH, application system, etc. In persimmon, the effect of some antioxidants has been studied as natural inhibitors of PPO enzyme purified from the fruit (Núñez-Delicado et al., 2003; Özen et al., 2004) and preliminary work from our group showed some improvement delaying browning of fresh-cut tissue (Pérez-Gago et al., 2009).

In the literature, browning evaluation is generally based on reflectance measurement (L*, a*, b*) on fresh–cut surface of fruits and

vegetables during storage (*in vivo* studies). Nevertheless *in vitro* studies, involving extraction of soluble browning products and measurement of absorbance at particular wavelengths, have also been suggested as pre-screening to determine the potential effect of antioxidant agents controlling enzymatic browning of fruits and vegetables tissues, such as apples and pears (Eissa et al., 2006; Arias et al., 2008; Chiabrando and Giacalone, 2012). Because not all PPO products are water soluble, Amiot et al. (1992) suggested that to estimate the susceptibility of apple to browning, the absorbance at 400 nm of the supernatant and lightness (L*) of the pellets obtained after centrifugation should be measured as a value of soluble and insoluble browns pigments, respectively. Therefore, the aim of this work was to study the potential to control enzymatic browning of a wide range of antioxidant agents at different concentrations in the extracts and precipitates of 'Rojo Brillante' persimmon (*in vitro* studies). Then the most effective antioxidants were studied on fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC (*in vivo* studies).

2. Material and methods

The study was divided in two parts. In the first part, enzymatic browning was determined in persimmon extract and precipitate, meanwhile the second part was carried out in fresh-cut persimmon.

2.1. Plant material and antioxidants

'Rojo Brillante' persimmons were provided by the Cooperative 'Nuestra Señora de Oreto' (l'Alcudia, Valencia, Spain). Astringency was removed by maintaining the fruit at 20 ºC in closed containers with 95% CO_2 for 24 hours. After removal from the containers, the fruit was stored in air at 15 ºC for 1 day until processing. The antibrowning agents tested included ascorbic acid (AA) and citric acid (CA) from Quimivita (Barcelona, Spain), peracetic acid (PA) from Fluka (Sigma Co., Barcelona, Spain), calcium chloride ($CaCl_2$), hexametaphosphate (HMP), cyclodextrin (CD), cysteine (Cys), and 4-hexylresorcinol (Hexyl) from Sigma-Aldrich (St. Louise, MO, USA).

2.2. Determination of enzymatic browning in persimmon extract and precipitate (in vitro studies)

Persimmons were cleaned, peeled, cut into small pieces, frozen with liquid nitrogen, and crushed with a blender (Braun, Model MR350, Kronberg im Taunus, Germany). The ground samples were stored at -20 ºC till analysis, that was done within 2 weeks, to avoid browning of the tissue.

For the analysis, 3 g of frozen samples were introduced in a centrifuge tube that contained 30 ml of the antioxidant solution. An initial concentration of 10 mM was tested for all the antioxidants and concentrations were increased or decreased depending on absorbance and reflectance measurements. The study was concluded when a concentration provided the complete inhibition of soluble and insoluble brown pigments or when an increase in the concentration of the antioxidant did not show a significant improvement to control browning. Table 1 shows the antioxidant concentrations tested expressed as mM and % (w/v). A reference sample or 'blank' was prepared with AA at 113 mM. This concentration provided a complete inhibition of soluble and insoluble brown pigments. Water was used as untreated control. Samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland), left 1 hour at 20 ºC and then centrifuged for 10 min at 12.000 rpm at 5ºC. Absorbance of extracts was determined at 450 nm with a UV spectrophotometer (Thermo Electron Corporation, Auchtermuchty, Fife, UK). This absorbance corresponded to the maximum difference observed among samples in the range 360-500 nm. The graphical abstract shows the spectrum of the reference sample o 'blank' and the untreated control (no antioxidant). The precipitate was poured into a petri dish and L* (lightness), a* (red to green), and b* (yellow to blue) values were measured with a Minolta chromameter (Model CR-300, Ramsey, NY, USA) on the bottom of the dish. A standard white calibration plate was employed to calibrate the equipment. Data were reported as the total color difference with the control sample (c) treated with no antioxidant as:

$$\Delta E = ((L^*-L_c^*)^2 + (a^*-a_c^*)^2 + (b^*-b_c^*)^2)^{½}$$

Both extract and precipitate were evaluated visually by three trained judges using the next interval scale for enzymatic browning: 0 = totally

browned, 1 = partially browned, 2 = slightly browned, 3 = no presence of browning (ISO 4121:2003; ISO 6658:2005). A treatment was considered effective when both extract and precipitate were visually scored as 3.

2.3. Determination of enzymatic browning in fresh-cut persimmons (in vivo studies)

After washing, persimmons were peeled and cut into rectangular pieces (approximately 5 cm x 3.5 cm x 1.5 cm) using a sharp stainless-steel knife. The pieces were dipped in the antioxidant solutions for 3 min and allowed to drain and dry under cold conditions. The tested antioxidants were AA, CA, $CaCl_2$, and Hexyl at the concentrations reported in Table 2. Once dried, 4 pieces were placed on polypropylene trays that were heat-sealed with microperforated films (35 µm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure that the gas composition within the package remained near ambient concentration and study only the effect of the antibrowning agents, the polypropylene film was additionally perforated with a needle (4 perforations of 1 mm diameter). During storage, the gas composition in the package headspace was monitored with an O_2/CO_2 analyzer (CheckMate 3, PBI Dansensor Inc., Denamark). Finally, samples were stored 7 days at 5 ºC. A maximum of 15 persimmons were processed at the same time to minimize their exposure to oxygen and the whole process was carried out in a temperature-controlled room at 10±1 ºC under suitable hygienic conditions. A total of 3 trays were prepared per treatment and storage time.

Color measurements were taken periodically with a Minolta chromameter (Model CR-300, Ramsey, N.Y., USA) on 12 persimmon pieces per treatment using the CIE L* a* b* color space. Each measurement was taken randomly at three different locations of each sample piece. A standard white calibration plate was employed to calibrate the equipment. The data were reported as hue angle calculated as $\tan^{-1}$ (b*/a*) and the color difference (ΔE*) between L* a* b* values at the time of analysis and those measured just after processing. The results were expressed as the means of the 12 measured samples per each sampling day.

Table 1. Effect of antioxidant type and concentrations on browning of 'Rojo Brillante' persimmon extract and precipitate.

Treatment [a]	Concentracion mM	% (w/v)	pH	Extract effectiveness		Precipitate effectiveness		Global effectiveness
				Abs_{450}	Visual[b]	ΔE [x]	Visual[c]	
AA	10	0.18	4.56	0.223 b	1	3.94 a	2	NO
	25	0.45	3.97	0.126 a	3	8.64 b	3	YES
CA	2	0.04	4.83	0.322 b	0	4.74 a	3	NO
	10	0.21	3.77	0.121 a	3	6.01 a	3	YES
PA	10	0.08	4.90	0.234 b	1	2.19 a	0	NO
	25	0.20	4.57	0.203 b	1	3.28 a	0	NO
	50	0.62	4.32	0.146 a	2	7.02 b	2	NO
$CaCl_2$	2	0.02	5.92	0.175 b	3	2.88 a	2	NO
	10	0.12	5.74	0.080 a	3	8.06 b	3	YES
CD	10	1.14	5.68	0.351 b	0	3.74 a	2	NO
	25	2.84	5.95	0.205 a	1	4.22 a	2	NO
	50	5.69	6.02	0.264 a	1	9.55 b	3	NO
Cys	10	0.12	5.50	0.317 a	0	3.59 ab	0	NO
	25	0.31	5.92	0.300 a	0	5.47 b	2	NO
	50	0.62	5.74	0.286 a	0	4.56 b	2	NO
	75	0.92	5.41	0.281 a	0	2.06 a	2	NO
HMP	10	0.10	6.27	0.403 a	0	2.95 a	1	NO
	25	0.26	6.19	0.437 a	0	5.35 b	2	NO
	50	0.52	5.88	0.491 b	0	4.09 ab	2	NO
Hexyl	2	0.04	5.87	0.172 b	3	7.11 a	2	NO
	10	0.20	5.97	0.048 a	3	13.54 b	3	YES
CONTROL	0	0	5.88	0.300	0	----	0	NO
BLANK	113	2.00	3.20	0.117	3	8.23	3	YES

[a] AA: Ascorbic acid; CA: Citric acid; PA=Peracetic acid; $CaCl_2$: Calcium chloride CD: Cyclodextrin; Cys: Cysteine: HMP: Hexametaphosphate; Hexyl: 4-hexylresorcinol; Blank: reference sample prepared with AA at 113 mM, which provided complete inhibition of browning in extract and precipitate.

[b] Visual evaluation: 0 = totally browned, 3 = not presence of browning.

[c] Color difference with control sample $\Delta E = ((L^*-L_c^*)^2 + (a^*-a_c^*)^2 + (b^*-b_c^*)^2)^{½}$.

For each antioxidant, means values with the same letter are not different ($p \leq 0.05$).

Table 2. Antioxidant type and concentrations tested in 'Rojo Brillante'persimmon tissue.

Antioxidant	Concentration (%, w/v)
AA	0.45 – 1.12 – 2.25
CA	0.21 – 0.52 – 1.05
$CaCl_2$	0.12 – 0.30 – 0.60
Hexyl	0.01 – 0.02 – 0.05

AA: Ascorbic acid; CA: Citric acid; $CaCl_2$: Calcium chloride; Hexyl: 4-hexylresorcinol.

During storage, persimmon pieces were also evaluated visually by 10 judges. Each treatment was coded, presented in random order and the judges had to rank each sample from lowest to highest degree of browning. The visual quality in each treatment based on general visual appearance was also determined using a visual scale, where: 9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny et al., 1999). A color photograph of the samples rated with this scale was provided to the judges. The results for ranking based on color and visual quality, performed by the same panel members, were the average of the scores.

2.4. Statistical analysis

Statistical analysis was performed using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences between means were determined by least significant difference (LSD). Specific differences for color obtained by sensory evaluation were determined by Friedman test, which is recommended with ranking (UNE 87 023, 1995). Significant differences were defined at $p \leq 0.05$.

3. Results and discussion

3.1. Effect of antioxidants on color and visual quality of persimmon extract and precipitate (in vitro studies)

Table 1 shows the effect of the antioxidant agents at different concentrations for extract and precipitate of 'Rojo Brillante' persimmon based on absorbance at 450 nm and color difference compared to the control (ΔE), respectively. The global effectiveness in the extract and precipitate was also assessed by visual evaluation, where the judges evaluated the samples according to browning.

The extract and precipitate of the control (water) presented an intense brown color, which translated in high absorbance (Table 1) and low L* value (data not shown); whereas, the reference sample or 'blank' prepared with 113 mM AA provided a complete inhibition of soluble and insoluble brown pigments. The best treatment to control enzymatic browning in persimmon extract and precipitate was Hexyl at 10 mM showing the lowest and highest Abs_{450} and ΔE values, respectively. At the same concentration, $CaCl_2$ and CA were also effective to control soluble and insoluble brown pigment formation. In the extract and precipitate, both treatments showed absorbance values similar to the blank, whereas in the precipitate, 10 mM $CaCl_2$ was more effective in preventing the formation of insoluble brown pigments (higher ΔE) than CA at the same concentration. Nevertheless, both treatments were evaluated by the judges as not brown. A higher concentration of AA (25 mM) was required to control browning in the extract and precipitate; whereas Cys, PA, CD, and HMP were not effective even at concentrations of 50-75 mM. Since an increase in the concentration of these antioxidants from 10 mM to 50 mM did not show a significant improvement at controlling brown pigments in the extract and precipitate, higher concentrations were not studied. In similar studies carried out in artichoke cv. Blanca de Tudela and eggplant cv. Telma, Cys was one of the most effective treatments to control browning in the extract and precipitate, even at very low concentrations (Ghidelli et al., 2013a,b).

The pH optimum for persimmon PPOs activity has been reported to be dependent on the substrate. The enzyme was active in a narrow pH range in 4-methylcatecol and 3-(3,4-dihydroxyphenyl)propionic acid substrates, having pH optimum of 7.5 and 5.5, respectively. In cathecol

and l-3,4-dihydroxyphenylalanine, the enzyme catalyzed the oxidation over a wider pH range of 6.5-8.5 with a pH optimum of 7.5 for each substrate (Özen et al., 2004). In the case of 4-tert-butylcatechol (TBC) and in absence of sodium dodecyl sulfate (SDS) the activity increased at acidic pH; however the presence of SDS increased the optimum pH to 5.5 (Núñez-Delicado et al., 2003). In the present work, a reduction of pH below 4 could be the reason for the complete inhibition of soluble and insoluble brown pigments at 10 mM CA and at 25 mM AA (Table 1). Nevertheless, AA usually acts reducing the initial quinone to the original diphenol before it undergoes the secondary reactions that lead to browning, rather than as an enzyme inhibitor by lowering the pH (Whitaker, 1972). AA has also been an effective inhibitor of persimmon PPO activity when added in a final concentration of 1 mM (Núñez-Delicado et al., 2003) and 0.1 mM (Özen et al., 2004).

Contrary to our findings, other works reported that thiol containing compounds such as Cys and metabisulfite were effective to inhibit the PPO activity of persimmon, suggesting an addition reaction taking place with the quinones to form stable colorless products and/or a binding to the active center of the enzyme in the case of metabisulfite (Núñez-Delicado et al., 2003; Özen et al., 2004). However, Núñez-Delicado et al. (2003) found that the activity of thiol compounds was only significant in the presence of 1mM SDS, indicating that these compounds are more effective in the SDS-activated form of the enzyme. In that work, CD also decreased persimmon PPO activity. Its effect depended on the concentrations of CD and the substrate used (TBC). CD is a group of naturally occurring cyclic oligosaccharides with cavities that form inclusion complexes with a wide range of organic guest molecules, including (poly)phenols, thereby preventing their oxidation to quinones and subsequent polymerization to brown pigments (Cai et al., 1990). Because TBC is a diphenolic compound with a hydrophobic group, the persimmon PPO activity was reduced with increased concentration of CD and decreased concentration of TBC. In our work, CD did not result an effective inhibitor of enzymatic browning either in the extract, or in the precipitate, probably because a higher concentration was required.

Hexyl and $CaCl_2$ at 10 mM showed pH values close to the optimum of persimmon PPOs activity. In this case, browning reduction can be attributed to the specific inhibition of the PPO enzyme by these

antioxidants. Hexyl is considered an inhibitor of the browning reaction acting as a competitive inhibition of PPO due to structural resemblance to phenolic substrates (Monsalve-González et al., 1995). $CaCl_2$ is mainly used for tissue firming, however it has been demonstrated that the chloride ion could act as an inhibitor of the PPO enzyme (McEvily et al., 1992).

3.2. Color changes on persimmon fresh-cut tissue (in vivo studies)

Antioxidant type and concentrations tested in fresh-cut tissue were selected from the best results obtained in the extracts and precipitates (25 mM AA, 10 mM CA, and 10 mM $CaCl_2$). Considering the differences between the nature of the samples in in vitro and in vivo studies (i.e. ground versus fresh-cut tissue), two higher concentrations were also tested in which corresponded to 2.5 and 5 times those concentrations. In the case of Hexyl, the concentrations selected were smaller from the optimum (10 mM Hexyl), since bibliography works have reported tissue damage in fresh-cut artichoke and eggplant at 10 mM Hexyl (0.2% Hexyl), recommending smaller concentrations (Ghidelli et al., 2013a,b). Table 2 shows the concentrations studied in %.

Increased enzymatic browning in persimmon pieces during storage was accompanied by an increase in a* values and a decrease in lightness (L*) and b* (data not shown), which translated in a decrease in hue angle and an increase in ΔE* between L* a* b* values at the time of analysis and those measured just after processing (Fig. 1, 2). AA, CA and $CaCl_2$ were the most effective antioxidants to reduce browning, showing higher hue values and the lower ΔE* than control samples for all the concentrations tested. The results confirmed the effectiveness of AA, as observed previously in *in vitro* studies, in reducing the quinone products to their original polyphenol compounds and, therefore, in preventing browning. However, although AA concentration did not affect negatively hue values, the increase in AA concentration to 2.25% significantly increased ΔE* due to a decrease in L*, suggesting that high AA

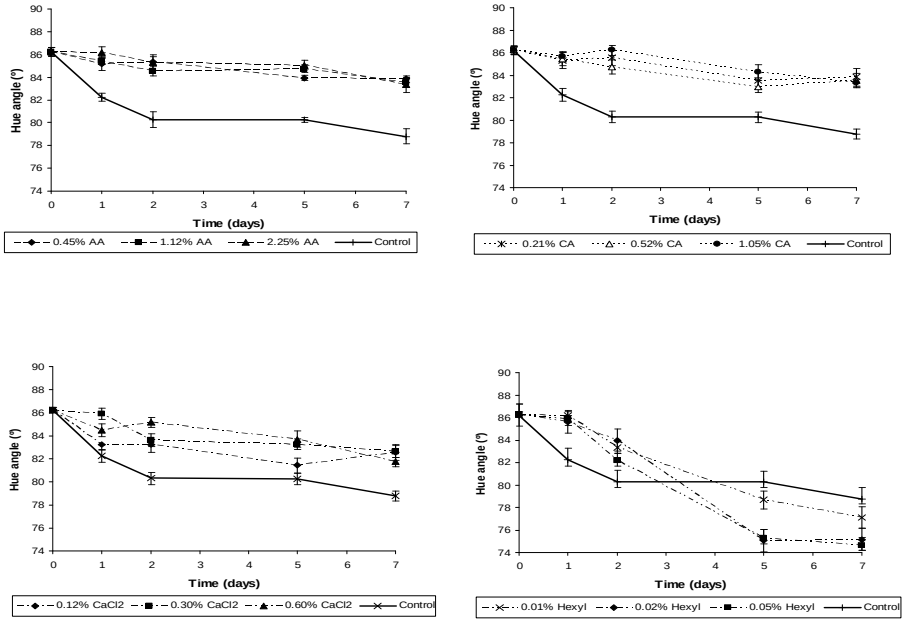

Fig. 1. Effect of antioxidant type and amount on hue angle of fresh-cut persimmon 'Rojo Brillante' stored at 5 ºC. Data shown are mean ± standard error. AA: Ascorbic acid; CA: Citric acid; $CaCl_2$: Calcium chloride; Hexyl: 4-hexylresorcinol.

concentrations might negatively affect persimmon tissue. An oxidative damage of the tissue with cell disruption and subsequent decompartmentalization of enzymes has been reported by the application of high concentrations of AA in Chinese water chestnuts (Jiang et al., 2004), fresh-cut 'Fuji' apples (Larrigaudière et al., 2008), and pears (Oms-Oliu et al., 2006).

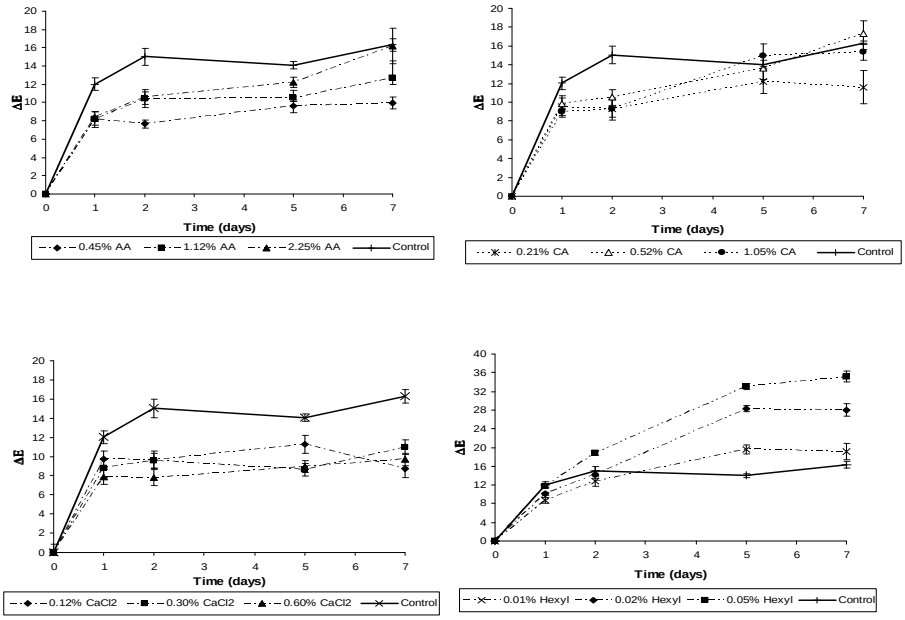

Fig. 2. Effect of antioxidant type and amount on color difference (ΔE*) with the initial values of fresh-cut persimmon 'Rojo Brillante' stored at 5 ºC. Data shown are mean ± standard error. AA: Ascorbic acid; CA: Citric acid; $CaCl_2$: Calcium chloride; Hexyl: 4-hexylresorcinol.

Samples treated with CA presented hue values significantly higher than control samples during all the storage period. Whereas, for ΔE* the differences with the control samples decreased at day 5, being 0.21% the most effective concentration. In the literature, the use of acidic browning inhibitors, at similar concentration that those used in our work, finds widespread application in other fresh-cut fruits. Application of 1% and 2% AA significantly reduced surface browning and extended the shelf life of fresh-cut apple (Gil et al., 1998; Chiabrando and Giacalone, 2012) and pear (Gorny et al., 2002). Pérez-Gago et al. (2006) reported a higher effectiveness of 0.5% and 1% AA compared to Cys and Hexyl in apple slices. Moreover, Rocha et al. (1998) observed that the only application

of 0.75% AA was more effective in controlling browning of fresh-cut apple than when AA was mixed with CA and $CaCl_2$.

$CaCl_2$ also reduced enzymatic browning of fresh-cut persimmon showing higher hue and lower ΔE* than the control samples, but its effectiveness was lower than AA and CA, with lower hue values. During the first 2 days of storage, the higher concentration applied (0.62%) was the most effective in reducing browning, but the differences at the end of storage among $CaCl_2$ concentrations disappeared. Calcium treatments are generally used as firming agents, however, it can also act as an antibrowning agent due to the presence of the chlorine ion (McEvily et al., 1992; Garcia and Barrett, 2002). Although, it is a weak PPO inhibitor, Rosen and Kader (1989) reported that 1% $CaCl_2$ was more effective at inhibiting browning of fresh-cut pear than AA and CA during 7 days of storage.

Hexyl was the least effective antioxidant for fresh-cut persimmon. Its application induced damage of the tissue which translated in samples with the highest ΔE* and the lowest hue values. Moreover, an increase in Hexyl concentration resulted in an increase in ΔE* and a decrease in hue, which become more evident after 2 days of storage, resulting in higher browning than the control. Those results contrast with the behavior observed in the extract and precipitate of 'Rojo Brillante' persimmon, where a concentration of 10 mM (0.20%) Hexyl controlled enzymatic browning, resulting the best antioxidant among the ones tested *in vitro*. Differences between *in vitro* (i.e. effect of antioxidants in the extract) and *in vivo* (i.e. effect of antioxidants in cut tissue) studies were also observed in artichokes and eggplants (Ghidelli et al., 2013 a,b), indicating that application of antioxidants has an effect not only in browning reactions, but also in the metabolic activity and cell wall changes during wound-induced reactions. In the bibliography, few studies have been carried out to show the effect of Hexyl in controlling enzymatic browning of fresh-cut fruits and vegetables and results vary depending on the produce, concentrations, and the combination or not with other antioxidants. González-Aguilar et al. (2000) reported that dipping mango slices in 1mM Hexyl did not reduce the browning and decay. Higher concentrations of Hexyl (0.5, 1, 1.5, 2%) did not preserve pear wedges from browning. Moreover, samples dipped in 2% Hexyl led to darkening even more rapidly than control (Oms-Oliu et al., 2006).

Similar results were observed in fresh-cut artichoke and eggplant (Ghidelli et al., 2013 a,b). On the other hand, Hexyl has been applied successfully in a concentration range between 0.01 and 0.02% in fresh-cut apple and pear (Monsalve-González et al., 1993, 1995; Luo and Barbosa-Cánovas, 1997; Arias et al., 2007b). Moreover, several authors have suggested that the effectiveness of Hexyl in controlling browning is improved by the combination with other antioxidant agents, such as AA (Monsalve-González et al., 1993; Dong et al., 2000; Arias et al., 2007a).

3.3. Visual quality on persimmon tissue

Browning of fresh-cut persimmons treated with the antioxidants was also assessed by a sensory panel with the objective of determining whether the color differences observed instrumentally could be observed visually. Figure 3 presents the time of fresh-cut persimmon to reach the limit of marketability for the different antioxidant treatments. Control samples and persimmon slices treated with $CaCl_2$ and Hexyl were evaluated below the limit of marketability, even by day 1 of storage, except 0.01% Hexyl and 0.6% $CaCl_2$ that reached the limit at day 1 and 2, respectively. Whereas, samples treated with AA and CA were evaluated as very good during the first 2 storage days. Samples treated with 2.25% AA were reached the limit of marketability after 5 days of storage, whereas at 1.12% AA the samples reached 7 days of storage at 5 ºC. Application of 0.21% CA also maintained the marketability during 7 days of storage. In general, higher concentrations of AA and CA had lower shelf life than lower concentrations. These results correlated with color parameters that showed AA and CA as the most effective treatments in controlling browning (Figs. 1 and 2).

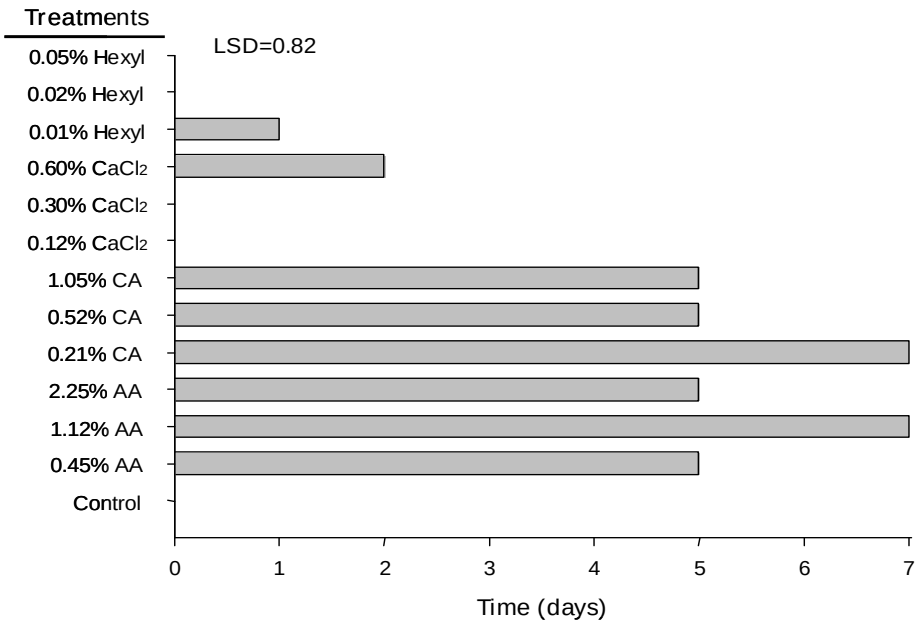

Fig. 3. Time of storage at 5 ºC of fresh-cut persimmon 'Rojo Brillante' to reach the limit of marketability for the different antioxidant treatments. LSD value at $p \leq 0.05$. AA: Ascorbic acid; CA: Citric acid; $CaCl_2$: Calcium chloride; Hexyl: 4-hexylresorcinol.

