UNIVERSIDAD POLITÉCNICA DE VALENCIA
Departamento de Tecnología de Alimentos

EFECTO DE TRATAMIENTOS POSCOSECHA NOVEDOSOS EN LA CALIDAD FISICOQUÍMICA, SENSORIAL Y NUTRICIONAL DE CÍTRICOS

TESIS DOCTORAL

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CERTIFICAN: Que la memoria titulada 'Efecto de Tratamientos Poscosecha Novedosos en la Calidad Fisicoquímica, Sensorial y Nutricional de Cítricos', que para aspirar al grado de Doctor en Ciencia y Tecnología de los Alimentos presenta Dña. Adriana Contreras Oliva, realizada bajo nuestra 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

AUTORIZAN: A la interesada a su presentación en el Departamento de Tecnología de Alimentos de la Universidad Politécnica de Valencia.

Y para que conste a los efectos oportunos, presentamos la referida memoria, firmando el presente certificado en Valencia a 28 de Septiembre de 2010.

Fdo. Dña. María Bernardita Pérez Gago

Fdo. Dña. Cristina Rojas Argudo

El amor a un hijo es el motor
para lograr lo imposible

A mi hijo Julio Andrés, por ser la razón de mi existir, el pilar de mi alegría para continuar con mi camino.

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En los últimos años, el consumo de cítricos ha ido en aumento propiciado por su elevado contenido en vitamina C y otros componentes bioactivos. Por ello, la prioridad del mercado es desarrollar nuevas tecnologías poscosecha respetuosas con el medio ambiente que permitan alargar la vida útil de los cítricos, manteniendo la calidad fisicoquímica, sensorial y nutricional de los mismos hasta llegar al consumidor.

En esta tesis se estudió el efecto de: (1) combinaciones de cuarentena por frío con atmósferas insecticidas (AI) e irradiaciones (Rayos X) en la calidad nutricional de mandarinas 'Clemenules', (2) AI aplicadas a altas temperaturas en la calidad fisicoquímica, sensorial y nutricional de naranjas 'Valencia', (3) la aplicación de recubrimientos comestibles de quitosano a distinto contenido en sólidos (CS) en la calidad fisicoquímica, sensorial y nutricional de mandarinas 'Oronules' y naranjas 'Valencia' y (4) la aplicación de recubrimientos comestibles compuestos a base de hidroxipropilmetil celulosa (HPMC)-lípido con distinto CS y proporción de cera de abeja:goma laca en la calidad fisicoquímica, sensorial y nutricional de mandarinas 'Oronules' y naranjas 'Valencia'.

La combinación del tratamiento cuarentenario por frío (1,5 ºC durante 6, 9 y 12 días) con AI (95% CO_2 a 20 ó 25 ºC) o irradiaciones ionizantes (0, 30, 54 y 164 Gy) no afectó negativamente a la capacidad antioxidante total, ni al contenido de ácido ascórbico total (AAT) de las mandarinas 'Clemenules'. Sin embargo, los contenidos de glucósidos de flavanona (FGs) y fenoles totales fueron ligeramente modificados.

En las combinaciones de cuarentena por frío (1 ºC durante 8, 16 y 24 días) con AI a altas temperaturas (95% CO_2 a 23, 28 ó 33 ºC), la AI a 28 ºC redujo la pérdida de peso y firmeza frente a las frutas control. El contenido en etanol aumentó en los frutos expuestos a AI aplicadas a 28 ó 33 ºC, sin llegar a afectar a la calidad sensorial. La combinación de AI con periodos de cuarentena de 8 ó 16 días no afectó el contenido en AAT de las naranjas, sin embargo, al aumentar el periodo de cuarentena a 24 días el contenido en AAT fue menor que en los frutos control.

La aplicación de un recubrimiento de quitosano con distinto CS (0,6, 1,2 ó 1,8%) redujo el intercambio gaseoso, modificando la atmósfera interna de las mandarinas 'Oronules' y naranjas 'Valencia' almacenadas durante 4 y 16 semanas a 5 ºC, respectivamente, seguido de 1 semana de almacenamiento a

20 ºC. Al aumentar el CS del recubrimiento se observó un aumento en el contenido en etanol de los cítricos. Sin embargo, la calidad sensorial de la fruta no se vio afectada por este comportamiento. De igual manera el CS del recubrimiento no afecto a la calidad nutricional de las naranjas 'Valencia', ni de las mandarinas 'Oronules'.

La efectividad de los recubrimientos comestibles compuestos de HPMC-lípido controlando la pérdida de peso de naranjas 'Valencia' fue limitada, mientras que en mandarinas 'Oronules' el recubrimiento más efectivo fue el de mayor CS y goma laca. Aunque los recubrimientos comestibles resultaron efectivos manteniendo la firmeza del fruto, no se observó una relación entre el CS y el ratio cera de abeja:goma laca con este parámetro de calidad. Por otro lado, estos factores si que afectaron a la atmósfera interna y al contenido en etanol durante el almacenamiento. Al aumentar el CS y de goma laca aumentó el contenido en etanol de los cítricos. Mientras que la calidad sensorial de las naranjas 'Valencia' no se vio afectada por la aplicación de los recubrimientos, la aplicación del recubrimiento al 8% CS a mandarinas 'Oronules' dio lugar a malos sabores. La calidad nutricional de los cítricos no se vio afectada de manera significativa por la aplicación de los recubrimientos.

En els últims anys, el consum de cítrics ha anat en augment propiciat pel seu elevat contingut en vitamina C i altres components bioactius. Per això, la prioritat del mercat és desenvolupar noves tecnologies postcollita respectuoses amb el medi ambient que permeten allargar la vida útil dels cítrics, mantenint la qualitat fisicoquímica, sensorial i nutricional dels mateixos fins a arribar al consumidor.

En esta tesi es va estudiar l'efecte de: (1) combinacions de quarantena per fred amb atmosferes insecticides (AI) i irradiacions (Raigs X) en la qualitat nutricional de mandarines ‘Clemenules', (2) AI aplicades a altes temperatures en la qualitat fisicoquímica, sensorial i nutricional de taronges ‘València’, (3) l'aplicació de recobriments comestibles de quitosan a distint contingut en sòlids (CS) en la qualitat fisicoquímica, sensorial i nutricional de mandarines ‘Oronules’ i taronges ‘València’ i (4) l'aplicació de recobriments comestibles compostos a base de hidroxipropilmetil cel·lulosa (HPMC)-lípid amb distint CS i proporció de cera d'abella:goma laca en la qualitat fisicoquímica, sensorial i nutricional de mandarines ‘Oronules’ i taronges ‘València'.

La combinació del tractament quarentenari per fred (1,5 ºC durant 6, 9 i 12 dies) amb AI (95% CO_2 a 20 o 25 ºC) o irradiacions ionitzants (0, 30, 54 i 164 Gy) no va afectar negativament la capacitat antioxidant total, ni el contingut d'àcid ascòrbic total (AAT) de les mandarines ‘Clemenules’. No obstant això, els continguts de glucòsids de flavanona (FGs) i fenols totals van ser lleugerament modificats.

En les combinacions de quarantena per fred (1 ºC durant 8, 16 i 24 dies) amb AI a altes temperatures (95% CO_2 a 23, 28 o 33 ºC), l'AI a 28 ºC va reduir la pèrdua de pes i fermesa en comparació amb els fruits control. El contingut en etanol va augmentar en els fruits exposats a AI aplicades a 28 o 33 ºC, sense arribar a afectar la qualitat sensorial. La combinació d'AI amb períodes de quarantena de 8 o 16 dies no va afectar el contingut en AAT de les taronges, no obstant això, a l'augmentar el període de quarantena a 24 dies el contingut en AAT va ser menor que en els fruits control.

L'aplicació d'un recobriment de quitosan amb distint CS (0,6, 1,2 o 1,8%) va reduir l'intercanvi gasós, modificant l'atmosfera interna de les mandarines ‘Oronules' i taronges ‘València' emmagatzemades durant 4 i 16 setmanes a 5 ºC, respectivament, seguit d'1 setmana d'emmagatzemament a

20 ºC. A l'augmentar el CS del recobriment es va observar un augment en el contingut en etanol dels cítrics. No obstant això, la qualitat sensorial de la fruita no es va veure afectada per este comportament. De la mateixa manera el CS del recobriment no va afectar la qualitat nutricional de les taronges 'València', ni de les mandarines 'Oronules'.

L'efectivitat dels recobriments comestibles compostos de HPMC-lípid en el control de la pèrdua de pes de taronges 'València' va ser limitada, mentres que en mandarines 'Oronules' el recobriment més efectiu va ser el de major CS i goma laca. Encara que els recobriments comestibles van resultar efectius per a mantenir la fermesa del fruit, no es va observar una relació entre el CS i el ràtio cera d'abella:goma laca amb este paràmetre de qualitat. D'altra banda, estos factors si que van afectar l'atmosfera interna i al contingut en etanol durant l'emmagatzemament. A l'augmentar el CS i la goma laca va augmentar el contingut en etanol dels cítrics. Mentres que la qualitat sensorial de les taronges 'València' no es va veure afectada per l'aplicació dels recobriments, l'aplicació del recobriment al 8% CS a mandarines 'Oronules' va donar lloc a mals sabors. La qualitat nutricional dels cítrics no es va veure afectada de manera significativa per l'aplicació dels recobriments.

Citrus are the most widely produced fruits and their market has increased in recent years favoured by their high content in vitamin C and other bioactive compounds, such as polyphenolic compounds, with high antioxidant properties. Nowadays new innovative environmentally friendly postharvest technologies are been studied to commercialize citrus fruit. These technologies should maintain the maximum physicochemical, sensory, and nutritional quality until the fruit reach the consumer.

This thesis studies the effect of: (1) two innovative quarantine treatments, such as insecticidal atmospheres (IA) and low doses X-ray irradiation in combination with short periods of cold-quarantine storage on the nutritional quality of mandarins 'Clemenules', (2) the combination of IA applied at high temperatures with cold-quarantine storage in the physicochemical, sensory, and nutritional quality of 'Valencia' oranges, (3) the application of a chitosan edible coating at different solid content (SC) on the physicochemical, sensory, and nutritional quality 'Oronules' mandarins and 'Valencia' oranges and (4) the application of edible composite coatings based on hydroxypropyl methylcellulose (HPMC)-lipid with different SC and beeswax:shellac ratio on the physicochemical, sensory, and nutritional quality 'Oronules' mandarins and 'Valencia' oranges.

Cold-quarantine treatment (1.5 ºC for 6, 9 and 12 days) combined with IA (95% CO_2 at 20 or 25 ºC) or X-ray radiation (0, 30, 54 and 164 Gy) did not affect negatively the total antioxidant capacity and total ascorbic acid (TAA) content of the 'Clemenules' mandarins. However, the flavanone glycosides (FGs) and total phenolic content were slightly modified.

Combinations of cold quarantine (1 ºC for 8, 16 and 24 days) and IA at high temperatures (95% CO_2 to 23, 28 or 33 ºC) did not affect negatively the quality of ‘Valencia’ orange. The exposure of oranges to the IA at 28 °C reduced the weight and firmness loss compared to control fruits. The ethanol content increased in fruit exposed to IA applied at 28 or 33 ºC, but sensory quality not adversely affected. Combination of IA and 8 or 16 days of cold storage did not affect the TAA content of oranges; however when cold quarantine period increased to 24 days, treated fruit had lower TAA content than control fruit.

Chitosan coating application with different SC (0.6, 1.2 or 1.8%) reduced gas exchange, modifying internal atmosphere of 'Oronules' mandarins and 'Valencia' oranges stored 4 and 16 weeks at 5 ºC plus 1 week

at 20 º C, respectively. Increasing the SC of the coating increased the ethanol content in juice. However, sensory quality was not affected. Similarly, the SC of coating did not affect of nutritional quality of 'Valencia' oranges and 'Oronules' mandarins

The effectiveness of composites edible coatings based on HPMC-lipid controlling weight loss of 'Valencia' orange was limited; while in 'Oronules' mandarins the coating with the highest SC and shellac content was effective controlling weight loss. Although edible coatings were effective maintaining fruit firmness, no relationship was observed between SC and beeswax:shellac ratio with this quality parameter. On the other hand, increasing SC and shellac content affected internal atmosphere and increased the ethanol content of the fruit during storage. Sensory quality of 'Valencia' orange was not affected by coating application. However, HPMC-lipid coatings with 8% SC induced off-flavor in 'Oronules' mandarins. The nutritional quality of citrus fruit was not significantly affected by coating application.

ABREVIATURAS / ABBREVIATIONS

AA	ácido L-ascórbico / L-ascorbic acid
AAT	ácido ascórbico total
AC	atmósfera controlada
AI	atmósfera insecticida
AM	atmósfera modificada
AT	acidez total
BW	beeswax
CA	controlled atmosphere
CC	cera comercial
Ch	chitosan
CMC	carboximetilcelulosa
CS	contenido en solidos
CTL	control
CW	commercial wax
DHA	L-dehydroascorbic acid
DID	didimina / didymin
DMSO	dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPPH$^{\bullet}$ RSC	DPPH$^{\bullet}$ radical-scavenging capacity
DTT	1,4-dithio-DL-threitol
FC	folin-ciocalteu
FGs	glucósidos de flavanonas / flavanone glycosides
GAE	gallic acid equivalents
GRAS	generally regarded as safe
Gy	gray
HES	hesperidina / hesperidin
HPLC	high performance liquid chromatography
HPMC	hidroxipropil metilcelulosa / hydroxypropyl methylcellulose
HR	humedad relativa
IA	insecticidal atmosphere
IM	indice de madurez
MC	metilcelulosa
MeOH	methanol
MI	maturity index
MPA	ácido *meta*-fosfórico / *meta*-phosphoric acid
Mw	molecular weight

ABREVIATURAS / ABBREVIATIONS

NAT	narirutina / narirutin
Q	quitosano
RH	relative humidity
SC	solid content
SERB	steam end rind breakdown
Sh	shellac
SSC	soluble solids content
SST	sólidos solubles totales
TA	total acidity
TAA	total ascorbic acid
TAC	total antioxidant capacity
TPC	total phenolic content

ÍNDICE

JUSTIFICACIÓN E INTERÉS DEL ESTUDIO

Gran parte de la producción de cítricos en España se destina para consumo en fresco y para su comercialización se aplican tratamientos postcosecha con el fin de preservar la calidad del fruto. Entre los tratamientos poscosecha más utilizados en la industria de cítricos se encuentran la desverdización para adelantar la campaña comercial de la fruta, la frigoconservación, la aplicación de ceras comerciales y los tratamientos cuarentenarios por frío cuando son exigidos por los países importadores. Otras tecnologías novedosas incluyen el uso de atmósferas insecticidas con alto contenido en CO_2, la aplicación de radiaciones ionizantes, el desarrollo de recubrimientos naturales y otros tratamientos térmicos, químicos y biológicos.

Tradicionalmente, la evaluación de la calidad poscosecha se ha dirigido a evaluar la calidad fisicoquímica de las frutas a través de parámetros como la pérdida de peso, firmeza, color, índice de madurez, pH y acidez, entre otros. Poco a poco, la evaluación sensorial de las frutas se ha ido incorporando en los trabajos para estudiar y evitar las alteraciones en las propiedades organolépticas durante la manipulación poscosecha. En la actualidad, la calidad nutricional ha pasado a tener gran interés siendo un componente de la calidad global muy valorado por el consumidor. Numerosos ensayos clínicos y estudios epidemiológicos han evidenciado que el consumo de frutas y verduras es beneficioso para la salud y contribuye a la prevención de los procesos degenerativos previniendo accidentes cerebrovasculares y cardiovasculares y bajando la tasa de incidencia y mortalidad de cáncer.

En particular, los cítricos constituyen una importante fuente nutricional de vitaminas, caracterizándose por su alto contenido en vitamina C, que es el mayor responsable de la capacidad antioxidante de los cítricos. Además, los cítricos son una fuente de compuestos polifenólicos con propiedades antioxidantes, como por ejemplo los flavonoides. La variedad y abundancia de compuestos antioxidantes en los cítricos posibilita la aparición de sinergias entre estos compuestos contribuyendo a la capacidad antioxidante total de estas frutas.

Es evidente que para conseguir extender la vida útil poscosecha de los cítricos no es suficiente la frigoconservación, siendo necesario la combinación con otras tecnologías. En la actualidad es importante desarrollar tecnologías poscosecha efectivas que alarguen y mejoren los

tiempos de almacenamiento, que sean respetuosas con el medio ambiente, sin olvidar que deben mantener también la calidad sensorial y funcional de los frutos hasta que éstos lleguen al consumidor. Por tanto, la principal motivación de esta tesis ha sido profundizar en los efectos que producen tratamientos poscosecha novedosos, potencialmente aplicables, sobre la calidad fisicoquímica, sensorial y nutricional de cítricos. En concreto, esta tesis doctoral estudia el efecto de tratamientos como son las atmósferas insecticidas y radiaciones ionizantes por rayos-X, y el desarrollo y aplicación de recubrimientos comestibles en combinación con la frigoconservación en la calidad poscosecha de naranjas 'Valencia' y mandarinas 'Oronules' y 'Clemenules'.

INTRODUCCIÓN

La producción de cítricos lidera el primer lugar en el mundo en relación a otras frutas. Durante el periodo 2006-2007, se registró una producción de aproximadamente 120 millones de toneladas (FAO, 2007). Los cítricos se cultivan comercialmente en más de 50 países. La contribución de la industria citrícola a la economía mundial se estima en más de 10 billones de dólares anualmente (Ladaniya, 2007).

A nivel mundial, España es el quinto país productor de cítricos (6.540.814 Tm en 2008/2009) después de Brasil, China, Estados Unidos y México (FAO, 2009). Además, es el principal exportador de cítricos frescos (3.352.6 Tm), correspondiendo el mayor porcentaje a mandarinas (60-80%), seguido por naranjas (40-60%) y limones (40-70%) (MARM, 2007). Dentro del ámbito nacional, la comunidad Valenciana es la principal zona productora de cítricos, con 59,28% de la producción, seguida de Andalucía con un 26,79% y la región de Murcia con un 9,59% (MARM, 2008).

La tendencia en la producción indica que las naranjas constituyen cerca del 60% del total de cítricos producidos, seguida por las mandarinas con un 20%, limones y limas con un 11-12%, y pomelos con un 5-6%. De esta producción, cerca a 68 millones de Tm se destinan al consumo en fresco y unos 27 millones de Tm como productos procesados (FAO, 2006). El incremento de la población mundial, proyectada en 10 billones de personas a mediados de siglo (Ladaniya, 2007), y la tendencia observada en los últimos años de un aumento del consumo de fruta cítrica fresca, indican la importancia de aumentar la producción y conservar la calidad natural de la fruta para consumo en fresco durante el periodo poscosecha, tanto para su comercialización en mercado interno como para su exportación.

1.1. Problemática de los cítricos en poscosecha

Un aspecto fundamental a tener en cuenta en el manejo poscosecha de frutas es que éstas continúan activas fisiológicamente aún después de cosechadas. De manera que la fruta cosechada continúa respirando, madurando e iniciando procesos de senescencia, todo lo cual implica una serie de cambios estructurales y bioquímicos que son específicos de cada fruta. Asimismo, el producto cosechado está constantemente expuesto a la pérdida de agua debido a la transpiración y a otros fenómenos fisiológicos.

Los frutos cítricos, en particular, presentan una serie de problemas tras su recolección derivados de la falta de aporte hídrico y de nutrientes desde

la planta, quedando así a expensas de su propio metabolismo. Esto da lugar a una pérdida gradual de calidad de la fruta en sus características organolépticas de textura, sabor y aroma a medida que avanza su estado de senescencia, determinando finalmente la muerte fisiológica. Este tipo de metabolismo limitado a sus propias reservas coloca además al fruto en una situación de debilidad frente a la deshidratación y las agresiones físicas externas tales como: fricción, golpes o heridas y también frente a las infecciones, especialmente de tipo fúngico. Por tanto, son normalmente las alteraciones fisiológicas y patológicas las que hacen inviable su comercialización mucho antes de que cese su actividad metabólica (Cuquerella, 1990). El conocimiento de la fisiología del fruto durante la etapa poscosecha es importante para entender el proceso de deterioro de la calidad.

1.1.1. Fisiología poscosecha

Respiración

La respiración es un indicador de la actividad metabólica y juega un papel significativo en la fisiología poscosecha y en el deterioro de la calidad de los alimentos. Es un proceso que implica la degradación oxidativa de los productos más complejos, normalmente presente en las células, como el almidón, los azúcares y los ácidos orgánicos, a moléculas más simples como el dióxido de carbono y el agua, con la consiguiente liberación de energía (Day, 1993; Kader, 2002).

La respiración, por tanto, involucra reacciones complejas que en condiciones normales requiere de la presencia de O_2 para la degradación de los compuestos (respiración aeróbica). Sin embargo, cuando los niveles de oxígeno son muy bajos, la respiración se desplaza hacia la ruta anaeróbica (Hagenmaier, 2000), generándose compuestos volátiles, como el acetaldehído y etanol, que pueden dar origen a malos sabores (Ahmed y Khan, 1987; Cohen et al., 1990; Ke y Kader, 1990).

Durante la respiración, la pérdida de reservas alimenticias almacenadas en el producto significa el aceleramiento de la senescencia conforme las reservas que proporcionan energía para mantener el estatus viviente del producto se agotan (Kader, 2002). Por tanto, la velocidad de deterioro de las frutas generalmente es proporcional a su velocidad de respiración. Adicionalmente, basados en sus patrones de respiración y producción de

etileno (C_2H_4) durante la maduración organoléptica, los frutos se clasifican en climatéricos y no climatéricos (Biale, 1960). Los frutos climatéricos muestran un pico respiratorio durante la maduración organoléptica con un incremento en la producción de CO_2 y de C_2H_4, mientras que los frutos no climatéricos no muestran cambios en sus velocidades de producción de CO_2 y de C_2H_4 y estas son generalmente bajas.

Los cítricos están considerados como frutos con una intensidad respiratoria baja. Pero al igual que en todos los frutos, la manipulación y temperatura estimulan la intensidad respiratoria de los mismos (Parker et al., 1984). Así por ejemplo, la intensidad respiratoria de los cítricos a 5 ºC está entre 5-10 mg CO_2/Kg h a 5 ºC, aumentando a valores entre 10-20 mg CO_2/Kg h a 10 ºC y 40-80 mg CO_2/Kg h a 20 ºC. Además, su comportamiento basado en su patrón de respiración y producción de C_2H_4 los clasifica como frutos no climatéricos, con una producción de C_2H_4 baja (<0,10 μL/Kg h en naranjas a 20 ºC) (Kader, 1985).

Transpiración

La transpiración es la principal causa de la pérdida de agua de las frutas y vegetales ocasionando pérdidas de peso, deterioro en la apariencia (marchitamientos y arrugamientos), disminución de firmeza (ablandamiento, pérdida de turgencia), cambios en la calidad nutricional, además de una mayor susceptibilidad a determinadas alteraciones tanto fisiológicas como patológicas (Mishra y Gamage, 2007).

La transpiración es un proceso por el cual los tejidos vegetales pierden agua en forma de vapor desde las células del interior hacia la atmósfera que los rodea. Las diferentes formaciones epidérmicas son las que regulan el flujo de vapor de agua hacia el exterior de los productos. El vapor de agua sale hacia el exterior desde los espacios intercelulares existentes entre las células del parénquima poroso, pasa a través de estomas, lenticelas, o microheridas, y atraviesa la epidermis y la cutícula. Las aperturas epidérmicas representan la principal vía de pérdida de agua, mientras que la transpiración a través de la cutícula representa alrededor del 5-10 % de la pérdida total (Taiz y Zeiger, 1998). Ben-Yehoshua et al. (1985) propusieron que la difusión de vapor de agua en cítricos se realiza tanto a través de las aperturas epidérmicas como a través de una fase acuosa líquida en la cutícula, contrariamente a los gases CO_2, O_2 y C_2H_4 cuya difusión se realiza esencialmente a través de los estomas.

La intensidad de la pérdida de agua depende de factores intrínsecos del fruto y de factores ambientales. Entre las variables intrínsecas al fruto, las más relevantes son la relación superficie/volumen, la estructura de la epidermis y el grosor y composición de la cera epicuticular. Las pérdidas de agua son directamente proporcionales a la relación superficie/volumen, por lo que los frutos de mayor volumen y más esféricos son los que presentan menor pérdida de agua.

Los factores ambientales que más influyen en la deshidratación son la temperatura, la humedad relativa (HR) y la velocidad de circulación del aire que rodea al fruto. En el almacenamiento a bajas temperaturas y altas HR se reduce el gradiente de presión del vapor de agua entre el fruto y la atmósfera de almacenamiento, con lo que disminuye la velocidad de pérdida de agua por transpiración (Martínez-Jávega, 1999). Además, en el almacenamiento y transporte, es importante una adecuada ventilación y velocidad del aire, puesto que incide sobre la capa de aire húmedo que rodea al fruto (Waks et al., 1985; Thompson, 2002). Por tanto, las frutas y vegetales suelen ser almacenados en un ambiente húmedo (90-98% HR), especialmente a bajas temperaturas y con una velocidad del aire adecuada para minimizar la pérdida de agua (Woods, 1990).

En los cítricos, la transpiración es la principal causa de deterioro durante la poscosecha (Ben-Yehoshua, 1969). Distintos estudios indican que se pueden alcanzar mermas de peso superiores a un 5% durante la comercialización, un 7% en la conservación frigorífica y un 16% en la frigoconservación durante periodos de tres meses (Jiménez-Cuesta et al., 1983).

1.1.2. Alteraciones fisiológicas

Las alteraciones fisiológicas pueden tener su origen en deficiencias nutricionales o condiciones climáticas adversas ocurridas durante el período precosecha y/o en una incorrecta manipulación en poscosecha, como por ejemplo el almacenamiento a temperaturas y HR no adecuadas, conservación en atmósferas no adecuadas, etc (Grierson, 1986, 2002; Kader, 1986).

Este tipo de alteraciones produce cambios indeseables en la piel de los cítricos y por lo tanto una pérdida del valor comercial (Agustí et al., 1997). Dentro de los desórdenes más comunes en las frutas cítricas se encuentran la

necrosis peripeduncular o SERB (Steam end rind breakdown) y los daños por frío.

El SERB es producido por una desecación de los tejidos situados alrededor del pedúnculo. En su fase inicial queda un anillo de 2 a 5 mm sin dañar, y al ir avanzando, el área afectada se hunde y cambia de color hacia tonos marrones. La alteración puede estar provocada por un desequilibrio nutricional, que involucra al nitrógeno y fósforo, y se desarrolla en el almacenamiento cuando hay condiciones propicias para la deshidratación (Martínez- Jávega y del Río, 1998).

Los cítricos, al igual que otros frutos tropicales y subtropicales, son sensibles a los daños por frío (chilling injury) cuando se almacenan a bajas temperaturas, aunque superiores al punto de congelación. Los daños por utilización de bajas temperaturas en el almacenamiento de cítricos se manifiestan externamente con picados, ennegrecimiento de glándulas oleíferas, bronceado y peteca. Asimismo la frigoconservación puede producir daños internos como descomposición acuosa y membranosis (Martínez-Jávega y del Río, 1998). El picado (pitting) aparece como depresiones más o menos redondeadas en la piel con ligera decoloración, las cuáles se oscurecen a medida que avanza el almacenamiento hacia tonalidades marrones. Los cítricos más susceptibles a esta alteración son pomelos y limones, mientras que las naranjas son las que presentan menor susceptibilidad. Entre las mandarinas, los cultivares ‘Nova’ y ‘Fortune’ son los más susceptibles. La peteca también forma depresiones en la corteza, pero tienen formas más circulares que las del picado y es más frecuente en limones (Martínez-Jávega y del Río, 1998; Roger, 1988).

1.1.3. Alteraciones patológicas

Una de las principales causas de las pérdidas económicas durante la poscosecha, son las alteraciones patológicas que limitan la vida útil de las frutas y hortalizas frescas. El porcentaje de frutos cítricos afectados por podredumbres durante una campaña normal oscila entre el 3 y el 6% del total manipulado, pudiendo alcanzar valores mayores (8-12%) en años con climatología anormal (Tuset, 1987).

En los cítricos, las alteraciones son provocadas casi exclusivamente por hongos y, principalmente, por los patógenos de heridas, como son *Penicillium digitatum*, causante de la podredumbre verde, y *P. italicum*,

causante de la podredumbre azul. Otros hongos que alteran los cítricos son *Alternaria citri*, *A. alternata*, *Botrytis cinerea*, *Colletotrichum gloesporioides*, *Geotrichum candidum*, *Rhizopus stolonifer*, *Rhizopus oryzae*, *Phytophthore citrophthora* (Tuset, 1999).

El ataque por patógenos generalmente sigue al daño físico o al deterioro fisiológico. En raras ocasiones, los patógenos pueden infectar tejidos aparentemente sanos y ser la causa primaria del deterioro. En concreto para que la podredumbre se produzca en los frutos, deben de darse las siguientes condiciones: a) nivel de inóculo suficiente en el ambiente; b) contacto entre el inóculo y la superficie de los frutos; c) entrada de la espora en el fruto a través de una herida (incluso no perceptible a simple vista); d) condiciones favorables para que la espora se desarrolle dentro de la herida; e) susceptibilidad del fruto a la alteración. Por lo tanto, la incidencia de las podredumbres depende de las características intrínsecas del fruto, de las condiciones ambientales, del manejo durante la recolección y posterior manipulación poscosecha (Kader, 1992).

La refrigeración se utiliza como método que ayuda a reducir la incidencia de las alteraciones patológicas en la fruta, ya que las bajas temperaturas reducen la germinación de las esporas y el crecimiento de patógenos y, además, al retrasar la senescencia del fruto, este mantiene un mayor contenido de los compuestos antifúngicos (e.g. fitoalexinas) que mejoran la resistencia fisiológica al ataque fúngico del fruto (Martínez-Jávega, 1995).

En el caso de los cítricos la principal estrategia de control de hongos es la aplicación de fungicidas de síntesis, como ortofenilfenato sódico, tiabendazol, imazalil o guazantina (Ben-Yehoshua y Porat, 2005). Sin embargo, el uso continuado de los fungicidas de síntesis presentan varias limitaciones importantes, como la aparición de cepas resistentes, las restricciones regulatorias sobre la aceptación de residuos en países importadores de cítricos y el posible efecto perjudicial de estos compuestos sobre la salud y el medio ambiente (Artés, 2000; Smilanick et al., 2006).

En la actualidad se están estudiando nuevas estrategias para el control de enfermedades poscosecha, que incluyen tratamientos físicos, químicos y biológicos. Así por ejemplo, se ha estudiado la aplicación de aire caliente y baños en agua caliente (Lurie, 1998; Paull y Chen, 2000), la aplicación de luz ultravioleta (254 nm) (Rodov et al., 1992), el uso de agentes químicos

naturales de baja toxicidad, como las sales inorgánicas, parabenos (Palou et al., 2001) y el desarrollo de agentes de control biológico (Wilson y Wisniewski, 1989).

1.2. Tratamientos poscosecha en cítricos: Efectos en la calidad fisicoquímica, sensorial y nutricional

Kader (2002) define la calidad como 'una combinación de características, atributos o propiedades que dan al producto el valor de alimento para el consumo humano'. Este conjunto de factores está relacionado tanto con la aceptación organoléptica y nutricional, como con su aspecto externo.

La evolución poscosecha de los cítricos tras la aplicación de distintos tratamientos puede alterar significativamente la calidad de los mismos (Echeverria e Ismail, 1987). En general, la calidad final de los frutos se puede mantener mediante el empleo de distintas tecnologías poscosecha, pero en todo caso siempre vendrá condicionada por la calidad inicial del fruto, siendo el grado de madurez en el momento de la recolección, uno de los factores que influye de manera decisiva en la calidad y conservación de la fruta (Brezmes et al., 1999). Los tratamientos poscosecha más utilizados en la industria de cítricos son la frigoconservación, la aplicación de ceras comerciales, la desverdización y aplicación de tratamientos cuarentenarios por frío cuando estos son exigidos por los países importadores. Otras tecnologías más novedosas en estudio incluyen el uso de atmósferas insecticidas con alto contenido en CO_2, la aplicación de radiaciones ionizantes, el desarrollo de recubrimientos naturales y otros tratamientos térmicos, químicos y biológicos.

Tradicionalmente la evaluación de la calidad poscosecha de los cítricos se ha dirigido a evaluar la calidad fisicoquímica de las frutas a través de parámetros como la pérdida de peso, firmeza, color, índice de madurez, pH y acidez, entre otros. Poco a poco, la evaluación sensorial de las frutas cobró importancia con el objetivo de estudiar y evitar la alteración de las características organolépticas del fruto durante la manipulación poscosecha. En la actualidad, la calidad nutricional ha pasado a tener gran interés, siendo un componente de la calidad global muy valorado por el consumidor. Numerosos ensayos clínicos y estudios epidemiológicos han evidenciado que el consumo de frutas y verduras es beneficioso para la salud y contribuye a la prevención de los procesos degenerativos, previniendo

accidentes cerebrovasculares y cardiovasculares y bajando la tasa de incidencia y mortalidad de cáncer.

En particular, los cítricos constituyen una importante fuente nutricional de vitaminas, caracterizándose por su alto contenido en vitamina C. La vitamina C es el mayor responsable de la capacidad antioxidante de los cítricos. Además, los cítricos son una fuente de compuestos polifenólicos con propiedades antioxidantes, como por ejemplo los flavonoides (Sánchez-Moreno et al., 2003). La variedad y abundancia de compuestos antioxidantes en los cítricos posibilita la aparición de sinergias entre estos compuestos contribuyendo a la capacidad antioxidante total de estas frutas.

Por tanto, la aplicación de las distintas tecnologías poscosecha deben mantener la calidad fisicoquímica, sensorial y nutricional de los frutos hasta que estos llegan al consumidor.

1.2.1. Frigoconservación

Teniendo en cuenta que la temperatura es el factor más importante en la vida poscosecha de los productos hortofrutícolas, el almacenamiento en frío es la tecnología poscosecha más extendida en los frutos cítricos. El objetivo es prolongar el periodo de comercialización del fruto y mantener su calidad durante el transporte a mercados distantes de las zonas productoras. Adicionalmente, la refrigeración también se aplica como tratamiento cuarentenario para el control de insectos, principalmente la mosca de la fruta del mediterráneo (*Ceratitis capitata*).

Sin embargo, los cítricos son frutos subtropicales y por tanto sensibles al frío, por lo que es necesario optimizar la temperatura de almacenamiento para evitar la aparición de daños por frío. Cada variedad y cultivar tiene unas condiciones óptimas diferentes para el almacenamiento dependiendo de su tolerancia a las bajas temperaturas, a la alta humedad, a la baja concentración de oxígeno, a la alta concentración de dióxido de carbono, etileno y a los daños mecánicos (Burdon, 1997).

La Tabla 1 muestra las temperaturas de almacenamiento recomendadas en el almacenamiento de algunos cítricos. Las naranjas son menos sensibles al frío que el resto de los cítricos. Entre las mandarinas, ‘Fortune’ y ‘Nova’ son las que presentan mayor susceptibilidad, y pomelos y limones presentan la máxima sensibilidad al frío (del Río y Martínez-Jávega, 1997; Roger, 1988). En general, se recomiendan temperaturas entre 2 y 10 ºC, una

humedad relativa cercana al 90% y concentraciones de etileno menores de 1 ppm y de dióxido de carbono inferiores al 0,25% durante el almacenamiento (Liu, 1992; Roger, 1988). Arpaia y Kader (2000) recomiendan temperaturas óptimas de 3-8 ºC para conservar naranjas durante 3 meses, dependiendo del cultivar, estado de madurez a la cosecha y área de producción.