The visual ranking confirmed the result obtained in the color analysis (data not shown). The control and the samples treated with Hexyl were evaluated with the highest degree of browning; whereas, samples treated with AA and CA were ranked with the lowest degree of browning during the storage period.

4. Conclusion

The effectiveness of an antioxidant depends on many factors, as it can be the application system, the pH, the substrate of application, storage condition etc. In *in vitro* studies, Hexyl was the best treatment reducing browning in both the extract and precipitate. However, *in vivo*

trials with fresh-cut 'Rojo Brillante' persimmon, Hexyl-treated samples resulted as the most browned, indicating that application of antioxidants has an effect not only in browning reactions, but also in the metabolic activity and cell wall changes during wound-induced reactions.

AA and CA were the most effective treatments in reducing enzymatic browning of fresh-cut 'Rojo Brillante' persimmon, reaching the limit of marketability in the range of 5-7 days. In particular, concentration of 1.12% AA and 0.21% CA seemed to be the most effective in controlling enzymatic browning of fresh-cut 'Rojo Brillante' persimmon. $CaCl_2$ also contributed to extend the shelf life of persimmon pieces, however its effectiveness was lower than AA and CA.

Future work will require a study of the effect of these antioxidants on the sensory quality of 'Rojo Brillante' persimmon and their combination with other technologies, such as modified atmosphere packaging and edible coatings.

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Chapter 4

Novel approaches to control enzymatic browning of fresh-cut artichoke: Effect of a soy protein-based edible coating and modified atmosphere packaging

CHRISTIAN GHIDELLI [1], MILAGROS MATEOS [2], CRISTINA ROJAS-ARGUDO [1] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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Abstract
A soy protein isolate (SPI):Beeswax (BW) edible coating was optimized based on BW and cysteine (Cys) content to reduce enzymatic browning of fresh-cut artichoke. The effect of this optimized coating combined with different modified atmospheres (MA) on extending shelf life of cut artichokes was studied during storage at 5 ºC. MAs were obtained by fluxing two gas mixtures (MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2) or by conventional passive MA (MA-P). Atmospheric conditions were used as control. The use of 0.3% Cys combined with a SPI-BW edible coating (40% BW, dry basis) helped controlling enzymatic browning and improved the quality of fresh-cut artichokes, which reached a 4-day commercial shelf life without providing off-odors. The combination of the coating with MAs did not extend the shelf life of artichoke slices, but helped maintain the antioxidant capacity of the product as compared to control packaging conditions.

Keywords: Minimally processed artichokes; soy protein edible coating; superatmospheric oxygen packaging; antioxidant capacity.

1. Introduction

Artichoke cv. Blanca de Tudela is one of the main cultivars grown in Spain. The nutritional benefits and healthy gastronomic properties attributed to artichoke have increased its demand, making it necessary to find appropriate postharvest technologies that extend its distribution range without negatively affecting its marketability to consumers. Processing operations, such as washing, removing external leaves, slicing, and packaging, can offer clear advantages for artichoke commercialization. However, these operations involve quality deterioration provoked by factors such as water loss, softening, microbial contamination, increase of respiration, and enzyme activity. Among them, enzymatic browning is the major problem that shortens the shelf life of fresh-cut artichoke (Amodio et al., 2011). The control of enzymatic browning can be achieved by the combination of chemical and physical methods, such as the use of antioxidant agents, modified atmosphere packaging (MAP) and a proper temperature control.

For ‘Blanca de Tudela’, artichoke heads were prepared by removing the inedible parts (leaves, stalks, and out bracts). The use of MAP with

reduced O_2 (5-10 kPa) and/or elevated CO_2 (5-18 kPa) levels had little or no effect on the visual quality if compared to storage under normal atmospheric conditions. In all cases, artichokes were considered acceptable after 8 days (Gil-Izquierdo et al., 2002) and 10 days of storage at 4-5 ºC (Giménez et al., 2003). However, the samples stored under the 2.8 kPa O_2 and 26.3 kPa CO_2 conditions presented a shorter shelf life due to the presence of necrotic zones caused by anoxic conditions (Giménez et al., 2003). The different types of MAP studied affected the vitamin C and phenolic content (Gil-Izquierdo et al., 2002) and the microbial quality (Giménez et al., 2003). The food safety analysis showed that the microbial counts in those batches where the equilibrium atmosphere was anaerobic were below the legal limit, whereas some batches with an acceptable sensory quality were above it, which indicates the need to look for alternatives to reach acceptable sensory and microbial quality. Some studies have proposed the use of elevated O_2 concentrations as an alternative to low O_2 atmosphere in order to reduce polyphenol oxidase enzyme (PPO) activity, inhibit anaerobic fermentation, control microbial growth and maintain the fresh-like quality of some fresh-cut products. The effectiveness of superatmospheric O_2 treatment, however, is dependent on factors such as type of commodity, temperature, storage duration, etc. (Kader and Ben-Yehoshua, 2000).

The process of cutting the edible part of artichoke into wedges and slices significantly increases browning reactions as compared to minimally processed artichoke heads, reducing the shelf life of this product. Recent works have found that, from a wide range of compounds, cysteine (Cys) was the most effective antioxidant for fresh-cut ‘Blanca de Tudela’ (Ghidelli et al., 2013) and ‘Catanese’ (Amodio et al., 2011) artichoke. However, the application of this antioxidant did not provide sufficient shelf life for commercialization.

A recent approach to prolong the shelf life of fresh-cut fruits and vegetables is the use of edible coatings alone or combined with MAP. Edible coatings can provide a semipermeable barrier to gases and water vapor by reducing respiration, enzymatic browning and water loss (Pérez-Gago et al., 2005), and their protective function may also be enhanced with the addition of ingredients such as antioxidants. The basic ingredients of edible coatings are proteins, polysaccharides, and lipids. Del Nobile et al. (2009) showed that a sodium alginate coating

containing citric acid had the best performance extending the shelf life of artichoke heads, although it was very limited. Among proteins, soy protein isolate (SPI) coatings have been able to preserve freshness of apple slices (Kinzel, 1992), control browning in potato slices, and reduce moisture loss in carrots and apple slices (Shon and Haque, 2007). Therefore, the aim of this work was to: (1) develop a SPI-based edible coating containing Cys as an antioxidant and (2) study the combined effect of this coating with MAP, including superatmospheric O_2 conditions, on postponing enzymatic browning of fresh-cut artichoke 'Blanca de Tudela'.

2. Materials and methods

2.1. Materials

Beeswax (BW) (Brillocera, S.A., Valencia, Spain) was selected as the lipid component of the soy protein isolate (SPI) emulsion film. SPI (SUPRO 760 IP) was supplied by Solae (Ieper, Belgium). Food-grade glycerol was from Panreac Quimica, S.A. (Barcelona, Spain). Cys was from Sigma-Aldrich (Barcelona, Spain).

2.2. Coating formulations

To accomplish the objectives of this work, two experiments were conducted with coating formulations varying in BW and Cys content. In the first set of coatings, the BW content of the formulations was 20% (dry basis, db) and the Cys content was 0.1, 0.3, 0.5% (wet basis, wb). In the second experiment, the coating was 40% (db) BW and 0.3% (wb) Cys. The formulations were prepared with a total solid content of 7.5 and 10% (w/v) for the first and second experiment, respectively. Table 1 shows the composition of the coatings for both experiments.

Table 1. Edible coating formulations based on soy protein isolate (SPI), Beeswax (BW) and Cysteine (Cys).

Ingredients	Experiment 1	Experiment 2
BW (%, w/w, dry basis)	20	40
Cys (%, w/v, wet basis)	0, 0.1, 0.3, 0.5	0.3
Total solid content (%,w/v)	7.5	10

SPI:glycerol ratio 2:1.

To prepare the coatings, aqueous solutions of 5 and 7% (w/v) SPI for the first and second experiment respectively, were prepared and denatured for 30 min in a 90 ºC water bath. Glycerol was added as plasticizer at a SPI to glycerol ratio of 2:1 and this ratio was kept constant throughout the study. The BW was added to the hot SPI-glycerol mixture at the selected concentration. Samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland) for 4 min at 30,000 rpm. After homogenization, the emulsions were placed in an ice bath to prevent further denaturation of the protein and to crystallize the lipid particles. Finally, the antioxidant was incorporated into the emulsion coating by magnetic agitation.

2.3. Preparation of artichokes

Artichokes (*Cynara scolymus* L., cv. Blanca de Tudela) were purchased at a local market (Valencia, Spain) and were stored at 5 ºC for 24 h until processing. After washing, artichoke external bracts, leaves and stalk were removed. Artichoke hearts were cut into slices (approximately 5 mm wide) using a sharp stainless-steel knife. A maximum of 15 artichokes were processed at the same time to minimize their exposure to oxygen and the whole process was carried out in a temperature-controlled room at 10±1 ºC under suitable hygienic conditions.

2.4. Application of edible coatings

Artichoke slices were dipped in the coating solutions or the aqueous solutions of the antioxidants for 3 min. As a control, samples were dipped in a water solution under similar conditions. An additional control was used in the first experiment by dipping samples in a SPI:BW coating without the antioxidant added. After draining and drying under cold conditions, 4 pieces (80±5 g) were placed in polypropylene trays (17.4 x 12.9 x 3.6 cm, Ilpra Systems, Barcelona, Spain) and were heat-sealed with microperforated polypropylene film (35 µm P-Plus film, 35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure that the atmosphere in the tray was not modified and to study only the effect of the treatments, the polypropylene films were also perforated with a needle (4 perforations of 1 mm in diameter). Samples were stored for 7 days at 5 ºC.

2.5. Modified atmosphere packaging

Four artichoke slices (80±5 g), either dipped into the SPI+0.3% Cys coating selected from the second experiment or uncoated (water dipped), were placed in the polypropylene trays and heat-sealed with the 35 µm P-Plus polypropylene film (35 PA 200) that had an O_2 transmission rate of 1,100 cm^3 m^{-2} day^{-1}, CO_2 transmission rate of 30,000 cm^3 m^{-2} day^{-1} at 25 ºC and 0% RH, and moisture vapor transmission rate of 0.9 g m^{-2} day^{-1} (Amcor Flexibles, Barcelona, Spain). MA conditions were obtained by flushing the trays with two gas mixtures (MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2, with the balance being N_2) or by conventional storage in atmospheric conditions with the same film to reach a passive MA (MA-P). For the control, the film was perforated with a needle (4 perforations of 1 mm in diameter) to ensure that the gas composition within the package remained near ambient oxygen concentration (Control). Thermosealing was done in an ULMA-Smart 300 packing machine (Oñati, Spain). All the samples were stored at 5 ºC for quality evaluation.

2.6. Headspace gas analysis

The gas composition in the package headspace during storage was determined in a gas chromatograph (GC valve ThermoFinnigan, Milan, Italy) equipped with a thermal conductivity detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). The temperatures of the injector, oven, and detector were 125, 35, and 180 ºC, respectively. Helium was used as a carrier gas at a flow rate of 22 ml min^{-1}. One ml of the gas sample from the headspace atmosphere of 5 trays per treatment was measured. Data are expressed in kPa of CO_2 and O_2.

2.7. Color measurement

Color measurements were made with a Minolta (Model CR-300, Ramsey, N.Y., USA) on 12 artichoke pieces per treatment and sampling day using the CIE L*a*b* color space. Each measurement was taken randomly at three different locations of each sample piece. A standard white calibration plate was employed to calibrate the equipment.

2.8. Sensorial analysis

During storage, artichoke pieces were evaluated visually by 10 judges. Each treatment was coded, presented in random order and evaluated based on general visual appearance using a scale, where: 9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny *et al.*, 1999). A color photograph of the samples rated with this scale was provided to the judges.

2.9. Determination of antioxidant capacity

The antioxidant capacity of fresh-cut 'Blanca de Tudela' artichokes was studied by the determination of the free radical scavenging effect of antioxidants on 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical. Extraction was performed following the method of Chen et al. (2008) with minor modifications. Briefly, 2 g of frozen sample (-80 ºC) was mixed with 30 ml of 80 ml/100 ml methanolic solution to be then homogenized at 20,000 rpm for 2 min (Ultraturrax, IKA, Staufen, Germany), followed by boiling in a water bath for 20 min to inactivate the PPO. After the extraction of the homogenate with an ultrasonic machine for 15 min at room temperature, the homogenate was centrifuged at 10,000 rpm for 20 min and at 5 ºC. The resultant supernatant was then filtered and a second extraction was done. The two supernatants were used as the extract to analyze the antioxidant capacity.

For the measurement, 75 µl of extract was pipetted into 225 µl of $DPPH^{\bullet}$ (24 ppm) to start the reaction and stored in the dark at room temperature for 20 min. Change in absorbance was measured at 520 nm using a multiplate reader (Multiskan Spectrum, Thermo Fisher Scientific, Finland). The $DPPH^{\bullet}$ radical scavenging activity was expressed as effective concentration (EC_{50}), being the amount of fresh-cut artichoke required to lower the initial $DPPH^{\bullet}$ concentration by 50% (g/g $DPPH^{\bullet}$), thus lower EC_{50} values mean higher antioxidant capacity.

2.10. Statistical analysis

Statistical analysis was performed with STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). Specific differences between means were determined by the least significant difference (LSD) applied after the analysis of variance (ANOVA). Significant differences were defined at $p \leq 0.05$.

3. Results and discussion

3.1. Effect of SPI:BW:Cys edible coatings on the color of fresh-cut artichoke

Two experiments were carried out to optimize the SPI-based coating formulation (Table 1). Fig. 1 shows the effect of the coatings and the aqueous Cys solutions from experiment 1 on color parameters L*, a*, and b* of artichoke pieces stored for 7 days at 5 ºC. The increase in enzymatic browning during storage was accompanied by an increase in a* and a decrease in L* and b* values. Application of Cys reduced enzymatic browning (higher L* and lower a* values), and also resulted in higher b* values (yellowness), which increased as the Cys concentration increased. The yellow color that developed on the surface of cut artichoke by Cys application has been attributed to the possible formation of a Cys-copper complex (Ghidelli et al., 2013).

The artichoke pieces dipped into the SPI:BW coating without antioxidant did not show significant differences as compared to the control samples (water), having the lowest L* and the highest a* values among the treatments tested. The application of protein-based coatings has been used in fresh-cut products, mainly because of their low permeability to O_2 and CO_2 (Pérez-Gago et al., 2006). However, the effectiveness of protein coatings depends on the nature of the cut vegetable or fruit. A SPI coating has proven more effective than sour whey protein (SWP) and calcium caseinate coatings at controlling the browning of potato slices, whereas the SWP coating was the most effective for apple slices (Shon and Haque, 2007). Similarly, whey protein isolate (WPI):BW emulsion coatings without antioxidants have also been found to be more effective than hydroxypropyl methylcellulose at reducing the browning of fresh-cut apples (Pérez-Gago et al., 2005). In the present work, although the application of the SPI:BW coating was not effective at controlling the browning of cut artichokes, the addition of Cys to this coating improved the quality of minimally processed artichokes as compared to the application of the antioxidant in an aqueous solution. A similar effect has been reported in fresh-cut apples for WPI-based (Pérez-Gago et al., 2006) and alginate and gellan-based coatings (Rojas-Graü et al., 2008) through the incorporation of Cys into the formulation.

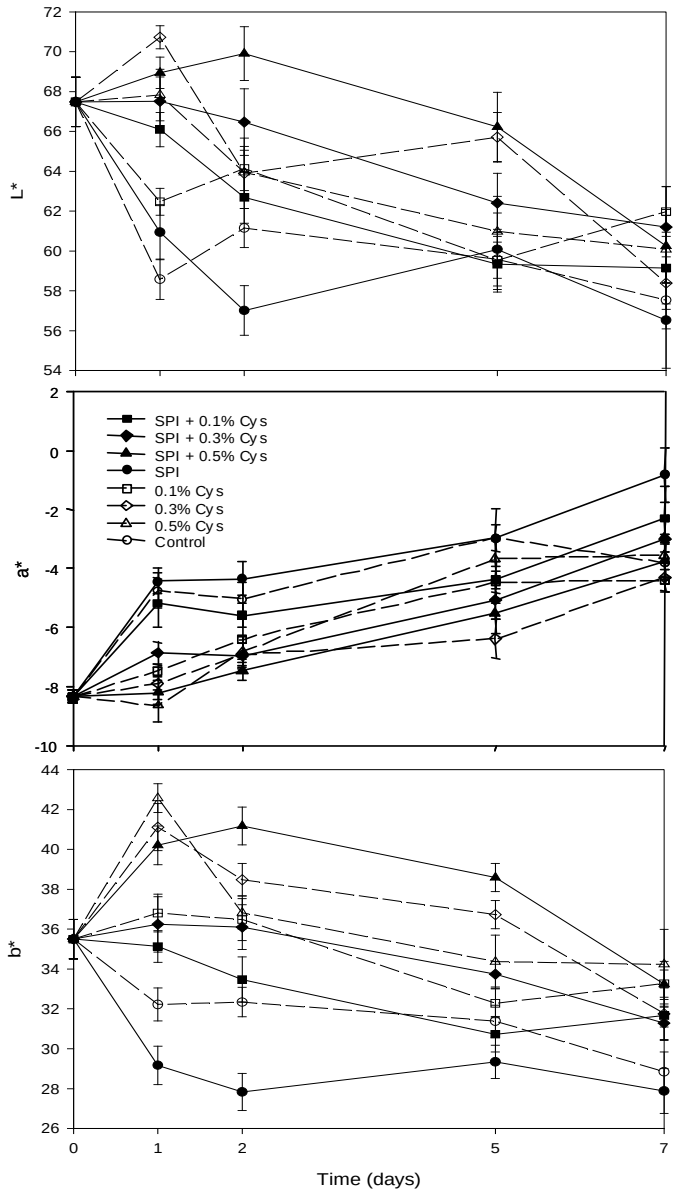

Fig. 1. Effect of Cys alone or incorporated to the soy protein isolate (SPI)-based edible coating on the color parameters (L*, a*, b*) of fresh-cut 'Blanca de Tudela' artichokes stored at 5 ºC. Vertical bars represent standard errors. Coating formulations from Table 1-Experiment 1.

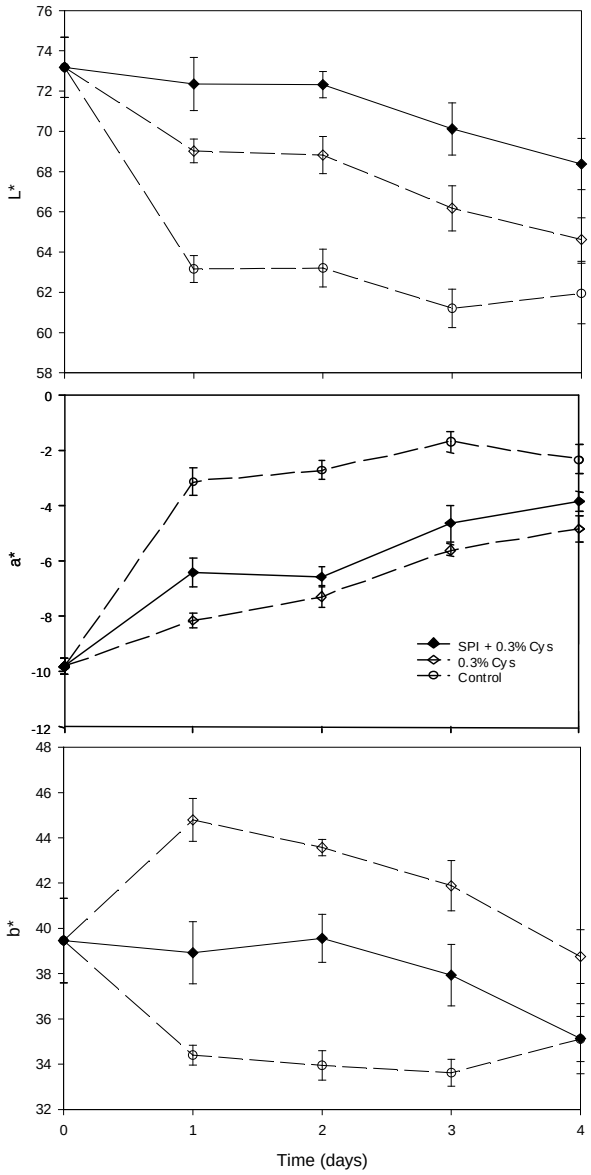

Fig. 2. Effect of Cys alone or incorporated to the soy protein isolate (SPI)-based edible coating on the color parameters (L*, a*, b*) of fresh-cut 'Blanca de Tudela' artichokes stored at 5 ºC. Vertical bars represent standard errors. Coating formulations from Table 1-Experiment 2.

The most effective Cys concentrations for reducing browning were 0.3 and 0.5% (wb), with minor or no significant differences found between them in L* and a* values, respectively. However, those samples prepared with the higher Cys concentration presented off-odors, which are characteristic of sulphur compounds. Therefore, the lower concentration was selected to optimize the coating formulation. Optimization implied an increase in the BW content from 20 to 40% (db) to reduce the yellow color that resulted from the Cys application to artichoke tissue. This coating (SPI+0.3% Cys) significantly reduced enzymatic browning of minimally processed artichoke, achieving higher L* and lower a* values than the control (water dipped) and the 0.3% Cys-treated samples, and also diminished the yellow color of the samples, as reflected by the lower b* values of the coated samples *vs.* the Cys-treated samples (Fig. 2).

3.2. Effect of SPI:BW:Cys edible coatings on the visual quality of fresh-cut artichoke

In the first experiment, the samples treated with 0.1% Cys (in the coating and aqueous solution) and the control samples fell bellow the limit of marketability within 1 day of storage, while those treated with 0.3% reached 2 days of marketability (Fig. 3). Although the addition of Cys to the SPI:BW coating did not significantly contribute to improve the visual quality of artichoke slices, the samples dipped into the SPI+0.5% Cys coating reached the limit of marketability after 5 day storage at 5 ºC.

Similar behavior was observed in the second experiment (Fig. 4). When considering that the coating tested in this experiment had 0.3% Cys, the increase of the BW content from 20 to 40% (db) in the SPI emulsion contributed to prolong the shelf life of the artichoke slices, with them reaching the limit of marketability within 4 days of storage at 5 ºC, whereas the application of the antioxidant aqueous solution had a lower limit of 2-3 days of storage. A similar 4-day shelf life was achieved in 'Blanca de Tudela' artichoke slices dipped into 0.5% Cys aqueous solutions (Ghidelli et al., 2013). Therefore, this SPI-based coating slightly improved as the formulation had a lower Cys concentration, thus reducing the risk of altering artichoke flavor. Del Nobile et al. (2009) reported that minimally processed artichoke heads dipped into sodium alginate-based edible coating containing citric acid reached 3 days of

storage, whereas the shelf life of the control ranged from 1 to 2 days at 5 ºC.

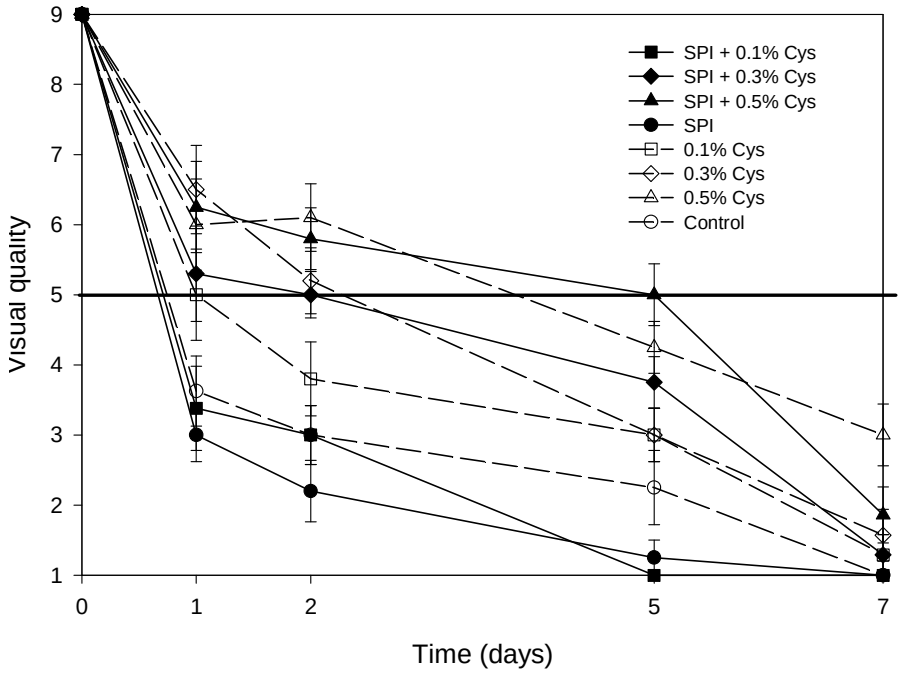

Fig. 3. Effect of Cys alone or incorporated to the soy protein isolate (SPI)-based edible coating on the visual quality of fresh-cut 'Blanca de Tudela' artichokes stored at 5 ºC. Visual quality was based on the visual scale: 9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible. Vertical bars represent standard error. Coating formulations from Table 1-Experiment 1.