Tabla 1. Temperatura y tiempo de vida útil de almacenamiento en la frigoconservación de cítricos

Especie/cultivar	Temperatura (ºC)	Vida útil almacenamiento (meses)
Pomelo	12-13	2-3
Limas	9-10	1.5-2.5
Limones		
Fino	11-12	3-4
Verna	13-14	4-5
Naranjas		
Navelina	2-3	2.5-3.5
Washington Navel	2-3	2.0-2.5
Navelate	3-4	2-3
Lanelate	2-3	2.5-3.5
Blanca común	2-3	2.5-3.5
Salustiana	2-3	3-4
Valencia Late	2-3	3-4
Mandarinas		
Satsuma	2-3	1.0-1.5
Clementina	4-5	1.5-2.5
Híbridos		
Mandarina Fortune	9-10	1.0-1.5
Mandarina Nova	9-10	0.5-1.0
Tangelo Minneola	9-10	0.5-1.5
Tangor Ellendale	5-6	2.0-2.5
Tangor Ortanique	5-6	2.5-3.0

Fuente: Martínez-Jávega et al., 1999

Los beneficios de la refrigeración reduciendo el deterioro poscosecha en cítricos han sido extensamente estudiados. Pozzan et al. (1993) indicaron

que la calidad de las naranjas 'Navelina' puede ser mantenida en condiciones de frío presentando poca variación en la pérdida de peso e índice de madurez. Abad et al. (2003) reportaron cambios mínimos de la calidad del sabor durante la frigoconservación de naranjas 'Delta' y 'Midknight'. Meier et al. (2004) al estudiar diferentes temperaturas de almacenamiento (2-5 ºC) para mandarinas 'Murcott' y naranjas 'Valencia Late' encontraron que la menor temperatura de almacenamiento en frío determinó los menores niveles de acetaldehído y de etanol en el zumo.

Teniendo en cuenta que la refrigeración constituye la base de conservación de los cítricos y su alta susceptibilidad a manifestar daños por frío, se han realizado numerosos estudios en los que se combina la refrigeración con otras tecnologías con el objetivo de reducir los daños fisiológicos y extender la vida útil de los mismos (Vázquez y Martínez-Jávega, 1999).

A nivel nutricional, muchos trabajos en la literatura muestran que el contenido de ácido ascórbico de las frutas y verduras disminuye con la aplicación de altas temperaturas y el almacenamiento prolongado (Lee y Kader, 2000; Thompson, 2004). Sin embargo, el almacenamiento a bajas temperaturas también puede acelerar la pérdida de vitamina C en frutas sensibles al frío, incluso antes de que los daños por frío sean evidentes (Miller y Heilman, 1952). Por ejemplo, Ito et al. (1974) observó que en mandarinas 'Satsuma' almacenadas en atmósferas controladas (AC) con bajo contenido de O_2 y altas concentraciones de CO_2 a 1-4 °C redujo el contenido de ácido ascórbico gradualmente, mientras que el contenido de ácido dehidroascórbico aumentó. Sin embargo, Palma et al. (2005) no observaron cambios en el ácido ascórbico total, ni en la capacidad antioxidante total de mandarinas 'Fortune' después de 90 días de almacenamiento a 5 ºC.

1.2.2. Tratamientos químicos

Entre los tratamientos químicos estudiados en poscosecha para prolongar la vida útil de cítricos se incluyen los reguladores del crecimiento (e.g. ácido giberélico) (Wills et al., 1984), las poliaminas que tienen actividad antioxidante (Kramer et al., 1991; Ponappa et al., 1993) y los metil-jasmonatos que se utilizan para prevenir los daños por frío (Meir et al., 1996). Con el objetivo de controlar podredumbres durante poscosecha se utilizan fungicidas de síntesis, como el ortofenilfenato sódico, el

tiabendazol, el imazalil o mezclas de estas materias activas. Con el mismo objetivo, a nivel experimental se están estudiando agentes químicos naturales de baja toxicidad, como las sales inorgánicas, parabenos, etc (Palou et al., 2001) y agentes de control biológico (Wilson y Wisniewski, 1989).

1.2.3. Pretratamientos térmicos

En combinación con la frigoconservación se han aplicado con éxito pretratamientos térmicos con el fin de proteger a las frutas de posibles daños por frío y/o reducir alteraciones patológicas, como el acondicionado (Cuquerella et al., 1988), el curado, calentamientos intermitentes y los baños en agua caliente (Schirra y Mulas, 1995; Schirra y D'Hallewin, 1997).

El curado es un tratamiento previo a la refrigeración y consiste en someter al fruto a altas temperaturas (35 ºC, 72 horas) durante un corto periodo de tiempo para inducir la producción de proteínas de resistencia a las bajas temperaturas (Heat shock proteins, HSP) (Laurie y Klein, 1991; Laurie et al., 1993; Whitaker, 1993). Los calentamientos intermitentes se basan en el carácter reversible de algunos daños por frío en la fase de latencia de la alteración. Consiste en someter al fruto a calentamientos intermitentes en el curso de la conservación frigorífica convencional. La eficacia de los calentamientos intermitentes se atribuye a una serie de respuestas fisiológicas del fruto durante los mismos que incluyen: la restauración de las membranas celulares dañadas por el frío, la eliminación de metabolitos tóxicos acumulados a bajas temperaturas, y la síntesis de metabolitos indispensables para el correcto funcionamiento celular (Marcelin y Ulrich, 1983; Artés, 1995). Se ha demostrado que los calentamientos intermitentes restablecen la respiración normal de cítricos, melocotones y tomates dañados por frío, y la emisión de etileno y el equilibrio de la actividad pectinesterasa y poligalacturonasa en melocotones que también presentaban daños por frío (Marcelin y Ulrich, 1983; Artés et al., 1996). En cultivares de cítricos sensibles al frío se ha observado una reducción de los daños mediante distintos tratamientos con calor (Schirra y Mulas, 1995; Schirra y D'Hallewin, 1997). Wild y Hood (1989) reportaron la reducción de los daños por frío de naranjas 'Valencia' almacenadas 15 semanas a 1 ºC mediante baños con agua caliente.

1.2.4. Almacenamiento en atmósferas modificadas (AM) y controladas (AC)

Las AC y AM son técnicas en las que se altera la composición del aire que rodea al fruto con el fin de retrasar su deterioro (Kader, 1992). La diferencia entre ambos métodos, está en el grado de control de las concentraciones gaseosas. En general, la atmósfera se modifica con un incremento en la concentración de CO_2 y una disminución de O_2. Sin embargo, también se han realizados estudios con altas concentraciones de O_2 (superatmósferas) y con altas concentraciones de CO_2 (choques gaseosos). En el caso de que se fijen y controlen los valores de composición de los gases durante el almacenamiento se habla de AC, mientras que cuando la atmósfera de almacenamiento evoluciona con el tiempo se habla de AM. En este último caso la atmósfera se ve afectada por la actividad metabólica del fruto y, en el caso de frutos envasados o recubiertos, por la permeabilidad de los envases o coberturas que envuelven al fruto.

Algunas ventajas de las AC y AM son la reducción de la tasa respiratoria, la disminución de los efectos del etileno en la senescencia, la retención de firmeza y la reducción del desarrollo de hongos (Wills et al., 1998). Sin embargo, las frutas presentan diferentes tolerancias al O_2 y CO_2 según la especie y cultivar, de acuerdo con su tasa respiratoria y permeabilidad de la piel (Balwin, 1994). En cítricos, bajas concentraciones de O_2 y/o altas concentraciones de CO_2 pueden dar lugar a la acumulación de etanol y acetaldehído y el desarrollo de malos sabores (Ke y Kader, 1990). La tolerancia mínima, sin embargo, pueden verse modificados en función de la combinación de gases (e.g. los niveles de tolerancia al CO_2 disminuyen si se reduce el O_2 y a la inversa), del tiempo de exposición y la temperatura de almacenamiento (Beaudry, 1999).

Aunque la conservación en AC o AM en los cítricos no proporciona en general beneficios importantes por la baja tolerancia que presentan al CO_2 (inferior al 3% en naranjas y mandarinas y al 5% en pomelos y limones) (Artés, 2000), la aplicación de atmósferas insecticidas (AI) han resultado efectivas como tratamiento cuarentenario contra la mosca del mediterráneo (*Ceratitis capitata*) y otras plagas (Alonso et al., 2005a; Follett y Neven, 2006; Neven y Rehfield-Ray, 2006; Palou et al., 2008). Las AI son un caso particular de las AC en las que se aplican altos niveles de CO_2 (superiores al 50%) y muy bajos de O_2 (inferiores al 1%) (Mitchell y Kader, 1992). La

efectividad de las mismas depende de la temperatura, la humedad relativa, la duración de la exposición y de la fase de vida del insecto.

Diferentes trabajos han investigado el uso de AI antes o después de la exposición al frío de los cítricos, con el fin de reducir la duración del tratamiento estándar de cuarentena en frío contra *C. capitata* y reducir así los problemas de daños por frío (Alonso et al., 2005a, b; Palou et al., 2008). Así pues, en mandarinas 'Clementinas' almacenadas primero a 1,5 ºC durante 3 días, y después tratados con AI (CO_2 al 95%) a 25 ºC se consiguió la mortalidad total de *C. capitata* sin efectos negativos sobre la calidad fisicoquímica y sensorial de la fruta tras un almacenamiento posterior 7 días a 20 ºC (Palou et al., 2008). De manera similar, la exposición de mandarinas 'Fortune' a altos niveles de CO_2 (95%) a 22 ºC durante 20 h tampoco afectó negativamente la calidad de la fruta, indicando que la selección de las condiciones óptimas de aplicación (temperatura – tiempo de exposición) son de vital importancia para mantener la calidad de la fruta (Alonso et al., 2005a).

A nivel nutricional, la aplicación a frutas y hortalizas de AC se ha visto que tiene un efecto retardando la pérdida de la clorofila, la biosíntesis de carotenoides y antocianinas, y la biosíntesis y oxidación de compuestos fenólicos. También, se ha visto que promueven la retención del ácido ascórbico y otras vitaminas con lo que se mejora la calidad nutricional, incluyendo la actividad antioxidante de frutas y hortalizas (Kader, 2003; Artés, 2006). Así por ejemplo, Delaporte et al. (1971) observaron que la pérdida de ácido ascórbico en manzanas se puede reducir mediante el almacenamiento en una atmósfera con bajo nivel de O_2. Sin embargo, la aplicación de oxígeno ultra bajo dio lugar a una disminución del ácido ascórbico en diferentes cultivares de manzana en comparación al almacenamiento en aire normal (Haffner et al., 1997). Por otro lado, incrementar la concentración de CO_2 por encima de un determinado umbral parece tener un efecto negativo sobre el contenido de vitamina C en manzanas y grosellas rojas (Bangerth, 1977), fresas y moras (Agar et al., 1997), pimiento (Wang, 1977), peras (Veltman et al., 1999) y un efecto moderado en grosellas negras, rojas y frambuesas (Agar et al, 1997). Wang (1983) estudió el efecto de la reducción de la concentración de O_2 en la atmósfera de almacenamiento en presencia de alto CO_2 manteniendo el contenido en ácido ascórbico de col china, observando sólo un efecto beneficioso de las bajas concentraciones de O_2 con concentraciones de CO_2

inferiores al 10%. En el caso de cítricos existen escasos trabajos que estudien el efecto de AC y AM durante el almacenamiento, así como la aplicación de AI por un periodo más corto. Ito et al. (1974) informó que en mandarinas 'Satsuma', la aplicación de AC con bajo contenido de O_2 y altas concentraciones de CO_2 a 1-4 °C redujo el contenido de ácido ascórbico gradualmente, mientras que el contenido de ácido dehidroascórbico aumentó.

1.2.5. Irradiación

La irradiación de alimentos es un método físico de conservación que consiste en exponer el producto a la acción de las radiaciones ionizantes (radiación capaz de transformar moléculas y átomos en iones, quitando electrones) durante un tiempo determinado, proporcional a la cantidad de energía que deseamos que el alimento absorba. Actualmente se utilizan cuatro fuentes de energía ionizante: rayos gamma provenientes de Cobalto o Cesio radioactivo, rayos X y electrones acelerados.

La aplicación de irradiaciones en poscosecha tiene por objeto la desinfección de plagas mediante la destrucción de larvas y huevos (Hallman, 1999), la inactivación de organismos patógenos (Gladon et al., 1997) y la reducción del metabolismo del fruto, disminuyendo la actividad respiratoria, la síntesis de etileno y la pérdida de agua (Dharkar y Sreenivasan, 1971; Lu et al., 1991).

Algunos trabajos muestran la aptitud de esta técnica para mejorar y alargar la conservación de ciertas frutas y hortalizas (El-Samahy et al., 2000; Martínez-Solano et al., 2001). Otros muestran un efecto negativo de las radiaciones ionizantes en algunos cultivares de fresas y arándanos que se manifiesta con una reducción de firmeza (Yu et al., 1996; Gladon et al., 1997). Los efectos de la radiación en las frutas depende de la especie y cultivar (Miller et al., 2000). Por otra parte, los tratamientos de irradiación han demostrado que el aumento o disminución del contenido antioxidante de los vegetales frescos depende de la dosis administrada, tiempo de exposición, y materia prima utilizada. El aumento de la capacidad antioxidante en productos vegetales después de la irradiación se atribuye principalmente al aumento de la actividad enzimática (e.g. fenilalanina amonio liasa y peroxidasa) (Tomás-Barberán y Espín, 2001; Bhat et al., 2007; González-Aguilar et al., 2007 a, b).

En cítricos, la irradiación se ha estudiado con el fin de eliminar insectos como *Ceratitis capitata* (mosca del mediterráneo). La tolerancia de los cítricos a la irradiación depende del cultivar. Miller et al. (2000) encontraron que determinadas dosis (450 Gy) de irradiación para tratamientos de cuarentena de larvas de la mosca de la fruta pueden dañar a algunos cítricos. La aplicación de irradiación gamma en mandarinas 'Clemenules' (Mahrouz et al., 2002) y en naranjas 'Navel' (600-850 Gy) (O'Mahony et al., 1985) ha dado buenos resultados.

Entre las diferentes fuentes de radiación ionizante, el uso de rayos X ha sido aprobado por la US Food and Drug Administration para la irradiación de alimentos (US FDA, 2004). En un principio, una dosis mínima absorbida de 225 Gy para fines de cuarentena contra *C. capitata* fue establecida por el USDA y una nueva norma establece un tratamiento genérico de dosis de 100 Gy contra la mosca de la fruta (USDA, 2002, 2006; Follett y Armstrong, 2004). En mandarinas 'Clemenules', la aplicación de rayos X a dosis bajas (195 y 395 Gy) reduce el período de cuarentena en frío suficiente para lograr la mortalidad total de *C. capitata* sin efectos perjudiciales sobre la calidad de la fruta (Alonso et al., 2007; Palou et al., 2007b). La completa mortalidad de insectos, sin efectos negativos sobre la calidad del fruto después de 7 días a 20 °C como periodo de vida útil, se obtuvo en mandarinas primeramente irradiadas con rayos X (30-164 Gy) y posteriormente expuestas a 1 °C durante 2 días. Esta combinación de tratamientos, reduce considerablemente el tiempo de cuarentena si se compara con los tratamientos estándar de cuarentena en frío (1,1-2,2 °C durante 14-18 días) y por lo tanto promete ser un tratamiento comercial potencial para las exportaciones españolas de cítricos (Palou et al., 2007a).

A nivel nutricional, la irradiación de 'Clementinas' con dosis de 300 y 500 Gy combinados con un tratamiento de agua caliente y almacenadas durante 3 semanas a 17 ºC contenían niveles más altos del ácido ascórbico total (AAT) que las muestras control (Abdellaoui et al., 1995). Sin embargo, en pomelos una dosis de 1.500 Gy disminuyó el contenido de AAT, mientras que una dosis de 250 Gy no afectó el contenido del AAT (Moshonas y Shaw, 1984). Girennavar et al. (2008) reportó en pomelos que una dosis de 1.000 Gy no afectó el contenido en AAT, mientras que una dosis de 2.500 Gy redujo significativamente el contenido del mismo. Patil et al. (2004) indicó que la irradiación de pomelos de principio de temporada con una dosis de 700 Gy y almacenadas 35 días a 10 ºC no afectó el

contenido de AAT, mientras que en frutas de estación tardía una irradiación mayor o igual a 200 Gy causó una marcada reducción en el contenido de AAT. Estos autores sugirieron que en frutas de temporada temprana, el mecanismo de defensa principal de la fruta contra el estrés oxidativo inducido por la irradiación con rayos gamma no afecta al ácido ascórbico, mientras que en los frutos de fin de temporada el estrés inducido por la irradiación junto con el estrés por bajas temperaturas afectan el contenido de AAT. Por lo tanto, la susceptibilidad a modificar el contenido de AAT en los cítricos podría evitarse mediante la selección de la fruta en un estado de madurez óptimo.

La irradiación aumenta significativamente el contenido de flavonoides de mandarinas 'Clementinas' (Oufedjikh et al., 1998, 2000). Vanamala et al. (2005) reportó que dosis bajas (300 Gy) de irradiación en pomelos aumentó los niveles de naringina y narirutina. Patil et al. (2004) encontró que la concentración total de flavonoides se incrementó cuando los pomelos de principios de temporada fueron expuesta a bajas dosis de irradiación (70 y 200 Gy) seguido de 4 semanas de almacenamiento a 10 ºC mas 1 semana a 20 ºC, mientras que los niveles de naringina (el flavonoide más abundantes en pomelo) y de narirutina disminuyeron a medida que se incrementó la dosis de irradiación por encima de 200 Gy.

1.2.6. Aplicación de recubrimientos: ceras comerciales y recubrimientos comestibles

La aplicación de recubrimientos o *encerado* es una práctica habitual en la industria citrícola para reponer las ceras eliminadas durante las etapas de lavado y manipulación de los frutos. Su aplicación permite alargar la vida útil durante el almacenamiento al reducir la pérdida de humedad y ralentizar la maduración de los frutos, ya que actúan como barrera al intercambio gaseoso. Además, otro objetivo de la aplicación de los mismos es aportar brillo al fruto, confiriéndole un aspecto más apetecible en el punto de venta.

En el caso de los cítricos, la aplicación de recubrimientos también reduce la susceptibilidad de los mismos a daños por frío o 'pitting' y la incidencia de SERB (Chace, 1969; Davis y Hofmann, 1973b; Ben-Yehoshua, 1987; Ben-Yehoshua et al., 1981). La efectividad del encerado de frutos cítricos reduciendo la incidencia de SERB se ha visto directamente relacionado con un menor grado de deshidratación del fruto (Cuquerella et al., 1988). De igual manera, la menor susceptibilidad al picado se cree

relacionado con la capacidad de reducción de pérdida de agua de los tejidos, ya que inhibe el colapso de las células de la epidermis (Wang, 2000).

Teniendo en cuenta que la aplicación de recubrimientos afecta al intercambio gaseoso entre el fruto y la atmósfera que lo rodea, si la barrera al intercambio gaseoso es muy alta se puede inducir una respiración anaeróbica, con el consecuente incremento de volátiles, como etanol y acetaldehído, responsables de la aparición de malos sabores. Así por ejemplo, Davis y Hofmann (1973a) determinaron que la cantidad de cera no debe de exceder de 0,2-0,3 mg/cm^2 para evitar la respiración anaeróbica.

Ceras comerciales

Las primeras referencias del uso del encerado, en naranjas y limones, se remontan a los siglos XII –XIII en China (Hardenburg, 1967). Las primeras patentes de formulaciones de ceras mencionan la inclusión de parafina como material céreo y, junto a ella, diversas mezclas de otros componentes como: carnauba, candelilla y otras ceras, goma laca, aceites vegetales o minerales u otras grasas y emulsificantes y surfactantes (Plotto y Baker, 2005).

Dado que los primeros recubrimientos de cítricos estaban compuestos por ceras (ésteres de ácidos carboxílicos y alcoholes grasos de cadena lineal), este término genérico se ha venido utilizando durante años para designar a los recubrimientos que se aplican a la fruta, a pesar de que en muchas de las formulaciones existentes en el mercado en la actualidad contienen poca proporción o nada de ceras (Hall, 1981).

Actualmente el tipo de recubrimientos comerciales empleados en la industria citrícola son ‘ceras al agua’ que consisten en disoluciones/dispersiones de una o más resinas y/o ceras emulsionadas (Hall, 1981). Estas ceras han ido desplazando a las ‘ceras solventes’ que utilizan solventes orgánicos, por el peligro y la contaminación medioambiental que conllevan. Las formulaciones de ‘ceras al agua’ requieren generalmente medios alcalinos para emulsionar la cera y la disolución de la resina (generalmente goma laca), por lo que está extendido el uso de álcalis como el hidróxido potásico, el amoníaco o morfolina en su formulación. Las ceras mayoritariamente empleadas son ceras sintéticas del tipo polietileno oxidado, empleándose en mucha menor medida las ceras vegetales del tipo carnauba y prácticamente testimonial es el uso de ceras de origen animal, como la cera de abeja. Para formar la microemulsión también

se añade emulsificantes como el ácido esteárico, palmítico, oleico o acetilglicéridos (ésteres de ácidos grasos con glicerol) (Baldwin et al., 1997; Hagenmaier, 1998). Asimismo, las ceras comerciales en muchos casos incorporan fungicidas sintéticos, como imazalil, tiabendazol u ortofenil fenato sódico, para controlar la podredumbre verde y azul, que son las principales enfermedades poscosecha de los cítricos.

Los componentes permitidos varían de unos países a otros, debido a diferencias en la legislación y a las exigencias de cada mercado (Llovera et al., 2002), así por ejemplo la legislación en Estados Unidos autoriza el uso de morfolina (CFR 172.235) y la colofonia modificada con anhídrido maleico y esterificada con pentaeritritol (CFR 172.210), mientras que la legislación Europea (Directiva europea 95/2/CE y posterior modificación 98/72/CE) no permite su uso y la morfolina es reemplazada por amoniaco (Llovera et al., 2002).

En función de la finalidad de su uso, se pueden definir dos tipos de encerado: los de conservación y los de comercialización, que se diferencian por el contenido en sólidos en la aplicación. El encerado de conservación se utiliza antes del almacenamiento en las cámaras frigoríficas y su objetivo es mantener el peso, la firmeza y las propiedades organolépticas, no siendo necesario, en general, que mejore la apariencia del fruto. El encerado de comercialización se aplica a la fruta antes de su envío al mercado de consumo, con la finalidad de mejorar el aspecto externo y mantener el peso. Por tanto, el contenido de sólidos en el caso del encerado de conservación no supera al 10-12%, mientras que en el caso de comercialización el contenido de sólidos no supera el 18% (Cuquerella et al., 2004).

Recubrimientos comestibles

El creciente interés de los consumidores hacia productos sanos y naturales ha orientado las investigaciones en el campo de los recubrimientos hacia el desarrollo de nuevos recubrimientos formulados a partir de compuestos naturales, seguros desde el punto de vista alimentario, apareciendo lo que se denominan ‘recubrimientos comestibles’. La expansión de los recubrimientos comestibles en cítricos va precedida de un aumento de su aplicación en otros frutos que se consumen con piel. Sin embargo, el futuro desarrollo de recubrimientos comestibles que eviten el uso de ceras sintéticas como el polietileno, o el uso de amoniaco, resulta un aspecto muy importante frente a las nuevas tendencias del mercado.

Los principales componentes utilizados en la preparación de estos recubrimientos naturales son proteínas, polisacáridos y lípidos. Además de estos componentes básicos, se añaden otros aditivos alimentarios, como plastificantes, emulsificantes, surfactantes, conservantes, antioxidantes..., que ayudan a mejorar la integridad mecánica, la calidad, aroma y valor nutricional de los alimentos.

Los ***Polisacáridos*** son los hidrocoloides más utilizados como recubrimientos de frutas y hortalizas (Kester y Fennema, 1986; Krochta y De-Mulder Johnston, 1997) y forman parte de la mayoría de las formulaciones que actualmente existen en el mercado. Los polisacáridos presentan buenas propiedades barrera a los gases y pueden adherirse a las superficies de frutas y hortalizas troceados, pero su carácter hidrófilo hace que presenten una baja barrera a la humedad.

Los polisacáridos utilizados habitualmente en los recubrimientos comestibles son derivados de celulosa, alginatos, carragenatos, pectinas, almidón, pullulan, quitosanos y gomas (Han y Gennadios, 2005).

Entre los polisacáridos de uso más extendido en la formulación de recubrimientos comestibles se encuentran los derivados de la celulosa (poli-β-(1→4)-D-glucopiranosa). Debido a la disposición de los grupos hidroximetil en la cadena polimérica, la celulosa presenta una estructura cristalina compacta que impide su solubilidad en sistemas acuosos. Sin embargo, su solubilidad puede ser aumentada mediante la inclusión de grupos funcionales en la cadena a través de reacciones de esterificación, interfiriendo la formación de la estructura cristalina. Cuando se trata la celulosa con álcali, seguido de ácido cloroacético, cloruro de metilo u óxido de propileno se obtiene carboximetilcelulosa (CMC), metilcelulosa (MC) e hidroxipropil metilcelulosa (HPMC), respectivamente (Kester y Fennema, 1986). El aumento de la solubilidad de estos compuestos ha impulsado el desarrollo de recubrimientos comestibles a base de los mismos (Wu et al., 2002).

El quitosano (polímero de β-1,4-glucosamina) es un componente de la pared celular de los crustáceos, capaz de formar películas semipermeables y que se encuentra entre los polisacáridos utilizados como recubrimientos comestibles. Su aplicación como recubrimiento ha proporcionado buenos resultados en cuanto a reducción de pérdida de peso y mejora de la calidad en diferentes frutas y hortalizas. En concreto, su aplicación en cítricos ha

mostrado resultados positivos sobre parámetros como pérdida de peso, firmeza y control de podredumbres (Salvador et al., 2003; Galed et al., 2004; Chien et al., 2007). Asimismo, existen estudios previos que han puesto de manifiesto el efecto antifúngico del quitosano y derivados en otros frutos, como fresa, mango, melocotón (Bautista-Baños et al., 2006; Vargas et al., 2006).

La efectividad de los recubrimientos de quitosano se ha visto que depende, entre otros factores, del peso molecular y del grado de desacetilación (González-Aguilar et al., 2005; Bautista-Baños et al., 2006).

Las ***Proteínas***, al igual que los polisacáridos, presentan buenas propiedades barrera a los gases, a baja HR, y su carácter polimérico hace que presenten buenas propiedades mecánicas, sin embargo, su carácter hidrófilo hace que presenten una baja barrera a la humedad. Las proteínas utilizadas en la formulación de recubrimientos comestibles pueden ser de origen animal (caseínas y proteínas del suero lácteo) o de origen vegetal (zeína de maíz, gluten de trigo, y proteínas de soja, principalmente) y dependiendo de este origen muestran una amplia variedad de características moleculares. Así, las proteínas varían en su peso molecular, conformación, carga (dependiendo del pH), flexibilidad y estabilidad térmica y las diferencias en estas características moleculares determinarán su habilidad para formar recubrimientos así como las características de los recubrimientos formados.

Los ***lípidos*** por su naturaleza hidrofóbica, ejercen una buena barrera a la humedad; sin embargo, su carácter no polimérico hace que presenten peores propiedades mecánicas que los hidrocolides formando recubrimientos más quebradizos (Krochta, 1997). Los lípidos utilizados en las formulaciones de recubrimientos incluyen ceras naturales (cera de abeja, cera de candelilla y cera de carnauba), acilgliceroles y ácidos grasos. En ocasiones, en las formulaciones para frutos que se consumen sin piel, como los cítricos, se añade goma laca, que es una resina natural que tiene como principal objetivo aportar brillo.

Teniendo en cuenta las propiedades de los distintos grupos, la tendencia en el desarrollo de recubrimientos comestibles para frutas y verduras es combinar hidrocoloides y lípidos y así aprovechar las ventajas que ambos ofrecen, formando lo que se conoce como **‘recubrimientos comestibles compuestos’**. De esta manera, los lípidos aportan resistencia al vapor de

agua y los hidrocoloides, la permeabilidad selectiva al O_2 y CO_2, y una buena cohesión estructural o integridad.

En la bibliografía existen numerosos trabajos que estudian la habilidad de los distintos compuestos para formar películas y recubrimientos comestibles, estudiando los factores que afectan las propiedades mecánicas y barrera en películas aisladas. En el caso de películas comestibles compuestas de hidrocoloide-lípido, el lípido puede encontrase laminando el hidrocoloide en forma de doble capa o 'bicapa', o disperso en la matriz de hidrocoloide en forma de 'emulsión'. La formación de películas compuestas 'bicapa' y 'emulsionadas' presentan importantes ventajas y limitaciones que dependen de la naturaleza de los compuestos que forman las películas y de la morfología de la misma. En general, las películas 'bicapa' son más efectivas reduciendo la transferencia al vapor de agua que las películas 'emulsionadas'. Sin embargo, la obtención de estas películas mediante laminación requiere de dos etapas de laminado y secado, así como el uso de solventes orgánicos y/o altas temperaturas, que hace su obtención más costosa y menos segura que en el caso de las películas 'emulsionadas'. Las películas comestibles 'emulsionadas' requieren un solo paso en su formación , lo que las hace más adecuadas desde el punto de vista de su desarrollo a nivel industrial. Sin embargo, las propiedades barrera y mecánicas de las películas compuestas de hidrocoloide-lípido se ven afectadas por numerosas variables de composición (tipo de lípido e hidrocoloide, contenido de lípido, ...), técnica de preparación (condiciones de secado, homogeneización, ...) y estructura (Pérez-Gago y Krochta, 2005).

Cuando el recubrimiento se encuentra aplicado a frutas y hortalizas, aparte de la composición del recubrimiento, existen otros factores a tener en cuenta que afectan la efectividad de los recubrimientos, como son el tipo de fruta y cultivar, el grosor del recubrimiento (que se ve modificado por cambios en el contenido en sólidos y viscosidad de las formulaciones), la adhesión del recubrimiento a la superficie del fruto (que depende de la naturaleza de la superficie y de las características del recubrimiento) y de las condiciones de almacenamiento (temperatura y HR).

En la bibliografía se encuentran numerosos trabajos sobre recubrimientos comestibles compuestos aplicados a frutas. En cítricos, la aplicación de HPMC-lípido resultó efectiva reduciendo la pérdida de peso y manteniendo la firmeza de mandarinas 'Fortune' (Pérez-Gago et al., 2002), 'Clemenules' (Navarro-Tarazaga y Pérez-Gago, 2006) y 'Ortanique'

(Navarro-Tarazaga et al., 2008). En estos trabajos, la efectividad de los recubrimientos dependió de la composición del recubrimiento y de las condiciones de almacenamiento. Entre los factores estudiados, el contenido y tipo de lípido, así como el contenido en sólidos de las formulaciones fueron los más importantes determinando la calidad fisicoquímica de la fruta, con un efecto importante en la calidad sensorial al crear en algunos casos una barrera excesiva a gases. Baldwin et al. (1995) mostraron que la aplicación de un recubrimiento comercial a base de celulosa en naranjas 'Valencias' incrementó en menor medida el contenido de compuestos volátiles que la aplicación de una cera comercial a base de goma laca, aunque el recubrimiento no fue efectivo controlando la pérdida de peso de las naranjas.

Chen y Nussinovitch (2001) compararon en mandarinas 'Nova' el efecto de la aplicación de recubrimientos compuestos a base de ceras y gomas (xantana, goma de garrofín o guar) con el de sus equivalentes formulados únicamente a base de ceras sobre la atmósfera interna y la calidad sensorial de las frutas. Los frutos recubiertos con estas formulaciones presentaron buen sabor debido a que la incorporación de las gomas perturbó la estructura ordenada de las ceras produciendo un menor obstrucción de los estomas y una mayor permeabilidad a gases.

Rojas-Argudo et al. (2009) desarrollaron recubrimientos comestibles compuestos a base de goma de garrofín y lípidos que controlaron la pérdida de peso y mejoraron el brillo de mandarinas 'Fortune', pero que produjeron un mayor aumento de los niveles de etanol que la aplicación de una cera comercial. Con la disminución del contenido en sólidos de la formulación a la mitad o el aumento del plastificante se redujo el contenido en etanol de los frutos recubiertos, pero únicamente el aumento de la cantidad de plastificante consiguió conjuntamente controlar efectivamente la pérdida de peso, reducir los niveles de etanol y mejorar el brillo de las mandarinas 'Fortune'.

El efecto de los recubrimientos comestibles en la calidad nutricional de cítricos ha sido poco estudiado. Togrul y Arslan (2004), reportaron que la pérdida de ácido ascórbico después del almacenamiento se detuvo cuando las mandarinas se recubrieron con CMC. Este resultado se explicó por la barrera a los gases que ejercen los recubrimientos, creando una AM, lo que disminuyó el potencial de auto-oxidación del ácido ascórbico.

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OBJETIVOS

2.1. Objetivo general

El objetivo general de esta tesis doctoral es estudiar el efecto de distintos tratamientos poscosecha novedosos en la calidad fisicoquímica, sensorial y nutricional de cítricos. En este sentido, se plantean los siguientes objetivos específicos.

2.2. Objetivos específicos

- Estudio del efecto de una atmósfera insecticida (95% CO_2) aplicada a 20 ó 25 ºC tras un tratamiento cuarentenario en frío (1,5 ºC durante 6, 9 y 12 días) en la calidad nutricional de mandarinas 'Clemenules'.

- Estudio del efecto de radiaciones ionizantes (0, 30, 54 y 164 Gy) en combinación con tratamiento cuarentenario en frío (1,5 ºC durante 6, 9 y 12 días) en la calidad nutricional de mandarinas 'Clemenules'.

- Estudio del efecto de una atmósfera insecticida aplicada a diferentes temperaturas (95% CO_2 a 23, 28 ó 33 ºC), combinado con diferentes tiempos de almacenamiento en frío (1 ºC durante 8, 16 y 24 días) como tratamiento cuarentenario, en la calidad físico-química, sensorial y nutricional de naranjas 'Valencia'.

- Estudio del efecto de un recubrimiento de quitosano aplicado a distintos contenidos en sólidos (CS) (0,6, 1,2 ó 1,8%) en la calidad físico-química, sensorial y nutricional de naranjas 'Valencia' y mandarinas 'Oronules'.

- Estudio del efecto de recubrimientos comestibles a base de hidroxipropilmetil celulosa (HPMC)-lípido con distinto CS (4 y 8%) y proporción de cera de abeja-goma laca (1:3 y 3:1) en la calidad físico-química, sensorial y nutricional de naranjas 'Valencia' y mandarinas 'Oronules'.

RESULTADOS Y DISCUSIÓN

CAPITULO I

Effect of insecticidal atmosphere and low dose X-ray irradiation in combination with cold quarantine storage on bioactive compounds of Clementine mandarins cv. 'Clemenules'

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Abstract

Citrus fruits are a rich source of vitamins and polyphenolic compounds with antioxidant capacity, that need to be maintained during postharvest storage. The aim of this study was to determine the effect of two innovative quarantine treatments, such as insecticidal atmospheres (IA) (95% CO_2 and balance air) applied at 20 or 25 °C for 20 h and low doses X-ray irradiation (0, 30, 54 and 164 Gy), in combination with short periods of cold-quarantine storage on the nutritional quality of 'Clemenules' mandarins. Mandarins were stored at 1.5 ºC for 6, 9, or 12 d before the application of IA treatments or for 0, 6, or 12 d after the X-ray radiation. Nutritional quality of mandarins was determined after the corresponding combination of quarantine treatment (IA or X-ray) with cold quarantine followed by a shelf life period of 7 d at 20 ºC to simulate shelf life conditions. Cold quarantine treatment combined with IA or with X-ray radiation did not affect negatively total antioxidant capacity and total ascorbic acid content of 'Clemenules' mandarins. However, flavanone glycosides (FGs) and total phenolics content were slightly modified. Application of the IA at 20 ºC induced a greater inhibition of the FGs than application at 25 ºC. When X-ray irradiation was applied without a previous quarantine period the synthesis of the FGs increased as irradiation dose increased.

Keywords: Citrus, cold quarantine, CO_2 atmosphere, X-ray irradiation, nutritional quality

Introduction

Spain is the world's largest exporter of fresh citrus fruit. Among the Spanish cultivars, 'Clemenules' (syns.: 'Clementina de Nules', 'Nules') is the leading clementine mandarin (*Citrus reticulata* Blanco) produced around the world. Clementines are characterized by a high sensory quality, seedless, and very easy to peel, which has contributed to an increase in the export shipments to overseas markets such as the USA and Japan (Palou et al 2008).