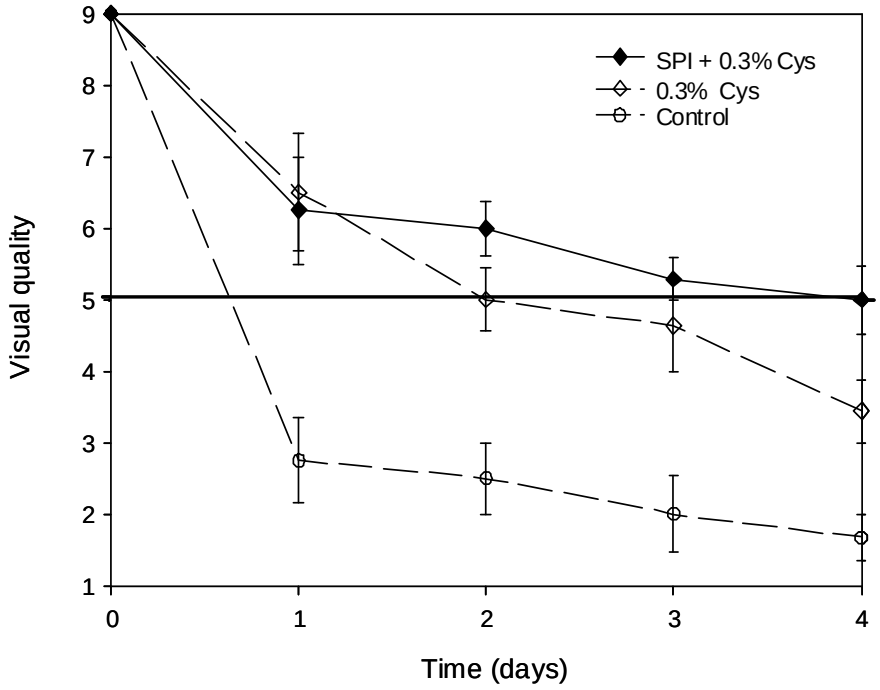

Fig. 4. Effect of Cys alone or incorporated to the soy protein isolate (SPI)-based edible coating on the visual quality of fresh-cut 'Blanca de Tudela' artichokes stored at 5 ºC. Visual quality was based on the visual scale: 9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible. Vertical bars represent standard errors. Coating formulations from Table 1-Experiment 2.

3.3. Effect of modified atmosphere packaging and optimized SPI-based edible coating on the headspace gas composition

Fig. 5 shows the changes in the headspace gas composition of the coated and uncoated fresh-cut artichokes packed under different MAs for 3 days of storage at 5 ºC. As expected, a sharp decrease in O_2 and increase in CO_2 concentration was observed during storage, except for those samples which were packed in the perforated film (Control), where

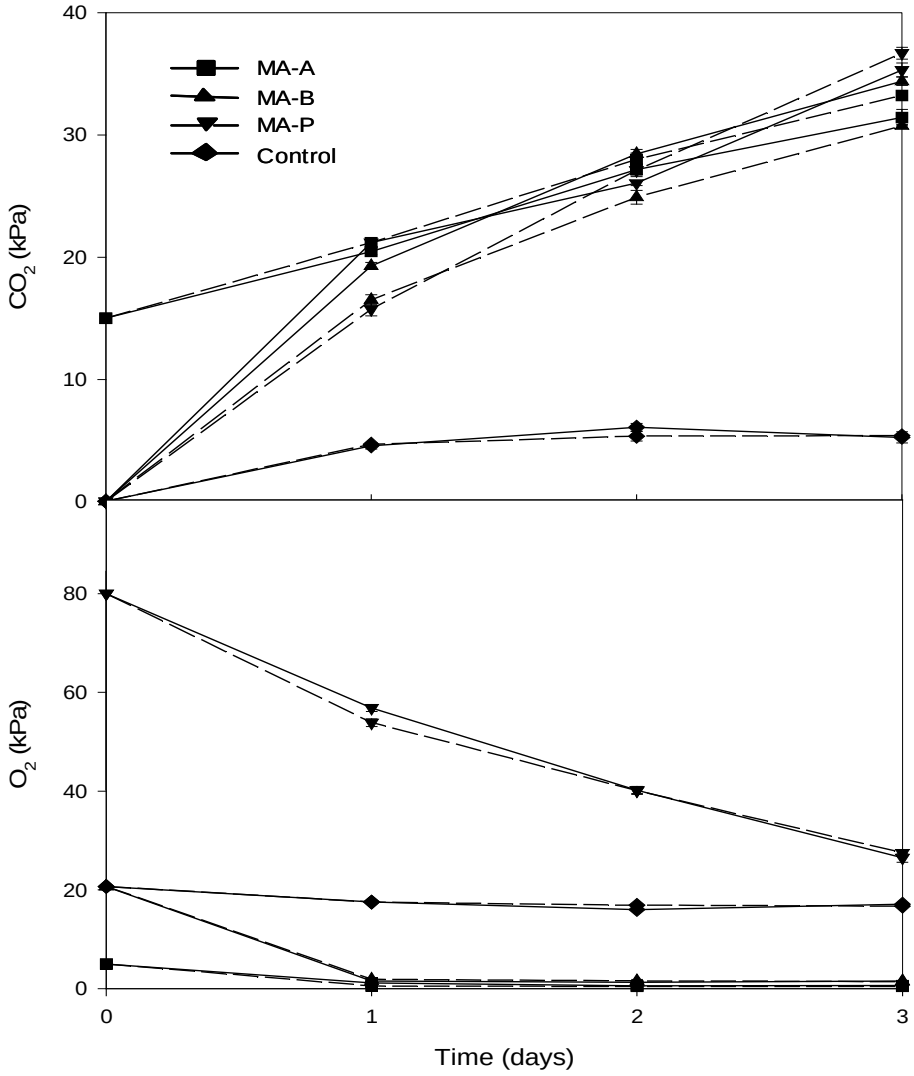

Fig. 5. Carbon dioxide and oxygen concentrations in the package headspace of fresh-cut 'Blanca de Tudela' artichokes stored under different modified atmospheres (MA) at 5 ºC. Vertical bars represent standard errors. Solid and dashed lines represent coated (SPI+0.3% Cys) and uncoated (water dipped) samples, respectively. MA-A (15 kPa CO_2 + 5 kPa O_2); MA-B (80 kPa O_2); MA-P (21 kPa O_2 + 0.03 kPa CO_2); Control (atmospheric conditions during storage).

O_2 was close to atmospheric values and CO_2 reached equilibrium after 1 day with a value of 4.6±0.4 kPa.

The O_2 concentrations rapidly decreased in the samples packed under MA-A (15 kPa CO_2 + 5 kPa O_2) and MA-P (ambient air) with P-Plus film, reaching the equilibrium on day 1 with a concentration below 1 kPa, whereas the CO_2 concentration constantly increased during the 3-day storage to reach a concentration of approximately 30 kPa. Gil-Izquierdo et al. (2002) classified 'Blanca de Tudela' artichoke as a cultivar with an extremely high respiration rate. Preparing artichoke heads by removing inedible parts led to a sharp decrease in O_2 and an increase in CO_2 (Gil-Izquierdo et al., 2002; Giménez et al., 2003). In these experiments, the packages with the highest film permeability reached equilibrium rapidly and the highest atmosphere modification was detected with a polypropylene film (P-Plus).

Application of superatmospheric O_2 may stimulate, have no effect, or lower the respiration rate of fresh-cut products (Kader and Ben-Yehoshua, 2000). In this experiment packaging under superatmospheric O_2 conditions (MA-B: 80 kPa O_2) decreased the O_2 concentration to 27±0.8 kPa and increased the CO_2 concentration to 36±0.5 kPa after 3 days of storage at 5 ºC, with the highest CO_2 concentration among the different packing conditions. Nevertheless, except for the Control packaging conditions, the final CO_2 concentrations reached for the different MAP conditions were between 30 and 34 kPa, irrespectively of the initial atmosphere inside the trays. The values obtained for the different packaging conditions suggest a high respiration rate of the 'Blanca de Tudela' artichoke slices.

The headspace O_2 composition for the different packaging conditions were not significantly influenced by coating application if compared to the uncoated samples (water-dipped). Likewise at the end of storage, no differences in the CO_2 concentrations were found between coated and uncoated artichokes under the Control packaging conditions, the superatmospheric condition (MA-B), and the low oxygen concentrations (MA-A). However the CO_2 concentrations of the coated artichoke packed under passive MAP conditions (MA-P) were higher than in the uncoated samples, indicating a slightly increased respiration rate due to coating application. Oms-Oliu et al. (2008a) also described that uncoated melon exhibited a lower modification in internal atmosphere than those pieces

coated with gellan-, pectin- or alginate-based formulations at the end of storage. Among the different polymer-based coatings, gellan coating modified the gas concentrations in the package headspace atmosphere to a greater extent, and the changes were attributed to the respiratory activity of gellan-coated melon rather than to the coating gas barrier properties. In our case, the low O_2 concentration reached under MA-A and MA-P may also affect the respiratory activity of coated artichoke pieces by increasing the CO_2 level in the package headspace.

3.4. Effect of modified atmosphere packaging and optimized SPI-based edible coating on the color of fresh-cut artichoke

Fig. 6 shows the effect of the SPI-based edible coating and MAP on the L*, a*, and b* values of artichoke slices during storage at 5 ºC, while Table 2 reflects the effect of MA and coating application on the *F*-ratio values for these parameters. During storage, fresh-cut artichoke browning was accompanied by a reduction of L* and an increase of a* values. The ANOVA analysis showed that the different MA conditions had not significant effect on the L* and b* values, whereas they affected the a* values of the samples (Table 2). However, the use of the SPI-based edible coating helped prolong the shelf life of fresh-cut artichoke, showing significantly higher L* and lower a* values than uncoated artichokes (water-dipped). Coated artichokes also had higher b* values than uncoated samples due to the effect of Cys on artichoke tissue, as previously described.

Among the coated samples, those packed under passive atmosphere (MA-P) and superatmospheric O_2 (MA-B) conditions presented the lowest a* values, whereas uncoated samples under atmospheric conditions (Control) presented the highest degree of browning with the lowest L* and the highest a* values. Packaging of both coated and uncoated fresh-cut artichoke pieces under MA-A did not significantly improve the quality of minimally processed artichokes as compared to atmospheric conditions (Control). Gómez di Marco et al. (2011) reported that the best combination to reduce artichoke heads browning was the application of high O_2 concentrations (80 kPa) and lemon juice as antioxidant. However when no antioxidant was applied, the passive MA was more effective than the high O_2 atmosphere. Similarly, potato slices dipped in citric acid and packed under high O_2 partial pressures also

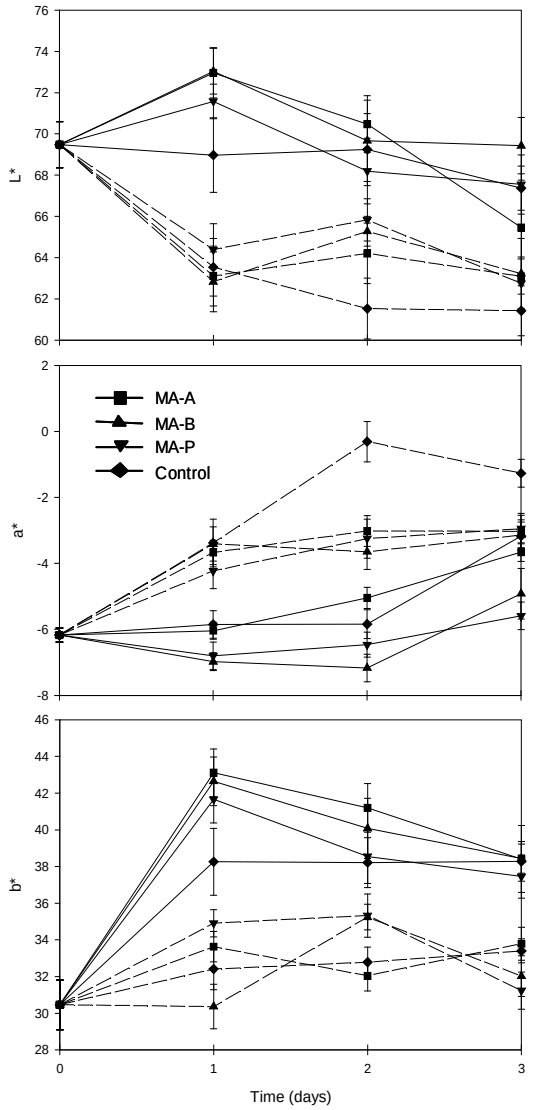

Fig. 6. Effect of SPI-based edible coating and modified atmosphere packaging on the color parameters L*, a*, and b* of fresh-cut 'Blanca de Tudela' artichokes stored at 5 ºC. Vertical bars represent standard errors. Solid and dashed lines represent coated (SPI+0.3% Cys) and uncoated (water dipped) samples, respectively. MA-A (15 kPa CO_2 + 5 kPa O_2); MA-B (80 kPa O_2); MA-P (21 kPa O_2 + 0.03 kPa CO_2); Control (atmospheric conditions during storage).

showed a reduction in PPO activity (Limbo and Piergiovanni, 2006). However, Poubol and Izumi (2005) reported that the application of superatmospheric O_2 concentrations (60 kPa) to fresh-cut mango showed a similar or higher degree of browning than the use of ambient air conditions at 5 ºC. Moreover, a decrease of the antioxidant effect due to dipping pear wedges in a thiol-containing solution has been observed when samples were packed in 70 kPa O_2 or low O_2 atmospheres (2.5 kPa O_2 + 7 kPa CO_2) with respect to those packed under passive MA (Oms-Oliu et al., 2008b, 2008c).

Table 2. Analysis of variance on the color index (L*, a*, b*), visual quality and antioxidant capacity (EC_{50}) of coated and uncoated fresh-cut 'Blanca de Tudela' artichokes.

	F-ratio				
	L*	a*	b*	Visual quality	EC_{50}
Treatments					
A: Atmosphere conditions	1.32^{NS}	6.29***	0.98^{NS}	4.39***	12.35***
B: Coating application	100.30***	116.32***	166.15***	87.62***	0.37^{NS}
Interactions					
AB	1.33^{NS}	2.24^{NS}	1.81^{NS}	2.10^{NS}	6.19***

F-ratios are shown for the sources of variations.
** Significant *F*-ratios at $p \leq 0.01$.
*** Significant *F*-ratios at $p \leq 0.001$.
NS = not significant.

3.5. Effect of modified atmosphere packaging and optimized SPI-based edible coating on the visual quality of fresh-cut artichoke

The visual quality of fresh-cut artichokes was significantly influenced by both the packaging conditions and dipping in the SPI-based edible coating (Table 2). Uncoated samples were evaluated to be below the limit of marketability by day 1 of storage (Fig. 7). The samples dipped in the SPI-based edible coating and packed under MA-A reached that limit after one day of storage. The maximum commercial shelf life achieved was 3 days of storage with the coated samples packed under superatmospheric O_2 (MA-B) and atmospheric conditions (Control), whereas those samples

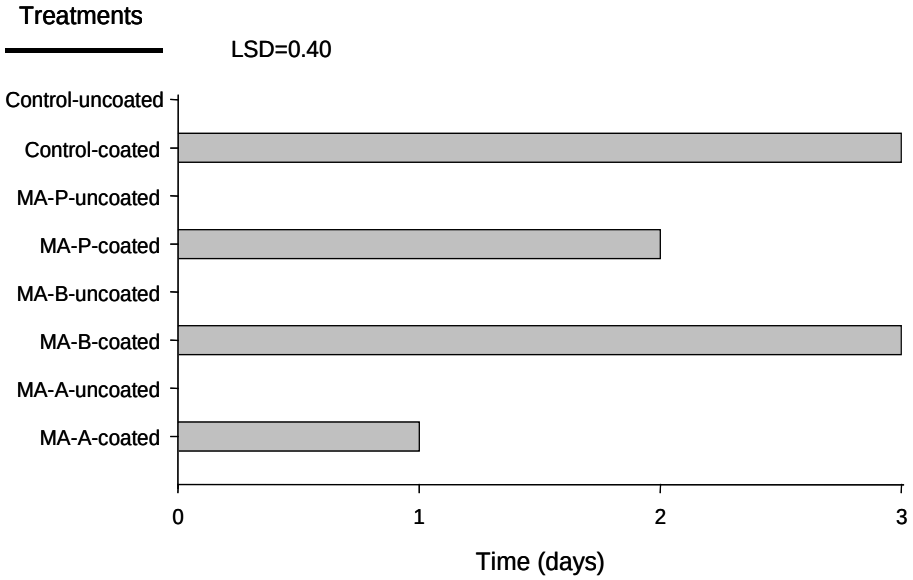

Fig. 7. Effect of SPI-based edible coating and modified atmosphere (MA) packaging on the marketable shelf life of 'Blanca de Tudela' artichoke slices stored at 5 ºC. Shelf life was define as the number of days to reach the limit of marketability (score of 5). MA-A (15 kPa CO_2 + 5 kPa O_2); MA-B (80 kPa O_2); MA-P (21 kPa O_2 + 0.03 kPa CO_2); Control (atmospheric conditions during storage).

in the passive atmosphere (MA-P) reached the limit of marketability within 2 days. Therefore, the combination of the SPI-based coating and the different MA conditions did not extend the shelf life of 'Blanca de Tudela' artichoke. These results contrast with the color analysis, which showed the passive modified atmosphere (MA-P) as having low a* and high L* values at the end of the storage (Fig. 6). These differences could be attributed to the heterogeneous surface of the artichokes slices.

3.6. Effect of modified atmosphere packaging and optimized SPI-based edible coating on the antioxidant capacity of fresh-cut artichoke

The antioxidant capacity of 'Blanca de Tudela' artichoke slices was measured on storage days 1 and 3. Since the results followed the same

trend for both storage periods, the day 3 data are only presented in Fig. 8. The ANOVA analysis showed that antioxidant capacity was significantly affected by packaging conditions, but was not affected by coating application (Table 2). Oms-Oliu et al. (2008d) also indicated that the use of alginate, pectin and gellan-based coatings containing N-acetylcysteine and glutatione did not seem to substantially contribute to enhancing the antioxidant capacity of fresh-cut pears, although the use of antioxidants maintained the antioxidant capacity of coated and uncoated pears.

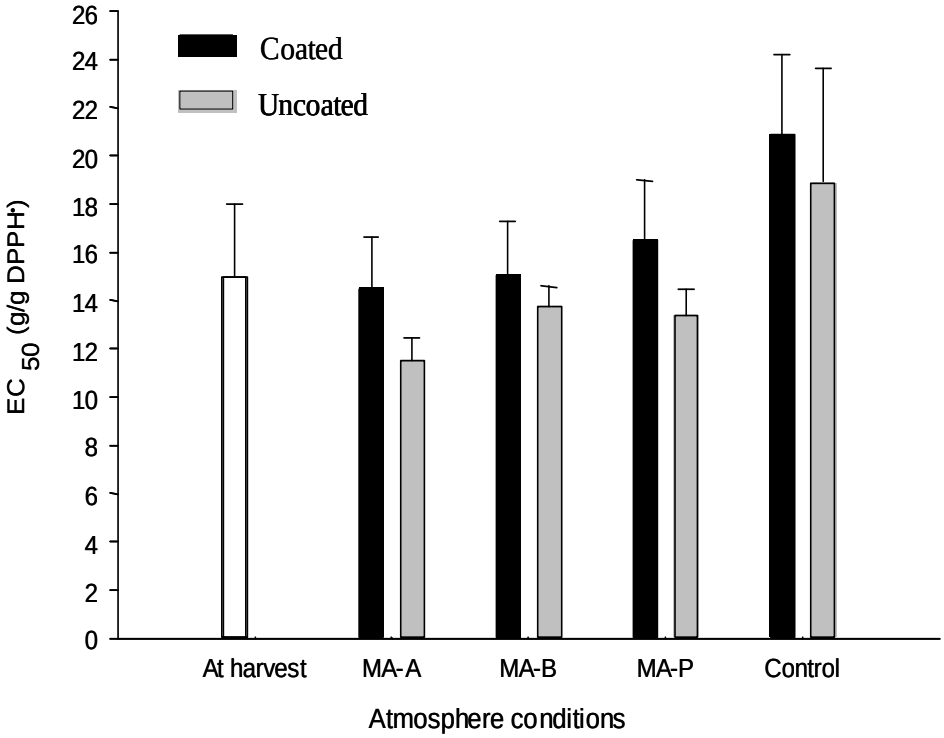

Fig. 8. Effect of SPI-based coating and modified atmosphere (MA) packaging on the antioxidant capacity (EC_{50}) of fresh-cut 'Blanca de Tudela' artichokes after 3 days of storage at 5 ºC. Vertical bars represent standard deviations. MA-A (15 kPa CO_2 + 5 kPa O_2); MA-B (80 kPa O_2); MA-P (21 kPa O_2 + 0.03 kPa CO_2); Control (atmospheric conditions during storage).

The antioxidant capacity of fresh artichoke before processing, expressed as EC_{50} (amount of sample required to reduce the initial $DPPH^{\bullet}$ by 50%) was 15.00±3.01 (g/g $DPPH^{\bullet}$). Although some differences were found among treatments, the antioxidant capacity of fresh-cut artichoke was maintained after processing and storage for those samples packed under MA conditions (MA-A, MA-B and MA-P), whereas packaging under atmospheric conditions (Control) significantly decreased the antioxidant capacity (higher EC_{50}) (Fig. 8). Gil-Izquierdo et al. (2002) observed that the content of phenolic compounds in 'Blanca de Tudela' artichokes was much higher in the internal than in the external portions. After storage, internal head portions had a higher phenolic content if compared with the value at harvest, whereas external portions had a lower content, which correlated with the use of phenolic compounds as PPO substrates in enzymatic browning. Storage with different MAP materials increased or maintained the total phenolic content of the internal and external portions, respectively. In our case, samples were processed as slices, meaning greater surface exposure to oxidation, which might explain the lower antioxidant capacity of the samples stored under atmospheric conditions (Control).

Very little information is available about the effect of high O_2 concentrations on the antioxidant capacity of fresh-cut commodities. Day (2001) reported that high O_2 atmosphere provoked a decrease of phenolic compounds in fresh-cut lettuce as compared with air or low O_2 MA, due to the loss of certain water soluble phenolic compounds. In fresh-cut pears and melon, samples stored in high O_2 MA also presented lower antioxidant capacity than those under low O_2 and high CO_2 MA (Oms-Oliu et al., 2008a, 2008b). However in 'Salambo' artichoke heads, the combination of antioxidants and high O_2 atmosphere (80 kPa) showed an increase in total phenols as compared to passive MA (Gómez di Marco et al., 2011). In our case, despite some differences being observed among the different MA conditions, probably attributed to the biological variation of samples, no change in antioxidant capacity was observed after 3 days of storage at 5 ºC in fresh-cut 'Blanca de Tudela' artichokes under the different MA conditions.

4. Conclusion

The use of cysteine at a concentration of 0.3%, combined with a SPI-BW edible coating, contributed to the control of enzymatic browning and improved the quality of fresh-cut artichokes, reaching 4 days of commercial shelf life at 5 ºC without off-odors. Combining this coating with the different MA packaging conditions studied in this work did not extend the shelf life of 'Blanca de Tudela' artichoke slices, but it helped with maintaining the antioxidant capacity of the product. Given the high degree of perishability of untreated fresh-cut artichoke, a 4-day commercial period could be considered adequate for the distribution of sliced produce to local markets. However, additional studies are required to enhance the quality and appearance of fresh-cut artichokes and further extend their shelf life.

Acknowledgements

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Chapter 5

Development of soy protein-beeswax edible coatings with antioxidant activity: effect on enzymatic browning of fresh-cut eggplants

CHRISTIAN GHIDELLI [1], MILAGROS MATEOS [2], SANCHÍS ELENA[1], CRISTINA ROJAS-ARGUDO [1] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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Abstract

Commercialization of fresh-cut eggplants is limited by enzymatic browning due to the oxidation of the phenolic compounds. The combination of edible coatings with antioxidant agents helps to reduce the respiration rate, weight loss and enzymatic browning of the fresh-cut tissue. The objective of this work was to study the effect of a new edible coating combined with different antioxidant agents in controlling enzymatic browning of fresh-cut eggplants. Edible coatings were prepared with soy protein isolate (SPI) and beeswax (BW). Ascorbic acid (AA) at 1% or cystein (Cys) at 0.5 % or 1% content were incorporated in the coating formulations as antioxidants. Fresh-cut eggplants were dip-coated either in the SPI-based coating or in the aqueous solution of the antioxidants. As a control, samples were dipped in a water solution under similar conditions. Samples were packed under atmospheric conditions and stored at 5 ºC for 9 days. Changes in color (CIEL*a*b*), visual quality, texture and weight loss were evaluated during storage. Cys-treated samples, either incorporated in the coating or applied in aqueous solution, showed the highest L* and the lowest a* values and reduced tissue softening compared to control samples. An increase in Cys concentration reduced enzymatic browning of fresh-cut eggplant. 1% AA was not effective in reducing enzymatic browning of fresh-cut eggplants. At the end of the storage, weight loss was below 1% and there were not significant differences among treatments. The samples coated with the SPI coating amended with 1% Cys were ranked by the sensory panel as the less brown and they reached the limit of marketability after 9 days of storage. These results show the potential of the SPI-Cys-based edible coating in controlling enzymatic browning of fresh-cut eggplants.

Keyword: fresh-cut eggplant, enzymatic browning, edible coating, antioxidant.

INTRODUCTION

Fresh-cut fruit and vegetables have a very short shelf-life due to tissue disruption and increased metabolism. Tissue browning due to processing is one of the main causes of quality loss. The problem is especially important in fresh produces with high content in phenolic compounds, such as eggplant. The main approach to inhibit browning is

the use of antibrowning agents. Chemical inhibitors are classified depending on whether they act on the enzyme polyphenol oxidase (PPO), the substrate or the products of the PPO reaction before these can lead to the formation of dark products. In a very recent work, Pérez-Gago et al. (2009) reported that ascorbic acid (AA) and cystein (Cys) were the most effective at controlling enzymatic browning of fresh-cut eggplants as compared with citric acid, peracetic acid or 4-hexylresorcinol. AA is probably the most widely used antibrowning agent. Its activity resides in its reducing character that reduces the *o*-benzoquinones back to *o*-diphenols. However, once the AA has been completely oxidized to dehydroascorbic acid by this reaction, quinones can again accumulate and undergo browning. Similarly, Cys is also a reducing agent that inhibits enzymatic browning preventing brown pigment formation by reacting with quinone intermediates to form stable, colorless compounds (Lamikanra, 2002).

Another approach to further increase the shelf life of fresh-cut products is the use of edible coatings (Pérez-Gago et al., 2005). Edible coatings offer the possibility to extend the shelf life of fresh-cut products by providing a semi-permeable barrier to gases and water vapour, and therefore, reducing respiration, enzymatic browning and water loss (Baldwin et al., 1995). Their protective function may also be enhanced with the addition of antimicrobials, antioxidants, flavors, nutrients, etc.