Many countries maintain strict quarantine measures against the mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). The most widely used postharvest disinfestation treatment of

citrus against this fruit fly involves exposure of the fruit to near-freezing temperatures. In the case of the USA, the U.S. Department of Agriculture (USDA) established a minimum exposure during overseas transit of 14 or 18 d below 1.1 or 2.2 ºC, respectively (USDA 2002a). Extensive research is currently focused on the development of alternative or complementary quarantine treatments for reducing cold quarantine storage specially for cold sensitive commodities such as citrus (Alonso et al 2005; Follett & Neven 2006; Palou et al 2008).

Insecticidal atmospheres (IA), with high CO_2 concentrations, and irradiation treatments are known to be effective against fruit flies and other pests (Hallman 1999; Follett & Neven 2006). Different studies have investigated the use of complementary CO_2 treatments previous or after cold exposure of citrus fruit, in order to reduce the duration of the standard cold disinfestation quarantine treatment against *C. capitata* and thus alleviate chilling injury problems (Alonso et al 2005; Palou et al 2008). Complete insect mortality of *C. capitata* with no negative effects on physicochemical and sensory quality of clementine mandarins after 7 d at 20 ºC of shelf life was obtained on fruit first exposed to 1.5 ºC for 3 d and second treated with 95 % CO_2 balanced with air at 25 ºC (Palou et al 2008).

Among the different ionizing radiation sources, the use of X-ray has been approved by the US Food and Drug Administration for food irradiation (US FDA 2004). A generic treatment dose of 100 Gy has been established for quarantine purposes against fruit flies (USDA 2002b). Palou et al (2007) reported complete insect mortality with no negative effects on fruit quality after 7 d at 20°C of shelf life on clementines firstly X-ray irradiated at 30-164 Gy and subsequently exposed to 1°C for 2 d. This combination of treatments considerably reduced quarantine time if compared to standard cold quarantine treatments (1.1-2.2°C for 14-18 d) and therefore showed promise as a potential commercial treatment for Spanish citrus exports.

Traditionally, postharvest quality assessment has been conducted by evaluating physico-chemical quality parameters, such as weight loss, firmness, colour, acidity, and maturity index, among others. Nowadays, nutritional and functional quality has gained great interest, being a component of the overall quality that is very much valued by consumers. Citrus fruits are an important source of vitamin C as well as bioactive compounds such as polyphenolic compounds, mainly flavonoids, with high antioxidant properties (Sánchez-Moreno et al 2003). Postharvest

technologies should maintain both nutritional and functional quality of fruits until they reach the consumer. Lee & Kader (2000) remarked the effects of storage temperature and time on vitamin C content of fruits and vegetables. The application of new quarantine treatments might also affect the physiology of the fruit altering their biochemical components. Recent studies show that irradiation of citrus fruit reduced significantly the total ascorbic acid (TAA) content when radiation doses were high (Patil et al 2004; Vanamala et al 2005; Girennavar et al 2008). However, information is still scarce on the effect of new quarantine treatments on nutritional quality of many citrus cultivars. Therefore, the aim of this work was to study the effect of two innovative quarantine treatments, such as IA (95% CO_2 balanced with air) applied at 20 or 25 °C and low doses X-ray irradiation (0, 30, 54 and 164 Gy), in combination with short periods of cold-quarantine storage on the nutritional quality of 'Clemenules' mandarins.

Material and methods

Fruit

Clementine mandarins (Citrus reticulata Blanco) cv. 'Clemenules' were hand-harvested at commercial maturity (MI=7.45) and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a mixed solution of imazalil (2,500 mg/L) and guazatine (800 mg/L) for 1.5 min. Fruit were allocated into homogeneous groups to apply, subsequently, each one of the combined quarantine treatments.

Materials

Reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH•), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). Methanol was from BDH Prolabo (Poole, UK). 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside, HES) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside,

NAT) and didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used for the analysis.

Cold and IA quarantine treatments

The mandarins were exposed to the standard cold-quarantine temperature of 1.5±0.5 ºC for 6, 9, or 12 d in a 40 m^3 cold room. Cold-treated fruit were allowed to warm in an air-atmosphere at room temperature (20±2 ºC) for 22–24 h before IA exposure. For each cold quarantine time, three groups of 150 fruit were exposed for 20 h to the following IA treatments: (T1) air-atmosphere at 20±1 ºC (control), (T2) atmosphere containing 95% CO_2 at 20±1 ºC and (T3) atmosphere containing 95% CO_2 at 25±1 ºC. In all cases, relative humidity (RH) was 85±5%. IA exposure chambers consisted of hermetic Perspex cabinets (82 cm x 62 cm x 87 cm), fitted with inlet and outlet ports through which CO_2 (Alphagaz, N38, Air Liquide S.A., Madrid, Spain) passed at a rate adjusted to yield a concentration of 95 % (*v/v*) inside the cabinet and balanced with air. Gas was allowed to escape from the outlet port through a bubble tube to maintain the proper gas mixture in the chamber. The desired gas concentrations were regularly reached after 25-30 min of closing the door of the cabinets. Levels of CO_2, O_2, temperature, and RH were continuously monitored by means of the system Control-Tec® (Tecnidex S.A., Paterna, Valencia, Spain). Cabinets were installed inside a 40 m^3 storage room that was also set to each experimental temperature (20 or 25 ºC). Once IA treatments were accomplished, mandarins were coated with a 10% total solids water wax containing polyethylene, shellac, and 0.5% of the fungicide thiabendazole (Brillaqua®, Brillocera S.A., Beniparrell, Valencia, Spain). Coated mandarins were stored 7 d at 20 ºC to simulate commercialization conditions.

X-ray irradiation and cold quarantine treatments

The mandarins were transported in a conditioned truck to the irradiation plant (Beta Gamma Service, BGM, Bruchsal, Germany). During transportation, the fruit were kept at 20±3 ºC. About 36 h later, the fruit were exposed to X-ray irradiation from a source with beam energy of 0.8 MeV and a conveyor speed of 5 m min^{-1}. The following theoretical doses were selected: 0 (control), 25, 50 and 150 Gy. Actual doses were determined

by placing 2 cm^2 radiochromatic dosimetry films (Gafchromic® HD-810, International specialty products, Wayne, NJ, USA) at three different heights within three different boxes. Readings (nine per dose) were made with a spectrophotometer at 560 nm and mean and standard error values were 30±1, 54±1, and 164±4 Gy for the respective theoretical doses. Control fruit were not irradiated; they were kept at 20 ºC until the application of the cold quarantine treatments.

Irradiated and non irradiated fruit were exposed to cold-quarantine at 1.5 ºC for 0 (control), 6 and 12 d followed by 7 d of shelf life at 20 ºC.

Determination of bioactive compounds of citrus

Nutritional quality of mandarins was determined at harvest (initial quality) and after the corresponding combination of quarantine treatment (IA or X-ray) with cold quarantine followed by a shelf life period of 7 d at 20 ºC to simulate prompt fruit commercialization. At the end of this period the juice from 3 replicates of 10 fruit each per treatment was obtained, transferred to vials with crimp-top caps and TFE/silicone septum seals and kept at –80 °C until the time of analysis.

Total antioxidant capacity (TAC)

The TAC was evaluated by the $DPPH^{\bullet}$ assay. Two mL of mandarin juice and 4 mL of methanol HPLC grade were mixed and centrifuged at 12,000 G for 15 min at 5 ºC. Five methanolic dilutions from the supernatant (0.075 mL) were mixed with 2.925 mL of $DPPH^{\bullet}$ (24 mg L-1) and kept in darkness for 40 min at 25±1 ºC. Afterwards, the change in absorbance was determined at 515 nm with a spectrophotometer (Thermo Electron Corporation, Auchtermuchty Fife, UK). The DPPH radical scavenging activity was expressed as effective concentration (EC_{50}), that is the amount of juice necessary to decrease the initial $DPPH^{\bullet}$ concentration by 50% (L juice/kg of $DPPH^{\bullet}$); thus, lower EC_{50} values mean higher antioxidant capacity (Sánchez-Moreno et al 2003).

Total ascorbic acid (TAA)

TAA was determined by the sum of ascorbic acid (AA) plus L-dehydroascorbic acid (DHA), by reducing DHA to AA with DTT. One mL mandarin juice was homogenized with 9 mL of MPA (2.5% w/v). Two mL

aliquot was mixed with 0.4 mL of DTT (20 mg mL-1) and allowed to react for 2 h in the dark at room temperature. Afterwards, samples were filtered through a 0.45 μm membrane filter and used for TAA determination by HPLC.

The HPLC system (Lachrom Elite, Merck Hitachi, Darmstadt, Germany) was equipped with an autosampler (Model L-2200), quaternary pump (Model L-2130), column oven (Model L-2300) and diode array detector (Model L-2450). A reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 μm-particle, Merck, Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. Injection volume was 20 μL and oven temperature 25 ºC. The mobile phase was 2% solution of KH_2PO_4, adjusted to pH 2.3 with H_3PO_4. The flow rate was fixed at 1 mL min-1 and the wavelength of measurement was 243 nm. AA was identified and quantified by comparison of peak areas with external standard and results were expressed as mg of TAA /100 mL of juice. Analysis was made by triplicate.

Flavanone glycosides (FGs)

The main FGs identified in citrus fruit, HES, NAT and DID were determined by HPLC. Two mL of juice were homogenized with 2 mL of DMSO:methanol (1:1 v/v) and centrifuged for 30 min, at 12,000 G and 4 ºC. The supernatant was filtered through one 0.45 μm nylon filter and analyzed by HPLC-DAD using the HPLC equipment described above and the chromatographic system conditions described by Cano et al (2008). The main FGs were identified by matching their respective spectra and retention times with those of commercially obtained standards. NAT, HES and DID contents were calculated by comparing the integrated peak areas of each individual compounds to that of its pure standards. Results were expressed as mg/100 mL.

Total phenolics content (TPC)

The TPC was determined using the Folin-Ciocalteu method (Singleton & Rossi 1965). 0.3 mL of mandarin juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted juice (0.4 mL) was mixed with 2 mL of Folin-Ciocalteu reagent (1:10, v/v diluted with water) and incubated for 1 min before 1.6 mL sodium carbonate (7.5%, w/v) was added. The mixture was incubated for 1 h at room temperature before absorption was measured at 765 nm with a spectrophotometer (Thermo Electron

Corporation, Auchtermuchty Fife, UK). TPC was expressed as mg gallic acid equivalents per 100 mL (mg GAE/100 mL). All extracts were analyzed in triplicate.

Statistical Analysis

A complete randomised design was used to perform the analysis of the samples. Statistical analysis of the data was performed using STATGRAPHICS Plus 2.1 (Manugistics, Inc., Rockville, Maryland, USA). Specific differences between means were determined by the Fisher's protected least significant difference test (LSD; $p \leq 0.05$) applied after an analysis of ANOVA.

3. Results and discussion

Cold and IA quarantine treatments

Total antioxidant capacity

Table 1 shows the EC_{50} values of treated mandarins. As mentioned earlier, the $DPPH^{\bullet}$ radical decreases by reacting with antioxidants present in the sample; therefore, a higher EC_{50} value indicates a lower TAC of the sample. In general, the TAC of the mandarins were not significantly affected by storage time or by the application of the different IA. Artés-Hernández et al (2007) found that the TAC in fresh-cut 'Lisbon' lemon products stored at different temperatures (0, 2, 5 or 10 ºC) remained constant during 12 d.

Total ascorbic acid

TAA content was not affected by the exposure to CO_2 or the increase in the cold quarantine period, except on mandarins exposed to the IA at 20 °C after 9 d of cold storage that had more TAA than the rest of the samples (Table 1). However, this difference although statistically significant was not observed for the rest of the storage periods and could be due to the intrinsic variability among samples.

Many studies in the literature show that AA content of fruits and vegetables decreases as the CO_2 concentration in the storage atmosphere

increases and these losses are usually accelerated by using high temperatures and long storage (Lee & Kader 2000; Thompson 2004). Storage at low temperature can also accelerate the loss of vitamin C in cold sensitive fruit, even before chilling injury is evident. For example, Ito et al (1974) reported that in 'Satsuma' mandarins, controlled atmosphere with low-O_2 and high-CO_2 concentrations at 1-4 °C reduced the AA level gradually, while the DHA content increased. In our study, mandarin exposure to 95% CO_2 was performed over a short period of time which could justify that the IA used did not affect TAA content and TAC. Although chilling injury can accelerate the loss of TAA in cold sensitive fruit, Palma et al (2005) did not observe changes in TAA and TAC of 'Fortune' mandarins after 90 d of storage at 5 ºC. Similarly in our work, storage at the cold quarantine temperature of 1.5 ºC did not affect the content of TAA and the TAC of the mandarins (Table 1).

Flavanone glycosides

Table 1 shows the content of the main flavonoids of 'Clemenules' mandarins after standard cold-quarantine periods and exposed to air or IA. The most abundant flavonoid was HES followed by NAT and DID. In general, HES content increased as cold storage time increased, being this increase less pronounced when the IA was applied at 20 °C. After 12 d of quarantine period, no differences were found in HES content between mandarins exposed to air-atmosphere and IA at 25 °C. Samples treated with 95% CO_2 at 20 °C after 9 and 12 d of storage had lower FGs content that control samples, which could indicate a slight inhibition in the synthesis of FGs by this treatment. Palma et al (2005) did not find differences in HES, NAT and DID in 'Fortune' mandarin juice during 90 d of storage at 5 ºC.

Total phenolic content

Table 1 shows the effect of cold quarantine periods and IA treatments on TPC of 'Clemenules' mandarins. TPC of 'Clemenules' mandarins ranged from 49.6 to 59.4 mg GAE/100 mL juice, which was in accordance with those reported in others studies for mandarin fruit (Wang et al 2007). TPC of the mandarins increased as cold quarantine storage increased. This result contrast with that reported by Palma et al (2005) that did not find differences in TPC of 'Fortune' mandarins during 90 d of cold storage at 5 ºC. In strawberry, an increase on the total phenols during storage time was observed although the fruits exposed to air + 20 kPa CO_2 contained lower

content of some specific phenolic compounds compared to those exposed to air, indicating that phenolic degradation may increase after exposition to CO_2-enriched atmospheres (Holcroft et al 1998). In our work, total phenols of 'Clemenules' mandarins increased slightly in the fruit kept in high CO_2 and exposed to cold quarantine temperature during 12 d.

Table 1. Total antioxidant capacity and bioactive compounds of 'Clemenules' mandarins exposed to cold quarantine at 1.5 ºC for 6, 9, or 12 d followed by 20-h exposure to air-atmosphere at 20 ºC (control) or insecticidal atmospheres (IA, 95 % CO_2) at 20 or 25 ºC.

Cold quarantine period (days)	IA treatment	TAC (EC_{50}) (L juice/kg DPPH)	TAA (mg/100 mL juice)	TPC (mg GAE/ 100 mL juice)	FGs (mg / 100 mL juice)		
					NAT	HES	DID
Initial (at harvest)		391.5 ± 41.1	32.73 ± 3.00	49.58 ± 1.37	2.52 ± 0.19	20.15 ± 0.76	0.33 ± 0.02
6	Control (air-20 ºC)	331.0 ± 26.5 a A	29.03 ± 2.70 a A	54.01 ± 1.27 a A	2.48 ± 0.19 a A	20.31 ± 1.16 ab A	0.30 ± 0.01 a A
	95% CO_2-20 ºC	355.8 ± 40.1 a A	29.74 ± 4.09 a A	55.45 ± 1.56 a A	2.53 ± 0.27 a A	19.68 ± 1.06 a A	0.29 ± 0.03 a A
	95% CO_2-25 ºC	395.5 ± 59.9 a A	29.75 ± 2.53 a A	59.06 ± 0.86 b B	2.89 ± 0.18 b B	21.09 ± 0.65 b AB	0.30 ± 0.01 a B
9	Control (air-20 ºC)	388.9 ± 18.0 a A	29.35 ± 2.59 a A	56.42 ± 0.14 a B	2.72 ± 0.15 b A	22.19 ± 0.41 b B	0.31 ± 0.01 b A
	95% CO_2-20 ºC	376.8 ± 66.5 a A	35.98 ± 1.79 b A	56.98 ± 1.90 a AB	2.38 ± 0.16 a A	21.19 ± 0.99 b B	0.25 ± 0.02 a A
	95% CO_2-25 ºC	408.5 ± 28.6 a A	30.04 ± 0.58 a A	54.75 ± 1.25 a A	2.52 ± 0.10 a A	19.97 ± 0.91 a A	0.26 ± 0.01 a A
12	Control (air-20 ºC)	377.8 ± 25.0 a A	28.72 ± 1.60 a A	56.68 ± 0.27 a B	2.65 ± 0.12 b A	22.77 ± 1.05 b B	0.31 ± 0.01 c A
	95% CO_2-20 ºC	433.9 ± 22.9 a A	29.60 ± 4.05 a A	59.31 ± 0.69 b B	2.47 ± 0.08 a A	21.56 ± 0.49 a B	0.27 ± 0.00 a A
	95% CO_2-25 ºC	381.3 ± 46.8 a A	32.22 ± 2.00 a A	59.35 ± 0.57 b B	2.64 ± 0.03 b AB	22.74 ± 1.14 b B	0.30 ± 0.01 b B

TAC=total antioxidant capacity, TAA=total ascorbic acid, TPC=total phenolic content, FGs=flavanone glycosides, NAT=narirutin, HES=hesperidin, DID=didymin

Previous to TAC, TAA, TPC and FGs determinations, treated fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Results present means ± standard deviation (n=3). For each cold quarantine period, mean values followed by different lower case letter indicate statistical differences among IA treatments according to Fisher's protected LSD test ($p \leq 0.05$). For each IA treatment, means with different capital letter indicate statistical differences among different quarantine periods according to Fisher's protected LSD test ($p \leq 0.05$)

X-ray irradiation and cold quarantine treatments

Total antioxidant capacity

Table 2 shows the changes in the TAC of irradiated and control 'Clemenules' mandarins at harvest and after the different quarantine periods. The EC_{50} values observed during the different storage periods were lower than the initial value measured at harvest, which indicates that the TAC of irradiated and non irradiated clementine mandarins increased after 7 d of storage at 20 ºC. The increase in the TAC might be due to an increase of the compounds of citrus fruit with high antioxidant properties such as TAA and polyphenols. However, this increase was not found in the same samples that were exposed to cold quarantine, followed by the IA treatments, and 7 d storage at 20 ºC (Table 1). In both works, control samples (non-irradiated and air-treated fruit) exposed to similar quarantine conditions and 7 d of storage at 20 ºC behaved differently. Differences in the behavior of the fruit could be due to differences in the handling of the fruit that had to be transported to the irradiation plant in Germany, which implied 4 additional d at 20±3 ºC. However, this should be confirmed with further studies. During storage, however, the TAC expressed as EC_{50} was not significantly affected by storage time at 1°C or by the dose of irradiation (30, 50 and 164 Gy).

Total ascorbic acid

TAA content of clementine mandarins ranged from 31.67±3.52 to 38.82±1.23 mg AA/100 mL juice (Table 2). These results are within the range of those reported in mandarins and other citrus fruit (Lee & Kader 2000; Cano et al 2008).

Application of low doses of X-ray irradiation combined with low-temperature quarantine storage did not affect negatively the TAA content of 'Clemenules' mandarins. Rather, an increase in TAA was observed in irradiated samples compared to control samples. This increase was higher in irradiated mandarins (30 or 54 Gy) stored directly at 20 ºC. Other authors have reported some increases in TAA of 'Clemenules' mandarins after storage at 20 ºC (Rojas-Argudo et al 2007) or gamma irradiation (Abdellaoui et al 1995). However, irradiation effect on TAA seems to depend on irradiation dose, fruit cultivar and maturity stage. Clementine fruits irradiated at 300 and 500 Gy doses along with hot water treatment and

stored for 3 weeks at 17 ºC contained higher TAA levels than control samples (Abdellaoui et al 1995). However, in grapefruit a dose of 1,500 Gy decreased TAA content, whereas a dose of 250 Gy did not affect the TAA content (Moshonas & Shaw 1984). Girennavar et al (2008) reported in grapefruit that a dose of 1,000 Gy did not affect the TAA content, whereas a dose of 2,500 Gy significantly reduced the TAA content. Patil et al (2004) reported that early season grapefruit irradiated at up to 700 Gy and stored 35 d did not affect TAA content, whereas in late season fruit an irradiation greater than or equal to 200 Gy caused a marked reduction in TAA content. These authors suggested that in earlier harvest fruit, vitamin C may not be the primary defence mechanism of fruit against the oxidative stress induced by gamma-irradiation, whereas in late season crops the stress induced by irradiation coupled with low temperature stress affecting the TAA content. Therefore, the susceptibility to modify the TAA content on citrus fruit might be avoided through selection of fruit in a optimum maturity stage.

Flavanone glycosides

In general, FGs content was affected by storage time at 1 ºC and by the irradiation dose applied (Table 2). X-ray irradiated mandarins stored 6 and 12 d at 1 ºC showed a decreased in FGs as the irradiation dose and storage time increased. When mandarins were not exposed to cold quarantine period, the FGs content increased as irradiation dose increased. Vanamala et al (2005) reported in grapefruits that low irradiation dose (300 Gy) increased naringin and NAT contents. Patil et al (2004), in early-season grapefruit, found that the total FGs concentration increased as the fruit was exposed to low doses of irradiation (70 and 200 Gy) followed by storage at 10 ºC for 4 weeks followed by 1 week at 20 ºC, whereas naringin (the more abundant FGs in grapefruit) and NAT levels decreased as the irradiation dose increased (above 200 Gy). The increase in FGs content at low irradiation doses was attributed to an increase in phenylalanine ammonia lyase (PAL) activity during low temperature storage. Whereas, the decline in FGs content of grapefruit at high doses of irradiation was related to their role in counteracting the oxidative stress induced by the gamma irradiation. Therefore, variations in the FGs content at different doses of irradiation may be a result of an equilibrium between gamma irradiation induced oxidative stress and *novo* synthesis of flavonoids by increased PAL activity (Patil et al 2004).

In the group of non-irradiated mandarins (control), HES content increased as quarantine storage increased. Patil et al (2004) also reported higher flavanoid content after cold storage of citrus fruit associated to an increase in the PAL activity during low temperature storage.

Total phenolics content

The TPC of 'Clemenules' mandarin juice is shown in Table 2. The TPC ranged from 50 to 60 mg GAE/100 mL juice, which was in accordance with those reported in others studies for mandarin fruit (Wang et al 2007). In general, our results show that low doses of X-ray irradiation did not significantly affect the TPC of 'Clemenules' mandarins, except for the second cold quarantine period (6 d at 1 ºC) where some differences were found among treatments, being 54 and 164 Gy irradiated mandarins the treatments with the highest TPC. In general, TPC increased as cold quarantine period increased with values from 50 mg GAE/100 mL juice at harvest to 58-60 mg GAE/100 mL juice after 12 d at 1 ºC followed by 1 week at 20 ºC.

Different stresses (irradiation, wounding, nutrient deficiencies, herbicide treatment, and viral, fungi, and insect attacks) have been shown to enhance either PAL synthesis or activity in different plants. PAL has been an indicative of rate-controlling enzyme in phenolic synthesis and wounding of citrus (Patil et al 2004). Many works have shown that irradiation influences phenolic biosynthesis as a response of plant tissue to abiotic stress and irradiation (Dubery 1992). Oufedjikh et al (2000) found that the TPC remained higher in irradiated fruits during 49 d at 3-4 ºC and this content was related to PAL activity, which also reached a maximum at 21 d of storage at 3-4 ºC. However, there were not always evidence of accumulation of phenolic compounds after the peak of PAL activity (Jones 1984; McDonald et al 2000).

Table 2. Total antioxidant capacity and bioactive compounds of 'Clemenules' mandarins irradiated with X-rays at 0, 30, 54, or 164 Gy and exposed to cold quarantine at 1.5 ºC for 0, 6, or 12 d.

Cold quarantine period (days)	X-Ray treatment	TAC (EC_{50}) (L juice/kg DPPH)	TAA (mg/100 mL juice)	TPC (mg GAE/100 mL juice)	FGs (mg / 100 mL juice)		
					NAT	HES	DID
Initial (at harvest)		391.5 ± 41.1	32.73 ± 3.00	49.58 ± 1.37	2.52 ± 0.19	20.15 ± 0.76	0.33 ± 0.02
0	Control	233.6 ± 16.2 a A	34.41 ± 1.88 a A	53.48 ± 0.33 a A	2.46 ± 0.19 ab A	20.84 ± 0.92 a A	0.32 ± 0.01 ab A
	30 Gy	227.2 ± 20.3 a A	37.60 ± 1.37 a A	52.73 ± 0.75 a A	2.42 ± 0.02 a A	20.71 ± 0.63 a A	0.31 ± 0.01 a A
	54 Gy	240.2 ± 51.1 a A	38.82 ± 1.23 a B	53.87 ± 1.12 a A	2.73 ± 0.07 bc A	22.33 ± 0.54 b A	0.34 ± 0.01 bc A
	164 Gy	272.9 ± 33.3 a A	35.92 ± 3.15 a A	54.84 ± 2.19 a A	3.01 ± 0.47 c B	24.58 ± 1.27 c A	0.36 ± 0.03 c B
6	Control	259.5 ± 16.8 a A	33.40 ± 1.72 a A	54.37 ± 1.00 a A	2.72 ± 0.17 a A	22.87 ± 1.69 a B	0.31 ± 0.03 a A
	30 Gy	244.5 ± 15.9 a A	31.67 ± 3.52 a A	56.42 ± 0.74 ab B	3.13 ± 0.36 b C	26.67 ± 2.76 b B	0.38 ± 0.06 b B
	54 Gy	275.2 ± 19.6 a A	32.67 ± 2.03 a A	58.00 ± 0.59 bc B	2.72 ± 0.19 a A	24.46 ± 0.94 a B	0.35 ± 0.01 ab B
	164 Gy	273.2 ± 53.9 a A	35.64 ± 1.96 a A	58.98 ± 1.73 c A	2.84 ± 0.06 a B	24.35 ± 0.64 a A	0.34 ± 0.01 a B
12	Control	271.2 ± 7.8 a A	32.55 ± 1.55 a A	57.43 ± 0.37 a B	2.65 ± 0.26 ab A	24.92 ± 0.40 b C	0.33 ± 0.03 b A
	30 Gy	278.5 ± 35.8 a A	35.23 ± 3.12 a A	59.89 ± 1.42 a C	2.81 ± 0.20 b B	24.83 ± 0.53 b B	0.35 ± 0.02 c B
	54 Gy	288.7 ± 12.3 a A	32.20 ± 0.98 a A	57.60 ± 1.32 a B	2.81 ± 0.09 b A	24.26 ± 0.93 b B	0.34 ± 0.01 bc A
	164 Gy	258.4 ± 31.0 a A	33.44 ± 2.40 a A	56.71 ± 4.27 a A	2.43 ± 0.23 a A	23.15± 1.25 a A	0.29 ± 0.02 a A

TAC=total antioxidant capacity, TAA=total ascorbic acid, TPC=total phenolic content, FGs=flavanone glycosides, NAT=narirutin, HES=hesperidin, DID=didymin

Previous to TAC, TAA, TPC and FGs determinations, fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Results present means ± standard deviation (n=3). For each cold quarantine period, mean values followed by different lower case letter indicate statistical differences among X-ray treatments according to Fisher's protected LSD test ($p \leq 0.05$). For each X-ray treatment, means with different capital letter indicate statistical differences among different quarantine periods according to Fisher's protected LSD test ($p \leq 0.05$).

4. Conclusion

Results indicate that innovative quarantine treatments, such as IA (95% CO_2, balanced with air) and X-ray irradiation at low doses (30, 54 and 164 Gy) in combination with short periods of cold-quarantine storage (6 to 12 d at 1.5 ºC) did not affect negatively the nutritional quality of 'Clemenules' mandarins. The TAC and TAA of mandarins was not affected by these treatments; whereas FGs synthesis was slightly inhibited by application of the IA and increased as X-ray irradiation dose increased.

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CAPITULO II

Effect of insecticidal atmosphere at high temperature combined with short cold-quarantine treatment on quality of 'Valencia' oranges

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Abstract

The combination of insecticidal atmosphere (IA) with short cold exposure periods have been effective in controlling the Mediterranean fruit fly, Ceratitis capitata (Wiedemann). In the present work, 'Valencia' orange quality was assessed on fruit exposed to insecticidal atmospheres (95% CO_2) at 23, 28 or 33 ºC for 20 h, next stored at 1 ºC for 8, 16 or 24 d, and then kept at 20 ºC for 7 d to simulate shelf life. Physicochemical, sensory and nutritional quality parameters were analyzed on treated and control (air-exposed) fruit. No significant negative effects on fruit quality were observed in IA-treated 'Valencia' oranges. In addition, the exposure of oranges to CO_2 at 28 ºC reduced the weight and firmness loss compared to fruit kept in air. Ethanol content increased in the fruits exposed to CO_2 at 28 or 33 ºC, but sensory quality was not adversely affected.

Additional index words: Ceratitis capitata, controlled atmosphere, flavor, vitamin C, phenolics

Introduction

Many countries maintain strict quarantine protocols against the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), which is one of the most damaging fruit pests worldwide and may be a major pest of citrus (White and Elson, 2004). The most widely used postharvest disinfestation treatment of citrus against this fruit fly involves exposure of the fruit to near-freezing temperatures. In the case of the USA, the U.S. Department of Agriculture established a minimum exposure during overseas transit of 14-18 days at 1.1-2.2 ºC (USDA, 2002). Nowadays, extensive research is currently focused on the development of alternative or complementary quarantine treatments, especially for cold sensitive commodities such as citrus (Jacas et al., 2008).

Controlled atmosphere (CA) treatments are known to be effective against fruit flies and other pests (Follett and Neven, 2006; Mitcham et al., 2003). Different works have investigated the use of insecticidal atmospheres (IA) consisting on high CO_2 shocks previous or after cold exposure of citrus fruit, in order to reduce the duration of the standard cold disinfestation quarantine treatment against *C. capitata* and thus alleviate chilling injury

problems (Alonso et al., 2005a, b; Palou et al., 2008a). In general, the effects of insecticidal treatments on treated produce considerably differ depending on species and cultivar (Follet and Neven, 2006). Therefore, the optimum combination of atmosphere gas composition, temperature, and length of application should be pursued for each pest-host system. For example, IA treatments consisting of exposure to 95% CO_2 at 20 ºC for 20 h (Alonso et al., 2005a), 98% CO_2 at 22 ºC for up to 24 h (Alonso et al., 2005a), and 95% CO_2 at 25 ºC for 20 h (Palou et al., 2008a) achieved complete insect mortality on 'Fortune' mandarins, 'Valencia' oranges, and 'Clemenules' mandarins, respectively, without affecting negatively fruit external appearance or organoleptic properties.

Nowadays, IA at curing temperature of 33 ºC on citrus are also been studied to control postharvest green mold of mandarins (Palou et al., 2008b). These new physical methods combining heat and gas shocks could be interesting to control established pathogenic infections and/or induce fruit resistance to postharvest diseases. However, little information is available for the effects of high CO_2 citrus exposure at high temperature, followed by cold-quarantine storage on overall fruit quality.

Conventionally, post-harvest quality assessment has been conducted to evaluate the physico-chemical quality of the fruit through parameters such as weight loss, firmness, maturity index and acidity, among others. Gradually, the sensory evaluation of fruit has been incorporated to study and prevent alterations in the organoleptic properties during postharvest handling. At present, nutritional quality is gaining interest, being a component of the overall quality very much valued by the consumer. In particular, citrus fruits are an important nutritional source of vitamin C and polyphenolic compounds with antioxidant properties, such as flavonoids (Sanchez-Moreno et al., 2003). The necessity of preserving the health properties of citrus recommends that post-harvest technologies would maintain both functional and nutritional quality until these reach the consumer. Lee and Kader (2000) reviewed the factors affecting postharvest content of vitamin C and concluded that temperature is the factor with more weight. In general, losses are accelerated by using high temperatures and long storage. However, low temperature storage can also accelerate the loss of vitamin C in cold sensitive fruit, even before chilling injury is evident (Miller and Heilman, 1952). Therefore, the exposure of citrus fruit to high CO_2 concentrations at different temperatures and the combination with

different periods of cold storage might affect the physiology of the fruit, altering the biochemical components of citrus. Therefore, the aim of this work was to study the effect of IA (95% CO_2) applied at 23, 28 and 33 °C combined with short cold quarantine storage on physicochemical, sensory and nutritional quality of 'Valencia' oranges.

Materials and methods

Fruit

'Valencia' oranges (Citrus sinensis) were hand-harvested with an average maturity index of 10.1 from a local grove in Valencia (Spain) and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a mixed solution of imazalil (1000 ppm) for 1 min. Subsequently, fruit were put into 12 homogeneous groups of 80 fruit each, which were placed in separate unlidded commercial cardboard boxes (40x29x27 cm).

IA treatments

For each period of time, four groups of 80 fruit were exposed for 20 h to the following IA treatments: (1) air atmosphere at 23±1 ºC (control), (2) IA containing 95% CO_2 at 23±1 ºC, (3) IA containing 95% CO_2 at 28±1 ºC, and (4) IA containing 95% CO_2 at 33±1 ºC. In all cases, RH was 85±5%. IA exposure chambers consisted of hermetic Perspex cabinets (82x62x87 cm), fitted with inlet and outlet ports through which CO_2 (Alphagaz, N38, Air Liquid S.A., Madrid, Spain) passed at a rate adjusted to yield a concentration of 95% (*v/v*) inside the cabinet and balance air. Gas was allowed to escape from the outlet port through a bubble tube to maintain the proper gas mixture in the chamber. Levels of CO_2, O_2, temperature, and RH were continuously monitored by means of the system of Control-Tec® (Tecnidex S.A., Paterna, Valencia, Spain). Cabinets were installed inside a 40 m^3 storage room that was also set to each experimental temperature (23, 28 or 33 ºC). Once IA treatments were accomplished, fruit were coated with a 10% total solids water wax containing polyethylene, shellac, and 0.5% of the fungicide thiabendazole (Brillaqua®, Brillocera S.A., Beniparrell, Valencia, Spain).

After waxing, the fruits were exposed to the standard cold-quarantine temperature of 1±0.5 ºC for 8, 16 or 24 d followed by 7 d of shelf life at 20 ºC to simulate prompt fruit commercialization. About 50 additional oranges were used to determine fruit quality at harvest (initial quality). Quality attributes were determined as follow.

Materials

Reagents 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu's phenolreagents, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside, HES) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside, NAT) and didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used.

Physicochemical quality

Weight Loss

Lots of 30 fruits per treatment were used to measure weight loss. The same fruit was weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage of initial weight loss.

Firmness

Firmness of 20 oranges per treatment was determined at the end of each storage time using an Instron Universal Testing Machine (Model 3343, Instron Corp., Canton, MA, USA). Each fruit was compressed between two flat surfaces closing together at the rate of 5 mm min^{-1}. The instrument gave the deformation after application of a load of 10 N to the equatorial region of the fruit. Results were expressed as percentage of deformation related to initial diameter.

Ethanol and acetaldehyde contents

Ethanol and acetaldehyde contents (EC and AC) in juice were determined by head-space gas chromatography. Ten fruits each in 3 replicates per treatment were analyzed. Five mL orange juice were transferred to 10 mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. EC and AC were analyzed in a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with an autosampler, a flame ionization detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). Temperatures of the oven, injector, and detector were 150, 175, and 200 ºC, respectively. Helium was used as the carrier gas at a flow rate of 28 mL min^{-1}. One mL sample of the head-space was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 ºC. EC and AC concentrations were calculated using peak areas of the samples relative to the peak areas of standard solutions. Results were expressed as mg/100 mL juice.

External disorders

Eighty fruit per treatment were inspected for external physiological disorders at the end of each storage period. The different degrees of disorders were rated as 0=none, 1=light, 2=moderate and 3=severe. Light was considered when less than 10% of fruit surface was affected and severe when more than 20% of fruit surface was affected. Results were converted to an average index.