The development of edible films and coatings has been focused upon barriers containing proteins, polysaccharides, and lipids, either alone or in combination. Soy protein coatings offer a high oxygen barrier and have been able to preserve freshness of apple slices (Kinzel, 1992). However, its hydrophilic nature requires the addition of lipid components to improve the moisture barrier. Beeswax (BW) is a natural wax classified as Generally Regarded as Safe (GRAS) compound, that has shown good compatibility with other coating-forming materials (Greener and Fennema, 1992). No work has been done with soy protein-based coatings to control enzymatic browning of fresh-cut eggplants. Therefore, the aim of this work was to study the effect of soy protein-BW edible coatings amended with antioxidant agents at reducing enzymatic browning on minimally processed eggplant.

MATERIAL AND METHODS

Beeswax (BW) (Brillocera, S.A., Valencia, Spain) was selected as the lipid component of the soy protein isolate (SPI) emulsion film. SPI (SUPRO 760 IP) was supplied by Solae (Ieper, Belgium). Food-grade glycerol was from Panreac Quimica, S.A. (Barcelona, Spain). Ascorbic acid (AA) was from Quimivita (Barcelona, Spain) and Cysteine (Cys) from Sigma-Aldrich (St. Louis, MO, USA).

To prepare the SPI-BW emulsion coatings, aqueous solutions of 5% (w/v) SPI were prepared and denatured for 30 min in a 90 ºC water bath. The BW was melted in the hot protein solution and glycerol was added as plasticizer. The protein plasticizer ratio was of 2 parts SPI to 1 part glycerol (dry basis, db), and this ratio was kept constant throughout the study. The BW was added to the SPI-glycerol mixture at 20% content (w/w, db). Water was added to bring the emulsion to 7.5% total solid content. Samples were homogenized with a high-shear probe mixer (Polytron, Model PT 2100, Kinematical AG Inc, Lucern, Switzerland) for 4 min at 30,000 rpm. After homogenization, the emulsions were placed in an ice bath to prevent further denaturation of the protein and to crystallize the lipid particles. Finally, AA at 1% (w/w, wet basis, wb) or Cys at 0.5 % or 1% (wb) content were incorporated into the emulsions by magnetic agitation. Table 1 shows the coating compositions.

Table 1. Edible coating composition of the soy protein isolate-beeswax edible coatings containing antioxidant agents (%, wet basis).

Components	SPI - 0.5% Cys	SPI - 1% Cys	SPI - 1% AA
SPI	3.7	3.3	3.3
BW	1.5	1.5	1.5
Glycerol	1.8	1.7	1.7
AA	0.0	0.0	1.0
Cys	0.5	1.0	0.0
Water	92.5	92.5	92.5

SPI: soy protein isolate; BW: beeswax; AA: ascorbic acid; Cys: cysteine.

Eggplants (*Solanum melongena* L., cv. Telma) were purchased at a local market (Valencia, Spain) and were stored in air at 10 ºC for 1 day until processing. Eggplants were cleaned, peeled, cut into rectangular pieces (approximately 5 cm x 3.5 cm x 1.5 cm) and dip-coated either in the SPI-BW-antioxidant coating or in the aqueous solution of the antioxidant for 3 minutes. As a control, samples were dipped in water under similar conditions. After dipping, eggplants pieces were drained and dried under cold conditions. A maximum of 15 eggplants were simultaneously processed to avoid an excessive exposure time to oxygen. A sharp stainless-steel knife was used throughout the process to reduce mechanical bruising and samples were processed in a temperature controlled room at 10 ± 1 ºC.

Eggplants pieces were then placed in polypropylene trays, heat-sealed with microperforated polypropylene films (35 µm P-Plus film, 35 PA 200, Amcor Flexibles, Barcelona, Spain) and stored for 9 days at 5 ºC. Films were additionally perforated with a needle to ensure no modification of the gas composition in the package.

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, NY, U.S.A.) on 12 eggplant pieces per treatment using the CIELAB color parameters, L*, a*, b*. Each measurement was taken at 3 locations for each sample piece.

Eggplants firmness was determined on 12 pieces per treatment by measuring the force required for an 8 mm probe to penetrate the sample to a depth of 2 mm at a speed of 5 mm/s using an Instron Universal testing machine (Model 3343, Instron Corp., Canton, MA, USA). The results were expressed as force (N).

Eggplant weight loss was determined at the end of the storage period by weighting 12 pieces per treatment. The results were expressed as the percentage loss of initial weight.

During storage, eggplant slices were evaluated visually by 10 judges. Each treatment was coded, presented in random order and evaluated based on general visual appearance using a scale where: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny et al., 1999). A color photograph of the samples rated with this scale was used by judges. Additionally, the judges were also asked to rank each sample from highest to lowest degree of browning.

Statistical analysis was performed using STATGRAPHICS 4.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.). Specific differences between means were determined by Fisher's protected least significant difference (LSD) applied after the analysis of variance (ANOVA) at $P \leq 0.05$. Significant differences for visual browning were determined by the Friedman test, which is recommended for ranking (ISO 8587:2006). Significant differences were defined at $P \leq 0.05$.

RESULTS AND DISCUSSION

Figures 1 and 2 show the effect of the coatings and the antioxidants on color L* and a* values of fresh-cut eggplants. Increased enzymatic browning of eggplant pieces during storage was accompanied by an increase in a* and a decrease in L*. In general, samples treated with Cys, either in the SPI-based coatings or in aqueous solutions, presented higher L* and lower a*values than control samples. Whereas,

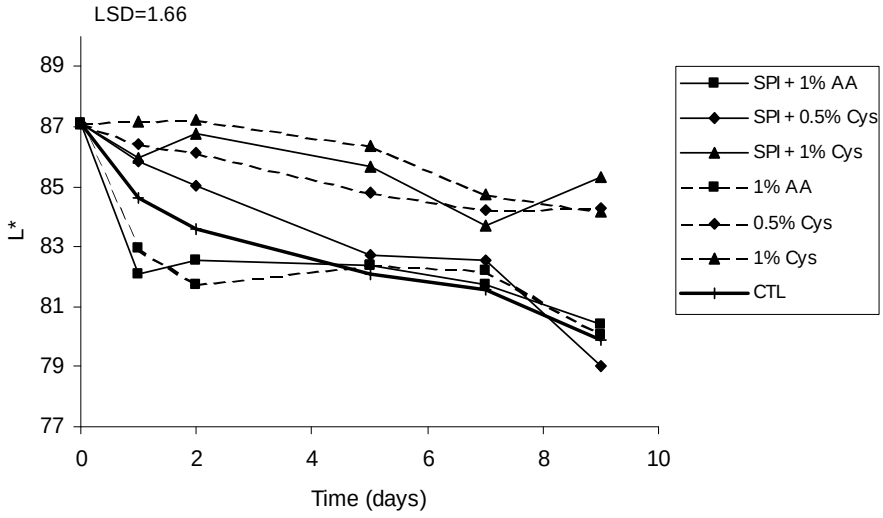

Fig. 1. Effect of antioxidants alone or incorporated to the soy protein isolate-beeswax edible coating on lightness (L*) of fresh-cut 'Telma' eggplants stored at 5 ºC. LSD value is at the 95% level.

eggplant slices treated with 1% AA presented a sharp decrease in L* and an increase in a* values at day 1 of storage. In a previous work, 0.35% and 0.88% AA aqueous solutions were effective at controlling enzymatic browning of fresh-cut eggplants (Pérez-Gago et al., 2009). However, an increase in tissue browning was observed when AA concentration increased to 1.5%. These results contrast with the behavior of AA in other cut tissues, such as apples and pears, where enzymatic browning decreases as concentration increases (Gorny et al., 2000). A similar behavior in controlling enzymatic browning has been reported with protein-based edible coatings containing AA in fresh-cut potatoes, carrots, mushrooms, and apples (Baldwin et al., 1995; Pérez-Gago et al., 2006).

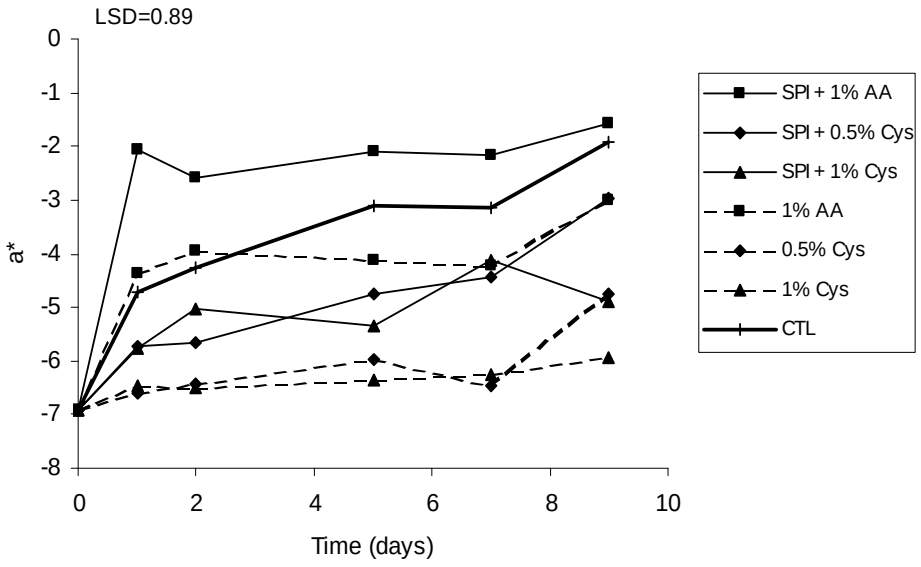

Fig. 2. Effect of antioxidants alone or incorporated to the soy protein isolate-beeswax edible coating on a* values of fresh-cut 'Telma' eggplants stored at 5 ºC. LSD value is at the 95% level.

When comparing Cys concentration, the enzymatic browning of 'Telma' fresh-cut eggplants was reduced as Cys increased from 0.5% to 1%. However, little differences on L* and a* values were observed when incorporated into the SPI-based edible coating. Contrarily, other works have reported that the incorporation of antioxidants to protein-based edible coatings resulted more effective in controlling enzymatic browning of fresh-cut produces, such as apples and persimmons, than the application of the antioxidants in aqueous solution (Pérez-Gago et al., 2005; 2006).

Browning of fresh-cut eggplants was assessed by a sensory panel with the objective of comparing if the color differences observed instrumentally could be observed visually. Visual appearance of eggplant slices, based on visual browning and general appearance, is shown in Fig. 3.

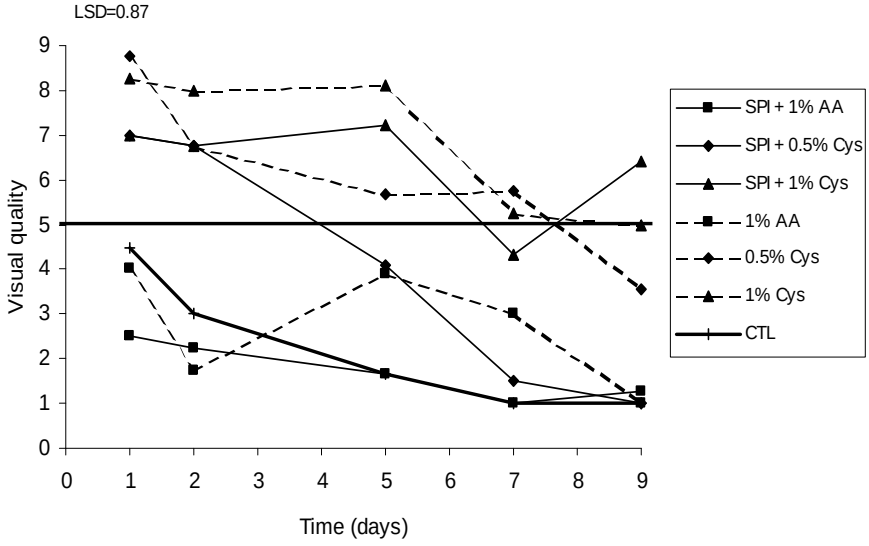

Fig. 3. Effect of antioxidants alone or incorporated to the soy protein isolate-beeswax edible coating on visual quality of fresh-cut 'Telma' eggplants stored at 5 ºC. LSD value is at the 95% level.

Samples treated with AA and control samples were evaluated below to be below the limit of marketability by storage day 1. Application of Cys, either alone or incorporated to the SPI-BW coating, improved the general visual quality of eggplant and extended the commercial shelf life to 7-9 days. Eggplant slices coated with the SPI-BW edible coating amended with 1% Cys were evaluated as less brown than the rest of the treatments. These samples reached the maximum commercial shelf life by day 9 of storage (Fig. 4).

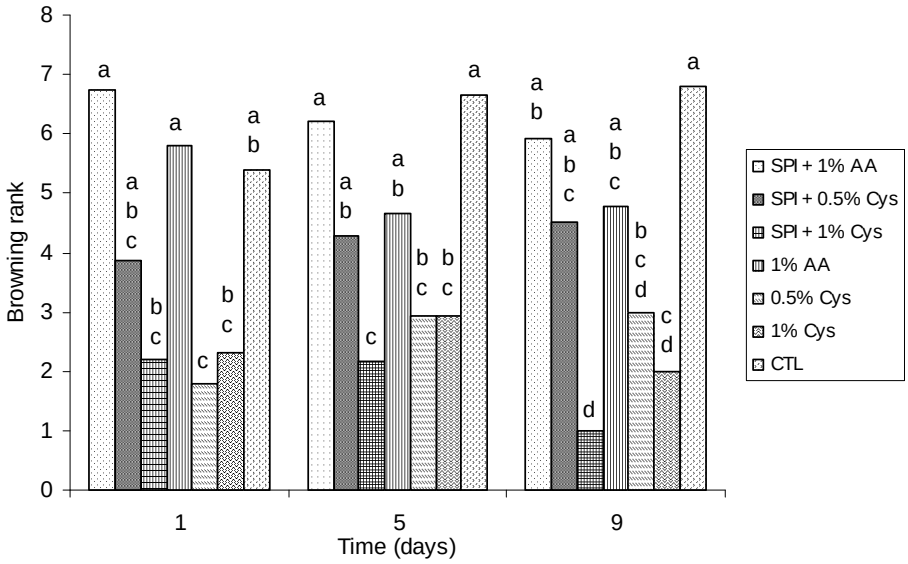

Fig. 4. Effect of antioxidants alone or incorporated to the soy protein isolate-beeswax edible coating on the visual browning of fresh-cut 'Telma' eggplant stored at 5 ºC. Judges ranked the eggplant pieces from 7 (more brown) to 1 (less brown) and were allowed to group those treatments that were considered similar. Means with the same letter are not significantly different ($p \leq 0.05$).

Texture and weight loss of fresh-cut eggplant was not affected by coating application (data not shown).

CONCLUSION

Samples treated with 1% Cys, either incorporated to the SPI coating or applied in aqueous solution, presented the best result in terms of color parameters with high L* and low a* values. AA at 1% was not effective at controlling enzymatic browning compared to control samples. In the visual assessment, the samples coated with the SPI-BW coating amended with 1% Cys were significantly less brown than the rest of the treatments and reached a commercial shelf life of 9 days at 5 ºC. Overall, SPI-BW edible coatings containing Cys as antioxidant could be a promising treatment to control enzymatic browning of fresh-cut 'Telma' eggplants.

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Chapter 6

Extending the shelf life of fresh-cut eggplant with a soy protein-cysteine based edible coating and modified atmosphere packaging

CHRISTIAN GHIDELLI [1], MILAGROS MATEOS [2], CRISTINA ROJAS-ARGUDO [1] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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Abstract

The effect of a soy protein-based edible coating with antioxidant activity, and conventional and superatmospheric modified atmosphere (MA) packaging, on the quality of fresh-cut 'Telma' eggplants was evaluated during storage. In a first experiment, eggplant pieces were dipped in either a coating composed of soy protein isolate (SPI) and 0.5% cysteine (Cys) or water as an uncoated control. Samples were packed in trays under atmospheric conditions to reach a passive MA (MA-P) or two gas mixtures (MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2) and were stored at 5 ºC. Atmospheric conditions were used as the control conditions (Control). The coated samples packed under MA-B and Control conditions obtained the highest whiteness index (WI) values during storage, whereas MA-A did not improve the shelf life of minimally processed eggplants and presented the lowest WI values. The MA-B and atmospheric control conditions helped to maintain firmness, whereas the coating helped to maintain the weight loss of fresh-cut eggplants packed under MA-A and MA-B. The maximum commercial shelf life was reached on day 6 for the coated samples packed under atmospheric conditions. In a second experiment, the commercial shelf life of fresh-cut eggplants was extended to 8 and 9 storage days by increasing the Cys content in the edible coating from 0.5% to 1% under MA-B and Control storage conditions, respectively.

Keywords: Minimally processed eggplants; enzymatic browning, soy protein edible coating; cysteine, superatmospheric oxygen packaging.

1. Introduction

Consumers demand fresh, healthy produces which must also be convenient and easy to prepare. This scenario has led to the increased consumption of minimally processed fruit and vegetables. Recently, eggplants (*Solanum melongena*), which are largely consumed as fresh and whole products, are attracting more interest as a minimally processed vegetable. However, wounding, slicing or chopping induces quality deterioration, which results in water loss, softening, microbial contamination, increased respiration and enzyme activity. Among these factors, the main limiting factor that reduces the shelf life of fresh-cut

eggplants is oxidation of phenolic compounds by polyphenol oxidase (PPO) (Barbagallo et al., 2012a).

The main approach to control enzymatic browning and to extend the shelf life of fresh-cut products is the combination of chemical and physical methods, like using antioxidant agents and modified atmosphere (MA) packaging. In fresh-cut 'Birgah' eggplants, Barbagallo et al. (2012b) reported a significant reduction in PPO relative activity by applying 0.5% or 1% L-ascorbic (-21%), benzoic (-15%), citric (-27%), ferulic (-43%), and L-glutamic (-32%) acids. These effects translated in browning index terms, which demonstrated the efficacy of the studied anti-browning treatments to extend the shelf-life of minimally processed eggplants. In a very recent work, we studied the effect of a wide range of antioxidants to inhibit the enzymatic browning of eggplant fresh-cut tissue. Overall, the best result for reducing enzymatic browning was obtained with 1% cysteine (Cys), which extended the commercial shelf life of this produce to 9 storage days at 5 ºC (Ghidelli et al., 2013). However at this concentration, off-flavor might be developed as reported for thiol-containing compounds such as Cys (García and Barrett, 2002).

The effect of low O_2 and high CO_2 MA packaging to control enzymatic browning has been reported for several fresh-cut fruit and vegetables (Rojas-Graü et al., 2009). The basic principle for using low O_2 and high CO_2 in fresh-cut products is that MAs are theoretically expected to control the physiological and quality changes in the product by reducing the respiration rate, ethylene production, browning, weight loss, etc. (Toivonen and DeEll, 2002). However, the response to different atmospheres depend largely on the commodity. In fresh-cut eggplants, Catalano et al. (2007) reported that, although MA improved quality preservation during storage at 4 ºC, an increase in CO_2 and a decrease in the O_2 concentration inside the package stimulated the PPO activity of the product.

Some studies have proposed the use of elevated O_2 concentrations as an alternative to low O_2 atmospheres to maintain the quality and to extend the storage life of some fresh-cut fruit and vegetables. The main benefits of superatmospheric O_2 are related with preventing microbiological spoilage and anaerobic fermentation, as observed in fresh-cut melon, cabbage, and baby spinach leaves (Allende et al., 2004; Oms-Oliu et al., 2008a; Lee et al., 2011). Moreover, high oxygen

atmospheres have been found to be particularly effective at inhibiting enzymatic discoloration and at maintaining the firmness of fresh-cut products, such as iceberg lettuces, mushrooms, potatoes and melons (Amanatidou et al., 2000; Day, 2001; Jacxsens et al., 2001; Limbo and Piergiovanni, 2006; Oms Oliu et al., 2008a). Nevertheless, the effect of superatmospheric O_2 treatment depends on certain factors such as commodity type, temperature, storage duration, etc. (Kader and Ben-Yehoshua, 2000). Thus storage under high oxygen MAP is not recommended for fresh-cut mango and pears as it induces enzymatic browning (Poubol and Izumi, 2005; Oms Oliu et al., 2008b,c).

A recent approach to prolong the shelf life of fresh-cut fruit and vegetables is the use of edible coatings either alone or combined with MA packaging. Edible coatings can provide a semipermeable barrier to gases and water vapor, reducing respiration, enzymatic browning and water loss (Pérez-Gago et al., 2005), and their protective function can also be enhanced with the addition of ingredients such as antioxidants. The basic ingredients of edible coatings are proteins, polysaccharides, and lipids. Among the proteins, soy protein isolate (SPI) coatings containing Cys have been seen to help control enzymatic browning of fresh-cut eggplants in a greater extend than Cys alone and extend the shelf life up to 9 storage days depending on the Cys content (Ghidelli et al., 2010). Other works have also demonstrated the effect of SPI-based coatings to preserve the freshness of apple slices (Kinzel, 1992), to control browning in potato slices, and to reduce moisture loss in carrots and apple slices (Shon and Haque, 2007). Therefore, this work aimed to study the effect of a soy protein-based edible coating containing two concentrations of Cys (0.5 and 1%, w/v) in combination with conventional and superatmospheric MA packaging to control the enzymatic browning of fresh-cut eggplants.

2. Materials and Methods

2.1. Materials

Beeswax (BW) (Brillocera, S.A., Valencia, Spain) was selected as the lipid phase of the soy protein isolate (SPI) emulsion film. The SPI (SUPRO 760 IP) was supplied by Solae (Ieper, Belgium). Food-grade glycerol was purchased from Panreac Quimica, S.A. (Barcelona, Spain). Cysteine (Cys) was acquired from Sigma-Aldrich (Barcelona, Spain).

2.2. Preparation of the coating formulation

Two experiments were conducted to study the effect of a SPI-based edible coating and MAs packaging on the shelf life of 'Telma' fresh-cut eggplant. The Cys content of the SPI-based coating was 0.5% and 1% (wet basis, wb) in the first and the second experiment, respectively.

To prepare the coatings, aqueous solutions of 5% (w/v) SPI were prepared and denatured for 30 min in a water bath at 90ºC. Glycerol was added as plasticizer at a SPI:glycerol ratio of 2:1, and this ratio was kept constant. BW was added to the hot SPI-glycerol mixture at a concentration of 20% (dry basis, db). Samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland) for 4 min at 30,000 rpm. After homogenization, emulsions were placed in an ice bath to prevent further protein denaturation and to crystallize the lipid particles. Finally, Cys was incorporated into the emulsion coating by magnetic agitation at the desired concentration. Both formulations were prepared with a total solid content of 7.5% (w/v).

2.3. Preparation of eggplants

Eggplants (*Solanum melongena* L., cv. Telma) were purchased in a local market (Valencia, Spain) and were stored at 5 ºC for 24 h until they were processed. After washing with chlorinate water (150 ppm), eggplants were peeled and cut into rectangular pieces (approximately 5 cm x 3.5 cm x 1.5 cm) using a sharp stainless-steel knife. A maximum of 15 eggplants were processed at the same time to minimize their exposure to oxygen. The whole process was carried out in a temperature-controlled room at 10±1 ºC under suitable hygienic conditions.

2.4. Application of edible coating and modified atmosphere packaging

Eggplant pieces were dipped into the coating or in water (uncoated-control) for 3 min at 5 ºC. After draining and drying under cold conditions, four pieces (75±5 g) were placed in polypropylene trays (17.4 cm x 12.9 cm x 3.6 cm, 470 ml, Ilpra Systems, Barcelona, Spain). Trays were heat-sealed with a 35 µm P-Plus polypropylene film (35 PA 200) with an O_2 transmission rate of 1100 cm^3 m^{-2} day^{-1}, a CO_2 transmission rate of 30,000 cm^3 m^{-2} day^{-1} at 25 ºC and 0% RH, and with a moisture vapor transmission rate of 0.9 g m^{-2} day^{-1} (Amcor Flexibles, Barcelona,

Spain). In the first experiment, MA conditions were obtained by flushing the trays with two gas mixtures (MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2) or by conventional storage under atmospheric conditions with the same film to reach a passive MA (MA-P). For the control, the film was perforated with a needle (four perforations, 1 mm in diameter) to ensure that the gas composition within the package remained near the ambient oxygen concentration (Control). In the second experiment, the assayed atmospheres were the MA-B and Control conditions. Table 1 shows the treatments for both experiments. Thermosealing was done in an ULMA-Smart 300 packing machine (Oñati, Spain). All the samples were stored at 5 ºC for quality evaluation during 8 and 9 days for the first and the second experiment, respectively.

Table 1. Soy protein-based coating and modified atmosphere (MA) packaging conditions.

	Experiment 1	Experiment 2
Cys (%, w/v, wet basis)[a]	0.5	1.0
MAs packaging conditions [b]	MA-A (15 kPa CO_2 + 5 kPa O_2)	------
	MA-B (80 kPa O_2)	MA-B
	MA-P (21 kPa O_2 + 0.03 kPa CO_2)	------
	Control (atmospheric conditions during storage)	Control

[a] Cysteine (Cys) content in the soy protein isolate (SPI)-Beeswax (BW) edible coating. BW content = 20% (dry basis); SPI:glycerol ratio of 2:1; total solid content = 7.5% (w/v).

[b] Initial gas mixtures in trays (balance N_2). Film: 35 µm P-Plus polypropylene film (35 PA 200).

2.5. Headspace gas analysis

The gas composition in the package headspace during storage was determined in a gas chromatograph (GC valve ThermoFinnigan, Milan, Italy) equipped with a thermal conductivity detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). The temperatures of the

injector, oven, and detector were 125 ºC, 35 ºC, 180 ºC, respectively. Helium was used as carrier gas at a flow rate of 22 ml min^{-1}. One ml of the gas sample from the headspace atmosphere of 5 trays per treatment was measured. Data are expressed in kPa of CO_2 and O_2.

2.6. Color measurements

Color measurements were taken with a Minolta (Model CR-300, Ramsey, N.Y., USA) on 12 eggplant pieces per treatment and sampling day. Each measurement was taken randomly at three different locations on each sample piece. A standard white calibration plate was employed to calibrate the equipment. The CIE L*a*b* values were determined and the whiteness index (WI) was calculated by the following equation (Amanatidou et al., 2000):

$$WI = 100 - \left((100 - L)^2 + (a^2 + b^2)\right)^{0.5}$$

2.7. Sensory analysis

During storage, eggplant pieces were evaluated visually by 10 judges at day 1, 3, 6, and 8 of storage. Each treatment was coded, presented in random order and evaluated based on general visual appearance using this scale: 9 = excellent, just sliced; 7 = very good, 5 = good, limit of marketability; 3 = fair, limit of usability; 1 = poor, inedible (Gorny et al., 1999). The shelf-life of the samples was defined as the number of days to reach the limit of marketability. A minimum of 3 trays per treatment was presented to account for biological variations. A color photograph of the samples rated by this scale was provided to the judges.

2.8. Weight loss determination

Weight loss was measured at the end of the storage period by weighing 12 eggplants pieces per treatment. The results were expressed as the percentage loss (%) of the initial weight.

2.9. Firmness determination

Eggplant firmness was determined on 12 pieces per treatment by measuring the force required for an 8-mm probe to penetrate the eggplant pieces to a depth of 2 mm and at a speed of 5 mm/s using an Instron Universal Machine (Model 3343; Instron Corp., Canton, MA, USA). The results were expressed as force (N).

2.10. Statistical analysis

Statistical analysis was performed using STATGRAPHICS Plus 4.1 (Manugistic Inc., Rockville, MD, USA). The specific differences between means were determined by least significant difference (LSD) applied after the analysis of variance (ANOVA). Significance differences were defined at $p \leq 0.05$.

3. Results and discussion

3.1 First experiment

3.1.1 Effect of MA packaging and SPI-based edible coating on the headspace gas composition

The consumption of oxygen and carbon dioxide production in fresh-cut products creates a MA in the packaging system, which is directly related with the respiration of the tissue and the packaging material characteristics. In this work, a sharp drop in O_2 and an increase in the CO_2 concentrations were observed in the headspace gas composition of both the coated and uncoated fresh-cut eggplants that had been packed under different MAs during storage at 5 ºC. Whereas, the samples packed in the perforated film (Control) maintained O_2 levels close to the atmospheric value and CO_2 levels below 5 kPa (Fig. 1). The samples packed under MA-A (15 kPa CO_2 + 5 kPa O_2) and MA-P (initial ambient conditions) with P-Plus film reached the equilibrium on day 3 and day 6, respectively, with the O_2 concentrations below 2 kPa and CO_2 concentrations of 15-18 kPa. Under superatmospheric O_2 (MA-B: 80 kPa O_2), the O_2 concentration lowered to 53±0.70 kPa and the CO_2 concentration increased to 12 and 16 kPa for the coated and the uncoated samples, respectively.

The headspace O_2 concentration was not significantly influenced by coating application as compared to the uncoated samples (water-dipped) for any packaging condition. However, significant differences were found in the CO_2 concentrations between the coated and uncoated eggplants stored under low oxygen concentration (MA-A) and superatmospheric O_2 (MA-B) conditions.

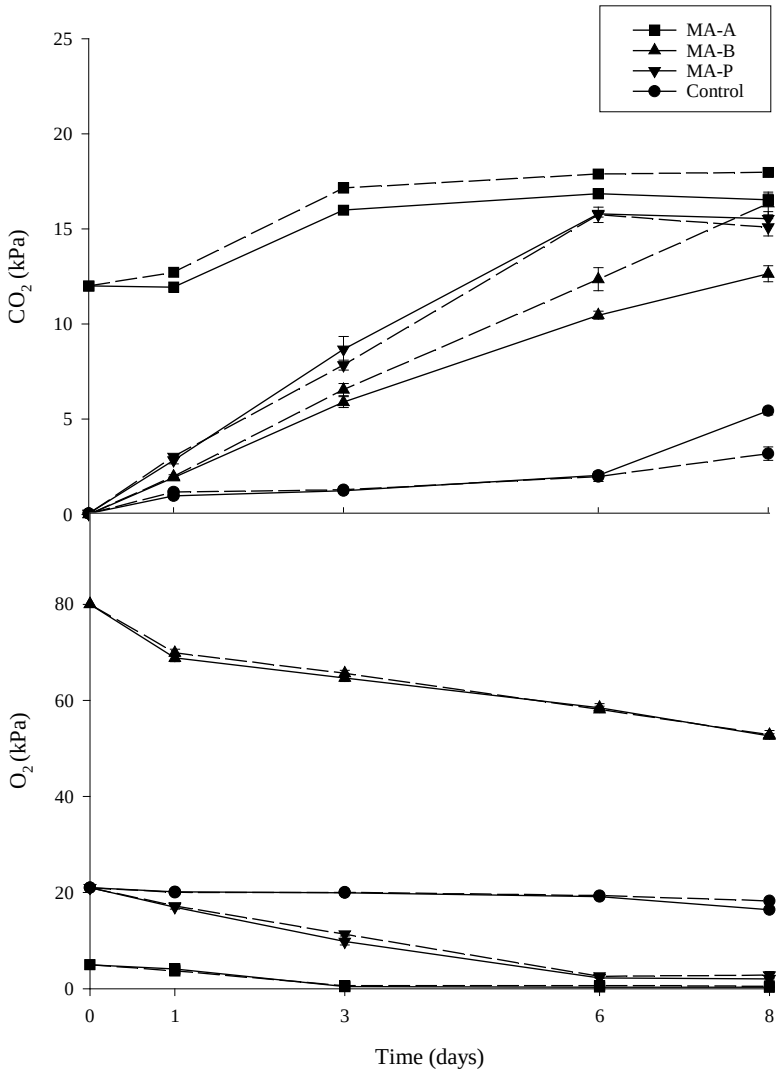

Fig. 1. Carbon dioxide and oxygen concentrations in the package headspace of fresh-cut 'Telma' eggplants stored in different modified atmospheres (MA) at 5 ºC. Vertical bars represent standard errors. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. Solid and dashed lines represent the coated (SPI + 0.5% Cys) and the uncoated (water-dipped) samples, respectively.

Several works have described the beneficial effect of polysaccharide and protein-based edible coatings on reducing the respiration rate of fresh-cut produces, which has been attributed to their good oxygen barrier (Olivas and Barbosa-Cánovas, 2005). Wong et al. (1995) observed a lower CO_2 production rate in apple pieces coated with several polysaccharide/lipid bilayer edible coatings. Lower CO_2 production has also been observed in fresh-cut apples and melons that had been dipped in an alginate-based edible coating if compared to uncoated samples (Rojas-Graü et al., 2007; Raybaudi-Massilia et al., 2008). Moreover, Lee et al. (2003) have shown that the application of whey protein concentrate decreased the CO_2 production of apple slices in a greater extend than a carrageenan coating.

3.1.2. Effect of MA packaging and SPI-based edible coating on the color of fresh-cut eggplants

Fig. 2 shows the effect of the SPI-based edible coating and MA packaging conditions on the WI of eggplant pieces during storage at 5 ºC. Table 2 presents the *F*-ratio values for this parameter as being affected by MA and coating application. The increase in enzymatic browning during storage was accompanied by a drop in the WI values. The ANOVA analysis showed that applying the edible coating and the different storage conditions had a significant effect on the WI (Table 2). In a previous work, Ghidelli et al. (2010) also observed that the SPI-BW edible coatings amended with Cys helped control the browning of fresh-cut eggplants.

The use of low O_2 and high CO_2 levels has been effectively proven to control enzymatic browning, firmness and decay of many fresh-cut fruit and vegetables (Rojas-Graü et al. 2009). However in the present work, the samples stored under MA-A (15 kPa CO_2 + 5 kPa O_2) and passive MA (MA-P) conditions gave a significantly lower WI than those stored under atmospheric (Control) and superatmospheric oxygen conditions (MA-B). Catalano et al. (2007) reported that an increase in CO_2 increased the PPO activity of fresh-cut eggplants. Similarly, atmospheres with high CO_2 accelerated tissue browning in fresh-cut pears as compared to the air control and CO_2 injury was reported to occur in a dose-responsive manner (Gorny et al., 2002).

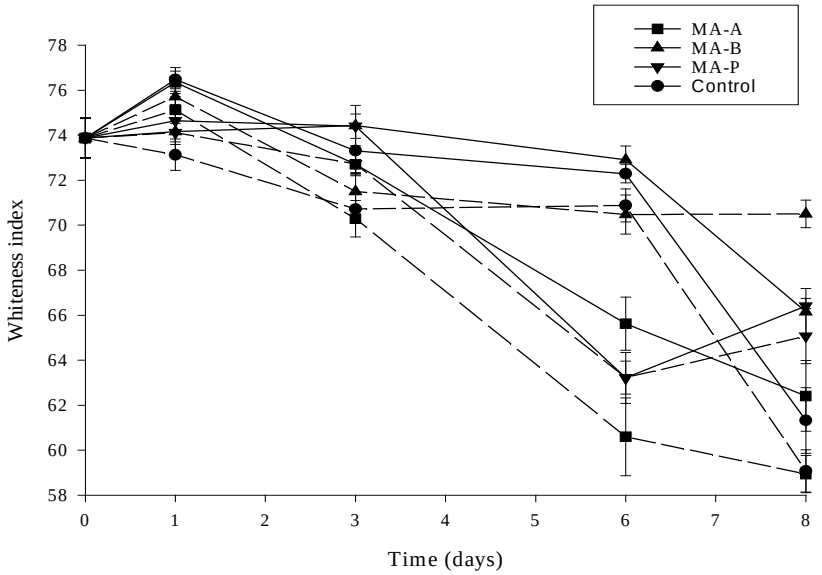

Fig. 2. Effect of SPI-based edible coating and modified atmosphere (MA) packaging on the whiteness index (WI) of fresh-cut 'Telma' eggplants stored at 5 ºC. Vertical bars represent standard errors. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. Solid and dashed lines represent the coated (SPI+0.5% Cys) and the uncoated (water-dipped) samples, respectively.

At the end of storage, the WI values significantly decreased in all the treatments, and the samples stored under surperatmospheric O_2 conditions where those with the highest values. Amanatidou et al. (2000) reported surface browning in fresh-cut carrots stored under 1 kPa O_2 + 10 kPa CO_2 atmosphere conditions, but not when the storage conditions were higher than 50% O_2 for storage lasting 12 days. Jacxsens et al. (2001) noted how high oxygen atmosphere packaging (70-95 kPa O_2) proved particularly effective at inhibiting enzymatic browning in sliced mushrooms as compared with low-oxygen atmosphere packaging. Limbo and Piergiovanni (2006) also showed the positive effect of high-oxygen

partial pressures combined with dipping in acid solutions to control enzymatic browning of fresh-cut potato.

Table 2. Analysis of variance on the whiteness index (WI), visual quality, weight loss, and firmness of coated and uncoated fresh-cut eggplants stored in different modified atmospheres at 5 ºC: results of experiment 1.

	F-ratio			
	WI	Visual quality	Weight loss	Firmness
Treatments				
A: Atmosphere conditions	9.21***	2.45 ^{NS}	87.52***	11.79***
B: Coating application	5.96*	126.76***	80.79***	0.54 ^{NS}
Interactions				
AB	1.70^{NS}	0.88^{NS}	51.76***	1.15^{NS}

F-ratios are shown for the sources of variations; NS = not significant.
* Significant *F*-ratios at $p \leq 0.05$.
*** Significant *F*-ratios at $p \leq 0.001$.

3.1.3. Effect of MA packaging and SPI-based edible coating on the visual quality of fresh-cut eggplant

The application of the SPI-BW coating amended with 0.5% Cys significantly affected the visual quality of fresh-cut eggplants (Table 2). Uncoated samples were evaluated to be below the limit of marketability by storage day 1, except for those stored in a passive modified atmosphere, which reached this limit by storage day 2 (Fig. 3). The samples coated and stored under atmospheric conditions (Control) reached the limit of marketability by storage day 6 at 5 ºC in accordance with the highest WI for this treatment (Fig. 1).

MA packaging did not significantly improve the visual quality of fresh-cut eggplants ($p>0.05$). Among the different MAs conditions tested, the coated samples stored under high O_2 atmosphere reached a commercial shelf life of 4 days, whereas those stored under passive and low oxygen MA were below the limit of marketability on storage day 3 at 5 ºC. The visual quality deferred from the color analysis as the coated and uncoated samples stored under superatmospheric conditions had the highest WI values on storage day 6. These differences indicate that it is

necessary to perform a visual analysis that considers not only color, but also the overall visual quality of samples to establish the product's shelf life.

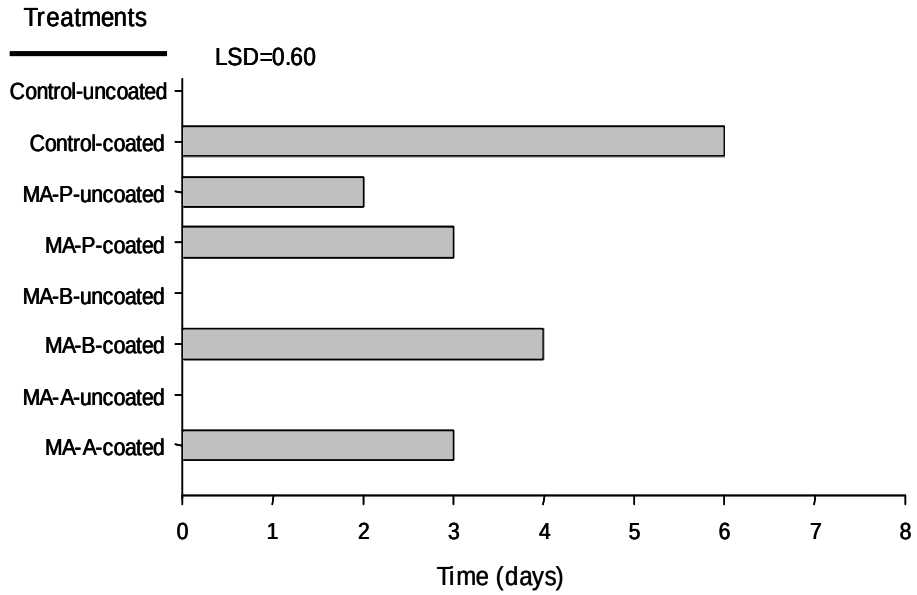

Fig. 3. Effect of a SPI-based edible coating amended with 0.5% cysteine and modified atmosphere (MA) packaging on the shelf life of the fresh-cut 'Telma' eggplants stored at 5º C. Shelf-life was defined as the number of days to reach the limit of marketability. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage.

3.1.4. Effect of MA packaging and SPI-based edible coating on the weight loss and firmness of fresh-cut eggplant

Table 3 shows the effect of the edible coating and MAs on the weight loss of fresh-cut eggplants on storage days 1 and 8 at 5 ºC. The application of the SPI-based edible coating and packaging conditions significantly affected the weight loss of fresh-cut eggplants during storage, and interaction between both factors was observed (Table 2).

The uncoated samples stored under superatmopsheric O_2 (MA-B) and low oxygen concentration (MA-A) conditions presented the greatest weight loss among the different treatments tested, with a weight loss of 4% after 8 days of storage. Nonetheless, no differences were found between the coated and uncoated samples stored under the Control or passive MA conditions. Several works have described the beneficial effect of edible coatings in reducing the weight loss of minimally processed fruit and vegetables, related to their barrier to moisture transfer. Thus, an SPI-based coating has been reported to reduce the moisture loss of carrots and apple slices (Shon and Haque, 2007). In our case, the application of the SPI-based coating prevented the significant increase in weight loss observed in those samples packed under MA-A and MA-B conditions, probably due to a decrease in respiration rate of the samples (Fig. 1).

Table 3. Effect of soy protein isolate (SPI)-based edible coating and modified atmosphere (MA) packaging on the weight loss (%) and firmness (N) of the fresh-cut eggplants stored at 5 ºC: results of experiment 1.

	Weight loss (%)		Firmness (N)	
	Storage time		Storage time	
Treatments	1 day	8 days	1 day	8 days
MA-A-coated	1.7±0.3 b	2.0±0.7 a	9.1±2.3 abc	5.3±1.8 a
MA-A-uncoated	2.7±0.9 c	4.1±1.0 b	9.2±3.1 abc	6.0±2.6 ab
MA-B-coated	0.9±0.3 a	1.0±0.3 a	11.0±2.1 c	7.3±2.1 bcd
MA-B-uncoated	4.3±1.0 d	4.5±1.2 b	10.2±3.2 bc	9.0±2.6 d
MA-P-coated	0.6±0.5 a	1.0±0.5 a	10.9±2.2 bc	6.6±2.9 abc
MA-P-uncoated	0.8±0.3 a	1.1±0.9 a	8.9±2.4 ab	5.0±0.8 a
Control-coated	0.6±0.2 a	1.3±0.9 a	8.1±2.0 a	7.4±2.7 bcd
Control-uncoated	0.8±0.5 a	1.5±0.8 a	8.7±2.4 ab	8.4±3.6 cd

Mean±SD (n=12). MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 80 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. SPI-based coating amended with 0.5% cysteine. Firmness before cutting was 11.0±1.5 N.

The firmness of fresh-cut eggplants diminished with storage and was significantly affected by the packaging conditions tested, but not by the edible coating application (Table 2). The effect of coatings on the firmness of fresh-cut commodities depends on many factors, such as coating composition, storage conditions, commodity, etc. Eswaranandam et al. (2006) reported that soy protein coated fresh-cut apples better maintained firmness than uncoated samples, whereas, Shon and Haque (2007) found that similar coatings had no effect on the textural quality of several cut fruit and vegetables, such as apples, carrots, potatoes and onions.

At the end of storage, the samples packed in the conventional (MA-A) and passive (MA-P) atmospheres presented lower firmness than those packed under superatmospheric O_2 and the Control conditions. The effect of the superatmospheric O_2 conditions to limit firmness loss has been reported in other fruit and vegetables, as sliced carrots, fresh-cut spinach, iceberg lettuces, and melons (Amanatidou et al., 2000; Day, 2001; Allende et al., 2004; Oms Oliu et al., 2008a). Firmness retention at high O_2 levels can be related with the lower activities of cell wall hydrolitic enzymes, as observed by Deng et al. (2005) in table grapes. The effect of conventional MA packaging, with low O_2 and high CO_2, on the firmness of fresh-cut commodities depends on the specific fruit or vegetable, and also on gas composition (Toivonen and DeEll, 2002). Thus an atmosphere containing 0.5 kPa O_2 has been found to prevent cut pears from softening (Rosen and Kader, 1989), whereas low O_2 ($\leq$4 kPa) and higher CO_2 (5, 10, 20 kPa) concentrations, either alone or in combination, did not prevent softening in fresh-cut pears and bananas (Gorny et al. 2002; Vilas Boas and Kader, 2006).

3.2 Second experiment

After considering the previous results, a second experiment was designed which aimed to further extend the shelf life of fresh-cut eggplants by increasing the Cys concentration from 0.5 to 1% in the SPI-based edible coating in combination with Control or superatmospheric MA packaging (MA-B) conditions.

3.2.1. Effect of MA packaging and SPI-based edible coating on headspace gas composition

Fig. 4 shows the changes in the headspace gas composition of the coated and uncoated fresh-cut eggplants packed under atmospheric conditions (Control) or superatmospheric O_2 conditions (MA-B) for 9 storage days at 5 ºC. At the end of the storage, the headspace CO_2 concentration for the Control samples reached 1±0.1 kPa, and the O_2 level was maintained near the atmospheric conditions. The samples packed in MA-B underwent a significant decrease in O_2 and an increase in the CO_2 concentrations during storage. Under this packing condition, the application of the SPI edible coating significantly lowered the respiration rate, thus confirming the results from the first experiment. At the end of the storage period, the headspace CO_2 composition was 10±1 kPa and 15±1 kPa for the coated and the uncoated samples, respectively. Furthermore, the headspace O_2 composition was 57±1 kPa and 48±1 kPa for the coated and the uncoated samples, respectively.

If compared to the first experiment, the increased in the Cys content in the SPI coating from 0.5 to 1% lowered the respiration rate of the samples, which resulted in lower CO_2 and O_2 concentrations in the package headspace. Ayranci and Tunc (2003) reported that an increase in ascorbic and citric acid concentrations in a methyl cellulose edible film improved the oxygen barrier property of the film. Moreover, Oms Oliu et al. (2008c) observed that the addition of N-acetylcysteine and glutathione to gellan, pectin, and alginate-based edible coatings reduced the O_2 exchange of pear wedges to a greater extent than for similar coatings without antibrowning agents.

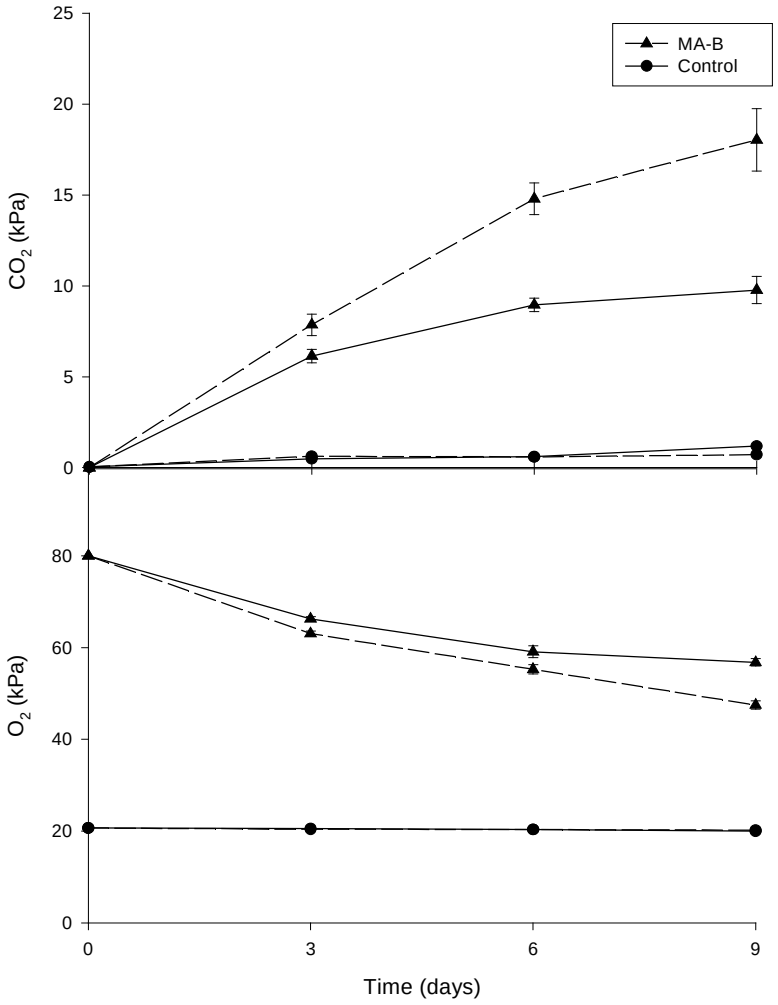

Fig. 4. Carbon dioxide and oxygen concentrations in the package headspace of fresh-cut 'Telma' eggplants stored in different modified atmospheres (MA) at 5 ºC. Vertical bars represent standard errors. MA-B: 80 kPa O_2; Control: atmospheric conditions during storage. Solid and dashed lines represent the coated (SPI + 1% Cys) and the uncoated (water-dipped) samples, respectively.

3.2.2. Effect of MA packaging and SPI-based edible coating on the color of fresh-cut eggplants

The coated samples packed under atmospheric conditions (Control) or at high O_2 concentrations (MA-B) had higher WI values than the uncoated ones (Fig. 5). No significant differences were observed between both packaging conditions, except for a sharp drop for the uncoated samples packed in MA-B after 9 storage days at 5 ºC.

When compared to the first experiment, the increase in the Cys content in the SPI edible coating reduced the enzymatic browning of fresh-cut eggplants, which resulted in higher WI values at the end of storage. Similar results have also been reported by Ghidelli et al. (2013) after increasing the Cys concentration of aqueous solutions, which reduced the enzymatic browning of fresh-cut eggplant.

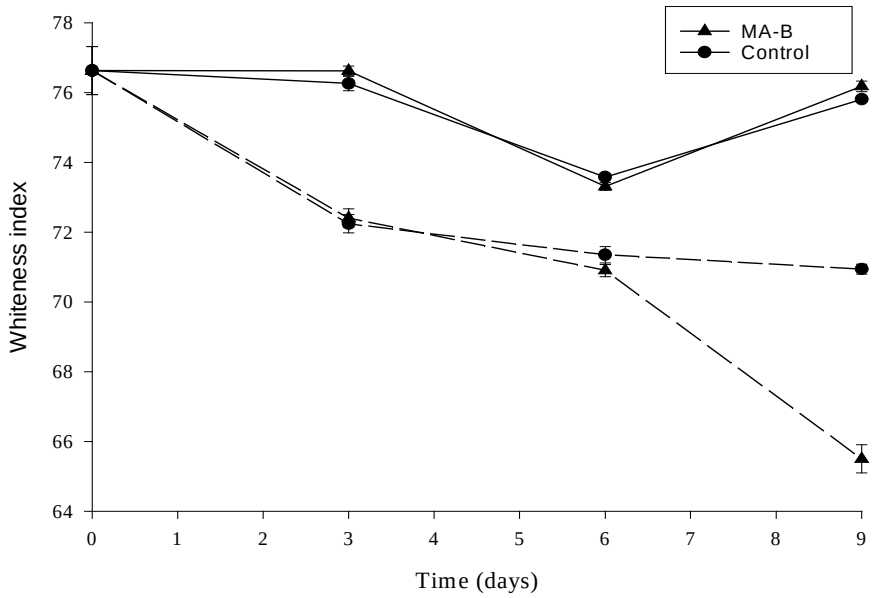

Fig. 5. Effect of SPI-based edible coating and modified atmosphere (MA) packaging on the whiteness index (WI) of fresh-cut 'Telma' eggplants stored at 5 ºC. Vertical bars represent standard errors. MA-B: 80 kPa O_2; Control: atmospheric conditions during storage. Solid and dashed lines represent the coated (SPI+1% Cys) and the uncoated (water-dipped) samples, respectively.

3.2.3. Effect of MA packaging and SPI-based edible coating on the visual quality of fresh-cut eggplants

The samples with SPI-edible coating containing 1% Cys and stored under atmospheric (Control) and superatmospheric O_2 conditions achieved a commercial shelf life of 9 and 8 days, respectively. Whereas, the uncoated samples were evaluated as not marketable by 1 day of storage (data not shown). These results confirm what was observed in the color analysis. An increase in the Cys concentration from 0.5 to 1% helps extend the commercial shelf life of eggplant pieces from 6 to 8-9 storage days.

3.2.4. Effect of MA packaging and SPI-based edible coating on the weight loss and firmness of fresh-cut eggplants

Table 4 shows the effect of the SPI-based edible coating with 1% Cys and the packaging conditions on the weight loss and firmness of fresh-cut eggplants by storage days 3 and 9 at 5 ºC. As observed in the first experiment, the uncoated samples stored in superatmopsheric O_2 presented the greatest weight loss, and no significant differences were found for the remaining treatments.