Internal quality parameters

Soluble solids content (SSC) was measured with a digital refractometer (Atago, Model PR1) and titratable acidity (TA) was determined by titration with 0.1 N NaOH and phenolphthalein indicator and expressed as g of citric acid per 100 ml of orange juice. The maturity index (MI) was calculated as SSC/TA ratio. The juice from three replicates of 10 fruit each was used to determine the above parameters.

Sensory Analysis

Sensory evaluation was conducted by 10 trained judges. Panelists rated flavor on a 9-point scale, where 1 to 3 represented a range of non-acceptable quality with the presence of off-flavor, 4 to 6 represented a range of

acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavor presence was evaluated using a 6 point scale where 0=absence of off-flavor and 5=high presence of off-flavor.

One sample consisted of whole segments taken from about 8 individual fruits. Samples were presented to panelist in trays labeled with 3-digit random codes and served at room temperature (25±1 ºC). The judges had to taste several segments of each treatment in order to compensate, as far as possible, for biological variation of material. Mineral spring water was provided for rinsing between samples.

Nutritional quality

DPPH• radical-scavenging capacity (DPPH• RSC)

The total antioxidant capacity (TAC) was evaluated by the $DPPH^{\bullet}$ assay. 0.4 ml of orange juice diluted with 0.8 mL of methanol was centrifuged at 12000 rpm and 4 ºC for 20 min. Six methanolic dilutions from the supernatant (0.075 mL) were mixed with 0.2925 mL of $DPPH^{\bullet}$ (24 mg L^{-1}) and kept in darkness for 40 min. Afterwards, the change in absorbance at 515 nm was measured in a Multiskan spectrum microplate reader (Thermo Labsystem, USA).

For each dilution, the percentage of remaining $DPPH^{\bullet}$ was determined on the basis of the $DPPH^{\bullet}$ standard curve. The amount of juice in each dilution was plotted against the amount of $DPPH^{\bullet}$ radical remaining. Using the curve obtained, the EC_{50} value was calculated. This result expressed the amount of orange juice (L) needed to reduce 1 kg of $DPPH^{\bullet}$ by 50%; thus, lower values mean higher antioxidant activity.

Total ascorbic acid (TAA)

TAA was determined by the sum of L-ascorbic acid (AA) plus L-dehydroascorbic acid (DHA), by using the reducing agent DTT (Sánchez-Mata et al., 2000). One mL of orange juice was diluted to 10 mL with 2.5% (*w/v*) MPA. Two mL of this solution were mixed with 0.4 mL of DTT (20 mg mL^{1}) for 2 h in darkness. Afterwards, the extracts were filtered through a 0.45 µm Millipore filter before being HPLC analyzed.

The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi, Germany) equipped with a L-2200 autosampler, L-2130 quaternary pump, L-2300 column oven and L-2450 diode array detector. System conditions were: injection volume 20 µL, oven 25 ºC, detector wavelength 243 nm, flow rate 1 mL min^{-1}, column Lichospher 100 RP-18 of 25x0.4 cm preceded by a precolumn (4x4 mm) 5 µm particle size (Merck, Darmstadt, Germany). The mobile phase was 2% KH_2PO_4 adjusted to pH 2.3 with H_3PO_4. Results were expressed as milligrams of AA per 100 mL of juice.

Flavanone Glycosides (FGs)

HES, NAT and DID (mg/100 mL) were determined by the method described by Cano et al. (2008) slightly modified. Two mL of orange juice were homogenized with 2 mL de DMSO:Methanol (1:1 *v/v*) and centrifuged for 30 min, at 12000 rpm and 4 ºC. The supernatant was filtered through one 0.45 µm nylon filter and analyzed by HPLC-DAD using the HPLC equipment described above. System conditions were: injection volume 10 µL, oven 25 ºC, detector wavelength 280 nm, flow rate 1 mL min^{-1}, column Lichospher 100 RP-18 of 25x0.4 cm preceded by a precolumn (4x4 mm) 5 µm particle size (Merck, Darmstadt, Germany). The mobile phase was acetonitrile (A):0.6% acetic acid (B) with initial condition of 10% A for 2 min, reaching 75% A in the following 28 min, then back to the initial condition in 1 min and held for 5 min prior to the next sample injection. The main FGs were identified by matching their respective spectra and retention times with those of commercially obtained standards. NAT, HES and DID contents were calculated by comparing the integrated peak areas of each individual compounds to that of its pure standards. Results were expressed as mg/100 mL.

Total phenolic content (TPC)

The orange juices were analyzed for total phenolics by the Folin-Ciocalteu colorimetric method. 0.3 mL of orange juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted extract (0.4 mL) was mixed with 2 mL of folin ciocalteau commercial reagent (previously diluted with water 1:10, *v/v*) and incubated for 1 min before 1.6 mL sodium carbonate (7.5% *w/v*) was added. The mixture was incubated for 1 h at room temperature. The absorbance of the resulting blue solution was measured spectrophotometrically at 765 nm (Thermo UV1, Thermo Electron Corporation, UK) and the concentration of total phenolics was expressed as

gallic acid equivalents per 100 mL (mg GAE/100 mL). All extracts were analyzed in triplicate.

Statistical Analysis.

Statistical analysis was performed using STATGRAPHICS Plus 4.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.). Significance between means was determined by least significant difference (LSD) at $p \leq 0.05$.

Results and discussion

Physico-chemical quality

The use of high temperatures is known to lead to an enhancement of the insecticidal activity of quarantine treatments (Vincent et al., 2003), but it also might increase fruit weight loss in the same way that conventional curing of citrus increases fruit weight loss (Plaza et al, 2003; Porat et al., 2000). Figure 1 shows the weight loss of 'Valencia' oranges exposed to the IA-treatments (95% CO_2) at 23, 28 or 33 ºC, followed by cold quarantine storage at 1 ºC for 8, 16 or 24 d and a shelf life period at 20 ºC for 7 d. The exposure of 'Valencia' oranges to the IA at different temperatures did not increase the weight loss compared to the control. Interestingly, oranges exposed to IA at 28 ºC had the lowest weight loss. It is known that exposure to moderate temperatures and high relative humidity induces wound healing by biosynthesis of lignin and other phenolic compounds (Mulas and Schirra, 2007; Nunes et al., 2007). Therefore, this mild heat treatment probably induced positive changes in the rind of the mandarins that helped reducing orange weight loss.

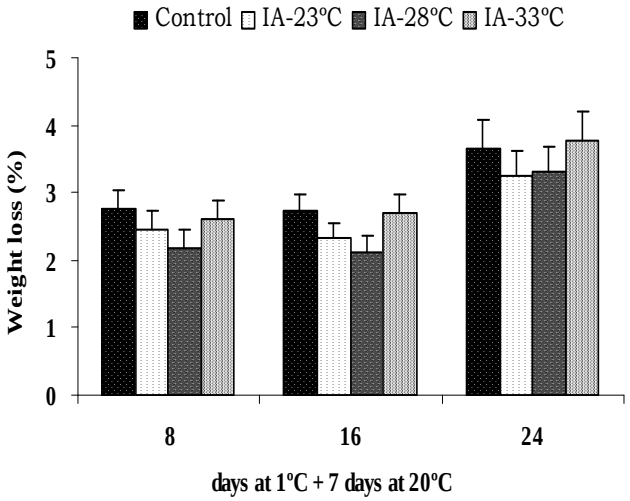

Figure 1. Weight loss of 'Valencia' oranges exposed to air (control) or an insecticidal atmosphere (IA, 95% CO_2) at 23, 28 or 33 °C for 20 h followed by a cold-quarantine storage at 1 ºC for 8, 16 or 24 d and a shelf life of 7 d at 20 ºC. Bars indicate SD value at $p \leq 0.05$.

Fruit firmness significantly decreased after 24 d of storage at 1 ºC (Table 1). Although no relevant differences were observed between IA-treated fruit and control fruit, fruit exposed to the IA at 28 or 23 ºC maintained higher firmness values than control fruit after 8 or 24 d of cold storage, respectively, which is in accordance with the lower weight loss of these treatments under those storage conditions (Figure 1). In general, the treatments had no harmful effect on fruit firmness and the maximal percentage of deformation remained below the 5% threshold established for firmness in citrus fruit (Martínez-Jávega et al., 1998). Similar results have been reported on mandarin and oranges exposed to combined cold and CA quarantine treatments (Alonso et al., 2005a, b; Palou et al., 2008a).

Table 1. Firmness of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO_2) at 23, 28 or 33 °C for 20 h followed by a cold-quarantine storage at 1 ºC for 8, 16 or 24 d and a shelf life of 7 d at 20 ºC.

Cold-quarantine period (days)	**IA treatment**	**Firmness (% deformation)**
Initial (at harvest)		2.24 ± 0.41
8	Control (air at 23 ºC)	2.28 ± 0.36 b
	IA – 23 ºC	2.24 ± 0.30 ab
	IA – 28 ºC	2.08 ± 0.20 a
	IA – 33 ºC	2.36 ± 0.27 b
16	Control (air at 23 ºC)	2.27 ± 0.36 a
	IA – 23 ºC	2.05 ± 0.41 a
	IA – 28 ºC	2.21 ± 0.38 a
	IA – 33 ºC	2.20 ± 0.36 a
24	Control (air at 23 ºC)	3.13 ± 0.38 b
	IA – 23 ºC	2.75 ± 0.44 a
	IA – 28 ºC	2.93 ± 0.49 ab
	IA – 33 ºC	3.14 ± 0.43 b

Previous to firmness measurement, fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Values give means ± SD (n=3). For each cold quarantine period, means with the same letter are not different at $p \leq 0.05$.

Figure 2 shows the EC and AC of 'Valencia' oranges exposed to combined IA and cold quarantine treatments. The EC and AC of CO_2-teated oranges remained fairly constant as cold quarantine storage time increased. 'Valencia' oranges exposed to high CO_2 had more EC and AC than those exposed to air. EC and AC were lower in fruit exposed to the IA at 23 ºC than in those exposed at 28 and 33 ºC. Under these high temperature conditions, EC exceeded slightly the limit, set up at 200 mg/100 mL juice, considered by some authors as the level of off-flavor build-up risk (Ke and Kader, 1990; Hagenmaier, 2002).

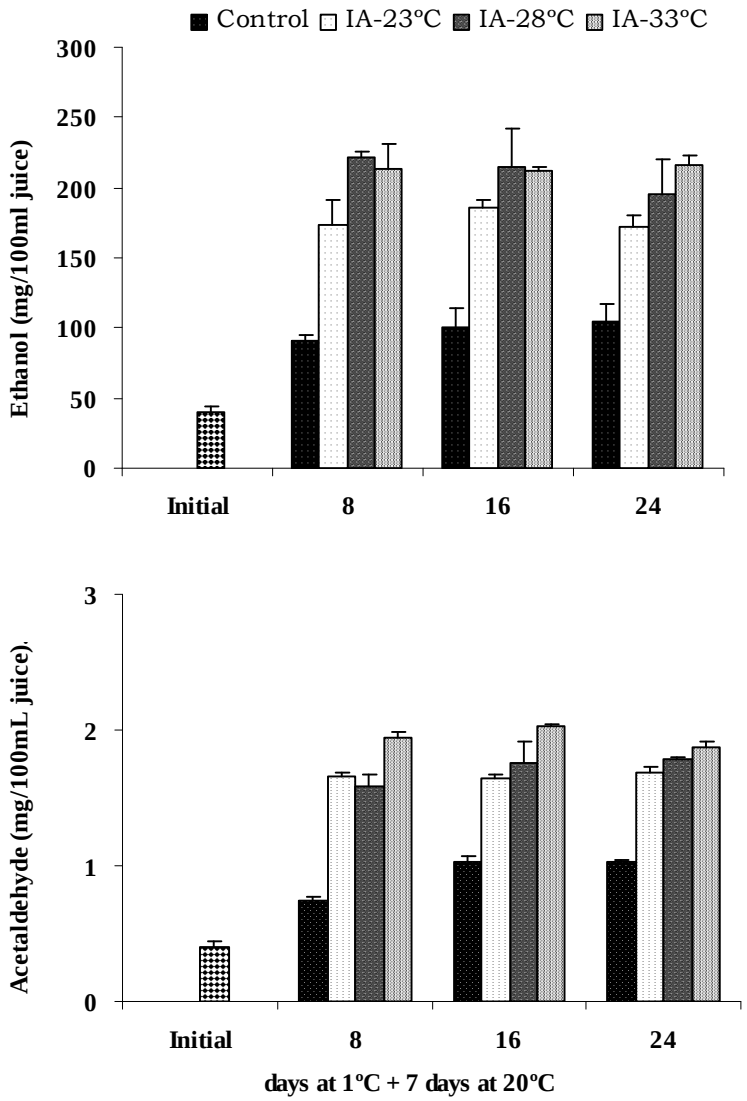

Figure 2. Ethanol and acetaldehyde contents of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO2) at 23, 28 or 33 °C for 20 h followed by a cold-quarantine storage at 1 ºC for 8, 16 or 24 d and a shelf life of 7 d at 20 ºC. Bars indicate SD value (n=3).

An increase in the concentration of these fermentative volatile compounds due to exposure to high CO2 and/or low O2 atmospheres has also been described in other citrus cultivars (Alonso et al., 2005a; Ke and Kader, 1990; Pesis and Avissar, 1989). Although the levels of volatile built-up as a consequence of CA exposure depends on fruit cultivar, treatment conditions and duration, these works report similar volatile built-up to the results found in our work when IA treatments were applied at temperatures below 25 ºC. In general, the level of ethanol, acetaldehyde, and other internal volatiles associated to anaerobic conditions increased as the temperature of application of the IA treatment increased.

The exposure of 'Valencia' oranges to the IA did not caused any rind damage. These results are in agreement with Alonso et al. (2005b) and Palou et al. (2008a) in 'Fortune' and 'Clemenules' mandarins, respectively. However, Ke and Kader (1990) described that longer expositions to high CO_2 atmospheres (9-14 d) induced severe rind injuries in 'Valencia' oranges.

Application of IA-treatments did not affect TA, SSC or MI (data not shown). Similarly, Alonso et al. (2005a) and Palou et al. (2008a) showed no effect of high CO_2 exposure for short storage periods on internal fruit quality parameters of 'Fortune' and 'Clemenules' mandarins, respectively. However, Pesis and Avissar (1989) showed that the exposure of oranges to high CO_2 atmospheres for 20-40 h decreased TA and increased MI.

Sensory Analysis

Overall flavor and off-flavors were not affected by the IA-treatment or the length of the cold quarantine period. Judges evaluated the oranges as acceptable at the end of storage, and having a very slight off-flavor (Table 2). Similarly, no negative influence on fruit sensory quality was found on Valencia' oranges or 'Fortune' mandarins exposed to IA atmospheres for short periods (Alonso et al., 2005a, b). However, the flavor of 'Clemenules' mandarins decreased after short term exposure to high CO_2 atmospheres (Palou et al., 2008a). These differences are due to the fact that several factors such as ethanol content, acidity, SSC and cultivar can influence the perception of off-flavors (Ke and Kader, 1990, Ke et al., 1991).

Table 2. Sensory analysis of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO_2) at 23, 28 or 33°C for 20 h followed by a cold-quarantine storage at 1 ºC for 8, 16 or 24 d and a shelf life of 7 d at 20 ºC.

Cold-quarantine period (days)	**IA treatment**	**Off-flavor (0-5)**	**Flavor (1-9)**
Initial (at harvest)		0.00 ± 0.00	7.12 ± 0.71
8	Control (air at 23 ºC)	0.67 ± 1.30 a	6.33 ± 1.44 a
	IA – 23 ºC	1.25 ± 1.42 a	5.58 ± 1.56 a
	IA – 28 ºC	1.08 ± 1.24 a	5.33 ± 1.61 a
	IA – 33 ºC	1.08 ± 1.44 a	5.00 ± 1.86 a
16	Control (air at 23 ºC)	0.82 ± 0.98 a	5.36 ± 1.80 a
	IA – 23 ºC	1.00 ± 1.26 a	5.09 ± 1.64 a
	IA – 28 ºC	1.36 ± 1.50 a	4.91 ± 1.76 a
	IA – 33 ºC	1.55 ± 1.21 a	5.00 ± 2.05 a
24	Control (air at 23 ºC)	0.46 ± 0.78 a	6.62 ± 1.19 a
	IA – 23 ºC	1.38 ± 1.45 a	5.54 ± 1.51 a
	IA – 28 ºC	1.23 ± 1.36 a	5.46 ± 1.66 a
	IA – 33 ºC	0.92 ± 1.26 a	5.92 ± 1.61 a

Previous to sensory evaluation, fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Values give means ± SD (n=3). For each cold quarantine period, means with the same letter are not different at $p \leq 0.05$.

Nutritional quality

Table 3 shows the DPPH• RSC of 'Valencia' oranges treated with IA at high temperature combined with short cold-quarantine periods. The DPPH• RSC of the oranges was not affected by the exposure to CO_2 or when the cold-quarantine period increased, except IA-33 ºC-treated oranges. In this treatment, EC_{50} values increased (i.e. DPPH• RSC decreased) at the end of the cold quarantine storage ($p \leq 0.05$). A high correlation between phenolic compounds and TAC in different fruit and vegetables has been widely reported (Kevers et al., 2007). In citrus fruit, AA in addition to phenolic content might contribute from 33% to 95% of the TAC (Gil-Izquierdo et al., 2002; Palma et al., 2005; Sánchez-Moreno et al., 2003). In our work, nor TAA neither total phenolics decreased in the IA-33 ºC-treated oranges, therefore, other phytochemicals, such as carotenoids, would be involved in the observed DPPH• RSC decrease.

TAA content of 'Valencia' oranges ranged from 34 to 42 mg/100 mL of juice (Table 3). The TAA content of 'Valencia' oranges was not affected by CO_2 exposure in fruits exposed to cold quarantine temperature for 8 or 16 d. However, after 24 d cold-quarantine storage, TAA increased in control fruit but no increase was observed in the rest of treatments. In accordance with our results, Rapisarda et al. (2008) reported an increase in the TAA content of 'Valencia' oranges during storage at 6 ºC. Therefore, exposure to CO_2 could diminish the capacity for the AA synthesis during fruit storage.

The FGs contents of 'Valencia' oranges exposed to IA and cold quarantine were in the range of those reported for citrus fruit (Table 3) (Dhuique-Mayer et al., 2005; Nogata et al., 2006). The HES, NAT and DID contents of 'Valencia' oranges were not affected by the increase in the cold quarantine period ($p \leq 0.05$). Palma et al. (2005) did not find significant differences in HES, NAT and DID in 'Fortune' mandarin during 3 months of storage at 5 ºC. In general the FGs contents were not affected by the exposure to CO_2, except on oranges exposed to the IA at 33 ºC after 16 d of cold quarantine storage that had more HES than the rest of the samples.

Table 3. DPPH• radical-scavenging capacity (DPPH• RSC) and bioactive compounds of 'Valencia' oranges exposed to air (control) or an insecticidal atmospheres (IA, 95% CO_2) at 23, 28 or 33 °C for 20 h followed by a cold-quarantine storage at 1º C for 8, 16 or 24 d and a shelf life of 7 d at 20 ºC.

Cold quarantine period (days)	**IA treatment**	**DPPH RSC (EC_{50}) (L juice/Kg DPPH)**	**TAA (mg/100 mL juice)**	**TPC (mg GAE/100 mL juice)**	**FGs (mg/100 mL juice)**		
					NAT	***HES***	***DID***
Initial (at harvest)		233.0 ± 14.2	34.71 ± 1.40	93.20 ± 4.82	2.86 ± 0.24	21.75 ± 1.21	0.97 ± 0.08
8	Control (air – 23 ºC)	224.3 ± 10.8 a A	39.15 ± 1.47 a A	91.23 ± 4.47 a A	3.26 ± 0.13 a A	27.83 ± 0.31 a A	1.02 ± 0.08 a A
	IA – 23 ºC	229.5 ± 7.1 a A	37.57 ± 1.23 a AB	90.21 ± 2.02 a A	2.85 ± 0.05 a A	27.47 ± 0.86 a A	0.96 ± 0.00 a A
	IA – 28 ºC	238.6 ± 19.5 a A	39.30 ± 1.18 a A	93.54 ± 1.64 a A	3.02 ± 0.53 a A	26.36 ± 1.36 a A	0.90 ± 0.11 a A
	IA – 33 ºC	206.3 ± 3.6 a A	36.21 ± 1.17 a A	98.70 ± 3.85 a A	2.95 ± 0.28 a A	26.88 ± 1.61 a A	0.87 ± 0.05 aA
16	Control (air – 23 ºC)	220.8 ± 7.6 a A	38.72 ± 1.10 a A	94.10 ± 0.38 a A	3.05 ± 0.20 a A	24.97 ± 1.09 a A	0.94 ± 0.08 a A
	IA – 23 ºC	228.7 ± 14.8 a A	35.50 ± 1.53 a A	99.74 ± 7.21 a A	2.84 ± 0.21 a A	25.08 ± 1.23 a A	0.84 ± 0.07 a A
	IA – 28 ºC	219.5 ± 35.6 a A	35.85 ± 1.29 a A	99.10 ± 5.28 a A	2.86 ± 0.22 a A	25.27 ± 0.28 a A	0.79 ± 0.07 a A
	IA – 33 ºC	194.9 ± 11.7 a A	36.21 ± 2.60 a A	107.19 ± 11.20 a A	2.97 ± 0.23 a A	27.51 ± 1.01 b A	0.80 ± 0.03 a A
24	Control (air at 23 ºC)	216.1 ± 2.1 a A	42.31 ± 1.65 b B	105.86 ± 4.99 a B	2.88 ± 0.18 a A	27.67 ± 0.61 a A	0.85 ± 0.07 a A
	IA – 23 ºC	227.0 ± 5.7 a A	38.84 ± 0.49 a B	100.66 ± 4.19 a A	2.94 ± 0.11 a A	26.66 ± 1.27 a A	0.88 ± 0.01 a A
	IA – 28 ºC	241.6 ± 17.2 a A	36.94 ± 2.22 a A	103.55 ± 9.38 a A	3.20 ± 0.11 a A	25.88 ± 0.64 a A	0.94 ± 0.06 a A
	IA – 33 ºC	234.8 ± 2.8 a B	38.01 ± 0.36 a A	107.80 ± 2.46 a A	3.16 ± 0.17 a A	26.11 ± 0.56 a A	0.90 ± 0.09 a A

DPPH• RSC=DPPH• radical-scavenging capacity, TAA=total ascorbic acid, TPC=total phenolic content, FGs=flavanone glycosides, NAT=narirutin, HES=hesperidin, DID=didymin.

Previous to DPPH• RSC and bioactive compounds determination, fruit was kept at 20 ºC for 7 d to simulate shelf life conditions.

Values give means ± SD (n=3). For each cold quarantine period, different treatments with the same lower case letter are not different at $p \leq 0.05$. For each treatment and different quarantine periods, means with the same capital letter are not different at $p \leq 0.05$

TPC of 'Valencia' oranges ranged from 90.21± 2.02 to 107.80±2.46 mg/100 mL juice (GAE) (Table 3). Gil-Izquierdo et al. (2002) found that TPC of orange juice and pulp following domestic and commercial squeezing were 87.8 and 71.7 mg/100 mL, respectively. Gardner et al. (2000) also found that total polyphenols ranged from 50.4±1.0 to 75.5±1.8 mg/100 ml (GAE) in three commercial orange juices.

In general there was an increase in the TPC of 'Valencia' oranges when the cold quarantine period increased. This result is accordance with Patil et al. (2004) which reported higher flavanoid content after cold storage of citrus fruit associated to an increase in the PAL activity during low temperature storage. In contrast, other works have shown that cold storage did not influence or decreased the citrus TPC. For example, Palma et al. (2005) did not find differences in the TPC of 'Fortune' mandarins after 90 d of storage at 5 ºC and Rapisarda et al. (2008) found a decrease of total phenolics in 'Valencia' oranges after 40 days of storage at 6 ºC attributed to senescence phenomena during storage.

Conclusion

It can be concluded from these results that the exposure of 'Valencia' oranges to 95% CO_2 at 23, 28 or 33 ºC combined with short cold-quarantine periods did not induce any harmful effect on physicochemical, sensory or nutritional citrus quality. Exposure of 'Valencia' oranges to 95% of CO_2 at 28 ºC for 20 h was beneficial to maintain the fruit quality, reducing weight and firmness loss. Combination of the IA and cold quarantine periods of 8 or 16 d did not affect the TAA content of the fruits; whereas when cold quarantine period increased to 24 d, fruit exposed to high CO_2 atmospheres had lower TAA content than control fruit. Therefore, these high CO_2 atmospheres could be applied as insecticidal treatments or, when combined with curing temperatures, for the control of citrus moulds without negatively affecting the quality of 'Valencia' orange.

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CAPITULO III

Effects of chitosan on physicochemical and nutritional quality of Clementine mandarins cv. 'Oronules'

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ABSTRACT

In the present study a commercial solution of chitosan was applied on mandarins 'Oronules' at different solid content (SC) (0.6, 1.2 and 1.8%). Additionally, one group of mandarins was coated with a commercial wax, and another group remained uncoated. Mandarins were stored at 5 ºC up to 28 days followed by 7 days at 20 ºC simulating retail conditions. One group of mandarins was stored at 20 ºC for 9 days simulating direct retail conditions. All coatings applied restricted gas exchange and modified internal atmosphere of the fruits compared to uncoated mandarins, with a greater effect at higher chitosan concentration. Chitosan-coated mandarins at the highest SC retained firmness after cold storage. In general, the internal quality and the health-related properties of mandarins were not affected by coating application. Chitosan application did not decrease effectively weight loss of mandarins during storage, whereas the commercial wax decreased weight loss of mandarins compared to control.

PRACTICAL APPLICATIONS

Edible coatings like commercial waxes are useful in maintaining citrus postharvest quality. Although it is well-known that coatings induced important changes in the fruit internal atmosphere, no information can be found about their effect on health-related compounds of clementine mandarins. This work shows that the internal quality and the functional properties of mandarins were not affected by coating application. Bioactive compounds were maintained during postharvest storage. In order to improve the water barrier properties of the chitosan coating, it would be necessary to add hydrophobic components to the formulation.

Keywords: citrus, wax, ethanol, weight loss, vitamin C

INTRODUCTION

Spain is the main exporter of clementine citrus mandarins, including in this group Oronules 'clementine'. This clementine cultivar is highly appreciated by its excellent organoleptic quality (Ortiz *et al.* 1998). Citrus fruit are coated in the citrus packinghouses to improve their appearance and extend their shelf life (Petracek *et al.* 1999). In general, commercial waxes

used by the citrus industry enhance shine, reduce water loss and act as a vehicle for fungicides. However, it has also been reported by many authors that waxing of citrus can adversely affect fruit flavor (Ke and Kader, 1990; Hagenmaier and Baker, 1993; Baldwin *et al.* 1995; Hagenmaier, 2002; Hagenmaier and Shaw, 2002; Porat *et al.* 2005), due to the overproduction of volatiles associated with anaerobic conditions when coatings offer a high barrier to gases.

Most of commercial waxes are microemulsions or wax suspensions containing natural waxes such as carnauba, petroleum-based waxes such as polyethylene wax, acetoglycerides, and oleic acid, or resins such as shellac to improve citrus shine or gloss (Baldwin *et al.* 1997). In recent years there has been an increased interest in "edible coating" development and application. The replacement of synthetic components used in commercial waxes, like polyethylene, by natural substances has advantages concerning to environmental protection and food safety.

Edible coatings incorporate proteins, polysaccharides and lipids (Baldwin, 1994). Polysaccharides have been widely used because of their ability to form films and their selective permeabilities to O_2 and CO_2 (Nisperos-Carriedo, 1994). Among polysaccharides, chitosan (poly β-(1→4)N-acetyl-D-glucosamine) has been used as coating of some fruits and vegetables, due to its antimicrobial and biostimulant activities, as well as film forming properties. In citrus fruit, significant reduction of postharvest penicillium decay and delay of fruit senescence during long-term cold storage of different citrus species and cultivars have been observed after the application of certain chitosan formulations (El-Ghaouth *et al.* 2000; Galed *et al.* 2004; Fornes *et al.* 2005; Chien and Chou, 2006; Chien *et al.* 2007).

Fruits and vegetables, especially citrus fruit, constitute an important nutritional source of health related compounds, mainly vitamin C, as well as polyphenolic compounds such as flavonoids. The variety and abundance of antioxidant compounds in the fruit makes synergy between these compounds possible, contributing to the total antioxidant capacity of the fruits (Sanchez-Moreno *et al.* 2003). The necessity of preserving the health properties of citrus recommends that post-harvest technologies would maintain both functional and nutritional quality until these reach the consumer. Despite this interest for maintaining the health-related quality of fruit, scarce works have studied the influence of citrus postharvest treatments and conditions on their bioactive compounds (Rapisarda *et al.*

2001; Del Caro *et al.* 2004; Patil, *et al.* 2004; Perez *et al.* 2005; Biolatto *et al.* 2005; Vanamala, *et al.* 2005, 2007; Girennavar *et al.* 2008; Rapisarda *et al.* 2008) and there is a necessity for determining possible effects of usual postharvest practices applied on citrus fruit such as waxing, especially when the coating formulation includes bioactive substances like chitosan. Although it is well-known that coatings induced important changes in the fruit internal atmosphere, no information can be found about their effect on health-related compounds of clementine mandarins.

The success of edible coatings for citrus mainly depends on the selection of appropriate formulations that not only reduced fruit weight and firmness loss, but also give a desirable internal gas composition. For a specific coating formulation, gas barrier can be affected by solid content (SC) of the formulation (Cisneros-Zevallos and Krochta 2003). Therefore, the objective of this work was to study the effect of a commercial chitosan formulation applied at SC on the physiology, sensory, and health-related quality of mandarins cv. 'Oronules' and compare its effect with a commercial coating.

MATERIALS AND METHODS

Materials

Crab-shell chitosan, Biorend®, was supplied by Idebio, S.L. This product is manufactured as a water solution (pH=4.8) of chitosan at 1.8 g/100 mL in acetic acid. Polysorbate 80 (Tween 80) was from Panreac Química, S.A. (Barcelona, Spain). 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). Methanol was from BDH Prolabo (Poole, UK). 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside) and didymin (isosakuranetin-7-rutinoside) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used for the analysis.

Sample preparation and coating application

Mandarins cv. 'Oronules' were harvested from a local grove in Valencia (Spain) and transported the same day to the research laboratory. Fruit were selected for size, color and absence of physical damage, and then dipped 1 min in 500 ppm imazalil solution as antifungal agent, following air drying.

Mandarins were coated on chitosan solutions containing 3 SC: 0.6%, 1.2% or 1.8% (0.6% Ch, 1.2% Ch and 1.8% Ch). The chitosan solutions contained 0.02% of Tween 80 to improve wetting of the coating to the citrus surface. Tween 80 was mixed in the chitosan solution by using a rotatory paddle mixer (Model RW16B; IKA-Werke GmbH & Co., Stanfen, Germany). Fruit were dip-coated by immersion in the chitosan solutions for 20 sec, drained of excess coating and dried in a tunnel at 55 ºC for 2 min. With the aim of comparing these chitosan coatings with commercial procedures, a commercial wax coating (CW) was applied by spraying with nozzles in the citrus processing line placed at the IVIA, followed by drying in a tunnel at the same conditions used with the chitosan coatings. CW consisted on a polyethylene/shellac mixture at total SC of 10%. After coating, fruit were stored for 28 days at 5 ºC and 90-95% RH, followed by 6 days at 20 ºC simulating retail handling conditions. Additionally, 1 group of fruit from each treatment was stored directly in retail conditions at 20 ºC and 75% RH for 8 days.

Weight loss

Three lots of 30 fruit per treatment were used to measure weight loss. The same fruit were weighted at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage loss of initial weight.

Fruit firmness

Firmness of twenty fruit per treatment was determined at the end of each storage period using an Instron Universal Testing Machine (Model 4301, Instron Corp., Canton, MA). Each fruit was compressed between two flat surfaces closing together at the rate of 5 mm/min. Results were expressed as the percentage of equatorial deformation, related to initial diameter, when a 10 N load was applied.

Internal gas composition

Ten fruit per treatment were used to calculate internal gas concentrations. Internal CO_2 and O_2 concentrations of each sample were obtained by withdrawing 1 mL internal gas sample from the mandarin central cavity with a syringe while the fruit was immersed under water. The gas sample was then injected into a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) fitted with a Porapak QS 80/100 (1.2 m x 0.32 cm) column, followed by a molecular sieve 5A 45/60 (1.2 m x 0.32 cm) column. Temperatures were 35, 125 and 180 ºC, respectively, for the oven, injector and thermal conductivity detector. Helium was used as carrier gas at 22 ml min^{-1} flow rate. Peak areas obtained from standard gas mixtures were determined before and after analysis of samples and results were expressed as percentage.

Ethanol and acetaldehyde content

Ethanol and acetaldehyde concentration in juice were determined by head-space gas chromatography according to the method described by Davis and Chace (1969). The juice from three replicates of 10 fruit each were analyzed. Five milliliters of juice were transferred to 10 mL vials with crimp-top caps and TFE/silicone septa seal. Volatiles were analyzed using a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) with a flame ionization detector and a 1.2 m x 0.32 cm Porapack QS 80/100 column. Temperatures of the oven, injector, and detector were 150, 175, and 200 °C, respectively. Helium was used as the carrier gas at a flow rate of 28 $mL \cdot min^{-1}$. A 1 mL sample of the headspace was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 °C. Ethanol and acetaldehyde were identified by comparison of retention times with standards. Results were expressed as mg L^{-1} juice.

Sensory evaluation

The effect of coatings on sensory quality of the samples, initially and after each storage period, was assessed by 10 to 15 judges with several year's experience tasting citrus products. Panelists rated flavor on a 9-point scale, where 1 to 3 represented a range of non-acceptable quality with the presence of off-flavor, 4 to 5 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavors presence was

evaluated using a intensity scale, where 0 represented absence of off-flavor and 5 high presence of off-flavor.

One sample consisted of segments taken from about 4 individual fruits. Samples were presented to panelists in trays labeled with 3-digit random codes and served at room temperature (25±1 ºC). The judges had to taste several segments of each sample in order to compensate, as far as possible, for biological variation of the material. Spring water was provided for rinsing between samples.

Fruit internal quality

Soluble solids content (SSC) was measured with a digital refractometer (Atago, Model PR1) and titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1 and expressed as g citric acid per L of juice. The maturity index (MI) was calculated as SSC/TA ratio. The juice from three replicates of 10 fruit each was used to determine the above parameters.

Bioactive compounds

The total antioxidant capacity was analyzed by the $DPPH^{\bullet}$ assay (Brand-Williams *et al.* 1995). The $DPPH^{\bullet}$ radical has a deep violet color due to its impaired electron, therefore radical scavenging can be followed by the loss of absorbance at 515 nm as the pale yellow non-radical form is produced. One-ml of juice diluted with 2 mL of methanol was centrifuged at 14,000 rpm and 4 ºC for 5 min. Five methanolic dilutions from the supernatant (7.5 µl) were mixed with 392.5 µl of $DPPH^{\bullet}$ (24 mg/L) and kept in darkness for 30 min. Afterwards, the change in absorbance at 515 nm was measured in a Multiskan spectrum microplate reader (Thermo Labsystem, USA). For each dilution, the percentage of remaining $DPPH^{\bullet}$ was determined on the basis of the $DPPH^{\bullet}$ standard curve. The amount of juice in each dilution was plotted against the amount of $DPPH^{\bullet}$ radical remaining. Using this curve, the EC_{50} value was calculated, which expresses the amount of mandarin juice needed to reduce 1 kg of $DPPH^{\bullet}$ by 50% (L juice/Kg of $DPPH^{\bullet}$); thus, lower EC_{50} values mean higher antioxidant capacity.