Table 4. Effect of SPI-based edible coating and modified atmosphere packaging on the weight loss (%) and firmness (N) of the fresh-cut eggplants stored at 5 ºC: results of experiment 2.

	Weight loss (%)		Firmness (N)	
	Storage time		Storage time	
Treatments	three days	nine days	three day	nine days
MA-B-coated	0.9±0.6 a	1.4±0.9 a	8.1±2.3 a	7.6±1.9 a
MA-B-uncoated	1.6±0.4 b	2.5±1.0 b	9.8±2.6 a	7.4±2.9 a
Control-coated	0.5±0.2 a	1.1±0.7 a	8.7±3.0 a	8.4±2.3 a
Control-uncoated	1.0±0.9 a	1.7±0.7 a	8.7±3.2 a	8.8±2.3 a

Mean±SD (n=12). MA-B: 80 kPa O_2; Control: atmospheric conditions during storage. SPI-based coating amended with 1% cysteine. Firmness before cutting was 12±2.8 N.

The firmness of the fresh-cut eggplants diminished from a initial value of 12.0±2.8 N to values which came close to 8 N after 9 days storage, and were similar to those obtained in the first experiment. These values were not affected by the coating application or the packaging conditions.

4. CONCLUSION

The obtained results indicate that applying a SPI-based edible coating amended with 1% Cys can help control enzymatic browning and maintains the visual quality of 'Telma' fresh-cut eggplants for up to 8-9 days at 5 ºC. Conventional MA packaging conditions (low O_2 and high CO_2) are not recommended for storage of fresh-cut eggplants under the studied conditions, since it induced damage of the tissue. Overall, the SPI-Cys coating under air atmospheric conditions provided the best and cheapest approach for extending the shelf life of fresh-cut eggplant.

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Chapter 7

Effect of a soy protein-based edible coating and modified atmosphere packaging on enzymatic browning of fresh-cut persimmons cv. Rojo Brillante

CHRISTIAN GHIDELLI [1], MILAGROS MATEOS [2], SANCHÍS ELENA[1], CRISTINA ROJAS-ARGUDO [1] AND MARÍA B. PÉREZ-GAGO [1,3*].

[1] Centro de Tecnología Poscosecha, Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain.
[2] PASAPTA. Universidad CEU-Cardenal Herrera. 46113 Moncada (Valencia), Spain.
[3] IVIA - Fundación AGROALIMED, 46113 Moncada, Valencia, Spain.

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Abstract

Persimmon fruit cv. Rojo Brillante can be marketed as a fresh-cut commodity after removal of the astringency by application of high levels of CO_2. However, the commercial success of the product is limited mainly by enzymatic browning. Therefore, the effect of an edible coating with antioxidant activity and modified atmosphere packaging (MA) has been investigated in this work. Persimmon pieces were dipped in a coating composed by soy protein isolate, citric acid and calcium chloride, or in water as a control. Fruit samples were then packed in trays under atmospheric conditions to reach a passive MA (MA-P) or two gas mixtures (MA-A: 15% CO_2 + 5% O_2 + 80% N_2; MA-B: 50% O_2 + 50% N_2), sealed with polypropylene films and stored at 5 ºC for 10 days. Atmospheric conditions were used as control conditions by macro perforating the polypropylene film. Changes in atmosphere composition, color (CIEL*a*b*), sensory analysis, texture, and antioxidant capacity were evaluated during storage. During storage, the headspace gas composition of the samples packed in MA-P and MA-B showed a sharp increase in CO_2 and a decrease in O_2. While headspace gas composition under atmospheric control conditions and MA-A were slightly modified during storage. Coated samples had lower a* values than uncoated samples. Browning was further reduced when coated samples were stored under MA-A. At the end of the 10 days of storage, these samples were evaluated above the limit of marketability. However, the MA-A reduced the persimmon antioxidant activity. Packaging in the MA-B damaged the tissue of the fruit, resulting in a decrease in L* and an increase in a* values. Persimmon firmness was not affected either by coating application or MA packaging. Coating application improved persimmon visual quality without affecting fruit taste. The results indicate that the combination of the MA-A with the soy protein-based coating offers a synergic effect in reducing enzymatic browning of fresh-cut 'Rojo Brillante' persimmon, extending the commercial shelf life.

Keywords: fresh-cut persimmons, enzymatic browning, soy protein edible coating, antioxidant capacity, modified atmosphere.

INTRODUCION

Persimmons fruit cv. Rojo brillante is an astringent variety with high production in the zone Ribera del Xuquer (Valencia, Spain). Application of high levels of CO_2 has been shown to be effective in removing astringency avoiding softening of the fruit (Arnal and del Río, 2003), which makes possible to market the fruit as a fresh-cut commodity. However, the commercial success is limited mainly by enzymatic browning and loss of firmness. The main approach to inhibit browning is the use of antibrowning agents, based on citric or ascorbic acid, and the use of modified atmosphere (MA) packaging. Recent works have reported that ascorbic acid, citric acid and cystein are effective antioxidants in controlling enzymatic browning of fresh-cut persimmon cv. 'Rojo Brillante' (Pérez-Gago et al., 2009; Ghidelli et al., 2013). Furthermore, the use of calcium salts has been proven to preserve fresh-cut tissue from softening, as well as to reduce enzymatic browning especially when pH solution is low (Garcia and Barrett, 2002; Ghidelli et al., 2013).

MA packaging with low O_2 concentrations and high CO_2 levels have been recommended for many fresh-cut products, since they reduce respiration rate and slow down browning reactions. Nevertheless, gas mixtures consisting of 2 kPa O_2 and 12 kPa CO_2 showed little effect on the shelf life of fresh-cut 'Fuyu' persimmons (Wright and Kader, 1997). Some studies have also shown that the use of superatmospheric O_2 conditions (>40 kPa) may have a beneficial effect on fresh-cut produces by reducing enzymatic browning and preventing anaerobic fermentation, but as in conventional MA packaging their effect greatly depends on the commodity, cultivar, physiological stage, storage conditions, etc. (Kader and Ben-Yehoshua, 2000).

Another approach to further increase the shelf life of fresh-cut fruits is the use of edible coatings. Edible coatings offer the possibility to extend the shelf life of fresh-cut produces by providing a semipermeable barrier to gases and water vapour, and therefore, reducing respiration, enzymatic browning, and water loss (Baldwin et al., 1995). Their protective function may also be enhanced with the addition of antimicrobials, antioxidants, flavors, nutrients, etc. The development of edible films and coatings has been focused upon barriers containing proteins, polysaccharides, and lipids. Among proteins, soy protein

coatings present a high oxygen barrier and have been able to preserve freshness of apple slices (Kinzel, 1992). However, no works have been published with soy protein-based coatings to control enzymatic browning of fresh-cut persimmons. Furthermore, the incorporation of antioxidants to soy protein coatings in combination with MA packaging may enhance their protective function. Therefore, the aim of this work was to study the effect of a soy protein edible coating containing citric acid and calcium chloride in combination with MA packaging, including superatmospheric O_2 conditions, to control enzymatic browning of fresh-cut 'Rojo Brillante' persimmon.

MATERIAL AND METHODS

Materials

Soy protein isolate (SPI) (SUPRO 760 IP) was supplied by Solae (Ieper, Belgium). Food-grade glycerol was from Panreac Quimica, S.A. (Barcelona, Spain). Citric acid was from Quimivita (Barcelona, Spain) and calcium chloride from Sigma-Aldrich (St. Louis, MO, USA).

Coating formulation

To prepare the coating, an aqueous solution of 7% (w/v) soy protein isolate (SPI) was prepared and denatured for 30 minutes in a 90 ºC water bath. Glycerol was used as plasticizer. The protein plasticizer ratio was 2 parts SPI to 1 part glycerol (dry basis). Citric acid and calcium chloride were incorporated to the solution at a concentration of 1% and 0.3% (w/w, wet basis), respectively. Water was added to bring the emulsion to 7.5% (w/v) total solid content.

Preparation of persimmons

'Rojo Brillante' persimmons were provided by the Cooperative 'Nuestra Señora de Oreto' in l'Alcudia (Valencia, Spain). Astringency was removed by maintaining the fruit at 20 ºC in closed containers with 95% CO_2 levels for 24 hours. After removal from the containers, the fruit was stored in air at 10 ºC for 1 day until processing. The persimmons were cleaned, peeled, and cut into rectangular pieces (approximately 5.5 cm x 3.5 cm x 1.5 cm), using a sharp stainless-steel knife to reduce mechanical bruising. A maximum of 20 persimmons were processed at the same time to minimize excessive exposure to oxygen and the whole

process was carried out in a temperature-controlled room at 10±1 ºC under suitable hygienic conditions.

Application of the SPI-based edible coating and modified atmosphere packaging

Persimmon pieces were dipped in the coating solution or in water solution, as a control, for 3 minutes. After draining and drying under cold conditions, 4 pieces (115±5 g) were placed in polypropylene trays and heat-sealed with the 35 µm P-Plus polypropylene film (35 PA 200) that had an O_2 transmission rate of 1,100 cm^3 m^{-2} day^{-1}, CO_2 transmission rate of 30,000 cm^3 m^{-2} day^{-1} at 25 ºC and 0% RH, and moisture vapor transmission rate of 0.9 g m^{-2} day^{-1} (Amcor Flexibles, Barcelona, Spain). MA conditions were obtained by flushing the trays with two gas mixtures (MA-A: 15 kPa CO_2 + 5 kPa O_2 + 80 kPa N_2; MA-B: 50 kPa O_2 + 50 kPa N_2) or by storage in atmospheric conditions with the same film to reach a passive MA (MA-P). For the control, the film was perforated with a needle (4 perforations of 1 mm in diameter) to ensure that the gas composition within the package remained near ambient oxygen concentration (Control). Thermosealing was done in an ULMA-Smart 300 packing machine (Oñati, Spain). All the samples were stored for 10 days at 5 ºC for quality evaluation.

Headspace gas analysis

Changes in the headspace gas composition (CO_2 and O_2) of the package were measured with a gas chromatograph (Thermo Finnigan GC 2000, Italy) equipped with a thermal conductivity detector as described by Ghidelli et al. (2013). An amount of 1 ml from the package headspace was withdrawn through an adhesive rubber septum. Measurements were made in 5 trays per treatment during storage.

Firmness

Persimmons firmness was determined on 12 pieces per treatment by measuring the force required for an 8 mm probe to penetrate the sample to a depth of 2 mm at a speed of 5 mm/s using an Instron Universal Machine (model 3343, USA).

Color measurement

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, NY, USA.) on 12 persimmon pieces per treatment using the CIE L* a* b* color space. Each measurement was taken at 3 locations for each sample piece.

Sensory analysis

The sensory analysis of the samples included a visual and taste evaluation. For the visual test, each treatment was coded, presented in random order and the judges had to rank them from the highest to the lowest degree of browning. Additionally, the general visual appearance of each treatment was evaluated based on the following scale: 9 = excellent, just sliced; 7 = very good; 5 = good, limit of marketability; 3 = fair, limit of usability; and 1 = poor, inedible (Gorny et al., 2002). A color photograph of sample rated with this scale was used by judges to score the samples.

To evaluate the effect of the SPI-based edible coating and MA packaging on taste of fresh-cut 'Rojo Brillante' persimmons, two tests were conducted, which included a triangle test and a descriptive quantitative analysis. The triangle test was performed to detect if panelists could be able to differentiate between coated and uncoated samples (ISO 4120:2004). This test was chosen as it allows one to distinguish between samples without having to specify the sensory characteristics that differ and it is also better at detecting small differences that are intensity ratings (Lawless and Heymann, 1998; Radovich et al., 2004). In each triad, the panelists compared between coated and uncoated samples that were packed under atmospheric control conditions (Control). The triangle test was repeated twice by the judges to improve the test power. The coated samples were prepared one day before and keep at 5 ºC until running the test, ensuring the complete drying of the coating before each session. Uncoated samples were prepared 3 hours before running the test. Under these preparation conditions samples could not be differentiated by color. The sensory panel consisted of 20 and 17 judges for the first and the second session, respectively. Panelist seated at partitioned booths were presented with three samples simultaneously, one different from the other two. The

presentation order of treatment comparisons was counter-balanced across panelists.

In the second sensory test, the panelist evaluated the effect of coating application and MA packaging conditions in off-flavors, characteristic flavor, firmness, and overall quality of fresh-cut persimmons at day 0, 3, and 6 of storage at 5 ºC. Off-flavors and characteristic flavor were rated on a 4 point scale, where 0 = no presence and 3 = marked presence. Firmness was rated in a 5 point scale, where 1 = very soft and 5 = very firm. Overall quality was rated on a 9 point scale, where 1 to 3 represented a range of poor quality, 4 to 6 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Two persimmon pieces per treatment were presented to judges in a random order, labeled with three-digit codes and served at room temperature (20±1 ºC). The sensory panel consisted of 20 trained members (ISO 4121:2003).

In both tests, to avoid discrimination due to the color, panelists were provided with glasses made with red and blue transparent paper and the booths were also illuminated with appropriate lights to completely mask browning. Spring water was used for palate cleansing between samples (ISO 6685:2005).

Antioxidant capacity

The antioxidant capacity of fresh-cut 'Rojo Brillante' persimmons was evaluated by determining the free radical scavenging effect of the samples on 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical. Extraction was performed following the method of Chen, et al. (2008) with minor modifications. Briefly, 2 g of frozen sample (-80 ºC) was mixed with 30 ml of 80 ml/100 ml methanolic solution to be then homogenized at 20,000 rpm for 2 min (Ultraturrax, IKA, Germany), followed by boiling in a water bath for 20 min to inactivate the PPO. After the extraction of the homogenate with an ultrasonic machine for 15 min at room temperature, the homogenate was centrifuged at 10,000 rpm for 20 min and at 5 ºC. The resultant supernatant was then filtered and a second extraction was done. The two supernatants were used as the extract to analyze the antioxidant capacity.

For the measurement, 75 µl of extract was added into 225 µl of $DPPH^{\bullet}$ (24 ppm) to start the reaction and stored in the dark at room

temperature for 20 min. Change in absorbance was measured at 520 nm using a multiplate reader (Multiskan Spectrum, Thermo Fisher Scientific, Finland). The $DPPH^{\bullet}$ radical scavenging activity was expressed as effective concentration (EC_{50}), being the amount of fresh-cut persimmon required to lower the initial $DPPH^{\bullet}$ concentration by 50% (g/g $DPPH^{\bullet}$), thus lower EC_{50} values mean higher antioxidant capacity.

Statistical analysis

Statistical analysis was performed using STATGRAPHICS 4.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.). Specific differences between means were determined by least significant difference (LSD) after the analysis of variance (ANOVA). Significant differences for visual browning were determined by the Friedman test, which is recommended with ranking (ISO 8587:2006). The significant effect on the triangle test sensory test was obtained from the one tailed binomial test (ISO 4120:2004). Statistical analysis on sensory taste data was performed using Fizz-Biosystemes software (Coutemon, France). Significant differences were defined at $p \leq 0.05$.

RESULTS AND DISCUSSION

Changes in the headspace gas composition

Figure 1 shows the O_2 and CO_2 concentrations in the package headspace of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC. For technical reasons, the MA-B (50 kPa of O_2) was not achieved in coated samples. The initial headspace O_2 concentrations for coated and uncoated samples packed in MA-B were 30 kPa and 50 kPa, respectively. After 10 days of storage, the CO_2 concentration of cut persimmons packed under this MA increased to 6 kPa and 8 kPa and the O_2 level decreased to 20 kPa and 35 kPa for coated and uncoated fresh-cut persimmons, respectively.

The samples packed in MA-A (15 kPa CO_2 + 5 kPa O_2) maintained the gas composition in the package headspace very close to initial values; thus, at the end of the 10 days storage period the CO_2 and O_2 concentrations were around 13 kPa and 2 kPa, respectively. This result indicates that there was an equilibrium between the film permeability and the O_2 consumption and CO_2 production of the fresh-cut persimmons.

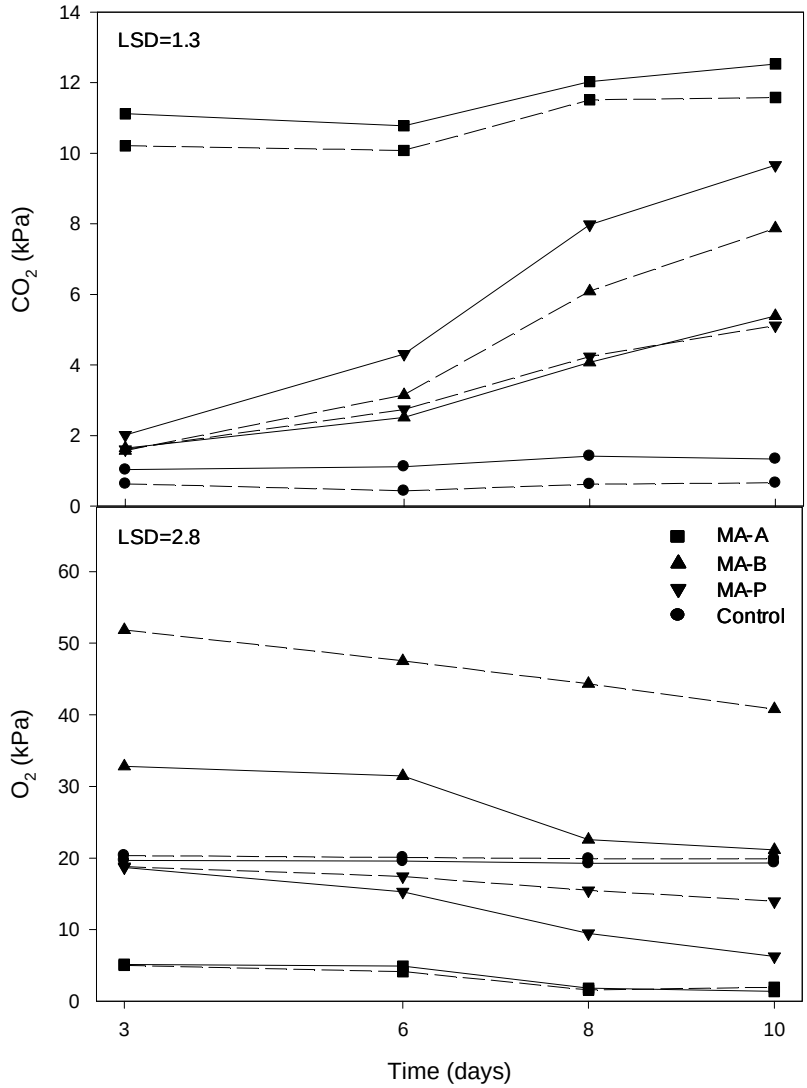

Fig. 1. Carbon dioxide and oxygen concentration in the package headspace of coated and uncoated fresh-cut 'Rojo Brillante' persimmon stored in different modified atmospheres (MA) at 5 ºC. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 50 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. Solid and dashed lines represent coated and uncoated samples, respectively. LSD values are at the 95% level.

The headspace gas composition of the samples packed under atmospheric control conditions (Control) was maintained at 21 kPa O_2 and <1 kPa CO_2 during storage, confirming the no modification of the atmosphere in the trays. Whereas, the headspace atmosphere in MA-P conditions showed a sharp increase in CO_2 and a decrease in O_2 concentrations as a consequence of persimmon respiration and the permeability characteristics of the polypropylene film that hinder gas exchange with the outside atmosphere. Under this packaging condition, coated samples consumed the O_2 of the headspace more rapidly than uncoated samples, reaching also higher levels of CO_2, which indicates a higher respiration rate than uncoated fresh-cut persimmon. Oms-Oliu, et al. (2008) also described that uncoated melon exhibited a lower modification in internal atmosphere than those pieces coated with gellan-, pectin- or alginate-based formulations at the end of storage.

Color changes on fresh-cut persimmon

Figures 2 and 3 show the effect of the SPI-based coating and the MAs packaging conditions on color a* and L* values of fresh-cut 'Rojo Brillante' persimmon. Increased enzymatic browning of persimmon pieces during storage was accompanied by an increase in a* and a decrease in L*. All the treatments presented a sharp decrease in L* and an increase in a* after 1 day of storage, while values were maintained fairly constant afterwards, except for uncoated persimmons stored under MA-B that showed a progressive decreased in L* value during storage. In general, coated samples had lower a* and higher L* values than uncoated samples, indicating that the SPI-citric acid coating was effective in controlling enzymatic browning of fresh-cut 'Rojo Brillante' persimmon.

The MA-A was the most effective atmosphere in reducing enzymatic browning of persimmon slices. Under this MA packaging condition, little or no differences were found in L* values between coated and uncoated samples, while a* values were lower for coated than for uncoated samples. Pérez-Gago et al. (2006) reported that a whey protein-based coating containing ascorbic acid was more effective at reducing enzymatic browning of fresh-cut 'Rojo Brillante' persimmons than the antioxidant aqueous solution.

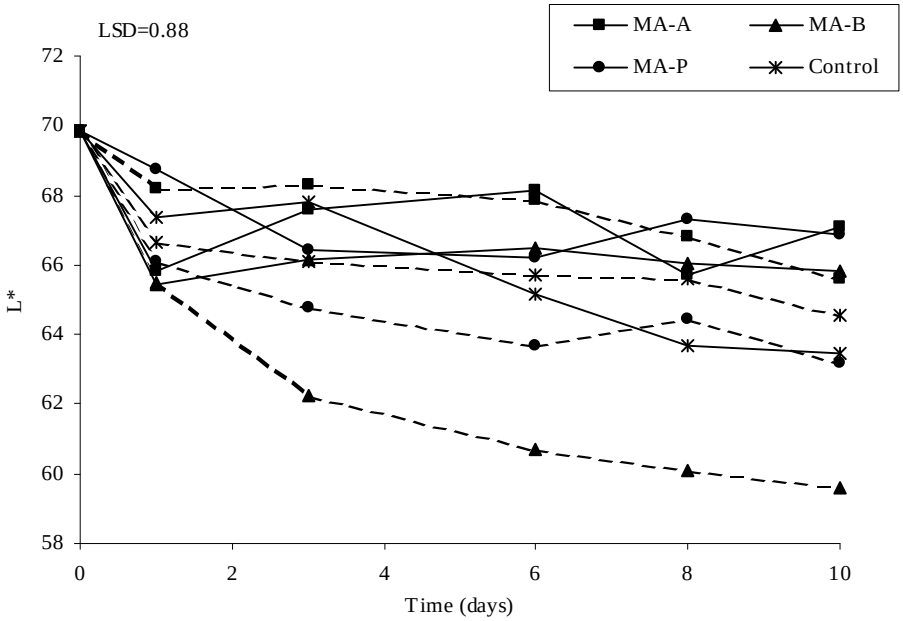

Fig. 2. Effect of a soy protein-based edible coating and modified atmosphere (MA) packaging on lightness (L*) of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 50 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. Solid and dashed lines represent coated and uncoated samples, respectively. LSD value is at the 95% level.

The MA-B was the least effective in controlling enzymatic browning of fresh-cut persimmons. Browning was induced in uncoated samples packed under MA-B compared to atmospheric control conditions, showing the lowest L* and the highest a* values. The application of the SPI-based coating increased L* and decreased a* values of fresh-cut persimmons packed under MA-B. This could be due to the effect of the coating to control enzymatic browning and/or to the lower CO_2 and O_2 concentration reached in the package headspace for coated samples compared to uncoated ones (Fig. 1). Some works have reported the positive effect of high O_2 atmospheres in controlling

enzymatic browning of fresh-cut products, such as melon (Oms-Oliu et al., 2008) and lettuce (Escalona et al., 2006). The combination of high O_2 atmospheres and citric acid has also been effective to reduce enzymatic browning of minimally processed potatoes (Limbo and Piergiovanni, 2006). However, in other products, such as pear or fresh-cut mango, exposure to high O_2 atmospheres induced browning of the tissue (Gorny et al., 2002; Poubol and Izumi, 2005).

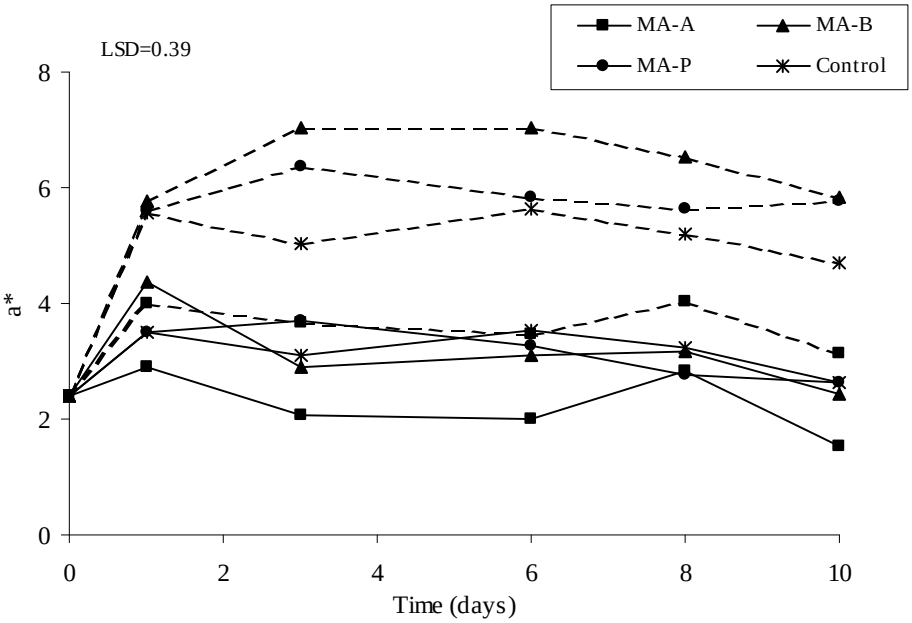

Fig. 3. Effect of a soy protein-based edible coating and modified atmosphere (MA) packaging on a* values of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 50 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control atmospheric conditions during storage. Solid and dashed lines represent coated and uncoated samples, respectively. LSD value is at the 95% level.

Uncoated persimmon pieces packed under passive modified atmosphere (MA-P) also showed enzymatic browning with lower L* and higher a* values than samples packed in atmospheric control conditions

(Control). These results might indicate that fresh-cut 'Rojo Brillante' persimmons are damaged by high CO_2 (7 kPa) and/or low O_2 levels (15 kPa). As observed in samples packed in MA-B, the application of the coating slightly reduced tissue browning, showing higher L* and lower a* values than uncoated samples under similar storage conditions.