Total ascorbic acid (TAA) was determined by the sum of ascorbic acid (AA) plus dehydroascorbic acid (DHA), by using the reducing agent DTT (Dhuique-Mayer *et al.* 2005). One-mL of sample was diluted to 10 mL with 2.5% (w/v) meta-phosphoric. Two-ml of this solution were mixed with 0.4

mL of DTT (0.02 g de DTT in 1 mL ultrapure water) for 2 h in darkness. Afterwards, the extracts were filtered through a 0.45 mm Millipore filter and analyzed by HPLC. The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi, Germany) equipped with a L-2200 autosampler, L-2130 quaternary pump, L-2300 column oven and L-2450 diode array detector. System conditions were: injection volume 20 μL, oven 25 ºC, detector wavelength 245 nm, flow rate 1 mL/min, column Lichospher 100 RP-18 of 25 x 0.4 cm with 5 μm particle size (Merck, Darmstadt, Germany). The mobile phase was 2% KH_2PO4 adjusted to pH 2.3 with H_3PO_4. Results were expressed as milligrams of AA per L of juice.

Flavanone glycosides, hesperidin, narirutin and didymin (mg L^{-1}) were determined by the method described by Cano *et al*. (2008) slightly modified. Two-mL of juice were homogenized with 2 mL of DMSO:MeOH (1:1 v/v) and centrifuged for 30 min at 12000 rpm and 4 ºC. The supernatant was filtered through a 0.45 μm nylon filter and analysed by HPLC-DAD using the HPLC equipment described above. System conditions were: injection volume 10 μL, oven 25 ºC, detector wavelength 280 nm, flow rate 1 mL/min, column Lichospher 100 RP-18 of 25 x 0.4 cm preceded by a precolumn (4 x 4 mm) with 5 μm particle size (Merck, Darmstadt, Germany). The mobile phase was acetonitrile (phase A):0.6% acetic acid (phase B) with initial condition of 10% A for 2 min, reaching 75% A in the following 28 min, then back to the initial condition in 1 min and held for 5 min prior to the next sample injection. The main flavanone glycosides were identified by matching their respective spectra and retention times with those of commercially obtained standards. Narirutin, hesperidin and didymin contents were calculated by comparing the integrated peak areas of each individual compound to that of its pure standards.

Mandarin juice was analyzed for total phenolic concentration by the Folin-Ciocalteu (FC) colorimetric method (Singleton and Rossi, 1965). 0.3 mL of mandarin juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted extract (0.4 mL) was mixed with 2 mL of FC commercial reagent (previously diluted with water 1:10, v:v) and incubated for 1 min before 1.6 mL sodium carbonate (7.5% w/v) was added. The mixture was incubated for 1 h at room temperature. The absorbance of the resulting blue solution was measured spectrophotometrically at 765 nm (Thermo UV1, Thermo Electron Corporation, UK) and the concentration of total phenolics was expressed as gallic equivalents (mg L^{-1}).

Antioxidant capacity, total vitamin C, flavonone glycosides and total phenolics were determined in juice from three replicates of 10 fruit each.

Statistical Analysis

Three replicates were used per treatment. Statistical analysis of the results was performed was performed by one-way analysis of variance (ANOVA) using STATGRAPHICS Plus 2.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.). Significant differences between means were determined by least significant difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Weight loss

Figure 1 shows weight loss of coated and uncoated 'Oronules' mandarins. As expected, weight loss increased as storage time increased, reaching values around 10%. For all storage periods, the commercial wax was the most effective treatment reducing weight loss of the mandarins; whereas, chitosan only reduced weight loss compared to the control in mandarins coated with 1.2 and 1.8% Ch after 9 d of storage at 20 ºC, and mandarins coated with 1.2% Ch after 1 week of storage at 5 ºC plus 1 week at 20 ºC. Commercial waxes used by the citrus industry are made of natural or synthetic waxes (beeswax, carnauba, polyethylene…), fatty acids, oils, shellac, emulsifier, plasticizers, anti-foam agents, and surfactants (Hagenmaier and Baker, 1994; Hagenmaier, 1998). The hydrophobic nature of these ingredients contributes to significantly reduce weight loss of coated fruit. Hydrophilic components, such as chitosan, do not offer the high moisture barrier that lipid coatings do, requiring the addition of hydrophobic components to reduce fruit weight loss. Nevertheless, some works have shown an effect of chitosan reducing weight loss of citrus fruit (Salvador *et al*. 2003; Galed *et al*. 2004; Chien *et al*. 2007), pepper and cucumbers (El Ghaouth *et al*. 1991), and strawberries (Hernandez-Muñoz *et al*. 2006). Differences with our results might be due to differences in the fruit type and cultivar, storage conditions and the chitosan nature. Previous works reported that similar hydroxypropyl methylcellulose-lipid edible coatings were effective reducing weight loss of 'Ortanique' mandarins, whereas they did not reduce weight loss of coated 'Valencia' oranges (Valencia-Chamorro,

2009; Valencia-Chamorro *et al*. 2009). Many works have also shown that effectiveness of chitosan applied as coating depends, among others factors, on the molecular weight (Mw) and the degree of deacetylation (Bautista-Baños *et al*. 2006, Chien *et al*. 2007). For instance, coating 'Murcott' mandarins with low Mw chitosan (15 kDa) was effective controlling fruit weight loss, whereas no differences were found between mandarins coated with high Mw chitosan (357 kDa) and control samples (Chien *et al*. 2007).

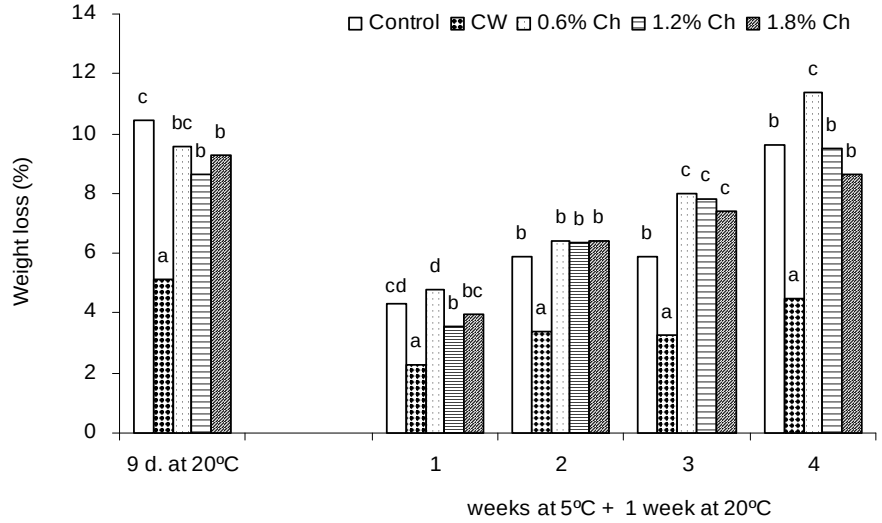

Figure 1. Weight loss (%) of coated and uncoated 'Oronules' mandarins during storage.
Means within each storage time with the same letter are not different ($p \leq 0.05$).
CW = commercial wax; Ch = chitosan coating

Fruit firmness

Firmness of uncoated mandarins after storage was lower than firmness at harvest, as can be seen by the higher percentage deformation of the fruit, whereas mandarins coated with the commercial wax maintained mandarin firmness during cold storage followed by 1 week storage at 20 ºC (Figure 2). After cold storage at 5 ºC followed by one week at 20 ºC, mandarins coated with 1.8% Ch were firmer (lower deformation) than uncoated mandarins.

However, after 9 days of storage at 20 ºC firmness of 1.8% Ch coated mandarins did not differ from control samples, and only 1.2% Ch showed a slight effect maintaining firmness.

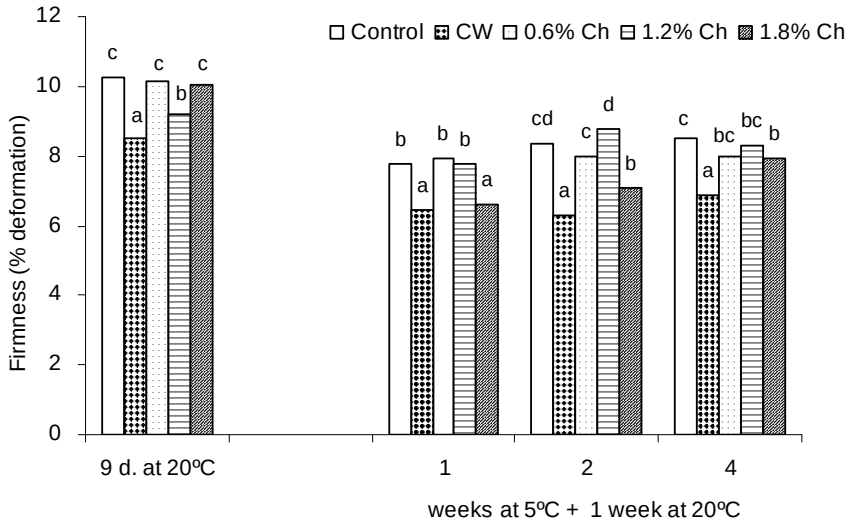

Figure 2. Firmness (% of deformation) of coated and uncoated 'Oronules' mandarins during storage.
Means within each storage time with the same letter are not different ($p \leq 0.05$).
CW = commercial wax; Ch = chitosan coating
Firmness at harvest was 6.7 % deformation

Rodov *et al*. (2000) reported that firmness of citrus fruit depends primarily on turgidity and weight loss rate. However, other authors have observed that not always there is a correlation between weight loss and firmness of citrus fruit (Hagenmaier, 2000; Perez-Gago *et al.* 2002; Navarro-Tarazaga *et al.* 2008). In this work, the lower weight loss observed on mandarins coated with the commercial wax correlates with a higher firmness of these mandarins. However, such a correlation between firmness and weight loss was not observed on mandarins coated with chitosan. Navarro-Tarazaga *et al.* (2008) concluded that for the coatings to affect fruit firmness significantly, they should induce a clear effect in fruit weight loss.

Apparently, factors such as coating type, storage condition, or fruit cultivar significantly influence the fruit firmness of coated samples.

The slight positive effect of the 1.8% Ch coating slowing down firmness loss of mandarins could be due to the biostimulating properties of chitosan, through an improvement of the rind of 'Oronules' mandarins. This cultivar, although very appreciated by its excellent flavor quality, shows a tendency to puffing after storage. Puffing is a physiological disorder characterized by the separation of the pulp from the rind that translates in a dramatic firmness loss (Burdon *et al.* 2007). Even though this defect did not become visible in this experiment, early stages of puffing could accelerate firmness loss of mandarins during storage. The beneficial effect of the high chitosan content (1.8% Ch) or commercial coating reducing firmness loss of 'Oronules' mandarins could help to slow down or minimize the appearance of puffing.

Internal gas composition

Figure 3 shows the internal CO_2 and O_2 content of coated and uncoated 'Oronules' mandarins. The application of the coatings modified the internal atmosphere in the fruit, increasing the CO_2 level and reducing the O_2 level compared to the control. Mandarins coated with 0.6% Ch presented the lowest internal CO_2 and the highest O_2 values among coated samples. As SC of the chitosan coating was increased, internal CO_2 level of mandarins increased and the O_2 level decreased. Many works have described a direct relation between the internal gas modification of coated fruit and coating thickness, which depends on SC, viscosity, and density of the coating formulation (Banks *et al.* 1993; Park *et al.* 1994; Cisneros-Zevallos and Krochta, 2003; Navarro-Tarazaga *et al.* 2006). In our case, as SC of the chitosan coating increased the internal CO_2 content increased by nearly a 30%, reaching values similar to mandarins coated with the commercial wax. Similarly, Salvador *et al*. (2003) also found no differences in the internal CO_2 content of 'Fortune' mandarins coated with a commercial wax and with a chitosan coating at 1.25% SC.

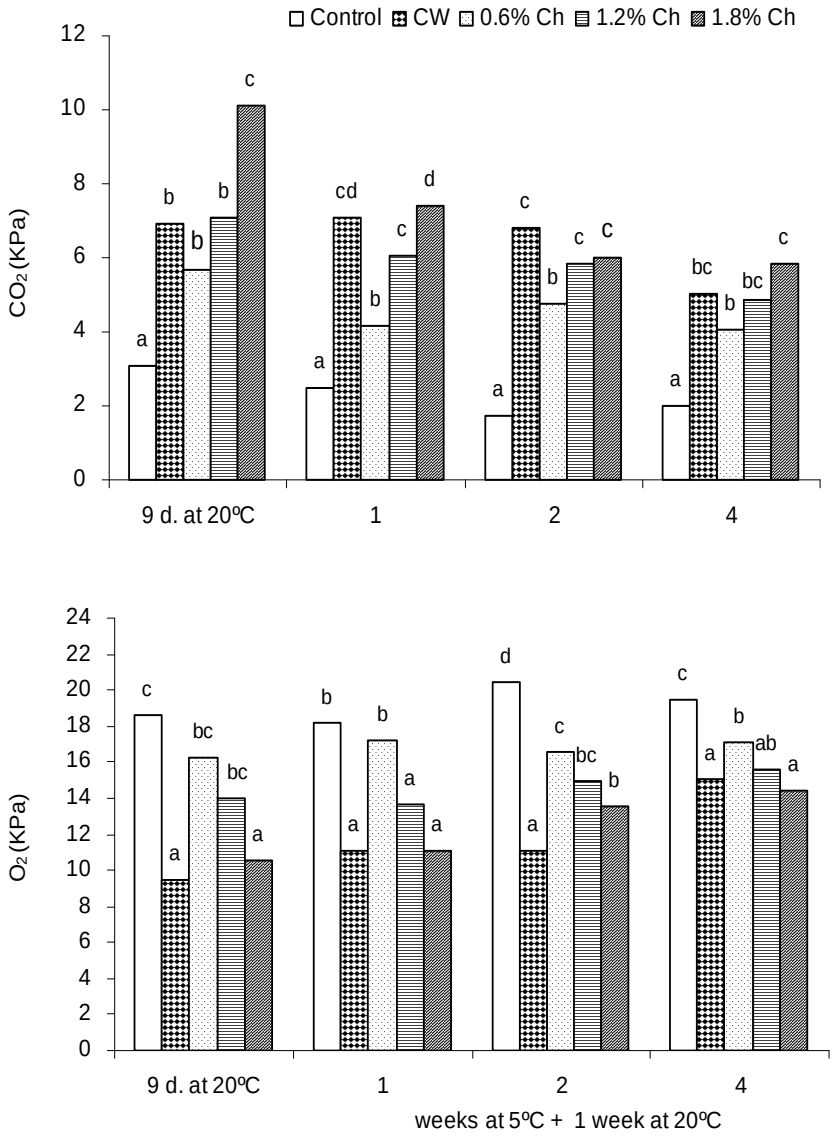

Figure 3. Internal CO_2 and O_2 contents of coated and uncoated 'Oronules' mandarins during storage.
Means within each storage time with the same letter are not different ($p \leq 0.05$).
CW = commercial wax; Ch = chitosan coating
At harvest, internal CO2 and O2 were 2.83 and 16.2 kPa, respectively

Among the different ingredients incorporated to coating formulations, shellac has been known to reduce gas exchange in a greater extend than waxes, creating in many cases an anaerobic/fermentative environment in the fruit (Baldwin *et al.* 1995; Hagenmaier, 2000; Hagenmaier and Baker, 1994). The commercial coating tested in this work consisted on a polyethylene-shellac mixture. At the end of the cold storage period the concentration of internal CO_2 and O_2 levels on mandarins coated with the commercial coating reached values around 6% and 15%, respectively. In general, these levels of internal O_2 could be considered not low enough to create anaerobic conditions inside the fruit (Baldwin *et al.* 1997).

Ethanol and acetaldehyde content

Fruit coating application induces an increase in the amount of internal volatiles associated with anaerobic conditions due to the gas barrier offered by coatings. In this work, mandarins coated with 0.6% Ch showed no differences on volatile content with uncoated mandarins. As SC of the chitosan coating increased, ethanol and acetaldehyde content of mandarins increased, which confirmed the creation of a modified atmosphere within the fruit. At the end of the storage period, the concentrations of ethanol and acetaldehyde of mandarins coated with 1.8% Ch and the commercial coating were the highest. These results correlate with changes in the internal gas composition (Figure 3).

In general, the concentration of ethanol in the juice of coated mandarins after 4 weeks of storage at 5 ºC followed by 1 week at 20 ºC was in the range of 100-700 mg L^{-1}. Different works have reported higher amount of ethanol content on coated citrus fruit after prolonged cold storage, with values that depended on citrus cultivar, coating type, and storage conditions (Baldwin *et al.* 1995; Hagenmaier and Baker, 1993; Hagenmaier, 2002; Hagenmaier and Shaw, 2002; Navarro-Tarazaga *et al*. 2008; Rojas-Argudo *et al.* 2009). The concentration of internal CO_2 and O_2 levels reached in this work could be considered not low enough to create anaerobic conditions inside the fruit, which translated in not very high volatile content (Baldwin *et al.* 1997).

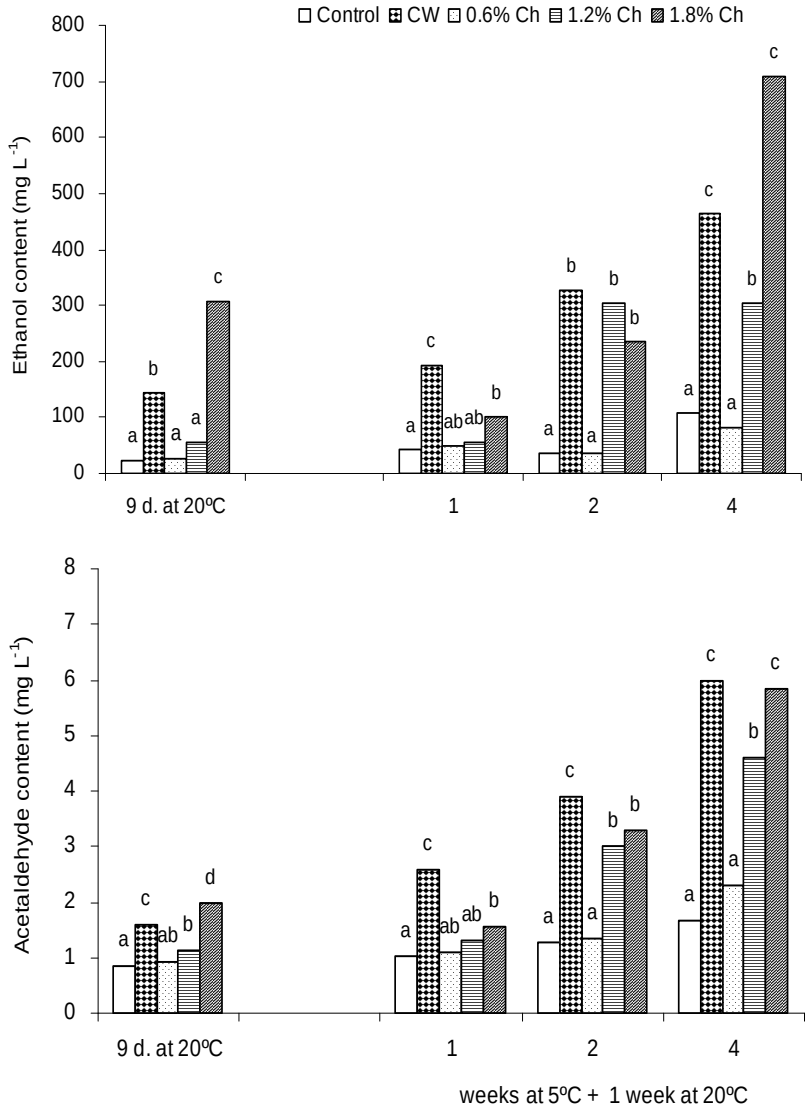

Figure 4. Acetaldehyde and ethanol contents of coated and uncoated 'Oronules' mandarins during storage.
Means within each storage time with the same letter are not different ($p \leq 0.05$).
CW = commercial wax; Ch = chitosan coating
At harvest, acetaldehyde and ethanol contents were 1.07 and 50.8 mg L^{-1}, respectively.

Sensory evaluation

Sensory quality of mandarins was evaluated within the range of acceptability after 4 weeks of storage at 5 ºC plus 1 week at 20 ºC, with values from 5.3 to 5.9 and no differences among treatments were found (data not shown). Several works showed that the contribution to off-flavor of volatile content depends on citrus cultivar. Ke and Kader (1990) established the minimum ethanol content associated with off-flavor in 'Valencia' oranges to be 2000 mg L^{-1}; whereas, Perez-Gago *et al*. (2002) found flavor degradation in 'Fortune' mandarin at an ethanol content above 3000 mg L^{-1} and Navarro-Tarazaga and Perez-Gago (2006) found that ethanol content of 1000 mg L^{-1} reduced flavor quality of 'Clemenules' mandarins. In our work, the highest ethanol content reached for 'Oronules' mandarins was 710 mg L^{-1} after 4 weeks of storage at 5 ºC (Figure 4), which was not enough to induce flavor degradation in this citrus cultivar.

Fruit quality

Coating application did not affect TA, SSC, or MI of 'Oronules' mandarins (data not shown). The effect of coating application on internal quality parameters has been shown to depend on coating type, fruit cultivar and storage conditions. Some authors have found no differences in these parameters after coating application on different citrus cultivars (Baldwin *et al.* 1995; Obenland *et al.* 2008); whereas others have found a decrease in SSC and TA losses compared to uncoated fruits, which was always related to a decrease in weight loss and respiration rate (Togrul and Arslan, 2004). Chien *et al*. (2007) observed that a low molecular weight chitosan coating beneficially influenced internal quality of 'Murcott' mandarins and coated fruit had higher acidity and lower SSC than uncoated fruit; whereas, a high molecular weight chitosan coating did not influence these parameters.

Bioactive compounds

Table 1 shows the content of the bioactive compounds analyzed of coated and uncoated 'Oronules' mandarins. The TAA of mandarins was not affected by coating application or the storage length. Togrul and Arslan (2004), however, reported that ascorbic acid loss after storage was delayed when mandarins citrus were coated with carboxymethyl cellulose. This result was explained by the gas barrier of the coatings which decreased the potential autoxidation of ascorbic acid in the presence of oxygen. In our

work, although commercial and chitosan coatings reduced the level of internal O_2 (Figure 3), these levels could be not low enough to affect the TAA of the mandarin.

Hesperidin was the more abundant flavanoid in mandarins 'Oronules' followed by narirutin and didymin (Table 1). The contents of the different flavonoids were not affected by storage length. In general coating application had not an important effect on the level of the different flavonoids, although some significant differences were found among treatments. Uncoated mandarins stored at 20 ºC for 9 days contained higher content of hesperidine, narirutin and didymin than coated mandarins, which could be related to the higher weight loss of uncoated mandarins under this storage condition.

In addition to flavanones, citrus fruit also contain other phenolic compounds, such as flavones and hydroxycinnamic acids (represented by ferulic, caffeic, synapic, and *p*-coumaric acids) that, although present in a lower concentration, contribute to the total phenolic concentration (Rapisarda *et al.* 1999; Gil-Izquierdo *et al.* 2002). Several authors have reported that chitosan act as an exogenous elicitor in plant tissue inducing different responses, such as the de novo biosynthesis of phenolic compounds (Benhamou *et al.* 1994; Lafontaine and Benhamou, 1996; Bautista-Baños *et al.* 2006, Meng *et al.* 2008). In this work, some significant differences were found among treatments depending on storage time, which makes difficult to withdraw any conclusion regarding the effect of coating composition (Table 1). Interestingly, at the end of the storage period studied, total phenolic content increased as SC of the chitosan coating increased, which could be an indication of the chitosan activity as an exogenous elicitor of plant tissue; however, this result should be corroborated for other storage periods.

Table 1. Total phenolics, flavonoids, ascorbic acid contents and antioxidant activity of coated and uncoated 'Oronules' mandarins after storage

		TAA (mg L^{-1})	Hesperidin (mg L-1)	Narirutin (mg L-1)	Dydimin (mg L^{-1})	Total phenolics (mg L^{-1})	EC_{50} (L juice/Kg DPPH)
At harvest		583.6 ± 24.7	220.1 ± 14.6	16.6 ± 1.8	3.8 ± 0.1	640.4 ± 17.6	187.83 ± 11.32
	Control	626.1 ± 31.2 a	255.7 ± 6.9 b	20.2 ± 1.0 c	4.5 ± 0.3 b	655.0 ± 12.2 a	187.96 ± 5.32 a
	CW	602.0 ± 21.7 a	193.5 ± 14.2 a	16.9 ± 1.7 b	3.4 ± 0.3 a	662.6 ± 24.0 a	179.98 ± 12.14 a
9 d 20C	0.6% Ch	580.3 ± 55.9 a	193.3 ± 12.2 a	16.1 ± 1.0 ab	3.3 ± 0.2 a	647.3 ± 31.8 a	193.91 ± 6.51 a
	1.2% Ch	598.6 ± 12.3 a	201.2 ± 2.6 a	14.4 ± 1.8 a	3.1 ± 0.1 a	655.2 ± 10.5 a	179.17 ± 6.78 a
	1.8% Ch	597.7 ± 4.3 a	188.5 ± 15.9 a	14.0 ± 0.6 a	3.4 ± 0.3 a	630.1 ± 22.0 a	179.37 ± 2.76 a
1 wk 5ºC+	Control	561.9 ± 35.3 a	204.6 ± 7.4 a	16.4 ± 1.9 a	4.0 ± 0.2 c	653.5 ± 0.8 b	-
+1 wk 20ºC	CW	528.8 ± 52.6 a	214.9 ± 19.4 a	16.7 ± 1.2 a	3.7 ± 0.2 bc	626.7 ± 8.0 ab	
	0.6% Ch	586.2 ± 52.2 a	211.2 ± 12.1 a	13.5 ± 1.7 a	3.4 ± 0.1 ab	652.7 ± 38.5 b	
	1.2% Ch	602.6 ± 55.3 a	202.5 ± 16.9 a	13.9 ± 2.7 a	3.3 ± 0.2 a	656.7 ± 38.1 b	
	1.8% Ch	527.1 ± 36.6 a	206.6 ± 5.7 a	16.2 ± 1.8 a	3.4 ± 0.1 ab	586.5 ± 1.1 a	
2 wk 5ºC+	Control	622.3 ± 45.9 a	179.6 ± 11.2 a	12.2 ± 1.5 a	3.1 ± 0.3 a	659.4 ± 29.1 a	-
+1 wk 20ºC	CW	623.4 ± 12.1 a	206.1 ± 6.8 b	14.4 ± 0.6 a	3.4 ± 0.1 a	669.2 ± 45.2 a	
	0.6% Ch	585.5 ± 11.3 a	174.6 ± 10.9 a	12.8 ± 1.3 a	3.1 ± 0.2 a	641.6 ± 23.1 a	
	1.2% Ch	629.2 ± 88.0 a	188.4 ± 10.0 a	12.6 ± 1.2 a	3.2 ± 0.4 a	674.3 ± 13.5 a	
	1.8% Ch	555.8 ± 43.9 a	189.9 ± 4.2 ab	13.0 ± 0.4 a	3.2 ± 0.1 a	686.2 ± 15.5 a	
3 wk 5ºC+	Control	566.9 ± 71.3 a	205.8 ± 8.0 a	14.8 ± 1.0 b	3.6 ± 0.1 b	643.0 ± 22.9 a	185.37 ± 3.16 b
+1 wk 20ºC	CW	543.3 ± 15.1 a	203.4 ± 9.1 a	13.5 ± 0.4 ab	3.1 ± 0.1 a	613.6 ± 11.6 a	189.79 ± 1.89 bc
	0.6% Ch	572.5 ± 53.7 a	193.3 ± 9.4 a	12.3 ± 0.8 a	3.0 ± 0.1 a	615.9 ± 26.5 a	159.33 ± 7.89 a
	1.2% Ch	565.8 ± 6.4 a	214.4 ± 3.7 a	14.7 ± 1.0 b	3.3 ± 0.0 a	616.7 ± 35.4 a	155.15 ± 6.38 a
	1.8% Ch	548.2 ± 37.4 a	225.2 ± 22.3 a	15.3 ± 1.5 b	3.2 ± 0.2 a	630.1 ± 30.3 a	196.39 ± 6.79 c
4 wk 5ºC+	Control	649.9 ± 67.8 a	221.3 ± 2.1 a	16.7 ± 1.2 a	3.3 ± 0.1 a	654.5 ± 6.4 b	158.12 ± 15.60 a
+1 wk 20ºC	CW	593.8 ± 19.4 a	236.5 ± 26.2 a	18.3 ± 2.3 a	3.1 ± 0.3 a	682.8 ± 9.2 bc	178.92 ± 3.29 b
	0.6% Ch	580.6 ± 61.7 a	203.3 ± 11.0 a	13.9 ± 1.2 a	2.8 ± 0.2 a	584.3 ± 27.8 a	218.72 ± 7.84 c
	1.2% Ch	529.7 ± 15.1 a	214.2 ± 21.3 a	14.6 ± 2.1 a	2.9 ± 0.2 a	653.2 ± 23.2 b	206.18 ± 4.63 c
	1.8% Ch	643.6 ± 63.0 a	225.7 ± 15.4 a	15.2 ± 2.3 a	3.0 ± 0.4 a	719.2 ± 41.3 c	180.20 ± 8.94 b

Means within each storage time with the same letter are not different ($p \leq 0.05$).
TAA = total ascorbic acid; CW = commercial wax; Ch = chitosan coating.

The antioxidant capacity was expressed as EC_{50} or juice quantity necessary to reduce by 50% the $DPPH^{\bullet}$, thus the lower the value the higher the antioxidant capacity of the citrus fruit. Table 1 shows the antioxidant capacity of the mandarins stored 9 days at 20 ºC and stored 3 or 4 wk at 5 ºC plus 1 week at 20 ºC. Coating application did not affect the total antioxidant capacity of samples stored 9 days at 20 ºC. After cold storage, some significant differences were found among treatments depending on storage time, which makes difficult to withdraw any conclusion regarding the effect of coating composition. After 4 weeks of cold storage plus 1 week at 20 ºC, mandarins coated with 0.6% Ch and 1.2% Ch presented the lowest antioxidant capacity (i.e. highest EC_{50}). These results could be related to the lowest total phenolic content of 0.6% Ch-coated mandarins ($p \leq 0.05$) or slightly lower TAA content of 1.2% Ch-coated mandarins ($p=0.08$). Previous works have found correlations between vitamin C and antioxidant capacity (Pretel *et al.* 2006) or phenolic content and antioxidant capacity of citrus (Rapisarda *et al.* 1999).

CONCLUSION

Commercial wax decreased weight and firmness loss of mandarins compared to uncoated samples, whereas chitosan coatings did not decrease effectively weight loss of mandarins during storage. Therefore, in order to improve the water barrier properties of the chitosan coating it would be necessary to add hydrophobic components to the formulation. Although the coatings applied restricted gas exchange and modified internal atmosphere of the fruits compared to uncoated mandarins, with a greater effect at higher chitosan concentration, sensory quality was not affected. Chitosan-coated mandarins at the highest SC retained firmness after cold storage. In general, the internal quality and the health-related properties of mandarins were not affected by coating application.

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CAPITULO IV

Efecto del quitosano aplicado como recubrimiento comestible en la calidad fisicoquímica, sensorial y nutricional de naranjas cv. 'Valencia'

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RESUMEN

El objetivo de este trabajo fue evaluar el efecto del quitosano aplicado como recubrimiento comestible a distinto contenido en sólidos (CS) (0,6, 1,2 o 1,8%) sobre la fisiología y calidad de naranjas 'Valencia'. Un grupo de naranjas se recubrió con una cera comercial (CC) de polietileno/goma laca al 10% de CS y otro grupo de naranjas se mantuvo como control, sin recubrir. Las naranjas se almacenaron a 5 ºC hasta 16 semanas seguido de 1 semana a 20 ºC, simulando un periodo de comercialización. Durante la frigoconservación se evaluó su calidad fisicoquímica, sensorial y nutricional. La aplicación de la CC disminuyó la pérdida de peso a partir de periodos de frigoconservación superiores a 5 semanas. La formulación de quitosano al 0,6% de CS mostró cierta eficacia reduciendo la pérdida de peso respecto de las naranjas sin recubrir, aunque fue menos eficaz que la CC; sin embargo, esta formulación modificó menos la atmósfera interna que la CC manteniendo los niveles de CO_2 y O_2 internos próximos a los de las naranjas sin recubrir. Las formulaciones de quitosano al 1,2 o 1,8% de CS modificaron la atmósfera interna de las naranjas alcanzando niveles de CO_2 y O_2 similares a los de las naranjas recubiertas con la CC. No obstante, la evaluación sensorial de las frutas no mostró un deterioro en el sabor de las naranjas recubiertas con los recubrimientos más restrictivos al intercambio gaseoso. En general, el contenido en vitamina C y la capacidad no se alteró debido a la aplicación de los recubrimientos o al almacenamiento. El contenido de los flavonoides mayoritarios analizados aumentó durante el almacenamiento sin detectar un efecto importante como consecuencia de la aplicación de los recubrimientos.

Palabras clave: postcosecha, cítricos, almacenamiento, calidad fisicoquímica, calidad nutricional, calidad sensorial

INTRODUCCIÓN

El quitosano (polímero de β-1,4-glucosamina) es un componente de la pared celular de los crustáceos, capaz de formar películas semipermeables a gases, que ha recibido un gran interés en los últimos años por su potencial como recubrimiento comestible. Su aplicación como recubrimiento ha proporcionado buenos resultados en cuanto a reducción de pérdida de peso y mejora de la calidad en diferentes frutas y hortalizas. En concreto, su

aplicación en cítricos ha mostrado resultados positivos sobre parámetros como pérdida de peso y firmeza (Salvador et al., 2003; Galed et al., 2004; Chien et al., 2007). Asimismo, existen estudios previos que han puesto de manifiesto el efecto antifúngico del quitosano y derivados en otros frutos, como fresa, mango y melocotón (Li y Yu, 2001; Srinivasa et al., 2002; Bautista-Baños et al., 2006; Vargas et al., 2006).

En la actualidad, existe un interés creciente en el estudio del efecto de los tratamientos poscosecha sobre los componentes funcionales de frutas y hortalizas (Cano et al., 2003). A los cítricos se les atribuye múltiples propiedades beneficiosas, asociadas sobre todo a su alto contenido en vitamina C y otros compuestos funcionales como flavonoides. El efecto de los recubrimientos de quitosano en la calidad fisicoquímica de distintas frutas y hortalizas se ha visto que depende, entre otros factores, del peso molecular y del grado de desacetilación (González-Aguilar et al., 2005; Bautista-Baños et al., 2006). Además, en general, la barrera a la humedad y a los gases que ejercen los recubrimientos comestibles depende del contenido en sólidos (CS) y de la viscosidad de las formulaciones (Cisneros-Zevallos y Krochta, 2003).

Dado que existen distintos factores que pueden modificar la calidad de un fruto recubierto, es necesario ampliar las investigaciones para establecer el efecto global que tiene el quitosano sobre la calidad de los cítricos, sobre todo en lo relacionado con parámetros de calidad que no han sido tradicionalmente objeto de estudio, como son las propiedades sensoriales y las nutricionales. En este sentido, el objetivo fundamental de este trabajo es determinar el efecto de un quitosano comercial aplicado a distinto CS sobre la fisiología, calidad nutricional y sensorial de naranjas ‘Valencia’.

MATERIAL Y MÉTODOS

Material vegetal

El material vegetal utilizado fue naranjas ‘Valencia’ recolectadas con madurez comercial. Tras una selección de frutos sanos, se formaron 5 grupos homogéneos de 80 frutos para cada uno de los tratamientos.

Preparación y aplicación del recubrimiento

Los recubrimientos se prepararon a partir de quitosano comercial (peso molecular intermedio) al 1,88% de CS en ácido acético (Biorend®, Idebio, S.L., Salamanca). Para facilitar la adhesión del recubrimiento se añadió 0,1% de Tween 80. Se prepararon tres formulaciones de quitosano al 0,6, 1,2 y 1,88% de CS (tratamientos 0,6% Q, 1,2% Q y 1,8% Q). La aplicación de cada formulación de quitosano se realizó por inmersión durante 15 seg. En otro lote de frutas se aplicó una cera comercial (CC) de polietileno al 10% de CS. Un grupo de frutos sin recubrir constituyó el tratamiento control. Todos los tratamientos fueron almacenados a 5 ºC durante 3, 5, 7, 9 y 16 semanas, seguido de 1 semana a 20 ºC simulando comercialización directa. Al término de cada periodo de almacenamiento se realizaron los correspondientes ensayos fisicoquímicos, sensoriales y nutricionales.

Calidad fisicoquímica

La pérdida de peso se realizó en 30 frutos por tratamiento. El resultado se expresó como porcentaje de pérdida de peso respecto al peso inicial.

La firmeza se determinó mediante un ensayo de compresión en el que se aplicó una fuerza de 1 Kg sobre la zona ecuatorial del fruto. La medida se realizó en 20 frutos por tratamiento con un texturómetro Instron Universal Machine (Modelo 3343; Instron Corp., Canton, MA, EE.UU.) utilizando una placa de 35 mm de diámetro y una velocidad de compresión de 5 mm/min. El resultado se expresó como porcentaje de deformación relativo al diámetro inicial del fruto.

El contenido de CO_2 y O_2 interno se determinó por cromatografía gaseosa, analizándose 10 frutos por tratamiento. Para ello se tomaron muestras de 1 mL extraído de la cavidad interna de las naranjas con una jeringuilla Hamilton 1001. La toma de muestras se hizo bajo agua para evitar la contaminación de la misma con aire externo. Dicha muestra fue posteriormente inyectada en un cromatógrafo de gases (Thermo mod. Trace, Thermo Fisher Inc., Waltham, MA, EE.UU.) equipado con un detector de TCD y columnas Poropak QS 80/100 (1,2 m x 0,32 cm) y tamiz molecular, 5 Å 45/60 (1,2 m x 0,32 cm). Las temperaturas del cromatógrafo fueron 125, 35 y 180 ºC para inyector, horno y detector, respectivamente y el caudal del gas portador (He) fue 22 mL/min. El resultado se expresó en porcentaje de CO_2 y O_2 interno.

Las concentraciones de etanol y acetaldehído en zumo, se determinaron por cromatografía gaseosa de espacio de cabeza. Se separaron 3 lotes de 10 frutos por tratamiento de los que se obtuvieron 3 zumos. Muestras de 5 mL de zumo se colocaron en viales de 10 mL, debidamente sellados con tapón de TFE/silicona, y se almacenaron a -18 ºC hasta su análisis. Previo análisis, las muestras se descongelaron a 20 ºC y los viales se incubaron a 30 ºC durante 12 minutos. Tras la incubación se inyectó 1 mL de gas del espacio de cabeza del vial en un cromatógrafo de gases (Mod. Trace; Thermo Fisher Scientific, Inc., Waltham, MA, EE.UU.) equipado con muestreador automático (Modelo HS 2000), detector de ionización de llama (FID) y columna Poropak QS 80/100 (1,2 m x 0,32 cm). Las temperaturas de trabajo del cromatógrafo fueron 175, 150 y 200 ºC para inyector, horno y detector respectivamente y el caudal de gas portador (He) fue 28 mL/min. Los análisis se realizaron por triplicado. El etanol y el acetaldehído de las muestras se identificó por comparación de tiempos de retención con soluciones patrón. Los resultados se expresaron en mg de etanol y acetaldehído por 100 mL de zumo.

Para las determinaciones de sólidos solubles totales (SST) y acidez total (AT), se separaron 3 lotes de 10 frutos por tratamiento de los que se obtuvieron 3 zumos. El contenido en SST se midió con un refractómetro digital (Atago, Modelo PR1), siendo expresados en ºBrix. Para la determinación de la AT, 5 mL de cada zumo se valoraron con NaOH 0,1 N hasta pH 8,1. La AT se expresó en g de ácido cítrico en 100 mL de zumo. El índice de madurez (IM) se calculó como el cociente SST/AT.

Calidad sensorial

La evaluación organoléptica de los frutos la realizó un panel entrenado de 10-12 jueces en una sala de análisis sensorial que cumple la norma UNE 87004 (AENOR, 1997). Para la preparación de las muestras se tomaron de cada tratamiento 10 frutos al azar que se pelaron y dividieron en gajos. Se escogieron 2 gajos procedentes de cada tratamiento y se presentaron a los jueces en recipientes desechables identificados con códigos de tres dígitos al azar. Los jueces se enjuagaron la boca con agua mineral antes y después de evaluar cada muestra. Para la evaluación de la calidad olfato-gustativa se utilizó una escala del 1 a 9, donde se agruparon los valores en tres grados de calidad (1-3 = calidad no aceptable, 4-6 = calidad aceptable y 7-9 = calidad

excelente). La evaluación de los malos sabores se realizó mediante una escala de 0 a 5, donde 0 es ausencia de malos sabores y 5 presencia acusada.

Calidad nutricional

La capacidad antioxidante total (EC_{50}) se evaluó mediante el método de captura de radicales libres del 2,2-difenil-1-picrilhidracilo ($DPPH^{\bullet}$) descrito por Brand-Williams et al. (1995). El método mide la reducción de la absorbancia a 515 nm de soluciones de $DPPH^{\bullet}$ al reaccionar con antioxidantes. Para el análisis se tomaron 2 mL de zumo y 4 mL de metanol grado HPLC (Merck, Alemania) y se centrifugó a 12000 rpm durante 15 min a 5 ºC. A partir del sobrenadante obtenido se realizaron diluciones del mismo con metanol con el fin de poder relacionar la disminución en la absorbancia del $DPPH^{\bullet}$ con la concentración de la muestra. Para ello, se mezcló en cubetas desechables 0,075 mL de cada una de las diluciones metanólicas de las muestras con 2,925 mL de una solución metanólica de $DPPH^{\bullet}$ (24 ppm) (Sigma-Aldrich, Alemania) y se dejó reaccionar durante 40 min en oscuridad a 23,5 ºC ± 1 ºC. Finalmente, se midió la absorbancia a 515 nm con un espectrofotómetro (Thermo Electron Corporation, UK). La capacidad antioxidante se expresó como EC_{50}, que es la cantidad de muestra necesaria para reducir al 50% los radicales libres de 1 Kg de $DPPH^{\bullet}$ (l zumo/Kg de $DPPH^{\bullet}$) (Sánchez-Moreno et al., 2003).

Los análisis y cuantificación del ácido ascórbico total (AAT) y glucósidos de flavanonas mayoritarios (narirutina, hesperidina y didimina) se realizaron mediante cromatografía líquida de alta resolución (HPLC) (Modelo Alliance 2996; Waters, USA) equipado con un modulo de separación (Modelo 2695) y un detector de fotodiodos (Modelo 2996). Se utilizó una columna de fase inversa C18 Tracer Excel 5 μm (250 mm x 4 mm) (Teknokroma, Barcelona, España), precedida de una precolumna (4 x 4 mm) con diámetro de partícula 5 μm (Merck, Alemania), alojadas en el horno de columnas a 25 ºC.

El AAT se determinó como la suma del ácido ascórbico más el ácido dehidroascórbico, mediante reducción de 1 mL de zumo con 200 μL de 1,4-dithio-DL-threitol (200 mg/mL) (Fluka, Barcelona). Las muestras se filtraron a través de un filtro de 0,45 μm y se analizaron por HPLC. Un volumen de 20 μL se inyectó en el equipo, utilizando una fase móvil en condiciones isocráticas de metanol: 0,6% ácido acético (95:5) a un flujo de 1 mL/min. El pico de ácido ascórbico se detectó a una longitud de onda de

253 nm y la cuantificación del AAT se realizó a partir de concentraciones conocidas de ácido ascórbico (Sigma Aldrich, Barcelona).

Para el análisis de los glucósidos de flavanonas, 1 mL de zumo se homogeneizó con 5 mL de dimetil sulfóxido:metanol (1:1) (Scharlau, Sentmenat, España) y se centrífugo a 12000 rpm durante 15 min a 4 ºC. Las muestras se filtraron a través de un filtro de 0,45 μm y se analizaron por HPLC. El volumen de inyección fue 10 μL. La fase móvil utilizada fue acetonitrilo (A):0.6% de ácido acético (B) con la condición inicial de un 10% de A durante 2 min, alcanzando un 50% de A en los siguientes 18 min, se mantuvieron estas condiciones durante 5 min y posteriormente se regresó a la condición inicial en 5 min. El flujo se mantuvo a 1 mL/min. Los glucósidos de flavanonas se identificaron y cuantificaron a partir de estándares comerciales de narirutina (Extrasynthese, Genay, France), hesperidina (Sigma Co., Barcelona, Spain) y didimina (ChromaDex (Irvine, CA, USA). La cuantificación se realizó a 280 nm.

El análisis de los compuestos fenólicos totales se realizó por el método colorimétrico que utiliza el reactivo Folin-Ciocalteu (Singlenton and Rossi, 1965). Para ello, se homogenizaron 0,3 mL de zumo de naranja con 1,7 mL de metanol acuoso al 80%. A continuación, 0,4 mL de la dilución metanólica se mezclaron con 2 mL de Folin-Ciocalteu (previamente diluido con agua 1:10 v/v) y se dejó reposar 1 min. Por último, se añadió 1,6 mL de Na_2CO_3 al 7,5%. La mezcla se dejó reposar durante 1 hora a temperatura ambiente y se midió la absorbancia en un espectrofotómetro (Thermo UV1, Thermo Electron Corporation, UK) a 765 nm. Los resultados se expresaron en base a una recta patrón de ácido gálico (mg/100 mL de zumo) (Sigma-Aldrich Chemie, Alemania).

Las determinaciones de compuestos bioactivos se realizaron por triplicado en 3 zumos obtenidos de 10 frutos por tratamiento cada uno que se mantuvieron a -80 ºC hasta su análisis.

Análisis estadístico

El análisis estadístico se realizó mediante análisis de la varianza (ANOVA) utilizando el paquete estadístico Statgraphics plus 4.1. Las diferencias significativas entre las medias se establecieron a través de intervalos MDS (Mínima diferencia significativa) con un nivel de confianza del 95%.

RESULTADOS Y DISCUSIÓN

Calidad fisicoquímica

La pérdida de agua es una de las principales causas de deterioro, no sólo en los cítricos sino en la mayoría de los productos hortofrutícolas. La figura 1 muestra la pérdida de peso de las naranjas 'Valencia' recubiertas con quitosano a diferente CS, la CC y el control. Al aumentar el tiempo de almacenamiento aumentó la pérdida de peso de las naranjas 'Valencia' hasta valores entorno al 6%. Tras 3 semanas de almacenamiento en frío más el período de comercialización (1 semana a 20 ºC), ni los recubrimientos de quitosano, ni la CC fueron efectivos controlando la pérdida de peso de la fruta respecto al control. A partir de 5 semanas de almacenamiento en frío, la CC fue efectiva controlando la pérdida de peso de las naranjas, mientras que entre las formulaciones de quitosano únicamente fue efectiva la formulación 0,6% Q después de 5 y 7 semanas de almacenamiento a 5 ºC más 1 semana a 20 ºC. Contreras et al. (2010) también observaron que la aplicación de quitosano como recubrimiento a diferentes CS no fue efectivo reduciendo la perdida de peso de mandarinas 'Oronules' durante frigoconservación.

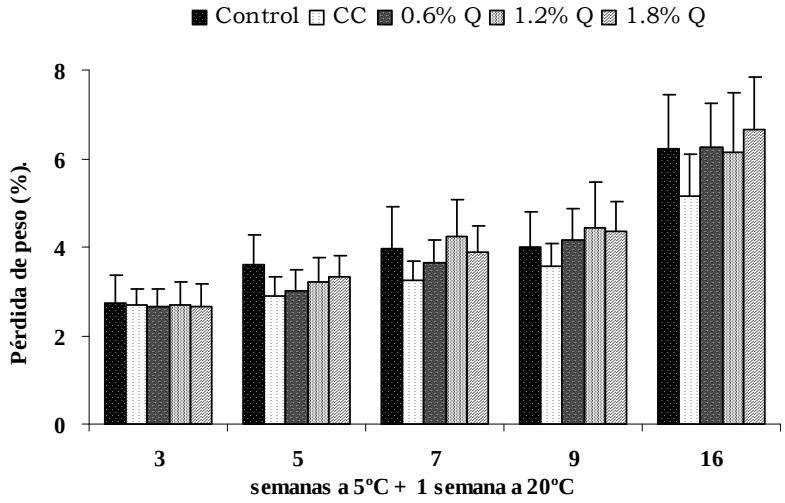

Fig. 1. Pérdida de peso (%) de naranjas 'Valencia' recubiertas y control. Las barras indican desviación estándar (n=30).

En general, los recubrimientos comestibles formulados a base de polisacáridos presentan una barrera a la humedad muy baja como consecuencia de su naturaleza hidrofílica. Por este motivo, los recubrimientos destinados a frutas incorporan lípidos, dando lugar a recubrimientos comestibles compuestos capaces de controlar la pérdida de peso de la fruta. En algunos trabajos, sin embargo, se ha visto que recubrimientos con quitosano, sin incorporar lípidos, redujeron la perdida de peso de frutos cítricos (Galed et al., 2004; Chien et al., 2007). Nuestros resultados, junto con los resultados observados por otros autores, podrían indicar que la efectividad de estos recubrimientos controlando la pérdida de peso de naranjas 'Valencia' depende del contenido en sólidos, y/o de la naturaleza del quitosano aplicado. Algunos trabajos indican que las propiedades del quitosano dependen del peso molecular, del grado de desacetilación y de la cristanilidad (Rege y Block, 1999). Blair et al. (1987) probaron que la permeabilidad al vapor de agua de películas de quitosano se reduce significativamente cuando disminuye el grado de desacetilación. Santos et al. (2006) confirmaron este resultado, indicando además que el peso molecular tenía el mismo efecto.

La figura 2 muestra los valores de textura expresados como % de deformación de las muestras recubiertas y control. Tanto los recubrimientos de quitosano como la CC ofrecieron un efecto limitado reduciendo la pérdida de firmeza de las naranjas 'Valencia' durante el almacenamiento. La aplicación de la CC tan solo resultó efectiva controlando la pérdida de firmeza en fruta almacenada 9 semanas a 5 ºC más 1 semana a 20 ºC, mientras que la aplicación de los recubrimientos de quitosano no redujo la pérdida de firmeza de las naranjas 'Valencia' respecto al control, observándose una mayor deformación de los frutos recubiertos con 1,2% Q al finalizar el almacenamiento (16 semanas a 5 ºC mas 1 semana a 20 ºC). Rodov et al. (2000) reportaron que la firmeza de los frutos cítricos depende fundamentalmente de la turgencia y de la pérdida de peso. Otros trabajos, sin embargo, no encontraron relación entre ambos parámetros (Hagenmaier, 2000; Pérez-Gago et al., 2002; Navarro-Tarazaga et al., 2008). Navarro-Tarazaga et al. (2008) concluyeron que para afectar significativamente la firmeza del fruto, los recubrimientos deben inducir un claro efecto en la pérdida de peso de los frutos. En este trabajo, tan sólo la CC resultó efectiva controlando la pérdida de peso de las naranjas 'Valencia' durante todo el periodo de almacenamiento, aunque no resultó efectiva reduciendo la firmeza del fruto recubierto. Aparentemente, factores como el tipo de

recubrimiento, condiciones de almacenamiento, o el cultivar influyen de manera significativa en la firmeza de las frutas recubiertas.

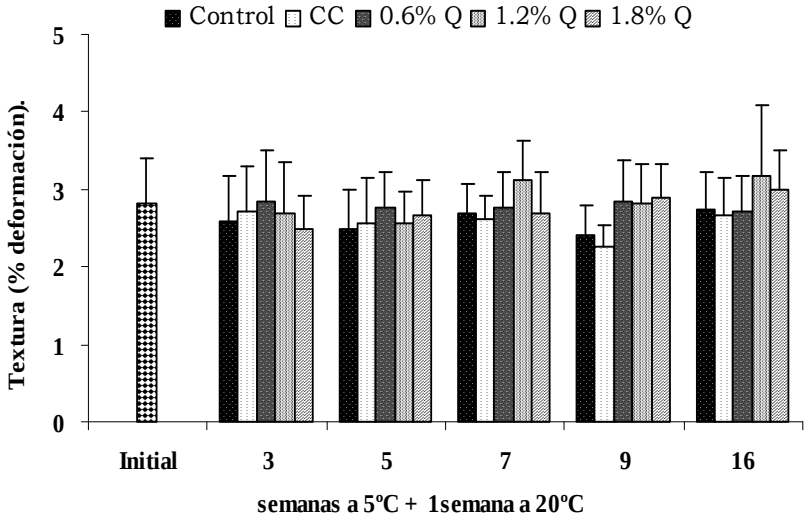

Fig. 2. Firmeza (% de deformación) de naranjas 'Valencia' recubiertas y control. Las barras indican desviación estándar (n=20)

Los recubrimientos aplicados en la superficie de los frutos aportaron una barrera adicional al intercambio de los gases CO_2 y O_2 a través de la cutícula. La figura 3 muestra el contenido en CO_2 y O_2 interno de las naranjas 'Valencia' recubiertas y control. La aplicación de los recubrimientos modificó la atmósfera en el fruto, aumentando los niveles de CO_2 y disminuyendo los niveles de O_2. Al aumentar el CS del quitosano se observó un aumento en el nivel de CO_2 interno de las naranjas y una reducción en el nivel de O_2 interno. Previamente a este estudio, se observó un comportamiento similar en mandarinas 'Oronules' durante su frigoconservación (Contreras et al., 2010). El aumento en el CS de la formulación pudo aumentar el grosor del recubrimiento (Cisneros-Zevallos y Krochta, 2003) y, por tanto, la distancia que los gases CO_2 y O_2 tienen que recorrer para su difusión, lo que aumentó la barrera de los recubrimientos a ambos gases. La fruta tratada con 0,6% Q dio valores de CO_2 muy bajos, que en algunos periodos de almacenamiento no fueron significativamente

diferentes del control. Salvador et al. (2003) en mandarinas 'Fortune' no encontraron diferencias significativas en el contenido de CO_2 interno entre la fruta recubierta con una CC y la tratada con quitosano con un CS de 1,2%. En nuestro trabajo, al aumentar el CS a un 1,8% se alcanzaron valores de O_2 y CO_2 similares a las naranjas recubiertas con la CC.

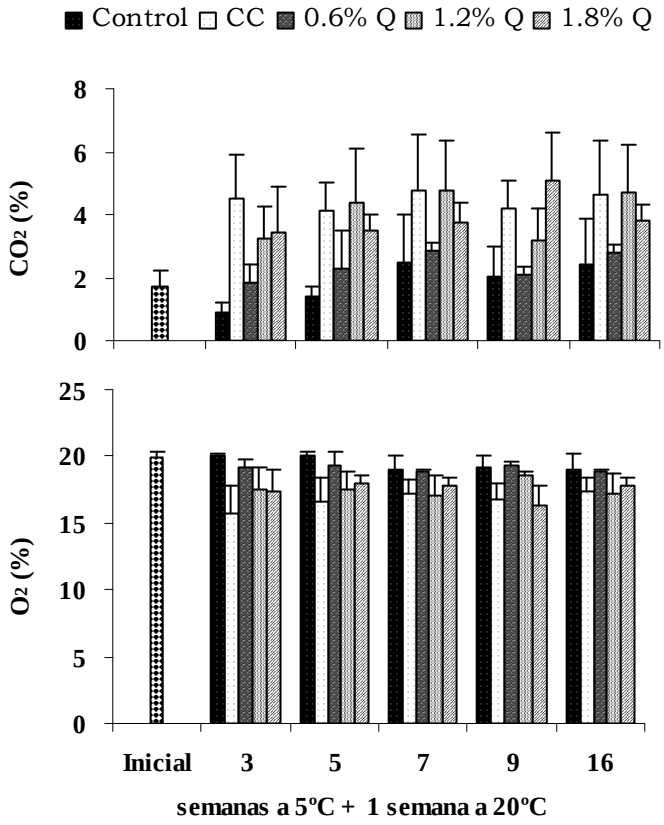

Fig. 3. CO_2 y O_2 interno de naranjas 'Valencia' recubiertas y control. Las barras indican desviación estándar (n=3)

La aplicación de recubrimientos comestibles a frutas induce un aumento en el contenido de compuestos volátiles como consecuencia de la barrera a los gases que los mismos ofrecen. En este trabajo se observó un incremento del contenido en etanol y acetaldehído al aumentar el tiempo de almacenamiento (Figura 4). En general, también se observó un aumento del

contenido en estos compuestos volátiles al aumentar el CS de los recubrimientos de quitosano, siendo el recubrimiento de 1,8% Q y la CC los que dieron lugar a los niveles más altos de etanol al final del periodo de frigoconservación. Estos resultados se correlacionan con los cambios en el O_2 y CO_2 interno de las naranjas recubiertas (Figura 3). Contreras et al. (2010) también observaron que los niveles de los compuestos volatiles en mandarinas'Oronules' aumentaron al incrementar el CS del quitosano, encontrando los niveles más altos en mandarinas recubiertas con 1,8% Q y la CC al finalizar el almacenamiento (4 semanas a 5 ºC mas una semana a 20 ºC).

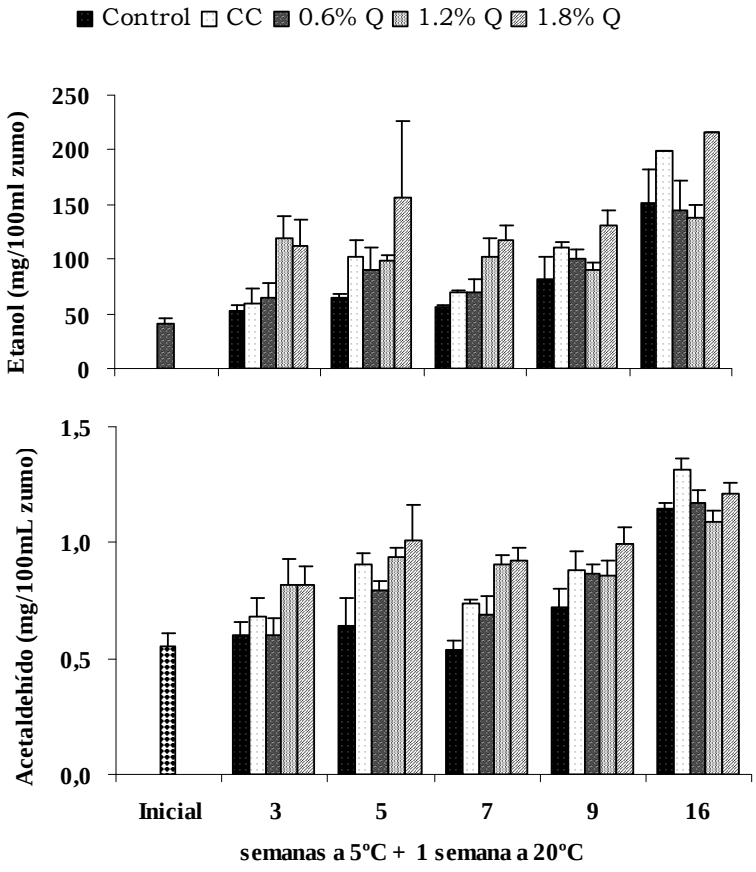

Fig. 4. Etanol y acetaldehído interno de naranjas 'Valencia' recubiertas y control. Las barras indican desviación estándar (n=3)

Distintos trabajos muestran un aumento en los niveles de etanol en los cítricos recubiertos después de un almacenamiento prolongado en frío, con valores que dependen del cultivar, tipo de recubrimiento, y las condiciones de almacenamiento (Baldwin et al., 1995; Hagenmaier y Baker, 1993; Hagenmaier y Shaw, 2002; Navarro-Tarazaga et al., 2008; Rojas-Argudo et al., 2009). En este trabajo al finalizar el almacenamiento, la concentración de etanol en el zumo de las naranjas varió entre 140-215 mg/100 mL de zumo. Siendo la CC y el recubrimiento de 1,2% Q los que dieron lugar a los niveles más altos. Sin embargo, los niveles alcanzados se pueden considerar bajos indicando que la restricción en el intercambio gaseoso provocada por los recubrimientos no fue lo suficientemente alta como para crear condiciones anaeróbicas dentro del fruto (Baldwin et al. 1997).

Durante el almacenamiento se observó una disminución de la AT y un aumento del IM. Sin embargo, no se observó un efecto de la aplicación de los recubrimientos de quitosano y la CC en la AT, SST, o IM de naranjas 'Valencia' (no se muestran datos). El efecto de la aplicación de recubrimientos comestibles sobre los parámetros de calidad interna depende del tipo de recubrimiento, del cultivar y de las condiciones de almacenamiento. Algunos autores no han encontrado diferencias en estos parámetros después de la aplicación de recubrimientos comestibles en diferentes cultivares de cítricos (Baldwin et al., 1995; Obenland et al., 2008); mientras que otros han encontrado una disminución en el contenido de SST y la pérdida de AT en comparación con los frutos sin recubrir, lo cual siempre se relacionó a una disminución en la pérdida de peso y la tasa de respiración (Togrul y Arslan, 2004). Chien et al. (2007) observaron que la aplicación de un recubrimiento de quitosano de bajo peso molecular mejoró la calidad interna de mandarinas 'Murcott', manteniendo mayor acidez y menor contenido de SST que los frutos sin recubrir, mientras que la aplicación de un recubrimiento de quitosano de alto peso molecular no afectó a estos parámetros de calidad.

Tabla 1. Sabor y malos sabores de naranjas ‘Valencia’ sin recubrir y recubiertas con quitosano a distinto contenido en sólidos y cera comercial.

tratamientos	Inicial		3 semanas a 5ºC + 1 semana a 20ºC		5 semanas a 5ºC + 1 semana a 20ºC		7 semanas a 5ºC + 1 semana a 20ºC		9 semanas a 5ºC + 1 semana a 20ºC		16 semanas a 5ºC + 1 semana a 20ºC	
	Malos sabores (0-5)	sabor (1-9)	Malos sabores (0-5)	sabor (1-9)	Malos sabores (0-5)	sabor (1-9)	Malos sabores (0-5)	sabor (1-9)	Malos sabores (0-5)	sabor (1-9)	Malos sabores (0-5)	sabor (1-9)
Control	0.35	6.63	0.52 a	6.67 c	0.00 a	6.47 c	0.75 a	5.94 a	1.05 a	5.16 a	1.20 a	5.20 a
CC	0.35	6.63	0.74 a	5.56 ab	1.03 bc	5.26 ab	0.92 a	5.88 a	1.53 a	4.79 a	1.13 a	5.33 a
0.6%Q	0.35	6.63	0.61 a	6.50 c	0.68 b	5.79 bc	0.63 a	5.55 a	1.00 a	5.47 a	1.53 a	4.00 a
1.2%Q	0.35	6.63	1.20 a	5.15 a	0.79 b	5.84 bc	1.26 a	5.04 a	1.26 a	5.21 a	1.23 a	5.33 a
1.8%Q	0.35	6.63	0.52 a	6.04 bc	1.53 c	4.68 a	1.08 a	5.10 a	1.11 a	5.11 a	1.53 a	5.07 a

En cada periodo de almacenamiento, valores seguidos de la misma letra no difieren significativamente (MSD, p≤0,05).

Calidad sensorial

Los tratamientos aplicados produjeron cambios mínimos en las características organolépticas evaluadas por el panel de jueces (Tabla 1). Tan sólo se encontraron diferencias significativas en el segundo periodo de almacenamiento (5 semanas a 5 ºC más 1 semana a 20 ºC), donde los frutos recubiertos con quitosano al 1,8% y la CC fueron evaluados con peor sabor que el resto, aunque dentro del rango considerado como aceptable. El sabor de las naranjas disminuyó al prolongarse en tiempo de almacenamiento. No obstante, al finalizar el almacenamiento todos los tratamientos presentaron un sabor aceptable (valoraciones entre 6 y 4). Estos resultados indican que los recubrimientos empleados permiten prolongar el almacenamiento a 5 ºC de las naranjas 'Valencia' manteniendo una buena calidad organoléptica. De igual manera, la aplicación de quitosano en mandarinas 'Fortune' y 'Oronules' no afectó a la calidad organoleptica tras su alamacenamiento (Contreras et al., 2010; Salvador et al., 2003).

Algunos trabajos muestran que, en general, concentraciones de etanol en torno a 200 mg/100mL dan lugar a malos sabores en cítricos (Ahmad y Khan, 1987) y en concreto en naranjas 'Valencia' los malos sabores se han detectado a partir de concentraciones de etanol en torno a 80 mg/100mL (Ke y Kader, 1990). El contenido en etanol, es un indicador de malos sabores en cítricos. Sin embargo, la percepción de los malos sabores no puede atribuirse exclusivamente al contenido de este compuesto en el fruto puesto que existen otros factores involucrados en el desarrollo y detección de los malos sabores. Estos factores son propios del tipo de fruto y de la variedad estudiada, relacionados por ejemplo con la presencia de otros compuestos aromáticos y con la interacción de todos ellos con otros componentes del fruto. Así por ejemplo, en naranjas 'Valencia', Navarro-Tarazaga et al. (2007) encontraron que el umbral de detección de malos sabores (ligeramente perceptible) se correspondió con niveles de etanol en torno a 70 mg/100mL y el máximo de malos sabores detectado fue medianamente perceptible y se correspondió con niveles de etanol en torno a 100 mg/100mL. Valencia-Chamorro et al. (2009) encontraron en naranjas 'Valencia' malos sabores muy ligeramente perceptibles correspondiendo a valores de etanol en torno a 120 mg/100mL. Mientras que en experiencias realizadas con mandarinas 'Clemenules', Navarro-Tarazaga y Pérez-Gago (2006) encontraron que el umbral de detección de los malos sabores se dio a concentraciones de etanol en torno a 50 mg/100mL, y concentraciones de

etanol de 100mg/100mL dieron malos sabores bastante perceptibles. En este trabajo el umbral de detección de los malos sabores (ligeramente perceptibles) se correspondió con niveles de etanol en torno a 100mg/100mL, manteniéndose el umbral de detección de los malos sabores como ligeramente perceptibles hasta el final del periodo de almacenamiento, a pesar de que los niveles de etanol alcanzaron valores ligeramente superiores a 200 mg/100mL.

Calidad nutricional

La capacidad antioxidante fue expresada como EC_{50}, es decir la cantidad de zumo necesario para reducir en un 50% el contenido en $DPPH^{\bullet}$. Por tanto, cuanto menor es el valor de EC_{50} mayor es la capacidad antioxidante de la fruta. La tabla 2 muestra la capacidad antioxidante de las naranjas 'Valencia' almacenadas 3, 5, 7, 9 y 16 semanas a 5 ºC más 1 semana a 20 ºC. En general, al finalizar el almacenamiento los valores EC_{50} no se vieron modificados de manera significativa respecto al valor inicial. La aplicación de recubrimientos no afectó a la capacidad antioxidante total de las muestras almacenadas 7 y 16 semanas a 5 ºC seguidas de 1 semana a 20 ºC, mientras que se encontraron algunas diferencias significativas entre tratamientos que dependieron del tiempo de almacenamiento (3, 5 y 9 semanas a 5 ºC mas 1 semana a 20 ºC), lo que hace difícil determinar el efecto de la composición del recubrimiento en la capacidad antioxidante de las naranjas 'Valencia'. Así pues, las naranjas recubiertas con la CC presentaron los menores valores de capacidad antioxidante durante los dos primeros periodos de almacenamiento, mientras que a las 9 semanas de almacenamiento la capacidad antioxidante de estas muestras fue mayor que el resto de tratamientos. Trabajos anteriores han encontrado correlaciones entre la vitamina C (AAT) y la capacidad antioxidante (Pretel et al., 2006) o el contenido de compuestos fenólicos y capacidad antioxidante de los cítricos (Rapisarda et al., 1999). En nuestro caso, no se observó una correlación entre estos parámetros. Junto a la vitamina C y compuestos fenólicos, otros compuestos bioactivos, como carotenoides, limonoides, presentes en cítricos pueden contribuir a la capacidad antioxidante.

El valor inicial de AAT de las naranjas fue de 41 mg/100 mL de zumo. El almacenamiento, en general, no afectó al contenido en AAT. En referencia al efecto de los diferentes tratamientos sobre el contenido en vitamina C, los frutos control no mostraron diferencias significativas con

respecto a los recubiertos con la CC, excepto tras 5 semanas a 5 ºC seguido de 1 semana a 20 ºC, cuando se encontraron valores superiores de vitamina C en las frutas control. Aunque se observó diferencias significativas entre los frutos recubiertos con quitosano en los distintos periodos de almacenamiento ensayados, no se observó una tendencia clara en función del CS del recubrimiento de quitosano. Li y Yu (2001) observaron que el contenido de ácido ascórbico en melocotón fue más alto en frutos tratados con quitosano que en frutos control después de un período de almacenamiento de 12 días, lo que se relacionó con una modificación de la atmósfera interna en el fruto. Por otra parte, los daños por frío pueden acelerar las pérdidas de vitamina C en frutos sensibles al frío (Miller y Heilman, 1952). En este trabajo, no se observó una pérdida de vitamina C tras una frigoconservación prolongada. Palma et al. (2005) no observaron cambios en el contenido de AAT en mandarinas 'Fortune' después de un almacenamiento de 90 días a 5 ºC.

El efecto de los recubrimientos sobre los glucósidos de flavanonas fue variable, haciendo difícil encontrar una tendencia en el comportamiento de los mismos que permita extraer conclusiones del efecto de los recubrimientos con quitosano. En general, se observó un aumento del contenido en narirutina, hesperidina y didimina al aumentar el tiempo de almacenamiento. Palma et al. (2005), sin embargo, no encontraron diferencias significativas en hesperidina, narirutina y didimina en zumo de mandarinas 'Fortune' durante almacenamiento prolongado a 5 ºC.

Los cítricos contienen junto a flavanonas otros compuestos fenólicos, como flavonas y ácidos hidroxicinámicos (representados por los ácidos ferúlico, cafeico, sinapico y p-cumárico) que, aunque presentes en una concentración más baja, contribuyen al contenido en fenoles totales (Rapisarda et al., 1999; Gil-Izquierdo et al., 2002). Tras el primer periodo de almacenamiento el contenido en fenoles totales aumentó en las naranjas recubiertas con 1,8% Q. En general este tratamiento fue el que presentó mayor contenido en fenoles totales (Tabla 2). Trabajos previos han señalado que el quitosano actúa como un desencadenante exógeno en el tejido vegetal induciendo diferentes respuestas como la biosíntesis de los compuestos fenólicos (Lafontaine y Benhamou, 1996; Bautista-Baños et al., 2006; Meng et al., 2008). Al finalizar el almacenamiento, el contenido de fenoles totales se incrementó al aumentar el CS del recubrimiento de quitosano (1,8% Q),

lo que podría ser una indicación de la actividad del quitosano como un desencadenante exógeno del tejido vegetal.

Tabla 2. Capacidad antioxidante (EC_{50}), ácido ascórbico total (AAT), glucósidos de flavanonas y compuestos fenólicos totales de naranjas 'Valencia' recubiertas y control.