Fruit firmness

Firmness o the samples decreased from 25±4.0 N at day 0 of storage to 12±4.2 N at the end of the storage. But, it was not affected by the coating application or the MA packaging conditions (data not shown).

The effect of conventional MA packaging with low O_2 and high CO_2 in fresh-cut commodities seems to depend on the specific fruit or vegetable and the gas composition (Toivonen and DeEll, 2002). For example, an atmosphere containing 0.5 kPa O_2 was found to prevent softening of cut pears (Rosen and Kader, 1989); whereas, low O_2 ($\leq$ 4 kPa) and higher CO_2 (5, 10, 20 kPa) concentrations did not prevent softening in fresh-cut pear and banana (Gorny et al 2002, Vilas Boas and Kader, 2006). Similarly, the use of high O_2 levels has been reported to have a positive effect at maintaining firmness in sliced carrot, fresh-cut spinach, iceberg lettuce and melon (Amanatidou et al. 2000; Day 2001; Allende et al. 2004; Oms Oliu et al., 2008); whereas, showed not effect in other fresh-cut products, such as mango (Poubol and Izumi, 2005).

Firmness loss can also be prevented with the use of edible coatings. However, its effectiveness depends on many factors, such as coating composition, storage conditions, commodity, etc. For example, Eswaranandam et al. (2006) reported that the firmness of fresh-cut apple coated with a soy protein-base edible coating was higher than uncoated samples. Whereas, Shon and Haque (2007) found in preliminary studies no effect of similar coatings in texture of several cut vegetables and fruits, such as apples, carrots, potatoes, and onions.

Sensory quality

Browning of fresh-cut persimmons was also assessed by a sensory panel with the objective of comparing if the color differences observed instrumentally could be observed visually. The visual quality of the persimmon slices, based on color and general appearance, is shown in Fig. 4.

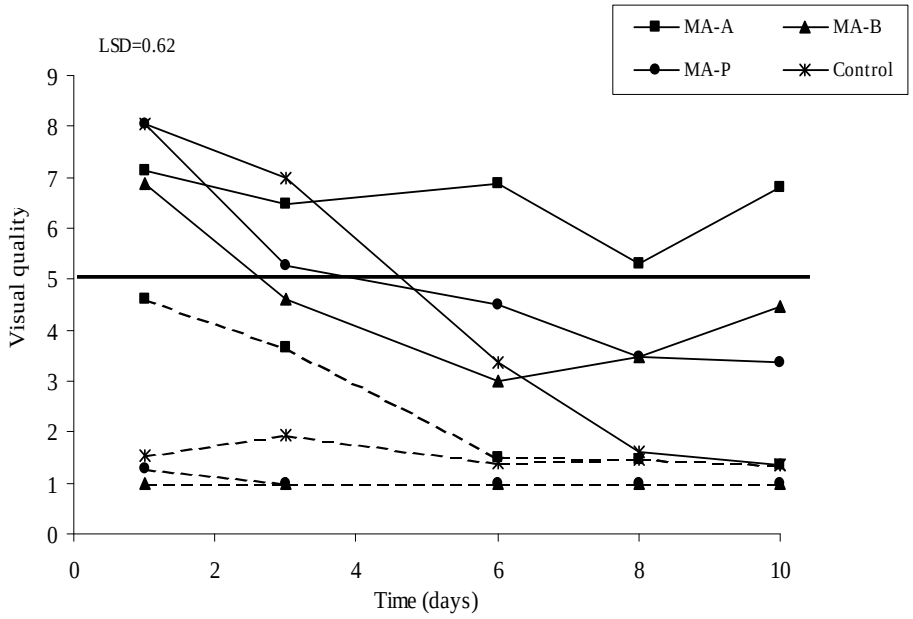

Fig. 4. Effect of a soy protein-based edible coating and modified atmosphere (MA) packaging conditions on visual quality of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 50 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. Solid and dashed lines represent coated and uncoated samples, respectively. LSD value is at the 95% level.

Independently of the packaging conditions, uncoated samples were evaluated below the limit of marketability by day 1 of storage; whereas, all the coated samples were still above this limit by day 3 of storage. Coated fresh-cut 'Rojo Brillante' persimmons packed under MA-A reached the maximum commercial shelf life, with a limit of marketability of 8-10 days of storage at 5 ºC. These results were confirmed in the ranking test based on the degree of browning (Fig. 5). In general, coated samples were classified with lower degree of browning

than uncoated samples. After 10 days of storage at 5 ºC, coated samples packed in MA-A and MA-B were ranked with the lowest degree of browning; whereas, uncoated samples packed in MA-B were ranked with the highest degree of browning. These results correlated with the color data (Figs. 2 and 3).

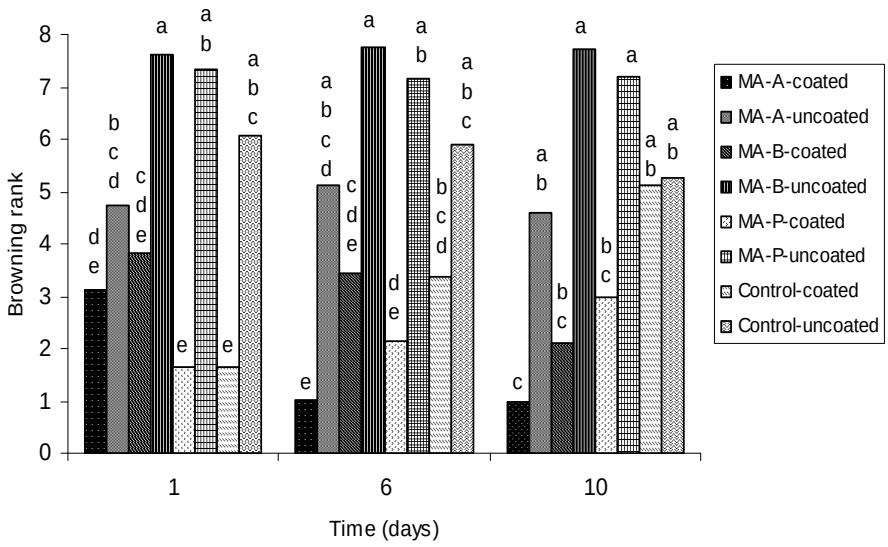

Fig. 5. Effect of a soy protein-based edible coating and modified atmosphere (MA) packaging conditions on the degree of browning of fresh-cut 'Rojo Brillante' persimmon during storage at 5 ºC. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 50 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage. Judges ranked the persimmon pieces from 8 (highest browning) to 1 (lowest browning) and were allowed to group those treatments that were considered similar. Means values with the same letter are not significantly different ($p \leq 0.05$).

To determine if panelists could detect the edible coating a triangle test was performed between coated and uncoated samples. The triangle test was repeated in two sessions to improve test power (Dacremont and Sauvageot, 1997). Table 1 shows the number of correct responses identifying the odd sample in both triangle tests. The results showed that panelists were able to differentiate between coated and uncoated samples ($p<0.01$). This could be because the SPI provided a distinctive flavor to persimmon slices or to the presence of citric acid in the formulation. In this sense, some members of the panel indicated differences in acidity between samples. Therefore, it may be useful to set up a similar experiment comparing coated samples versus samples dipped in the antioxidant aqueous solution to identify if differences were due to the soy protein flavor or the citric acid.

Table 1. Number of correct responses identifying the odd persimmon slice sample in the triangle test: Comparison between samples dipped in the soy protein-based edible coating (coated) and samples dipped in water (uncoated).

Comparison	Session 1	Session 2
Coated vs. Uncoated	14/20***	13/17***

*** Indicates that the differences are significant at 0.1% level.

The sensory quality attributes of 'Rojo Brillante' persimmons were evaluated before processing and after 3 and 6 days of storage at 5 ºC (Tables 2 and 3). Before processing, fresh persimmons were evaluated as very firm, without off-flavor, with a moderate 'characteristic flavor to persimmon' and very good overall quality (Table 2).

Table 2. Sensory evaluation of fresh 'Rojo Brillante' persimmons before processing.

Taste attributes	Values
Off flavor	0.0±0.0
Characteristic flavor	2.3±0.8
Firmness	3.9±0.4
Overall quality	7.3±0.9

Off-flavors and characteristic flavor rated from 0 (no presence) to 3 (marked presence). Firmness rated from 1 (very soft) to 5 (very firm). Overall quality rated from 1 (very poor) to 9 (excellent).

After processing and storage at 5 ºC, the samples maintained their characteristic flavor as moderate, the firmness decreased, as well as the overall quality of the samples. In any case the application of the SPI-based coating or the different packaging conditions induced off flavors to the samples. Although some significant differences were found among treatments after 3 days of storage in characteristic flavor, firmness and overall quality, differences disappeared after 6 days of storage at 5 ºC (Table 3).

Table 3. Effect of a SPI-based edible coating and modified atmosphere (MA) packaging conditions on sensory quality attributes of fresh-cut 'Rojo Brillante' persimmon after 3 and 6 days of storage at 5 ºC.

Treatments	Off flavor		Characteristic flavor		Firmness		Overall quality	
	3 d	6 d	3 d	6 d	3 d	6 d	3 d	6 d
MA-A-coated	0.3 a	0.4 a	1.4 c	1.5 a	3.1 abc	2.6 a	5.6 c	5.2 a
MA-A-uncoated	0.1 a	0.1 a	1.8 abc	1.5 a	3.5 a	3.1 a	6.3 abc	5.7 a
MA-B-coated	0.3 a	0.2 a	1.7 bc	1.4 a	3.0 bc	2.5 a	6.0 bc	5.2 a
MA-B-uncoated	0.1 a	0.2 a	2.1 ab	1.6 a	3.5 a	2.7 a	6.6 ab	5.5 a
MA-P-coated	0.2 a	0.2 a	1.7 c	1.8 a	2.8 c	3.0 a	5.5 c	5.7 a
MA-P-uncoated	0.2 a	0.1 a	2.1 a	1.8 a	3.5 ab	3.2 a	6.6 ab	6.1 a
Control-coated	0.4 a	0.4 a	1.6 abc	1.7 a	2.7 c	2.8 a	5.6 c	5.5 a
Control-uncoated	0.1 a	0.2 a	2.2 ab	2.1 a	3.4 ab	2.9 a	7.0 a	6.1 a

SPI = soy protein isolate. MA-A (15 kPa CO_2 + 5 kPa O_2); MA-B (50 kPa O_2); MA-P (21 kPa O_2 + 0.03 kPa CO_2); Control (atmospheric conditions during storage). Off-flavors and characteristic flavor rated from 0 (no presence) to 3 (marked presence). Firmness rated from 1 (very soft) to 5 (very firm). Overall quality rated from 1 (very poor) to 9 (excellent).

These results indicate that, although the judges were able to detect the coating (Table 1), these treatments do not affect negatively the sensory quality of the fresh-cut 'Rojo Brillante' persimmon.

Antioxidant capacity

Fig. 6 shows the total antioxidant capacity of fresh-cut 'Rojo Brillante' persimmon measured at day 0 (at harvest) and 6 of storage at 5 ºC. The lowest antioxidant capacity was observed in samples packed under MA-A (low O2 and high CO_2 levels), showing a decrease around 50% compared to the initial value. Whereas, persimmon slices packed in atmospheric conditions (Control) and passive MA (MA-P) maintained the antioxidant capacity after 6 days of storage at 5 ºC.

There is scarce information related with the antioxidant capacity of fresh and minimally processed persimmon. Chen et al. (2008) determined the antioxidant capacity of 'Mopan' persimmon showing higher values than those obtained in our work with 'Rojo Brillante' persimmon. De Ancos et al. (2000) also reported higher antioxidant capacity values than those determined in our work for fresh 'Rojo Brillante' persimmon. Nevertheless, the values obtained by De Ancos et al. (2000) corresponded to astringent fruits. The application of high CO_2 concentrations to remove the astringency provokes the insolubilization of the tannins, which might have caused a decrease of the antioxidant capacity of the product.

Several works have described that the application of high CO_2 concentrations (Testoni, 2002; Wright and Kader, 1997) and operations as peeling and cutting of fruits and vegetables (Lee and Kader, 2000) might provoke the degradation of antioxidant compounds like polyphenols and vitamin C during the first hours of cold storage. In our case, the use of active MA packaging with low O_2 and high CO_2 levels negatively affected the total antioxidant capacity of the samples; whereas, passive MA packaging and atmospheric conditions where the best packaging conditions. The application of the SPI-based coating did not affect the total antioxidant capacity of the samples, except for those packed in MA-A. However, more studies are needed to understand the effect of edible coatings and MA packaging on the antioxidant capacity of fresh-cut 'Rojo Brillante' persimmon.

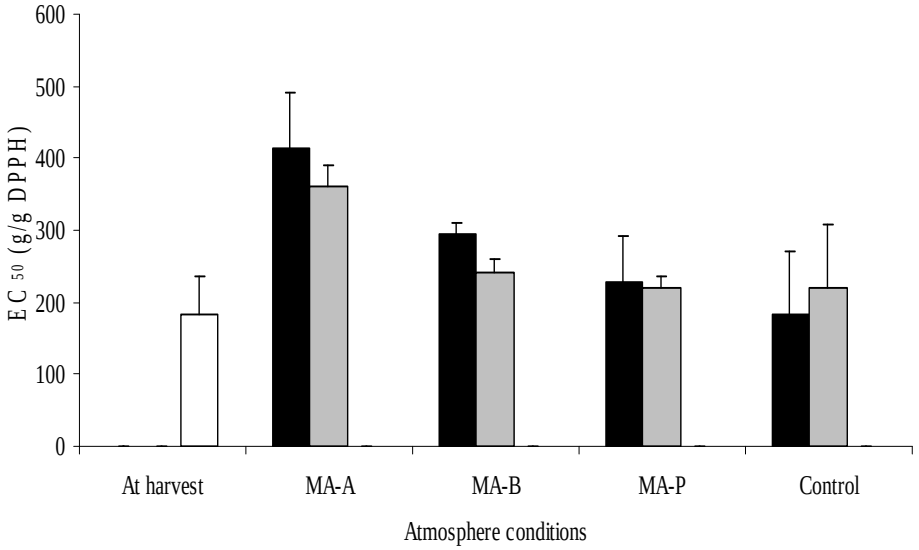

Fig. 6. Effect of a soy protein-based coating and modified atmosphere (MA) packaging conditions on the antioxidant capacity (EC_{50}) of fresh-cut 'Rojo Brillante' persimmon after 6 days of storage at 5 ºC. Vertical bars represent standard deviations. ■,coated; ▭ uncoated. MA-A: 15 kPa CO_2 + 5 kPa O_2; MA-B: 50 kPa O_2; MA-P: 21 kPa O_2 + 0.03 kPa CO_2; Control: atmospheric conditions during storage.

CONCLUSION

The combination of the SPI-based coating with the active MA-A packaging (15 kPa CO_2 + 5 kPa O_2) showed a synergic effect in controlling tissue browning of fresh-cut 'Rojo Brillante' persimmon, maintaining the general visual quality above the limit of marketability up to 8-10 days of storage at 5 ºC. However, this packaging condition reduced the total antioxidant capacity of fresh-cut persimmons after 6 days of storage at 5 ºC. On the contrary, the application of high O_2 atmospheres (>30-50 kPa) is not recommended for persimmons slices, since it induced tissue browning. Although the sensory panel was able to

discriminate between coated and uncoated samples, the application of the SPI-based coating did not affect negatively the overall quality of fresh-cut persimmon, which makes this coating a potential treatment to extend the commercial shelf life of minimally processed 'Rojo Brillante' persimmon.

AKNOWLEDGEMENTS

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GENERAL DISCUSSION

1. Effect of antioxidants in controlling enzymatic browning of minimally processed artichoke, eggplant and persimmon

The general objective of the present doctoral Thesis was to develop soy protein-based edible coatings with antioxidant activity to control enzymatic browning of fresh-cut 'Blanca de Tudela' artichoke, 'Telma' eggplant, and 'Rojo Brillante' persimmon and to study the combined effect of selected edible coatings and modified atmosphere packaging conditions on the quality and shelf life of these products, which are characterized by a relatively short shelf life due to a rapid onset of enzymatic browning.

Firstly, a wide range of antioxidant agents with different mechanism of action were studied in extracts and precipitates of selected fruit and vegetables as pre-screening to determine the potential effect of the antioxidants to control enzymatic browning of fresh-cut fruits and vegetables (*in vitro* studies) (Amiot et al., 1992; Eissa et al., 2006; Arias et al., 2008; Chiabrando and Giacalone, 2012). The antioxidants tested included acidulants as citric and peracetic acid (CA, and PA), reducing agents as cysteine (Cys) and ascorbic acid (AA), chelating and complexing agents as hexametaphosphate (HMP) and cyclodextrin (CD), or enzymatic inhibitors as 4-hexylresorcinol (Hexyl) and calcium chloride ($CaCl_2$). An initial concentration of 10 mM was tested for all the antioxidants and the concentrations were either increased or decreased depending on absorbance and reflectance measurements obtained for each antioxidant, as a value of soluble and insoluble brown pigments.

AA is probably the most widely used antibrowning agent, and in addition to its reducing properties, it also slightly lowers pH (Garcia and Barrett, 2002). In *in vitro* studies, AA was effective at controlling enzymatic browning on extracts and pellets of artichoke, eggplant, and persimmon at concentrations of 10, 20, and 25 mM, respectively, showing the potential of this antioxidant for these commodities.

Cys at 10 mM completely inhibited soluble and insoluble brown pigments of eggplant. The application of a lower concentration (1 mM Cys) also reduced soluble and insoluble brown pigments of the extract and precipitate, that were evaluated by the judges as slightly browned, showing the potential of this antioxidant to control browning of fresh-cut eggplants. In artichoke a higher concentration of Cys (50 mM) was

required to control browning of the extract and precipitate; whereas in persimmon extracts and precipitates, Cys was not effective even at the highest concentrations tested (50-75 mM), suggesting that this antioxidant is not appropriated to control enzymatic browning of 'Rojo Brillante' persimmon. Contrary to this finding, other works have shown that thiol containing compounds such as Cys and metabisulfite were effective in inhibiting persimmon polyphenol oxidase (PPO) activity (Núñez-Delicado et al., 2003; Özen et al., 2004). However, the activity of thiol compounds was only significant in the presence of 1 mM sodium dodecyl sulfate (Núñez-Delicado et al., 2003).

The use of chemicals such as CA and PA to lower the product pH of the product below the optimum for PPO activity is also a normal practice in fresh-cut fruits and vegetables. In this Thesis, CA at 10 mM was effective at controlling browning in persimmon extracts and precipitates; whereas, a higher concentration of CA (50 mM) was needed to prevent browning on artichoke and eggplant extract and precipitate. A reduction of pH below 4 could be the reason for the complete inhibition of soluble and insoluble brown pigments of persimmon at 10 mM CA. However, the results suggested a higher stability of the PPO in artichoke and eggplant, since even at pHs values below the optimum for PPO activity brown pigments were not completely inhibited by CA application.

On the other hand, the use of PA was only effective at high concentration (50 mM) in the extract and precipitate of eggplant. Whereas, PA was not effective at controlling browning for artichoke and persimmon at the higher concentrations tested, even though pHs were below the optimum PPOs activity. This is supported by several works that describe variations in the effect of different acids on PPO (Almeida and Nogueira, 1995; Garcia and Barrett, 2002; Todaro et al., 2011).

4-Hexyl is one of the antioxidants that has been described as having the highest potential for application to fresh-cut produces. In eggplant and persimmon extracts and precipitates, Hexyl at 10 mM was effective to inhibit soluble and insoluble brown pigments. This antioxidant, although prevented the formation of brown pigments in the precipitate and extract of artichoke, provoked a sharp pinkish color in the precipitate and a translucent appearance in the extract. Nevertheless, a concentration of 50 mM was considered effective by the judges at controlling browning of the extract and precipitate.

Although $CaCl_2$ is usually used for tissue firming, it has been demonstrated that the chloride ion can act as an inhibitor of PPO activity (McEvily et al., 1992). In the *in vitro* study, $CaCl_2$ at 10 Mm was effective at preventing the formation of browning pigments both in persimmon extracts and precipitates; whereas it was not effective for artichoke and eggplant even at the highest concentrations tested.

The other antioxidants tested (CD and HMP) were not effective even at concentrations of 50-75 mM. Since an increase in the concentration of these antioxidants from 10 mM to 75 mM did not show a significant improvement at controlling brown pigments in the extract and precipitate, higher concentrations were not studied.

The most effective antioxidant type and concentrations in *in vitro* studies were selected to be tested in fresh-cut tissue of artichoke, eggplant and persimmon (*in vivo* studies). Considering the differences between the nature of the samples in *in vitro* and *in vivo* studies (i.e. ground versus fresh-cut tissue), these concentrations were either increased (2.5 and 5 times those concentrations) or decreased, depending on the antioxidant, to values close to those reported in the bibliography for other fresh-cut commodities.

Although antioxidants reduced enzymatic browning of **fresh-cut 'Blanca de Tudela' artichokes** compared to non-treated samples, shelf life was still low compared to fresh-cut eggplants and persimmons. In extracts and precipitates, AA, Cys and Hexyl were the most effective antioxidants preventing browning. However, in fresh-cut tissue only Cys at concentrations above 0.5% significantly extended shelf life till 4 days of storage at 5 ºC. However, Cys treatments also resulted in an increase in b* values (yellowness), that increased as Cys concentration increased. Similarly to our results, yellowness was also observed by Amodio et al. (2011) in fresh-cut 'Catania' artichoke as Cys content increased, indicating the formation of some color compounds at high Cys concentration. According to Cavallini et al. (1969), the addition of excess Cys to an alkaline Cu^{II} solution resulted in the appearance of a yellow compound identified with a Cys-copper complex. Considering that artichoke is a vegetable with a significant copper content (United States Department of Agriculture, 2011), the addition of high Cys concentrations could be a reason for the formation of these yellow compound.

Application of AA in a concentration range of 0.5-2% and Hexyl at 0.002 and 0.005% induced browning. These results in fresh-cut artichokes contrast with those found in the extract, where AA and Hexyl were effective at reducing soluble and insoluble browning products at different concentrations. These differences might be due to the effect of the antioxidants not only in browning reactions, but also in the metabolic activity and cell wall changes during wound induced reactions. Our results in fresh-cut 'Blanca de Tudela' artichoke are confirmed by works that have also reported higher browning in minimally processed artichokes treated with AA (Palma et al., 2004; Amodio et al., 2011) and Hexyl (Amodio et al., 2011) compared to untreated samples. Furthermore, Todaro et al. (2010) observed that depending on artichoke cultivar, PPO activity could be stimulated at high AA concentrations.

In **fresh-cut eggplant**, only Cys resulted effective at controlling browning *in vitro* and *in vivo*; whereas, Hexyl was only effective *in vitro* and induced browning in fresh-cut tissue. Similarly, the application of AA, CA, and PA at the concentrations tested also resulted in an increase in tissue browning compared to untreated samples. The maximum commercial shelf-life for fresh-cut eggplant was 9 days of storage at 5 ºC when Cys was applied at a concentration of 1%. Dipping eggplants in high concentrations of AA (>0.88%) and other low-pH solutions such as CA and PA is not recommended as it increased browning. A similar behavior was observed in fresh-cut 'Blanca de Tudela' artichokes, which has been attributed by other authors to a possible oxidative damage of the tissue with cell disruption and decompartmentalization (Jiang et al. 2004; Larrigaudière et al. 2008).

Contrary to the results observed in fresh-cut artichokes and eggplants, AA and CA were the most effective antioxidants at reducing enzymatic browning of **fresh-cut 'Rojo Brillante' persimmon**, reaching the limit of marketability in the range of 5-7 days. In particular, concentrations of 1.12% AA and 0.21% CA seemed to be the most effective in controlling enzymatic browning. Nevertheless, an increase in AA or CA concentrations to 2.25% or 1.0% slightly decreased lightness of persimmon tissue. $CaCl_2$ also contributed to extend the shelf life of persimmon pieces, however its effectiveness was lower than AA and CA.

As observed with artichoke and eggplant, Hexyl was the least effective antioxidant for fresh-cut persimmon and its application induced

damage of the tissue. In *in vitro* studies, Hexyl resulted effective in reducing browning in the extracts and precipitates of all the commodities tested. However, in *in vivo* trials with fresh-cut tissues, Hexyl-treated samples were the most browned, indicating that the application of the antioxidants has an effect not only on browning reactions, but also on metabolic activity and cell wall changes during wound-induced reactions.

2. Effect of soy protein isolate (SPI)-based edible coatings with antioxidant activity in controlling enzymatic browning of minimally processed artichoke, eggplant and persimmon

It is widely known that edible coatings can provide significant benefits in extending shelf-life and enhancing quality of fresh-cut fruits and vegetables by providing a semi-permeable barrier to gases and water vapor, and by acting as carriers of food ingredients such as antioxidants, antimicrobials, flavors, nutrients, etc. (Baldwin et al., 1995). Nevertheless, the use of edible coatings on a wide range of fresh-cut products and on a commercial scale is still limited by several factors. The success of an edible coating is based on the physicochemical and barrier properties of its components (proteins, polysaccharides, lipids) and the effect of minor ingredients. Determining the proper composition and proportions of the components is of prime importance in order to extend the shelf-life and enhance the quality of fresh-cut fruit and vegetables. In particular, soy protein isolate (SPI) coatings have been able to preserve freshness of apple slices (Kinzel, 1992), to control browning in potato slices, and to reduce moisture loss in carrots and apple slices (Shon and Haque, 2007). Nevertheless, its hydrophilic nature requires the addition of hydrophobic components to improve the moisture barrier of the coating. Among lipid components, beeswax (BW) has shown good compatibility with many coating-forming materials (Greener and Fennema, 1992). Therefore, in this work our objective was to develop SPI-BW edible coatings amended with selected antioxidant agents to maintain the quality and extend the shelf life of minimally processed ‘Blanca de Tudela’ artichoke, ‘Telma’ eggplant, and ‘Rojo Brillante’ persimmon.