		EC_{50} (Lzumo/Kg DPPH)	**AAT (mg/100 mL zumo)**	**Narirutina (mg/100 mL zumo)**	**Hesperidina (mg/100 mL zumo)**	**Didimina (mg/100 mL zumo)**	**Fenoles Totales (mg/100 mL zumo)**
Inicial		232.01± 4.44	41.07± 3.01	5.41± 0.52	23.6± 0.09	1.51± 0.08	77.7± 1.79
	Control	205.7± 6.9 a	39.8± 1.3 a	6.32± 0.19 a	21.9± 1.9 a	1.77± 0.07 a	80.0± 6.3 a
3 semanas a 5ºC	CC	286.3±12.8 c	38.7± 0.9 a	5.81± 0.43 a	23.4± 1.6 a	1.58± 0.15 a	79.5± 5.5 a
+	0.6% Q	279.8±11.4 bc	39.8± 1.4 a	5.91± 0.39 a	25.4± 0.6 a	1.59± 0.12 a	86.4± 2.4 ab
1 semana a 20ºC	1.2% Q	264.1± 9.1 b	42.1± 3.7 a	5.26± 0.61 a	23.1± 1.4 a	1.48± 0.19 a	85.4± 3.2 ab
	1.8%Q	266.8± 1.1 b	38.5± 0.8 a	5.65± 0.25 a	24.8± 0.7 a	1.69± 0.03 a	92.0± 2.4 b
	Control	284.9±13.1 bc	40.8± 0.1 c	6.72± 0.40 bc	28.8± 1.1 c	2.04± 0.16 c	88.8± 0.4 ab
5 semanas a 5ºC	CC	301.4±24.4 c	35.7± 1.6 a	5.73± 0.30 a	25.0± 0.5 a	1.71± 0.06 ab	86.0± 3.9 a
+	0.6% Q	263.2±19.7 ab	38.2± 1.0 b	5.94± 0.13 ab	27.1± 1.8 bc	1.61± 0.08 a	92.5± 1.8 cd
1 semana a 20ºC	1.2% Q	258.8± 9.0 ab	40.3± 0.8 c	6.97± 0.84 cd	26.9± 0.8 ab	1.63± 0.01 a	90.3± 1.1 bc
	1.8%Q	238.8±23.5 a	35.5± 0.7 a	7.65± 0.33 d	28.4± 0.2 bc	1.87± 0.08 bc	95.1± 0.7 d
	Control	240.4± 5.2 a	41.9± 0.7 a	5.67± 0.48 a	26.3± 3.2 a	1.77± 0.16 b	96.9± 5.5 b
7 semanas a 5ºC	CC	226.8± 5.5 a	40.1± 2.2 a	6.02± 0.45 a	27.1± 0.9 a	1.79± 0.12 b	90.4± 2.5 ab
+	0.6% Q	218.5±12.3 a	39.5± 1.8 a	6.11± 0.31 a	23.1± 0.9 a	1.45± 0.02 a	91.9± 3.4 b
1 semana a 20ºC	1.2% Q	219.3±14.9 a	38.3± 1.3 a	6.04± 0.19 a	25.1± 0.2 a	1.56± 0.05 a	84.2± 3.2 a
	1.8%Q	209.3±15.9 a	38.1± 3.4 a	5.66± 0.11 a	25.3± 0.2 a	1.63± 0.08 ab	93.4± 2.5 b
	Control	213.0± 9.4 b	40.9± 0.8 b	6.46± 0.38 a	31.7± 2.5 a	1.94± 0.14 a	93.9± 0.7 bc
9 semanas a 5ºC	CC	190.6± 1.8 a	40.8± 1.0 b	7.61± 0.92 a	33.7± 2.1 a	2.25± 0.21 a	96.6± 2.8 c
+	0.6% Q	217.5± 2.8 b	37.7± 3.1 a	7.11± 0.48 a	32.2± 1.0 a	1.95± 0.06 a	85.9± 2.9 a
1 semana a 20ºC	1.2% Q	221.5± 9.0 b	36.4± 0.6 a	6.61± 0.26 a	31.1± 2.7 a	1.84± 0.10 a	89.9± 2.1 ab
	1.8%Q	215.6± 9.9 b	36.1± 0.9 a	6.57± 0.59 a	29.4± 2.3 a	1.99± 0.19 a	94.4± 2.4 c
	Control	229.2± 2.8 a	42.4± 1.2 b	7.41± 0.06 a	31.4± 0.9 a	2.06± 0.05 a	92.9± 6.2 a
16 semanas a 5ºC	CC	261.9±49.2 a	41.7± 1.7 b	7.06± 0.49 a	31.6± 1.1 a	2.01± 0.12 a	92.9± 4.4 a
+	0.6% Q	243.4±25.4 a	41.8± 0.4 b	7.27± 0.43 a	32.9± 0.7 a	2.05± 0.11 a	90.8± 3.8 a
1 semana a 20ºC	1.2% Q	237.6±10.5 a	42.9± 1.5 b	6.79± 0.95 a	32.3± 2.6 a	1.99± 0.39 a	91.6± 1.1 a
	1.8%Q	253.2± 1.2 a	36.7± 1.2 a	7.94± 0.94 a	33.9± 3.2 a	2.43± 0.29 a	99.8± 1.2 a

En cada periodo de almacenamiento, valores seguidos de la misma letra no difieren significativamente (MSD, $p \leq 0,05$).
Valores medios ± desviación estandar (n=3)

CONCLUSIONES

La aplicación de la CC disminuyó la pérdida de peso de las naranjas en comparación con las muestras control, mientras que la aplicación del recubrimiento de quitosano comercial a distintos CS no resultó efectivo controlando la pérdida de peso de las naranjas. Por lo tanto, con el fin de mejorar la barrera a la humedad del recubrimiento de quitosano sería necesario añadir a la formulación componentes hidrofóbicos. Los recubrimientos aplicados restringieron el intercambio gaseoso y modificaron la atmósfera interna de los frutos en comparación a las naranjas sin recubrir, con un efecto mayor al aumentar el CS del quitosano, aunque la calidad sensorial de las naranjas no se vio afectada. En general, la calidad interna de las mandarinas y los compuestos bioactivos no se vieron afectados por la aplicación de estos recubrimientos.

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CAPITULO V

Effect of solid content and composition of hydroxypropyl methylcellulose-lipid edible coatings on physicochemical, sensory and nutritional quality of 'Oronules' mandarins

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Abstract

'Oronules' mandarins were coated with edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW) and shellac. Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components (BW/shellac)-oleic acid (5:1) (db) were kept constant throughout the study. BW and shellac content was 60 % (db), but formulations were prepared at two different BW:shellac ratios (1:3 and 3:1) and two different percentages of total solid content (SC) (4 and 8%). A commercial wax (polyethylene-shellac) at a 10% SC, as a control of coated fruit, and an uncoated control were also tested. Mandarins were stored for 0, 1, 2, 3 and 4 wks at 5 ºC, followed by 1 wk at 20 ºC simulating retail storage conditions. Samples were periodically analyzed for physicochemical, sensory and nutritional quality. Coating application had little effect controlling weight loss of 'Oronules' mandarins. No tendency was observed between SC and BW:shellac ratio of coating formulation and firmness. However, these factors affected the internal mandarin atmosphere and the ethanol content during storage. Increasing SC and shellac content in the formulation had a greater effect in fruit internal atmosphere and significantly increased the level of ethanol, showing the importance of controlling this parameters when coating mandarins. In general, nutritional quality was not affect by the application of the different treatments; however, sensory quality was affected by the application of coatings with 8% SC.

Keywords: edible coating, commercial wax, beeswax, shellac, postharvest quality.

Introduction

Consumers demand higher quality and longer shelf-life in foods, while reducing disposable packaging materials and increasing recyclability. Such concerns have caused an increased interest in the development of new edible films and coatings. Coatings are used in fresh fruits to retard moisture loss, improve appearance, act as carriers for natural antimicrobials, and create a barrier for gas exchange between the commodity and the external atmosphere (Grant and Burns, 1994). However, if the coating offers a high gas barrier, anaerobic conditions can be induced with the build-up of

volatile compounds and the development of off-flavor (Hagenmaier, 2002; Hagenmaier and Baker, 1993).

Edible fruit coatings are made with food-grade ingredients, generally regarded as safe (GRAS) for human consumption. Major components include polysaccharides, proteins, and lipids (Kester and Fennema, 1986). They present advantages and disadvantages when used as coating ingredients. Generally, lipids offer a good moisture barrier due to their hydrophobic nature, reducing water loss, shriveling, and shrinkage of coated fruit. However, their non-polymeric nature limits their ability to form cohesive films. Proteins and polysaccharides are good film-formers and present an intermediate O_2 barrier at medium-high relative humidity. However, their hydrophilic nature makes them poor moisture barriers. For this reason, most natural coatings for fruits contain a combination of ingredients forming what is called "edible composite coatings".

In the literature, many works report the effect of edible composite coatings, on the postharvest quality of citrus fruits. The combination of hydroxypropyl methylcellulose (HPMC) and lipids has been shown to reduce weight loss and retain firmness of different citrus fruit cultivars (Pérez-Gago et al., 2002; Navarro-Tarazaga and Pérez-Gago, 2006; Navarro-Tarazaga et al., 2007, 2008a). In these works, coating performance depended on composition, storage conditions and fruit commodity. Lipid type and content, and solid content (SC) seemed to be the main factors affecting the final quality of coated citrus fruits. In general, HPMC-beeswax (BW) coatings provided a good weight control of 'Fortune' mandarins (Pérez-Gago et al., 2002), 'Clemenules' mandarins (Navarro-Tarazaga and Pérez-Gago, 2006) and 'Ortanique' mandarins (Navarro-Tarazaga et al., 2008a). However, these coatings did not improved fruit appearance. Shellac, which is a natural resin, is usually used as ingredient of natural coatings in fruits that are not consumed with peel like citrus fruits in order to provide gloss (Rhim and Shellhammer, 2005). However, the higher gas barrier of resins compared to waxes may induce anaerobic conditions and increase the level of volatile components modifying fresh citrus flavor (Hagenmaier, 2002).

Nowadays, nutritional and functional quality has gained great interest, being a component of the overall quality that is very much valued by consumers. Citrus fruits are an important source of vitamin C, as well as other bioactive compounds such as polyphenolic compounds, mainly

flavonoids, with high antioxidant properties (Sánchez-Moreno et al., 2003). Therefore, postharvest technologies should maintain fruit nutritional and functional quality until they reach the consumer. Most of the works found in the literature provide information about the effect of edible coatings on the physicochemical and sensory quality, but few studies can be found on their effect on the nutritional quality of coated citrus fruits. Therefore, the objective of these work was to study the effect of SC and BW:shellac ratio of HPMC-lipid edible coatings on the physicochemical, sensory and nutritional quality of 'Oronules' mandarin.

Material and methods

Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). Shellac and BW (grade 1) were supplied by Fomesa Fruitech, S.L. (Beniparrell, Valencia, Spain). Oleic acid and glycerol were from Panreac Química, S.A. (Barcelona, Spain). Silicone antifoam (FG-1510) and ammonia (25%) were from Dow Corning® (Belgium) and Scharlau (Sentmenat, Barcelona, Spain), respectively.

Reagents 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu's phenolreagents, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). Methanol was from BDH prolabo (Poole, UK), 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside, HES) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside, NAT) and didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used for the analysis.

Coating formulation

Emulsion coatings consisted on HPMC and different ratios of BW and shellac suspended in water. Oleic acid and glycerol were added as

emulsifiers and plasticizer, respectively. Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components (BW/shellac)-oleic acid (5:1) (db) were kept constant throughout the study. BW and shellac content was 60% (db). Formulations were prepared at two different BW:shellac ratio (1:3 and 3:1) and two SC (4% and 8%). Table 1 and 2 show the coating treatments applied to 'Oronules' mandarins and the composition of the HPMC-based coatings, respectively.

Table 1. Treatments applied to 'Oronules' mandarins.

Treatment	BW:Shellac ratio	Solid Content (%)	Viscosity (cP)
T1: Uncoated	--		
T2: CW	--	10	
T3: 1:3 BW:Sh – 4% SC	1 : 3	4	15.5
T4: 1:3 BW:Sh – 8% SC	1 : 3	8	24.5
T5: 3:1 BW:Sh – 4% SC	3 : 1	4	11.5
T6: 3:1 BW:Sh – 8% SC	3 : 1	8	25.2

BW=beeswax; CW=commercial wax; HPMC=hydroxypropyl methylcellulose; Sh=Shellac.
T3, T4, T5 and T6: HMPC-based edible coatings.

Table 2. Composition on the HPMC:lipid edible coatings (%, wet basis).

Treatment	HPMC	BW	Shellac	Glycerol	Oleic acid
T3	0.75	0.60	1.80	0.37	0.48
T4	1.49	1.20	3.60	0.75	0.96
T5	0.75	1.80	0.60	0.37	0.48
T6	1.49	3.60	1.20	0.75	0.96

HPMC = Hydroxypropyl methylcellulose; BW = Beeswax.

Emulsion was made in 2-L a stirred pressure cell (Parr Instrument Co., Molline, IL), as described by Navarro-Tarazaga et al. (2007) with some modifications. Glycerol, oleic acid, BW, shellac, NH_3, and one-third of the water were added to the pressure cell. The mixture was initially stirred at 100 rpm until the temperature reached 60 ºC. Next, stirring was increased to 400 rpm until temperature reached 110 ºC and remained at these conditions for 30 min. Afterwards, the remaining water, previously heated to 90 °C, was pumped into the vessel mantaining the stirring conditions at 400 rpm for about 10-15 min after the water was incorporated. The emulsion was then removed from the pressure vessel and mixed with a 5% HPMC

solution previously prepared by dispersing the HPMC in hot water at 90 ºC and later hydration at 20 °C for 45 min. Finally, the emulsions were cooled under agitation to a temperature lower than 20 °C by placing them in an ice water bath. Water was added to a final SC of 4 or 8% depending on the treatment.

Emulsion viscosity

Emulsion viscosity was measured with a viscometer Synchro-Lectric viscometer Model LVF (Brookfield Engineering Laboratories, Inc.). Three measurements were made per emulsion and results were expressed as centipoises (cp). Sample viscosity was measured at 20 ºC.

Fruit preparation–coating application

'Oronules' mandarins were hand-harvested with an average maturity index of 11.8 ºBrix from a local grove in Valencia (Spain) and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a solution of imazalil (1,000 ppm) for 1 min.

The mandarins were randomly divided into 6 groups: 4 experimental coating treatments, 1 uncoated (control), and 1 commercial wax (CW) (polyethylene-shellac) applied at 10% SC as a control of coated fruit (Table 1). The fruits were dip-coated by inmersion in the coating solutions for 20 sec, drained of excess coating and dried in tunnel at 50 ºC for 2 min (Pérez-Gago et al., 2002). After coating, fruit were stored for 0, 1, 2, 3 and 4 weeks at 5 ºC and 90-95% RH, followed by 1 additional week at 20 ºC to simulate retail storage conditions.

Physicochemical quality

Weight Loss

Lots of 30 fruits per treatment were used to measure weight loss. The same fruit were weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage loss of initial weight.

Fruit firmness

Firmness of 20 mandarins per treatment was determined at the end of each storage time using an Instron Universal Testing Machine (Model 3343, Instron Corp., Canton, MA, USA). The instrument gave the deformation (length) after application of a compression load of 10 N to the equatorial region of the fruit at a rate of 5 mm/min. Results were expressed as percentage deformation related to initial diameter.

Internal gas concentration

Ten fruit per treatment were used to calculate internal gas concentrations. Internal CO_2 and O_2 concentrations of each sample were obtained by withdrawing 1 mL internal gas sample from the mandarin central cavity with a syringe while the fruit was immersed under water. The gas sample was then injected into a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) fitted with a Poropak QS 80/100 (1.2 m x 0.32 cm) column, followed by a molecular sieve 5A 45/60 (1.2 m x 0.32 cm) column. Temperatures were 35, 125 and 180 ºC, respectively, for the oven, injector and thermal conductivity detector. Helium was used as carrier gas at 22 ml/min flow rate. Peak areas obtained from standard gas mixtures were determined before and after analysis of samples and results were expressed as percentage.

Ethanol and Acetaldehyde content

Ethanol and acetaldehyde content in juice were determined by head-space gas chromatography according to the method described by Ke and Kader (1990). Ten fruits each in 3 replicates per treatment were analyzed. Five mL mandarin juice were transferred to 10 mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. Ethanol and acetaldehyde content were analyzed using a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with an autosampler, a flame ionization detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). Temperatures of the oven, injector, and detector were 150, 175, and 200 ºC, respectively. Helium was used as the carrier gas at a flow rate of 28 mL/min. A 1 mL sample of the head-space was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 ºC. Ethanol and acetaldehyde concentrations were calculated

using peak areas of the samples relative to the peak areas of standard solutions. Results were expressed as mg/100 mL juice.

Internal quality parameters

Soluble solids content (SSC) was measured with a digital refractometer (Atago, Model PR1) and titratable acidity (TA) was determined by titration with 0.1 N NaOH up to pH=8.1 and expressed as g of citric acid per 100 ml of mandarin juice. The maturity index (MI) was calculated as SSC/TA ratio. The juice from three replicates of 10 fruit each was used to determine the above parameters.

Sensory quality

Sensory evaluation was conducted by 10 trained judges (5 females and 5 males), 25 to 50 years old, at the end of each storage period. Panelists evaluated overall flavor and off-flavor of mandarins. Overall flavor was rated on a 9-point scale, where 1 to 3 represented a range of non-acceptable quality with the presence of off-flavor, 4 to 6 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavors presence was evaluated using a 6-point intensity scale where 0=absence of off-flavor and 5=high presence of off-flavor. Six fruit per treatment were pealed and separated into individual segments. Two segments from two different fruit were presented to judges in trays labeled with 3-digit random codes and served at room temperature (25±1 ºC). The judges had to taste several segments of each treatment in order to compensate, as far as possible, for biological variation of material. Mineral spring water was provided for rinsing between samples. External aspect of treated fruit (coating cracks, spots, etc.) was also evaluated by the panelist. A 3-point scale was used, in which the aspect was classified as 1=bad, 2=acceptable, and 3=good. Panelists were also asked to rank visually the treatments from highest to lowest gloss. Sum of rankings were calculated (UNE 87 023; AENOR, 1995). The lowest sum of ranking indicates the highest glossy treatment. For visual aspect (external aspect and gloss ranking), four intact fruit per treatment were placed in trays labeled with 3-digit random codes and presented to the judges under the same conditions (light intensity and temperature) to minimize variations in human perception.

Bioactive compounds

Total antioxidant capacity (EC_{50})

The total antioxidant capacity (EC_{50}) was evaluated by the $DPPH^{\bullet}$ assay. 0.4 mL of mandarin juice diluted with 0.8 mL of methanol was centrifuged at 12,000 rpm and 4 ºC for 20 min. Six methanolic dilutions from the supernatant (0.075 mL) were mixed with 0.2925 mL of $DPPH^{\bullet}$ (24 mg L^{-1}) and kept in darkness for 40 min. Afterwards, the change in absorbance at 515 nm was measured in a Multiskan spectrum microplate reader (Thermo Labsystem, USA). For each dilution, the percentage of remaining $DPPH^{\bullet}$ was determined on the basis of the $DPPH^{\bullet}$ standard curve. The amount of juice in each dilution was plotted against the amount of $DPPH^{\bullet}$ radical remaining. Using the curve obtained, the EC_{50} value was calculated. This result expressed the amount of mandarin juice (L) needed to reduce 1 kg of $DPPH^{\bullet}$ by 50%; thus, lower values mean higher antioxidant activity.

Total ascorbic acid (TAA)

TAA was determined as the sum of AA plus L-dehydroascorbic acid (DHA), by using the reducing agent DTT (Sánchez-Mata et al., 2000). One mL of mandarin juice was diluted to 10 mL with 2.5% (*w/v*) MPA. Two mL of this solution were mixed with 0.4 mL of DTT (20 mg mL^{1}) for 2 h in darkness. Afterwards, the extracts were filtered through a 0.45 µm Millipore filter before being HPLC analyzed.

The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi, Germany) equipped with autosampler (Model L-2200), quaternary pump (Model L-2130), column oven (Model L-2300), and diode array detector (Model L-2450). A reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 µm-particle, Merck, Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. System conditions were: injection volume 20 µL, oven 25 ºC, detector wavelength 243 nm, and flow rate 1 mL min^{-1}. The mobile phase was 2% KH_2PO_4 adjusted to pH 2.3 with H_3PO_4. AA was identified and quantified by comparison of peak areas with external standard and results were expressed as milligrams of AA per 100 mL of juice.

Flavanone glycosides (FGs)

The main FGs identified in citrus fruit (HES, NAT, and DID) were determined by the method described by Cano et al. (2008) slightly modified.

Two mL of mandarin juice were homogenized with 2 mL de DMSO:Methanol (1:1 *v/v*) and centrifuged for 30 min at 12,000 rpm and 4 ºC. The supernatant was filtered through one 0.45 μm nylon filter and analyzed by HPLC-DAD using the HPLC equipment described above. System conditions were: injection volume 10 μL, oven 25 ºC, detector wavelength 280 nm, and flow rate 1 mL min^{-1}. The column Lichospher 100 RP-18 of 25x0.4 cm was preceded by a precolumn (4x4 mm) with 5 μm particle size (Merck, Darmstadt, Germany). The mobile phase was acetonitrile (A):0.6% acetic acid (B) with initial condition of 10% A for 2 min, reaching 75% A in the following 28 min, then back to the initial condition in 1 min and held for 5 min prior to the next sample injection. The main FGs were identified by matching their respective spectra and retention times with those of commercially obtained standards. NAT, HES and DID contents were calculated by comparing the integrated peak areas of each individual compounds to that of its pure standards. Results were expressed as mg/100 mL.

Total phenolic content (TPC)

The mandarin juices were analyzed for TPC by the Folin-Ciocalteu colorimetric method. 0.3 mL of mandarin juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted extract (0.4 mL) was mixed with 2 mL of folin-ciocalteau commercial reagent (previously diluted with water 1:10, *v/v*) and incubated for 1 min before 1.6 mL sodium carbonate (7.5% *w/v*) was added. The mixture was incubated for 1 h at room temperature. The absorbance of the resulting blue solution was measured spectrophotometrically at 765 nm (Thermo UV1, Thermo Electron Corporation, UK) and the TPC was expressed as gallic acid equivalents per 100 mL (mg GAE/100 mL).

Total antioxidant capacity, TAA, FGs and TPC were determined in juice from three replicate of 10 fruit each.

Statistical Analysis

A complete randomized design was used to perform the analysis of the samples. Statistical analysis of the results was performed by one-way analysis of variance (ANOVA) using STATGRAPHICS Plus 4.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.). Significance differences between means was determined by least significant difference test (LSD;

p≤0.05) applied after the analysis of variance (ANOVA). For sensory gloss, specific differences were determined by Friedman test, which is recommended for ranking by the UNE 87 023 (AENOR, 1995). Significance differences were defined at p≤0.05.

Result and discussion

Physicochemical quality

Weight loss

Figure 1 shows the weight loss of coated and uncoated mandarins stored for 0, 1, 2, 3, and 4 weeks at 5 ºC, followed by 1 week at 20 ºC. Weight loss increased with storage time, increasing to nearly 25% after 4 weeks at 5 ºC plus 1 week at 20 ºC on uncoated samples. The CW was the most effective coating controlling weight loss of 'Oronules' mandarins during storage.

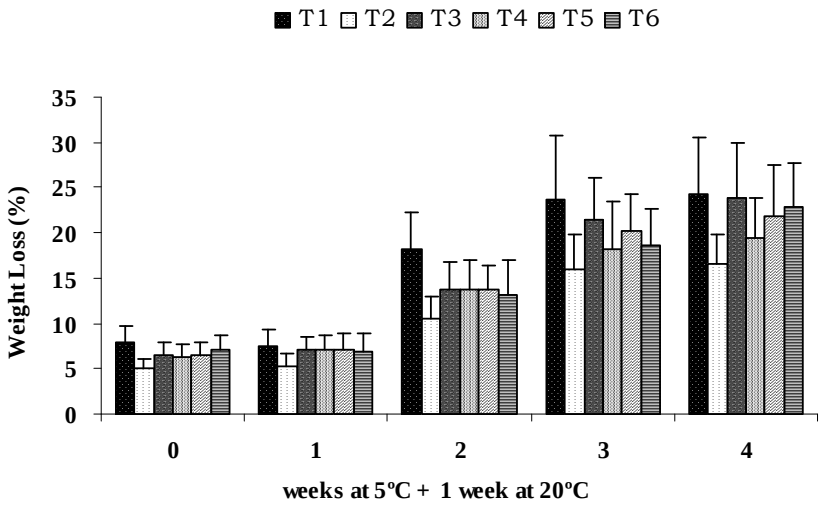

Figure 1. Weight Loss of coated and uncoated 'Oronules' mandarins during storage. Error bars indicate standard deviations (n=30).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

The HPMC-based coatings had no effect controlling weight loss of 'Oronules' mandarins stored 1 week at 5 ºC plus 1 week at 20 ºC. After 2 weeks at 5 ºC plus 1 week at 20 ºC, these coating reduced fruit weight loss by 30% compared to the control with no differences among treatments. However, for longer storage periods, the HPMC-based coatings lost effectiveness. Under these storage conditions, T4 (BW:shellac ratio 1:3 and 8% SC) was the most effective HPMC-based coating controlling weight loss of the fruit, whereas T3 (BW:shellac ratio 1:3 and 4% SC) showed no effect compared to control samples. All the HPMC-based coatings had the same content of hydrophobic components (BW-Shellac), but differed in the BW:Shellac ratio and SC. The small differences found among the HPMC-based treatments could be due to the similar content of hydrophobic components (BW-Shellac), indicating that changes in BW:Shellac ratio had little effect on weight loss control of the mandarins.

Application of HPMC-based edible coatings has been reported both with and without significant effects on weight loss of some fruit. For example, Pérez-Gago et al. (2002) reported that HPMC-lipid composite containing different types of lipids reduced weight loss of coated 'Fortune' mandarins. Other works reported that similar HPMC-lipid edible coatings were effective reducing weight loss of 'Ortanique' mandarins (Valencia-Chamorro, 2009), whereas they did not reduce weight loss of coated 'Valencia' oranges (Valencia-Chamorro et al., 2009). In 'Angeleno' plums, HPMC-BW coatings containing different types of plasticizers did not reduce weight loss of the fruit as compared with uncoated samples (Navarro-Tarazaga et al., 2008b). Similarly, HPMC coatings containing soybean oil or carnauba wax had minimal effect on water loss of coated cherries or cucumbers (Baldwin et al., 1997).

Fruit firmness

In general, the firmness of 'Oronules' mandarins was slightly improved by coating application compared to uncoated mandarins (Figure 2). Even though some significant differences in firmness were found among treatments, no tendency was observed between BW:shellac ratio or SC of coating formulations and firmness. The lack of tendency between coating type and fruit texture has also been reported by Rojas et al. (2002) in 'Fortune' mandarins.

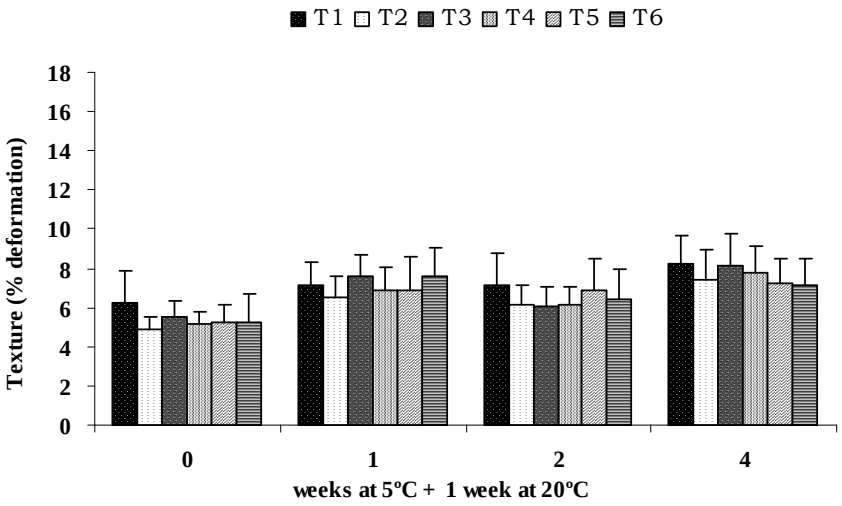

Figure 2. Firmness of coated and uncoated 'Oronules' mandarins during storage. Error bars indicate standard deviations (n=20).
T1=uncoated, T2=CW, T3=1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.
Firmness at harvest was 6.3% deformation

In our work, despite of the good control of weight loss by the CW, this coating did not showed any effect controlling firmness loss of 'Oronules' mandarins during storage. Some investigators have observed a correlation between citrus fruit weight loss and firmness (Ben-Yehoshua, 1985; Pozzan et al., 1993; Navarro-Tarazaga et al., 2008a), whereas others have found no correlation (Hagenmaier, 2000; Pérez-Gago et al., 2002). Differences in the results might indicate that in order to see an effect on fruit texture due to coating application, the coatings should provide sufficient weight loss control. Moreover, fruit cultivar and storage conditions could be a factor for the observed differences.

Internal gas concentration

Figure 3 shows the internal gas concentration of coated and uncoated 'Oronules' mandarins. The concentration of internal CO_2 and O_2 on coated

mandarins reached values around 6-11 and 4-12%, respectively, at the end of the storage.

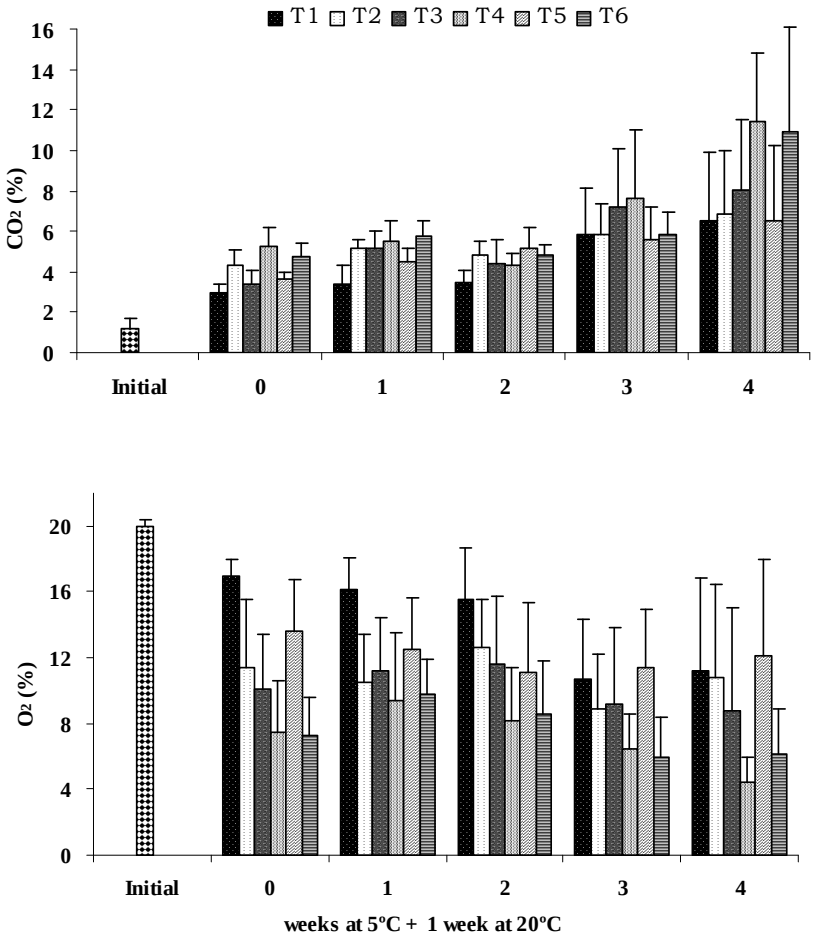

Figure 3. Internal CO_2 and O_2 contents of coated and uncoated 'Oronules' mandarins during storage. Error bars indicate standard deviation values (n=10).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

In general, the CW increased the internal CO_2 and decreased the O_2 level of coated mandarins stored up to 2 weeks at 5 ºC plus 1 week at 20 ºC compared to uncoated samples, whereas no differences were found for longer storage periods. For short storage periods (up to 2 weeks at 5 ºC plus 1 week at 20 ºC), slight differences were found between HPMC-based coatings and the CW. However, an important increased in CO_2 and a decreased in O_2 were observed as SC of HPMC-based coatings increased in coated mandarins stored 4 weeks at 5 ºC plus 1 week at 20 ºC. Many works have described a direct relation between the internal gas modification of coated fruit and coating thickness, which depends on SC, viscosity, and density of the coating formulation (Banks et al., 1993; Park et al., 1994; Cisneros-Zevallos and Krochta, 2003; Navarro-Tarazaga and Pérez-Gago, 2006; Contreras-Oliva et al., 2010b).

For similar SC, the BW:shellac ratio seemed to have little or no effect on the mandarin internal atmosphere. This contrasts with the higher gas barrier than resins, such as shellac, provide compared to waxes such as BW (Hagenmaier and Baker, 1994; Hagenmaier, 2000). Therefore, when comparing all the HPMC-based coatings, T4 and T6 were the coatings that induced the highest CO_2 and the lower O_2 accumulation in the fruit, indicating that SC of the HPMC-based coatings had a greater effect on internal atmosphere that the ratio of the hydrophobic ingredients. Mandarins coated with T3 and T5 coatings did not show differences in internal atmosphere with those coated with the CW and the control. However, in 'Valencia' oranges similar coatings with higher shellac content (BW:Shellac ratio 1:3) modified the internal atmosphere in a greater extend than coatings with lower content (BW:Shellac ratio 3:1), indicating the importance of fruit cultivar in the behavior of the coatings (Contreras-Oliva et al., 2010c).

Ethanol and acetaldehyde Contents

Figure 4 shows the ethanol and acetaldehyde levels in coated and uncoated mandarins with storage time. The HPMC-based and CW coatings increased both ethanol and acetaldehyde levels in mandarins coated compared to uncoated mandarins, which confirms the creation of a modified atmosphere in the fruit. As observed in the fruit internal atmosphere, the CW showed a moderate increase in ethanol level compared to some HPMC-based coatings. Comparing the HPMC-based coatings, an increase in SC significantly increased the ethanol level in the fruit, which correlated with the higher gas barrier that these coatings offered to the fruit. Citrus fruit

coated with shellac-based coatings generally have been reported as having higher ethanol content than those treated with wax-based coatings (Hagenmaier and Baker, 1994; Baldwin et al., 1995; Hagenmaier, 2000). In our experiment, in mandarins stored up to 2 weeks at 5 ºC plus 1 week at 20 ºC, we found that in coatings with 4% SC, an increase in shellac content did not affect the ethanol level of 'Oronules' mandarins; whereas, at 8% SC an increase in shellac content significantly increased the ethanol level. In general, mandarins coated with T4 (BW:shellac ratio 1:3 with 8% SC) had the highest levels of ethanol and mandarins coated with T5 (BW:shellac ratio 3:1 with 4% SC) had the lowest levels of ethanol. The same behavior is observed for acetaldehyde levels throughout the storage period, except for the end storage period where the highest acetaldehyde levels was in mandarins coated with T3 (BW:shellac ratio 1:3 with 4% SC) and the CW, whereas mandarins coated with the rest of the HPMC-based coatings had lower acetaldehyde levels than those coated with the CW.

At the end of the storage, the levels of ethanol in coated samples reached values between 1,650-2,460 mg L^{-1} juice. Different works have reported higher levels of ethanol on coated citrus after prolonged cold storage. For instance, 'Fortune' mandarins coated with HPMC:lipid (20% lipid content, db) reached ethanol values between 3,000 and 4,000 mg L^{-1} after 30 days at 9 ºC plus 7 days at 20 ºC (Pérez-Gago et al., 2002). In another study with 'Ortanique' mandarins coated with HPMC:BW, the ethanol content was higher than 4,000 mg L^{-1} after 45 days at 5 ºC plus 7 days at 20 ºC (Navarro-Tarazaga et al., 2008a). In this work, however, ethanol concentration in coated oranges did not exceed 3,000 mg L^{-1}.