In **fresh-cut ‘Blanca de Tudela’ artichokes**, the optimization of the SPI:BW edible coating was based on Cys and BW content to reduce

enzymatic browning. Although the application of the SPI:BW coating was not effective at controlling the browning of cut artichokes, the addition of Cys to the SPI-BW based edible coating, formulated with 20% BW (dry basis, db), improved the quality of minimally processed artichokes as compared to the application of the antioxidant in aqueous solution. The samples dipped into the SPI coating amended with 0.5% Cys (wet basis, wb) reached the limit of marketability after 5 day storage at 5 ºC. Optimization of the coating implied an increase in the BW content from 20 to 40% (db) to reduce the yellow color that resulted from the Cys application to artichoke tissue. The increase of the BW content in the SPI emulsion contributed to prolong the shelf life of the artichoke slices, as a lower Cys content (0.3%) allowed to reach the limit of marketability within 4 days of storage at 5 ºC whereas, the application of the antioxidant aqueous solution had a lower limit of 2 days of storage. A similar 4-day shelf life was achieved in 'Blanca de Tudela' artichoke slices dipped into 0.5% Cys aqueous solutions from previous optimization (Chapter 1). Therefore, this SPI-based coating slightly improved as the formulation had a lower Cys concentration, thus reducing the risk of altering artichoke flavor and fresh appearance. Del Nobile et al. (2009) reported that minimally processed artichoke heads dipped into sodium alginate-based edible coating containing citric acid reached 3 days of storage, whereas the shelf life of the control ranged from 1 to 2 days at 5 ºC.

No work has been found in the literature describing the effect of edible coatings on the shelf life of **fresh-cut eggplants**. The SPI coating containing 20% BW (db) was amended with Cys at 0.5 % or 1% (wb), as selected antioxidant from previous optimization. AA at 1% was also tested, since a concentration of 0.88% in aqueous solution maintained the limit of marketability around 5 days of storage at 5 ºC (Chapter 2). However, the SPI-BW coating containing 1% AA did not control enzymatic browning and the samples were evaluated below the limit of marketability after 1 day of storage, as control samples. The application of Cys, either alone or incorporated to the SPI-BW coating, helped at controlling enzymatic browning and extending the commercial shelf life of 'Telma' fresh-cut eggplants to 7-9 days. The incorporation of the Cys to the SPI-BW coating resulted in little or no differences in color parameters compared to the application of the antioxidant in aqueous

solution. Nevertheless, the visual assessment evaluated the samples coated with the SPI-BW-1% Cys coating as significantly less brown than the rest of the treatments, reaching a commercial shelf life of 9 days at 5 ºC. Contrarily, other works have reported that the incorporation of antioxidants to protein-based edible coatings resulted more effective in controlling enzymatic browning of fresh-cut produces, such as apples and persimmons, than the application of the antioxidants in aqueous solution (Pérez-Gago et al., 2005; 2006).

For **fresh-cut 'Rojo Brillante' persimmon**, the combination of 1% CA and 0.3% $CaCl_2$ was selected, based on previous results, as antioxidant treatment to be incorporated into the SPI-based coating (Chapter 3). In a preliminary screening based on fruit appearance, it was observed that the incorporation of BW to the formulation gave a slight whitish appearance to the fresh-cut persimmon that affected the visual quality (data not presented). Therefore, the selected coating formulation to be tested in combination with modified atmosphere packaging was SPI-CA+$CaCl_2$.

3. Combined effect of selected soy protein-based edible coatings with antioxidant capacity and modified atmosphere packaging (MAP) on the quality and shelf life of fresh-cut artichoke, eggplant, and persimmon

Post-processing treatments such as low temperature, antioxidant dips and low oxygen atmosphere packaging are the most common approaches to extend the shelf life of minimally processed fruits and vegetables. Other technologies such as edible coatings with natural additives and non-conventional atmospheres have gained a lot of interest as a possibility to extend the shelf life of these products. However, the high perishability of some products challenges in many cases their marketability by not achieving sufficient shelf life to survive the distribution system, requiring the combination of treatments to assure safety and quality.

Low O_2 and high CO_2 MAP has been widely used to extend fresh-cut fruits and vegetables shelf life by reducing the respiration rate, ethylene production, enzymatic browning, weight loss, etc. (Toivonen and DeEll, 2002). As an alternative to low O_2, some studies have proposed the use of elevated O_2 concentrations in order to reduce PPO activity, inhibit

anaerobic fermentation, control microbial growth and maintain the fresh-like quality of some fresh-cut products. Nevertheless, the effectiveness of conventional and high O_2 MAP are dependent on factors such as type of commodity, temperature, storage duration, etc. (Kader and Ben-Yehoshua, 2000). Therefore, the final objective of this Thesis was to evaluate the combined effect of selected soy protein-based edible coatings with antioxidant capacity and conventional or high oxygen MAP on the quality and shelf life of fresh-cut 'Blanca de Tudela' artichoke, 'Telma' eggplant, and 'Rojo Brillante' persimmon during storage at 5 ºC.

In **fresh-cut 'Blanca de Tudela' artichokes**, the maximum commercial shelf life achieved was 4 days of storage with the coated samples (optimized in previous works with 40% BW (db) and 0.3% Cys (wb)). Combining this coating with the different MAP conditions studied in this work did not extend the shelf life of 'Blanca de Tudela' artichoke slices, but it helped with maintaining the antioxidant capacity of the product. When comparing among MAP conditions, coated samples packaged under superatmospheric O_2 or atmospheric conditions (control) maintained a better quality that samples packaged in active conventional MAP (5 kPa O_2 + 15 kPa CO_2) and passive MAP, that reached the limit of marketability within 1 and 2 days, respectively. Gómez di Marco et al. (2011) reported that the best combination to reduce artichoke heads browning was the application of high O_2 concentrations (80 kPa) and lemon juice as antioxidant. However when no antioxidant was applied, the passive MAP was more effective than the high O_2 atmosphere.

Given the high degree of perishability of untreated fresh-cut artichoke, a 4-day commercial period could be considered adequate for the distribution of sliced produce to local markets. However, additional studies are required to enhance the quality and appearance of fresh-cut artichokes and further extend their shelf life.

For **fresh-cut eggplants**, two experiments were conducted to study the effect of SPI-based edible coatings and MAP on the quality and shelf life of the product. In the first experiment, the SPI-BW coating was prepared with 0.5% Cys. The headspace O_2 concentration was not significantly influenced by coating application as compared to the uncoated samples (water-dipped) for any packaging condition. However, significant differences were found in the CO_2 concentrations between the coated and uncoated eggplants stored under low oxygen concentration

and superatmospheric O_2 conditions. The results showed that conventional MAP conditions (low O_2 - high CO_2) induced damage of the tissue, not being recommended for storage of fresh-cut eggplants. This could be attributed to an increase in the PPO activity by high CO_2 levels (Catalano et al., 2007) or tissue damage by CO_2 injury as reported in fresh-cut pears (Gorny et al., 2002). The use high O_2 MAP also limited firmness loss, which could be related with the lower activity of cell wall hydrolytic enzymes, as observed by Deng et al. (2005) in table grapes.

In a second experiment conducted to further extend the shelf life of fresh-cut eggplants, the Cys concentration was increased from 0.5% to 1% in the SPI-based edible coating and coated samples were packed under atmospheric conditions or superatmospheric MAP conditions. If compared to the first experiment, the increased in the Cys content in the SPI coating lowered the respiration rate of the samples and extended the commercial shelf life from 6 to 9 days of storage at 5 ºC. Whereas, no differences were found between atmospheric conditions and high O_2 MAP in terms of produce shelf life. However, storage in a high O_2 atmosphere increased the weight loss of the samples, although the coating application helped maintain weight loss under this packaging condition. Overall, the SPI-Cys coating under air atmospheric conditions provided the best and cheapest approach for extending the shelf life of fresh-cut eggplant.

The visual quality of **fresh-cut 'Rojo Brillante' persimmon** was maintained above the limit of marketability for up to 8-10 days of storage at 5 ºC when samples were coated in the SPI-based coating and packaged under active MAP (5 kPa O_2 + 15 kPa CO_2). Whereas, the application of high O_2 atmospheres (>30-50 kPa) resulted in damage of the tissue, inducing browning. Although some works have reported the positive effect of high O_2 atmospheres in controlling enzymatic browning of fresh-cut products, in other products such as fresh-cut pear (Gorny et al., 2002) and mango (Poubol and Izumi, 2005) exposure to high O_2 atmospheres induced tissue browning. Conversely, the lowest antioxidant capacity was observed in samples packed in the active MAP, showing a decrease of around 50% of the initial value. Persimmon slices packaged under atmospheric conditions (Control), passive, or high O_2 MAP maintained the antioxidant capacity after 6 days of storage at 5 ºC. Nevertheless, more studies are needed to understand the effect of edible

coatings and MAP on the antioxidant capacity of fresh-cut 'Rojo Brillante' persimmon.

Before processing, fresh persimmons were evaluated by a sensory panel as very firm, without off-flavor, with a moderate 'characteristic flavor to persimmon' and very good overall quality. After processing and storage at 5 ºC the firmness decreased, as well as the overall quality of the samples, but in any case the application of the SPI-based coating or the different packaging conditions induced off-flavors to the samples. Although a triangle test showed that panelists were able to differentiate between coated and uncoated samples ($p<0.01$), coating application did not affect negatively the overall quality and acceptability of the samples, which makes this coating a potential treatment to extend the commercial shelf life of minimally processed 'Rojo Brillante' persimmon.

The results of this work confirm that the effect of MAP on fresh-cut fruits and vegetables depend upon a number of factors, such as the species, cultivar, postharvest handling/treatments, and storage conditions, among others (Zhuang et al., 2014). Therefore, the range of O_2 and CO_2 in the package must be defined for each product and handling/processing characteristic. For the commodities studied in this Thesis, active conventional MAP (5 kPa O_2 + 15 kPa CO_2) resulted beneficial for the storage of fresh-cut 'Rojo Brillante' persimmons, whereas 'Telma' eggplants and 'Blanca de Tudela' artichokes were susceptible to tissue damage when packaged under active and passive MAP with low O_2 and high CO_2 levels.

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CONCLUSIONS

GENERAL CONCLUSIONS

1. The use of antioxidants had a limited action controlling enzymatic browning of fresh-cut artichokes. In extracts and precipitates, only ascorbic acid (AA), cysteine (Cys) and 4-hexylresorcinol (Hexyl) at 10 mM, 50 mM and 50 mM, respectively, were effective to prevent browning. Whereas, citric acid (CA), peracetic acid (PA), $CaCl_2$, cyclodextrin (CD), and hexametaphosphate (HMP) were not effective even at the highest concentration tested (50 mM).

2. In fresh-cut 'Blanca de Tudela' artichokes, only Cys at concentrations above 0.5% significantly extended shelf life till 4 days of storage at 5 ºC. Whereas, AA and Hexyl in a concentration range of 0.5-2% and 0.002-0.005%, respectively, induced browning, probably due to an increase in metabolic activity or induced oxidative damage of the tissue.

3. In fresh-cut 'Telma' eggplants, only Cys resulted effective at controlling browning *in vitro* (extracts and precipitates) and *in vivo*; whereas, Hexyl was only effective *in vitro* and induced browning of the tissue. Similarly, the application of AA (>0.88%), CA (>1%), and PA (0.4-1%) also resulted in an increase in tissue browning compared to untreated samples.

4. The maximum commercial shelf-life for fresh-cut 'Telma' eggplants was 9 days of storage at 5 ºC when Cys was applied at a concentration of 1%.

5. In *in vitro* studies in extracts and precipitates of 'Rojo Brillante' persimmons, AA at 25 mM and CA, $CaCl_2$, and Hexyl at 10 mM were effective antioxidants to control soluble and insoluble brown pigments.

6. Application of AA and CA at 1% and 0.2%, respectively, reduced enzymatic browning of fresh-cut 'Rojo Brillante' persimmon, reaching the limit of marketability in the range of 5-7 days. $CaCl_2$ at 0.6% also contributed to extend the shelf life of persimmon pieces, but its effectiveness was lower than AA and CA. However, important

differences were observed in Hexyl treatments between *in vitro* and *in vivo* tests, since it induced browning of the cut tissue at the concentrations tested.

7. The optimized soy protein isolate (SPI)-beeswax (BW)-Cys coating for extending the shelf life of fresh-cut 'Blanca de Tudela' artichokes contained 40% BW (dry basis, db) and 0.3% Cys (wet basis, wb). This coating contributed to the control of enzymatic browning and improved the quality of fresh-cut 'Blanca de Tudela' artichokes, reaching 4 days of commercial shelf life at 5 ºC without off-odors. This formulation also reduced the yellow color developed on the surface of cut artichoke by Cys application.

8. The application of Cys, either alone or incorporated to the SPI-BW coating, helped at controlling enzymatic browning and extending the commercial shelf life of 'Telma' fresh-cut eggplants to 7-9 days at 5 ºC. Whereas, AA, alone and combined with the SPI-BW coating, induced damage of the tissue and increased browning.

9. Although the incorporation of Cys to the SPI-BW coating resulted in little or no differences in color parameters of fresh-cut eggplants compared to the application of the antioxidant in aqueous solution, the visual assessment evaluated the samples coated with the SPI-BW-1% Cys coating as significantly less brown than the rest of the treatments, reaching the maximum commercial shelf life of 9 days at 5 ºC.

10. The incorporation of 1% CA and 0.3% $CaCl_2$ to a SPI-based edible coating was effective at controlling enzymatic browning in fresh-cut 'Rojo Brillante' persimmons.

11. Although the sensory panel was able to discriminate between coated and uncoated fresh-cut persimmons, the application of the SPI-based coating did not affect negatively the overall quality of the product, which makes this coating a potential treatment to extend the commercial shelf life of minimally processed 'Rojo Brillante' persimmons.

12. In fresh-cut ‘Blanca de Tudela’ artichokes, the combination of the optimized SPI-BW-Cys edible coating and the different modified atmosphere packaging (MAP) conditions tested in this Thesis did not further extend the shelf life of the product, but they helped with maintaining the antioxidant capacity.

13. When comparing among MAP conditions in fresh-cut artichokes, coated samples packaged under superatmospheric O_2 (80 kPa) or atmospheric conditions (control) maintained a better quality that samples packaged in active conventional MAP (5 kPa O_2 + 15 kPa CO_2) and passive MAP, suggesting that low O_2 atmospheres should be avoided.

14. In fresh-cut eggplants, conventional MAP conditions (low O_2 - high CO_2) induced damage of the tissue, not being recommended for storage of fresh-cut eggplants.

15. The coating helped to maintain the weight loss of minimally processed eggplants packed under MAP conditions; whereas, the high O_2 atmosphere (80 kPa) and atmospheric control conditions helped to maintain firmness of the product.

16. Since no differences were found between atmospheric conditions and high O_2 MAP in terms of produce shelf life, the optimized SPI-BW-Cys coating under atmospheric conditions can be considered the best and cheapest approach for extending the shelf life of fresh-cut ‘Telma’ eggplant, reaching a commercial shelf life of 8-9 days at 5 ^{o}C.

17. The combination of the SPI-based coating with the active MAP packaging (15 kPa CO_2 + 5 kPa O_2) showed a synergic effect in controlling tissue browning of fresh-cut ‘Rojo Brillante’ persimmons, maintaining the general visual quality above the limit of marketability up to 8-10 days of storage at 5 ^{o}C. Whereas, the application of high O_2 atmospheres (>30-50 kPa) resulted in damage of the tissue, inducing browning.

18. The range of O_2 and CO_2 in the package must be defined for each product and handling/processing characteristic. Relevant results from this Thesis show that ‘Telma’ eggplants and ‘Blanca de Tudela’ artichokes are susceptible to tissue damage when packaged under active or passive MAP with low O_2 and high CO_2 levels, whereas high O_2 MAP (>30-50 kPa) resulted detrimental for the storage of fresh-cut ‘Rojo Brillante’ persimmons.

APPENDICES

ENTRADA CAQUI IV GAMA
NOMBRE:
FECHA:

MALOS SABORES	
0	Ausencia
1	Ligeramente perceptibles
2	Perceptibles
3	Presencia acusada

SABOR CARACTERÍSTICO	
0	Ausencia
1	Ligeramente perceptibles
2	Perceptibles
3	Presencia acusada

FIRMEZA	
1	Muy blando
2	Blando
3	Ni firme ni blando
4	Firme
5	Muy firme

SABOR GLOBAL Flavor (sabor+aroma)	
1	Mala calidad (no satisfactorio)
2	
3	
4	Calidad aceptable
5	
6	
7	Calidad excelente
8	
9	

CÓDIGO	MALOS SABORES	SABOR CARACTERÍSTICO	FIRMEZA	SABOR GLOBAL	OBSERVACIONES

Figure 1. Questionnaire for sensory evaluation of fresh-cut ‘Rojo Brillante’ persimmon.

EVALUACIÓN VISUAL (COLOR + ASPECTO GENERAL)

9 = excelente, recién cortado
7 = muy bueno, bastante fresco
5 = bueno, límite de comercialización
3 = adecuado, límite de consumo
1 = malo

Tratamiento	Puntuación	Observaciones

ANÁLISIS SENSORIAL

Nombre: Fecha:
Producto:

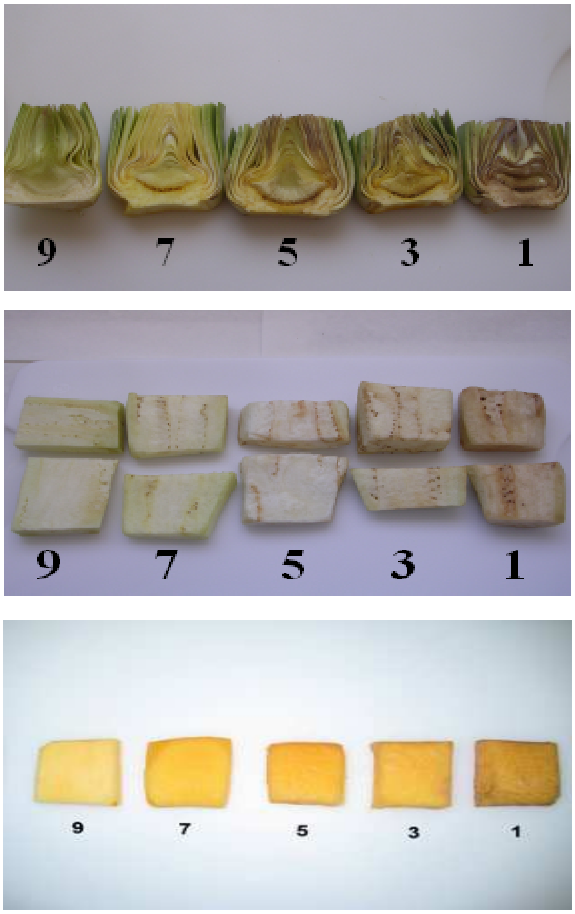
CLASIFICACIÓN POR ORDENACIÓN

Ordenar de MENOR A MAYOR (izquierda a derecha) el **pardeamiento** de las muestras estudiadas. Si algunas muestras presentan un pardeamiento similar se pueden agrupar indicando que se trata del mismo grupo

Menos pardeamiento				
				Mas pardeamiento

Colocar un tratamiento por casilla, y agrupar tratamientos con el mismo comportamiento con un círculo

Figure 2. Questionnaire for sensory evaluation of visual quality and browning ranking of fresh-cut produces.



9 = excellent, just sliced
7 = very good
5 = good, limit of marketability
3 = fair, limit of usability
1 = poor, inedible

Photo 1. Reference scale used by judges for the visual evaluation of fresh-cut artichoke, eggplant and persimmon samples.

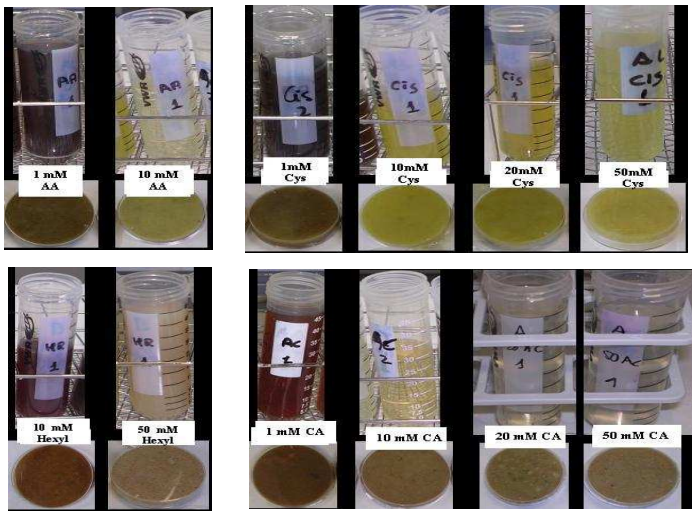


Photo 2. Effect of different antioxidants on extract and precipitate of fresh-cut 'Blanca de Tudela' artichokes. AA = ascorbic acid, Cys = cysteine, Hexyl = 4-hexylresorcinol, CA = citric acid.

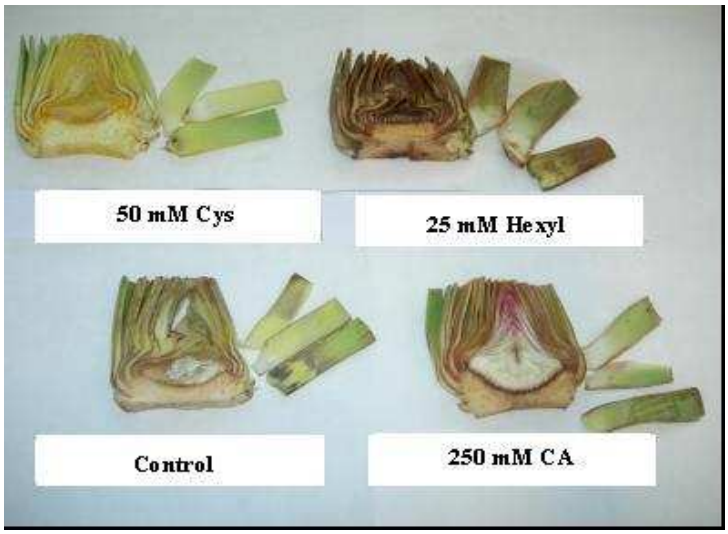


Photo 3. Effect of different antioxidants tested on fresh-cut 'Blanca de Tudela' artichokes stored at 5 ºC. Cys = cysteine, Hexyl = 4-hexylresorcinol, Control = water, CA = citric acid.

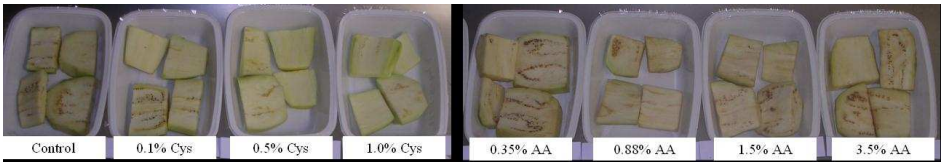


Photo 4. Fresh-cut 'Telma' eggplants treated with cysteine (Cys) or AA (ascorbic acid) and control (dipped in water) stored 5 days at 5 ºC.

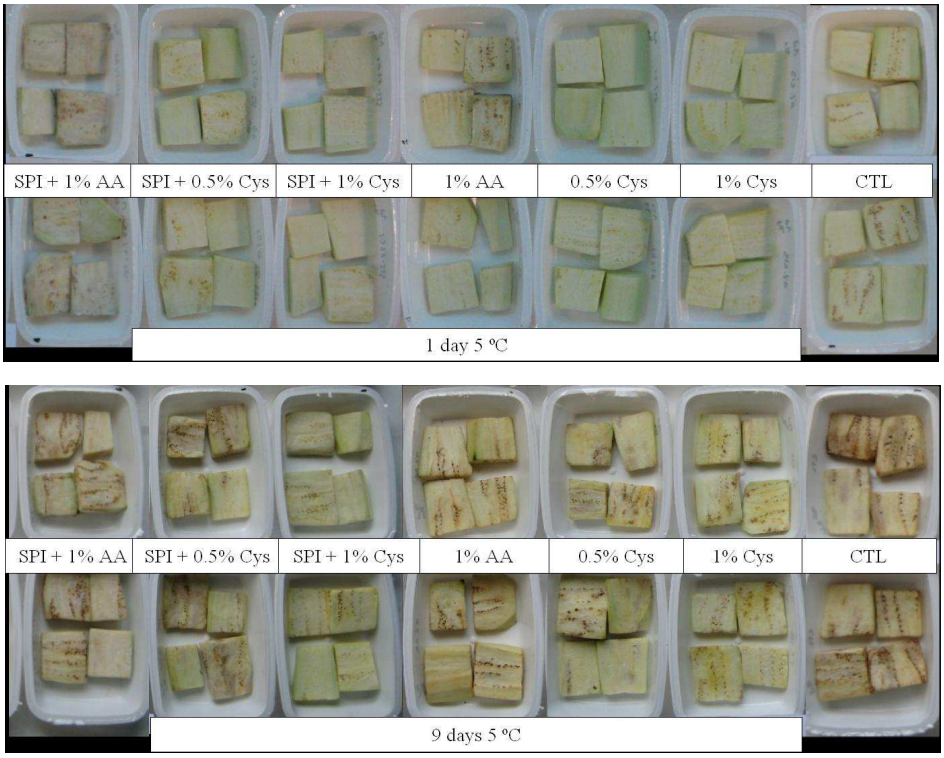


Photo 5. Effect of antioxidants alone or incorporated to the soy protein (SPI)-based edible coating on fresh-cut 'Telma' eggplants stored 1 and 9 days at 5 ºC.

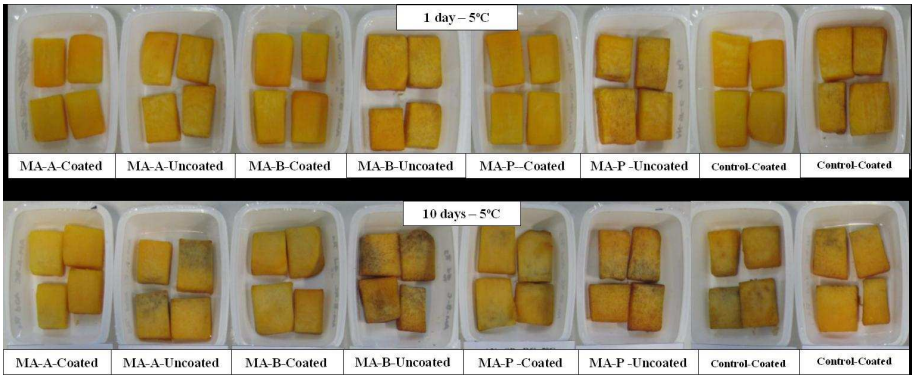


Photo 6. Effect of SPI-based edible coating and MA packaging on fresh-cut 'Rojo Brillante' persimmons stored 1 and 10 days at 5 ºC. MA-A (15 kPa CO_2 + 5 kPa O_2); MA-B (50 kPa O_2); MA-P (21 kPa O_2 + 0.03 kPa CO_2); Control (atmospheric conditions).