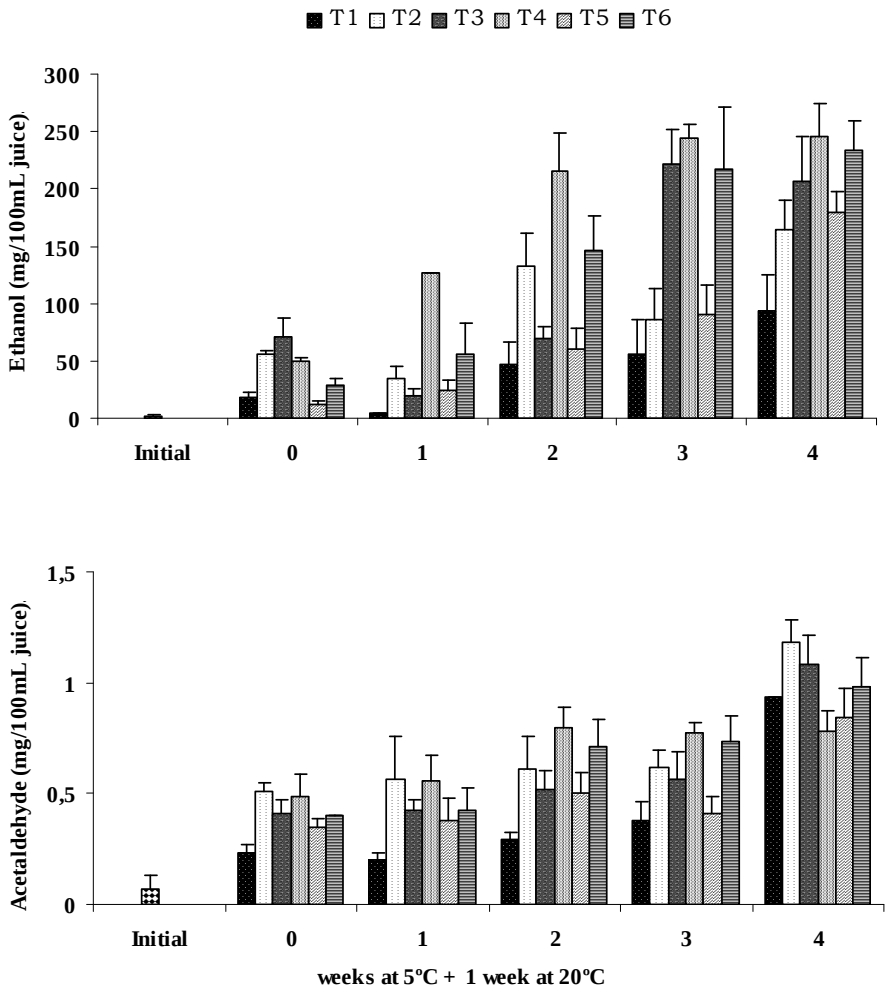

Figure 4. Ethanol and acetaldehyde contents of coated and uncoated 'Oronules' mandarins during storage. Error bars indicate standard deviation values (n=3).
T1=uncoated, T2=CW, T3=1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Internal quality parameters

Table 3 shows the effect of coating application on SSC, TA and MI of 'Oronules' mandarins after 4 weeks of storage at 5 ºC plus 1 week at 20 ºC. SSC of 'Oronules' mandarins was increased after 4 weeks of storage at 5 ºC plus 1 week at 20 ºC, except for those mandarins coated with 8% SC-coatings (T4 and T6) that maintained initial SSC values. Whereas, TA was reduced after 4 weeks of storage at 5 ºC plus 1 week at 20 ºC. However, the reduction in TA of mandarins coated with the HPMC-based coatings containing a BW:shellac ratio 1:3 was lower than in coatings with BW:shellac ratio 3:1. Although the MI of the mandarins was increased with storage, no differences were found between coated and uncoated fruit, with no differences among coatings. Some authors have found no differences in these parameters after coating application on different citrus cultivars (Baldwin et al., 1995; Obenland et al., 2008); whereas others have found a decrease in SSC and TA losses compared to uncoated fruits, which was always related to a decrease in weight loss and respiration rate (Togrul and Arslan, 2004).

Table 3. Soluble solid content, titratable acidity and maturity index of coated and uncoated 'Oronules' mandarins stored 4 weeks at 5 ºC followed by 1 week at 20 ºC.

Treatment	SSC (ºBrix)	TA (g citric acid / 100 ml)	MI
Initial (at harvest)	10.98±0.51	0.93±0.06	11.77±0.44
T1	11.85±0.25 d	0.68±0.06 a	17.45±1.25 a
T2	11.07±0.24 bc	0.67±0.02 a	16.45±0.66 a
T3	11.93±0.34 d	0.74±0.02 b	16.04±0.52 a
T4	10.90±0.44 ab	0.75±0.04 b	14.56±0.97 a
T5	11.50±0.17 cd	0.70±0.04 ab	16.53±1.19 a
T6	10.40±0.28 a	0.65±0.01 a	16.12±0.05 a

SSC= Soluble solid content; TA= titratable acidity; MI=Maturity index.
Mean values±standard deviations (n=3).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content

Sensory quality

Sensory quality of 'Oronules' mandarins were affected by coating and storage period (Table 4). Flavor evaluation of uncoated mandarins decreased with storage time from 7 at harvest time to 4 at the end of the storage. Several works showed that the contribution to off-flavor of volatile content depends on citrus cultivar. Ke and Kader (1990) established the minimum ethanol content associated with off-flavor in 'Valencia' oranges to be 2,000 mg L^{-1}; whereas, Pérez-Gago et al. (2002) found flavor degradation in 'Fortune' mandarin at an ethanol content above 3,000 mg L^{-1} and Navarro-Tarazaga and Pérez-Gago (2006) found that ethanol content of 1,000 mg L^{-1} reduced flavor quality of 'Clemenules' mandarins. In our experiment, mandarins coated with the HPMC-based coatings at 8% SC showed an important decrease in flavor and an increase in off-flavor compared to those coated at 4% SC at the end of the storage period. These coatings induced the highest ethanol production (Figure 4), exceeding slightly the limit observed by some authors to induce off-flavor. Therefore, the lower ethanol content for mandarins coated with the HPMC-based coatings at 4% SC, made them more appropriate to coat 'Oronules' mandarins under these storage conditions. The appearance of the mandarins was evaluated as acceptable throughout all the storage period, without differences among treatments (data not shown).

One of the aims of coating applications, together with the control of the weight loss, is the enhancement of external citrus appearance by conferring gloss. Panellists were asked to rank the five treatments on the basis of perceived gloss (1=the most glossy and 6=the least glossy) and the sum of the rank values was calculated (Figure 5). Therefore treatments with low scores represent shinier mandarins.

The CW was the coating that provided more gloss to 'Oronules' mandarins, while the HPMC-based coatings did not significantly improved fruit gloss compared to uncoated samples. Among the HPMC-based coatings, T3 (BW:shellac ratio 1:3 with 4% SC) was the most effective coating increasing mandarin gloss, approaching to the gloss provided by the CW coating. This could be related to its higher shellac content. It has been reported that shellac and other resins provide higher gloss to fruit than waxes, this being the main reason for their incorporation into many coating formulations (Baldwin et al., 1997; Hagenmaier and Baker, 1994).

Table 4. Flavor and off-flavor of coated and uncoated 'Oronules' mandarins after storage.

Treatments	Initial (At harvest)		0 wk 5ºC + 1 wk 20ºC		1 wk 5ºC + 1 wk 20ºC		2 wk 5ºC + 1 wk 20ºC		3 wk 5ºC + 1 wk 20ºC		4 wk 5ºC + 1 wk 20ºC	
	Flavor (1-9)	Off-flavor (0-5)	Flavor (1-9)	Off-flavor (0-5)	Flavor (1-9)	Off-flavor (0-5)	Flavor (1-9)	Off-flavor (0-5)	Flavor (1-9)	Off-flavor (0-5)	Flavor (1-9)	Off-flavor (0-5)
T1	7.00	0.00	6.13 a	0.46 c	5.43 a	0.61 a	5.28 a	0.96 b	4.57 a	1.57 abc	4.80 a	1.60 bc
T2	7.00	0.00	5.21 ab	0.83 c	4.22 a	1.74 a	5.20 a	1.24 b	4.43 a	1.52 abc	4.67 ab	1.40 c
T3	7.00	0.00	5.21 ab	1.04 bc	4.96 a	1.26 a	5.20 a	0.92 b	3.71 a	2.43 a	4.20 ab	1.93 bc
T4	7.00	0.00	4.04 c	2.08 a	4.13 a	1.78 a	3.40 b	2.72 a	3.19 a	2.33 ab	3.40 bc	2.73 ab
T5	7.00	0.00	5.88 a	0.83 c	4.52 a	1.57 a	5.16 a	1.16 b	4.76 a	0.86 c	4.00 ab	2.33 bc
T6	7.00	0.00	4.38 bc	1.83 ab	4.22 a	1.83 a	3.96 b	2.44 a	4.67 a	1.38 bc	2.47 c	3.80 a

Means within each storage with the same letter are not different ($p \leq 0.05$).T1=uncoated, T2=CW, T3=1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content

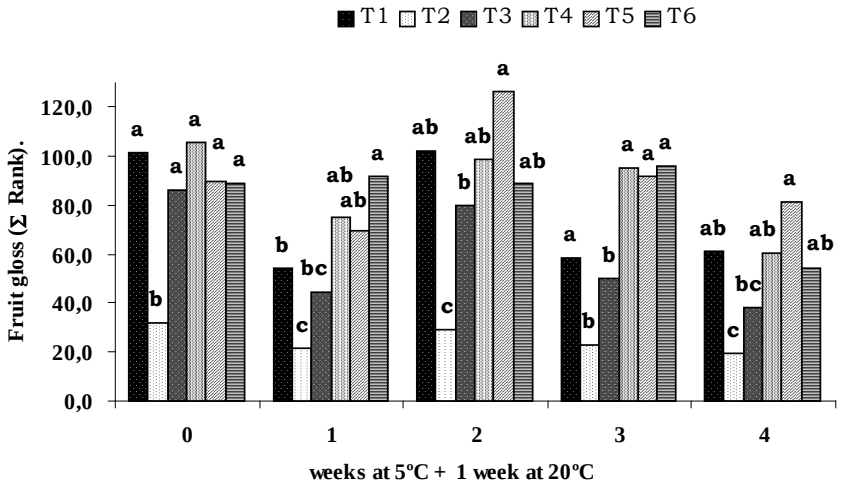

Figure 5. Gloss visual rank of coated and uncoated 'Oronules' mandarins during storage. Panelists ranked visually the treatments from highest (1) to lowest gloss (6) and the sum of the rank is presented. Within each storage period, bars with different letter are significantly different at $p \leq 0.05$.
T1=uncoated, T2=CW, T3=1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Many reports show a lower effectiveness of edible composite coatings providing gloss than commercial waxes. These differences could be related to differences in the lipid particle size. It has been observed that in order to obtain high gloss, wax coatings need to be prepared as microemulsions, so that when water evaporates the emulsion will have a smooth surface (Hagenmaier, 1998). The small lipid particle size of microemulsions makes the emulsion transparent to translucent (Prince, 1977) and as lipid particle size increases, emulsions lose transparency (Hernandez and Baker, 1991). In our experiment, all coating formulations were characterized by being translucent, and, therefore, it would be expected to have reduced gloss compared to commercial wax microemulsions. Although an increase in shellac content showed a slight increase in fruit gloss, the higher lipid particle size did not translate in a high gloss similar to the CW. In addition,

the increase in the SC of the coating would have translated in an increased coating thickness reducing transparency and gloss.

Bioactive compounds

Table 5 shows the content of the bioactive compounds analyzed of coated and uncoated 'Oronules' mandarins stored at 5 ºC for 0, 1, 2, 3, and 4 weeks plus 1 week at 20 ºC. The antioxidant capacity was expressed as EC_{50} or juice quantity necessary to reduce by 50% the $DPPH^{\bullet}$, thus the lower the value the higher the antioxidant capacity of the citrus fruit. The EC_{50} of mandarins was not affected by coating application or the storage length. In other study, coating application did not affect the total antioxidant capacity of 'Oronules' mandarins stored 9 days at 20 ºC; however, after 4 weeks of cold storage plus 1 week at 20 ºC, mandarins coated with a chitosan coating presented the lowest antioxidant capacity (Contreras-Oliva et al., 2010b). Artés-Hernández et al. (2007) found that the total antioxidant capacity in fresh-cut 'Lisbon' lemon products stored at different temperatures (0, 2, 5 or 10 ºC) remained constant during 12 days.

The TAA of 'Oronules' mandarins increased as storage time increased (Table 5). Although significant differences were found among treatments during storage, no tendency can be observed, which makes difficult to withdraw any conclusion regarding the effect of coating composition. This variability in the results during storage can be due to biological variation of the fruit. After 3 and 4 weeks of cold storage plus 1 week at 20 ºC, mandarins coated with T3 (BW:shellac ratio (1:3) with 4% SC) presented the highest TAA content. However, Contreras-Oliva et al. (2010b) reported that TAA of 'Oronules' mandarins was not affected by application of a chitosan coating or the storage length. Togrul and Arslan (2004) reported that AA loss after storage was delayed when mandarins were coated with carboxymethyl cellulose. This result was explained by the gas barrier of the coatings which decreased the potential autoxidation of ascorbic acid in the presence of oxygen.

The results showed that HES was the more abundant FGs in mandarins 'Oronules' followed by NAT and DID (Table 5). The contents of the different FGs were not affected by storage length. Similarly, these FGs were not affected after 3 months of storage at 5 ºC in 'Fortune' mandarin (Palma et al., 2005) or 24 days of storage at cold-quarantine temperature at 1 ºC in 'Valencia' oranges (Contreras-Olivas et al., 2010a). In general, coating

application had not an important effect on the level of the different FGs, although some significant differences were found among treatments for HES after 3 and 4 weeks at 5 ºC plus 1 week at 20 ºC. Application of a chitosan coating at different SC did not affect the FGs contents of 'Oronules' mandarins during storage at 5 ºC (Contreras-Olivas et al., 2010b).

Citrus fruit, in addition to flavanones, also contains other phenolic compounds, such as flavones and hydroxycinnamic acids (represented by ferulic, caffeic, synapic, and p-coumaric acids) that, although present in a lower concentration, contribute to the TPC (Rapisarda et al., 1999; Gil-Izquierdo et al., 2002). In the present work, the TPC is similar to those reported in 'Oronules' mandarin coated with chitosan coatings (58.43±27.8 to 71.92±41.3 mg/100 mL juice) (Contreras-Oliva et al., 2010b). Although some significant differences were found among treatments after 3 and 4 weeks of storage at 5 ºC, no tendency was found due to coating application, which makes difficult to withdraw any conclusion regarding the effect of coating composition.

After 1 week of storage at 20 ºC and 1 week at 5 ºC plus 1 week at 20 ºC, the TPC of 'Oronules' mandarins showed an increase over the initial value. However, during the next storage periods the TPC decreased to values close to the initial value. Some works have shown that cold storage either did not influence or decreased the citrus TPC. For example, Palma et al. (2005) did not find differences in the TPC of 'Fortune' mandarins after 90 d of storage at 5 ºC; whereas, Rapisarda et al. (2008) found a decrease of total phenolics in 'Valencia' oranges after 40 d of storage at 6 ºC, which was attributed to senescence during storage.

Table 5. Antioxidant activity (EC_{50}), total ascorbic acid (TAA), flavonoids and total Phenolics contents of coated and uncoated 'Oronules' mandarins after storage.

		EC_{50} (L juice/Kg DPPH)	TAA (mg/100 mL juice)	Narirutin (mg/100 mL juice)	Hesperidin (mg/100 mL juice)	Didymin (mg/100 mL juice)	Total phenolics (mg/100 mL juice)
Initial		339.52±10.93	45.1±1.9	0.87±0.15	18.9± 1.0	0.08±0.01	61.8±0.62
0 wk 5ºC + 1wk 20ºC	T1	301.3± 8.4 a	38.2± 3.1 a	1.00±0.12 a	18.6±1.1 a	0.09±0.01 a	67.2±4.0 a
	T2	294.0± 7.6 a	45.2± 4.4 ab	1.03±0.19 a	19.2±1.6 a	0.09±0.02 a	77.5±6.5 a
	T3	291.2±36.8 a	51.9± 2.8 bc	0.88±0.12 a	19.1±1.0 a	0.08±0.01 a	79.8±3.5 a
	T4	278.8±19.8 a	58.9± 8.6 c	0.97±0.05 a	22.5±1.0 c	0.09±0.01 a	74.1±3.1 a
	T5	282.5±16.9 a	43.4± 2.9 a	1.01±0.07 a	21.7±0.8 bc	0.10±0.01 a	69.1±9.5 a
	T6	260.2±17.0 a	40.1± 3.7 a	1.01±0.02 a	20.1±0.2 ab	0.10±0.00 a	68.4±2.6 a
1 wk 5ºC + 1wk 20ºC	T1	264.6±25.4 a	83.9± 2.1 c	1.05±0.01 a	21.2±0.9 ab	0.10±0.00 a	76.5±6.9 a
	T2	282.8± 4.7 a	64.1±13.6 b	1.03±0.12 a	20.1±0.6 a	0.09±0.00 a	78.1±2.5 a
	T3	298.4±16.4 a	75.9± 6.8 bc	1.06±0.15 a	20.5±1.4 a	0.10±0.01 a	81.6±6.4 a
	T4	290.6± 3.3 a	74.6±13.9 bc	1.26±0.10 a	22.1±0.3 bc	0.12±0.01 a	83.3±3.0 a
	T5	273.2±28.7 a	68.2± 5.0 bc	1.09±0.18 a	21.1±1.1 ab	0.11±0.02 a	79.9±6.7 a
	T6	270.5±25.7 a	37.5± 5.9 a	1.21±0.16 a	23.4±0.6 c	0.11±0.02 a	84.4±2.3 a
2 wk 5ºC + 1wk 20ºC	T1	271.9±23.4 a	76.7±11.7 b	0.89±0.05 a	20.4±0.9 a	0.11±0.00 b	62.7±2.1 a
	T2	260.7±21.4 a	59.9±22.8 ab	0.96±0.17 a	19.9±2.0 a	0.09±0.02 b	62.2±2.9 a
	T3	270.4± 6.1 a	56.3±17.2 ab	1.00±0.25 a	19.4±2.1 a	0.09±0.02 b	60.3±3.3 a
	T4	274.4±17.2 a	47.6±10.7 a	1.14±0.12 a	23.6±1.5 a	0.06±0.01 a	60.8±1.7 a
	T5	258.4±46.9 a	53.3±18.7 ab	1.11±0.17 a	23.7±2.2 a	0.11±0.01 b	61.5±1.9 a
	T6	246.3±33.1 a	110.9±3.6 c	1.05±0.13 a	23.2±2.7 a	0.09±0.01 b	63.2±0.9 a
3 wk 5ºC + 1wk 20ºC	T1	231.7±10.8 a	57.45± 2.9 a	1.02±0.04 a	23.2±1.0 c	0.11±0.00 a	68.5±2.9 b
	T2	279.5±34.6 a	78.5±14.6 b	0.92±0.12 a	18.4±1.9 ab	0.08±0.01 ab	61.9±0.5 a
	T3	297.6±50.2 a	119.3±14.9 c	0.96±0.10 a	19.5±1.0 ab	0.09±0.00 b	65.6±2.4 b
	T4	283.5± 4.3 a	64.16±9.5 ab	0.87±0.03 a	17.4±0.5 a	0.08±0.00 a	61.7±1.0 a
	T5	250.3±33.9 a	62.0±1.4 ab	0.99±0.13 a	19.6±1.5 ab	0.10±0.01 bc	67.0±0.8 b
	T6	252.4±16.2 a	60.6±1.3 a	0.98±0.05 a	20.6±1.0 b	0.09±0.01 ab	67.4±2.4 b
4 wk 5ºC + 1wk 20ºC	T1	271.9±36.0 a	98.0±1.01 ab	0.84±0.09 a	19.3±0.5 abc	0.08±0.01 a	66.8±1.5 bc
	T2	282.5±41.7 a	115.1±16.7 b	0.92±0.11 a	17.9±1.3 a	0.08±0.01 a	66.2±2.2 bc
	T3	286.2± 4.6 a	138.0± 4.5 c	1.05±0.03 a	20.8±0.5 bc	0.07±0.02 a	68.1±0.7 c
	T4	263.7±28.1 a	92.89±14.2 a	1.12±0.02 a	21.1±0.7 c	0.09±0.00 a	67.1±1.3 bc
	T5	251.1±18.6 a	92.8± 5.8 a	0.87±0.06 a	18.9±0.5 ab	0.08±0.00 a	64.8±1.9 ab
	T6	259.9±15.5 a	87.2± 6.1 a	0.96±0.21 a	20.3±2.1 bc	0.09±0.01 a	62.9±2.4 a

Means within each storage with the same letter are not different ($p \leq 0.05$).

T1=uncoated, T2=CW, T3=1:3 BW:Sh-4% SC, T4=1:3 BW:Sh-8% SC, T5=3:1 BW:Sh-4% SC, T6=3:1 BW:Sh-8% SC.

CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Conclusion

HPMC-coating application had little effect controlling weight loss of 'Oronules' mandarins. However, at the end of the storage period, the T4 coating (BW:shellac ratio 1:3 and 8% SC) was the most effective HPMC-based coating controlling weight loss, although it was less effective than the CW. Whereas, SC and the BW:shellac ratio had no clear effect on fruit weight and firmness loss, this factor affected the internal mandarin atmosphere and the ethanol content during storage. Increasing SC had a greater effect than the BW:shellac ratio in the fruit internal atmosphere, inducing off-flavor and reducing mandarin quality. Although appearance was slightly improved by increasing the shellac content in the formulation, the HPMC-based coatings did not provide similar gloss to commercial microemulsion waxes. These results showed the importance of controlling this parameters when selecting coating formulation. In general, the nutritional quality was not significantly affected by the application of the different coatings.

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CAPITULO VI

Effect of solid content and composition of hydroxypropyl methylcellulose-lipid edible coatings on physicochemical, sensory and nutritional quality of 'Valencia' oranges

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Abstract

'Valencia' oranges were coated with edible composite coatings based on hydroxypropyl methylcellulose (HPMC), beeswax (BW) and shellac. Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components (BW/shellac)-oleic acid (5:1) (db) were kept constant throughout the study. BW and shellac content was 60 % (db) and formulations were prepared at two different BW:shellac ratio (1:3 and 3:1) and two different percentages of total solid content (SC) (4 and 8%). A commercial wax (Polyethylene-shellac) at a 10% SC, as a control of coated fruit, and an uncoated control were also tested. Oranges were stored for 2, 4, 6, 8 and 16 weeks at 5 ºC, followed by 1 week at 20 ºC simulating retail storage conditions. Samples were periodically analyzed for physicochemical, sensory and nutritional quality. Coating application had little effect controlling weight loss of 'Valencia' oranges. Even though, no tendency was observed between SC and BW:shellac ratio of coating formulation and firmness, these factors affected the internal orange atmosphere and the ethanol content during storage. Increasing SC and shellac content in the formulation had a greater effect in fruit internal atmosphere and significantly increased the level of ethanol, showing the importance of controlling these parameters when selecting coating formulation. In general, sensory quality, coating appearance and the nutritional quality was not affect by the application of the different coatings.

Keywords: edible coating, commercial wax, beeswax, shellac, postharvest quality.

Introduction

In the citrus industry, fruit coating is a normal practice to replace the natural waxes that are generally removed during washing with the purpose to reduce fruit weight loss, shrinkage and improve appearance. Coating application has also been proven to reduce the incidence of chilling injury and other rind disorders in citrus (Porat et al., 2004; Bajwa and Anjum, 2007). However, it has also been reported by many authors that coating of citrus can adversely affect fruit flavor (Baldwin et al., 1995; Hagenmaier, 2002; Porat et al., 2005), due to the overproduction of volatiles associated with anaerobic conditions.

Consumer interest in health, nutrition, and food safety combined with environmental concerns has renewed efforts in the development of new coating formulations that avoid the use of synthetic components used in many commercial coatings, such as polyethylene wax, and the use of ammonia or morpholine in the formulations. Major components of edible coatings include proteins, polysaccharides, and lipids. Additionally, some authors include shellac, which is a natural resin, as ingredient of natural coatings for fruits that are not consumed with peel like citrus fruits, even though it is not included in the GRAS ingredient list (Rhim and Shellhammer, 2005). These groups present advantages and disadvantages when used as coating ingredients. Generally, lipids and resins offer a good moisture barrier due to their hydrophobic nature, reducing water loss, shriveling and shrinkage of coated fruit. However, their non-polymeric nature limits their ability to form cohesive films. Proteins and polysaccharides are good film-formers and present an intermediate oxygen barrier between lipid and resin coatings at medium-high relative humidity, which helps controlling the gas exchange between the fruit and the environment reducing the appearance of off-flavor compared to commercial waxes (Baldwin and Baker, 2002). However, their hydrophilic nature makes them poor moisture barriers. For this reason, most natural coatings for fruits contain a combination of ingredients forming what is called “edible composite coatings”. Several other compounds such as plasticizers and emulsifiers may be added to the formulations to improve coating integrity and form stable emulsions when lipids and hydrocolloids are combined.

In the literature, many works report the effect of edible composite coatings on the postharvest quality of citrus fruits (Hagenmaier et al., 2002; Hagenmainer and Shaw, 2002; Pérez-Gago et al., 2002; Hagenmaier, 2004; Porat et al., 2005; Navarro-Tarazaga and Pérez-Gago, 2006; Navarro-Tarazaga et al., 2007; Navarro-Tarazaga et al., 2008a; Rojas-Argudo et al., 2009). Most of these studies provide information about the effect of coating composition, formulation solid content (SC), storage conditions and fruit cultivar on the physicochemical and sensory quality, however little information can be found on their effect on the nutritional quality of citrus fruit. Nowadays, nutritional and functional quality has gained great interest, being a component of the overall quality that is very much valued by consumers. Citrus fruits are an important source of vitamin C as well as bioactive compounds such as polyphenolic compounds, mainly flavonoids, with high antioxidant properties (Sánchez-Moreno et al., 2003) and

postharvest technologies should maintain fruit nutritional and functional quality until they reach the consumer. Therefore, the objective of this work was to study the effect of coating composition and formulation SC of hydroxypropyl methylcellulose (HPMC)-lipid edible coatings on the physicochemical, sensory and nutritional quality of 'Valencia' oranges.

Material and methods

Materials

HPMC (Methocel E15) was purchased from Dow Chemical Co. (Midland, MI, USA). Shellac and beeswax (BW) (grade 1) were supplied by Fomesa Fruitech, S.L. (Beniparrell, Valencia, Spain). Oleic acid and glycerol were from Panreac Química, S.A. (Barcelona, Spain). Silicone antifoam (FG-1510) and ammonia (25%) were from Dow Corning® (Belgium) and Scharlau (Sentmenat, Barcelona, Spain), respectively.

Reagents 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), potassium dihydrogen phosphate (KH_2PO_4), *meta*-phosphoric acid (MPA), phosphoric acid (H_3PO_4), folin-ciocalteu's phenolreagent, sodium carbonate (Na_2CO_3), gallic acid and standard L-ascorbic acid (AA) were purchased from Sigma (Sigma-Aldrich Chemie, Steinhein, Germany). Acetic acid glacial and dimethyl sulfoxide (DMSO) were from Scharlau (Sentmenat, Spain). Methanol was from BDH Prolabo (Poole, UK), 1,4-dithio-DL-threitol (DTT) and hesperidin (hesperitin-7-0-rutinoside, HES) were obtained from Fluka (Sigma Co., Barcelona, Spain). Narirutin (naringenin-7-rutinoside, NAT) and didymin (isosakuranetin-7-rutinoside, DID) were purchased from Extrasynthese (Genay, France). All solvents used were of HPLC-grade and ultrapure water (Milli-Q) was used for the analysis.

Coating Formulation

Emulsion coatings consisted on HPMC and different ratios of BW and shellac suspended in water. Oleic acid and glycerol were added as emulsifier and plasticizer, respectively. Ratios of HPMC-glycerol (2:1) (dry basis, db) and lipid components (BW/shellac)-oleic acid (5:1) (db) were kept constant throughout the study. BW and shellac content was 60% (db), but formulations were prepared at two different BW:shellac ratio (1:3 and 3:1)

and two SC (4 and 8%). Table 1 and 2 show the coating treatments applied to the 'Valencia' oranges and the composition of the HPMC-based coatings, respectively.

Table 1. Treatments applied to 'Valencia' oranges.

Treatment	BW:Shellac ratio	Solid Content (%)	Viscosity (cP)
T1: *Uncoated*	--		
T2: CW	--	10	
T3: 1:3 BW:Sh - 4% SC	1 : 3	4	16.3
T4: 1:3 BW:Sh - 8% SC	1 : 3	8	30.2
T5: 3:1 BW:Sh - 4% SC	3 : 1	4	19.5
T6: 3:1 BW:Sh - 8% SC	3 : 1	8	34.0

BW=beeswax; CW=commercial wax; HPMC=hydroxypropyl methylcellulose; Sh=Shellac. T3, T4, T5 and T6: HMPC-based edible coatings.

Table 2. Composition of the HPMC:lipid edible coatings (%, wet basis).

Treatment	HPMC	BW	Shellac	Glycerol	Oleic acid
T3	0.75	0.60	1.80	0.37	0.48
T4	1.49	1.20	3.60	0.75	0.96
T5	0.75	1.80	0.60	0.37	0.48
T6	1.49	3.60	1.20	0.75	0.96

HPMC = Hydroxypropyl methylcellulose; BW = Beeswax.

Emulsions were made in a 2-L stirred pressure cell (Parr Instrument Co., Molline, IL), in which glycerol, oleic acid, BW, shellac, NH_3, and one-third of the water were added. The mixture was initially stirred at 100 rpm until the temperature reached 60 ºC. Next, stirring was increased to 400 rpm until temperature reached 110 ºC and remained at these conditions for 30 min. Afterwards, the remaining water, previously heated to 90 °C, was pumped into the vessel maintaining the stirring conditions at 400 rpm for about 10-15 min after the water was incorporated. The emulsion was then removed from the pressure vessel and mixed with a 5% HPMC solution previously prepared by dispersing the HPMC in hot water at 90 ºC and later hydration at 20 °C for 45 min. Finally, the emulsions were cooled under agitation to a temperature lower than 20 °C by placing them in an ice water bath. Water was added to a final SC of 4 or 8% depending on the treatment.

Emulsion viscosity

Emulsion viscosity was measured with a viscometer Synchro-Lectric viscometer Model LVF (Brookfield Engineering Laboratories, Inc.). Three measurements were made per emulsion and results were expressed as centipoises (cp). Sample viscosity was measured at 20 ºC.

Fruit preparation–coating application

'Valencia' oranges (*Citrus sinensis*) were hand-harvested with an average maturity index of 8.69 from a local grove in Valencia (Spain) and transferred to the IVIA postharvest facilities where they were selected, randomized, washed with tap water, and dipped in a solution of imazalil (1,000 ppm) for 1 min.

The oranges were randomly divided into 6 groups: 4 experimental coating treatments, 1 uncoated (CTL), and 1 commercial wax (CW) (Polyethylene-shellac) applied at 10% SC as a control of coated fruit (Table 1). The fruits were dip-coated by immersion in the coating solutions for 20 sec, drained of excess coating and dried in tunnel at 50 ºC for 2 min (Pérez-Gago et al., 2002). After coating, fruit were stored for 2, 4, 6, 8 and 16 weeks at 5 ºC and 90-95% RH, followed by 1 additional week at 20 ºC to simulate retail storage conditions.

Physicochemical quality

Weight loss

Lots of 30 fruits per treatment were used to measure weight loss. The same fruit were weighed at the beginning of the experiment and at the end of each storage period. The results were expressed as the percentage loss of initial weight.

Fruit firmness

Firmness of 20 oranges per treatment was determined at the end of each storage time using an Instron Universal Testing Machine (Model 3343, Instron Corp., Canton, MA, USA). The instrument gave the deformation (length) after application of a compression load of 10 N to the equatorial

region of the fruit at a rate of 5 mm/min. Results were expressed as percentage deformation related to initial diameter.

Internal gas concentration

Ten fruit per treatment were used to calculate internal gas concentrations. Internal CO_2 and O_2 concentrations of each sample were obtained by withdrawing 1 mL internal gas sample from the orange central cavity with a syringe while the fruit was immersed under water. The gas sample was then injected into a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA) fitted with a Porapak QS 80/100 (1.2 m x 0.32 cm) column, followed by a molecular sieve 5A 45/60 (1.2 m x 0.32 cm) column. Temperatures were 35, 125 and 180 ºC, respectively, for the oven, injector and thermal conductivity detector. Helium was used as carrier gas at 22 mL/min flow rate. Peak areas obtained from standard gas mixtures were determined before and after analysis of samples and results were expressed as percentage.

Ethanol and acetaldehyde content

Ethanol and acetaldehyde content (EC and AC) in juice were determined by head-space gas chromatography according to the method described by Ke and Kader (1990). Ten fruits each in 3 replicates per treatment were analyzed. Five mL orange juice were transferred to 10 mL vials with crimp-top caps and TFE/silicone septum seals and frozen until analysis. EC and AC were analyzed using a gas chromatograph (Thermo Fisher Scientific, Inc., Waltham, MA, USA) equipped with an autosampler, a flame ionization detector and fitted with a Poropak QS 80/100 column (1.2 m x 0.32 cm). Temperatures of the oven, injector, and detector were 150, 175, and 200 ºC, respectively. Helium was used as the carrier gas at a flow rate of 28 mL/min. A 1 mL sample of the head-space was withdrawn from each vial previously equilibrated in the autosampler incubation chamber for 10 min at 40 ºC. EC and AC concentrations were calculated using peak areas of the samples relative to the peak areas of standard solutions. Results were expressed as mg/100 mL juice.

External disorders

Eighty fruit per treatment were inspected for physiological disorders at the end of each storage period. The different degrees of physiological disorders were rated as 0=none, 1=light, 2=moderate and 3=severe. Light

was considered when less than 10% of fruit surface was affected and severe when more than 20% of fruit surface was affected. Results were converted to an average index.

Internal quality parameters

Soluble solids content (SSC) was measured with a digital refractometer (Atago, Model PR1) and titratable acidity (TA) was determined by titration with 0.1 N NaOH up to pH=8.1 and expressed as g of citric acid per 100 mL of orange juice. The maturity index (MI) was calculated as SSC/TA ratio. The juice from three replicates of 10 fruit each was used to determine the above parameters.

Sensory quality

Sensory evaluation was conducted by 10 trained judges (5 females and 5 males), 25 to 50 years old, at the end of each storage period. Panelists evaluated overall flavor and off-flavor of mandarins. Overall flavor was rated on a 9-point scale, where 1 to 3 represented a range of non-acceptable quality with the presence of off-flavor, 4 to 6 represented a range of acceptable quality, and 7 to 9 represented a range of excellent quality. Off-flavors presence was evaluated using a 6-point intensity scale where 0=absence of off-flavor and 5=high presence of off-flavor. Six fruit per treatment were pealed and separated into individual segments. Two segments from two different fruit were presented to judges in trays labeled with 3-digit random codes and served at room temperature (25±1 ºC). The judges had to taste several segments of each treatment in order to compensate, as far as possible, for biological variation of material. Mineral spring water was provided for rinsing between samples. External aspect of treated fruit (coating cracks, spots, etc.) was also evaluated by the panelist. A 3-point scale was used, in which the aspect was classified as 1=bad, 2=acceptable, and 3=good. Panelists were also asked to rank visually the treatments from highest to lowest gloss. Sum of rankings were calculated (UNE 87 023; AENOR, 1995). The lowest sum of ranking indicates the highest glossy treatment. For visual aspect (external aspect and gloss ranking), four intact fruit per treatment were placed in trays labeled with 3-digit random codes and presented to the judges under the same conditions (light intensity and temperature) to minimize variations in human perception.

Nutritional quality

Total antioxidant capacity (EC_{50})

The total antioxidant capacity was evaluated by the $DPPH^{\bullet}$ assay. 0.4 mL of orange juice diluted with 0.8 mL of methanol was centrifuged at 12,000 rpm and 4 ºC for 20 min. Six methanolic dilutions from the supernatant (0.075 mL) were mixed with 0.2925 mL of $DPPH^{\bullet}$ (24 mg L^{-1}) and kept in darkness for 40 min. Afterwards, the change in absorbance at 515 nm was measured in a Multiskan spectrum microplate reader (Thermo Labsystem, USA). For each dilution, the percentage of remaining $DPPH^{\bullet}$ was determined on the basis of the $DPPH^{\bullet}$ standard curve. The amount of juice in each dilution was plotted against the amount of $DPPH^{\bullet}$ radical remaining. Using the curve obtained, the EC_{50} value was calculated. This result expressed the amount of orange juice (L) needed to reduce 1 kg of $DPPH^{\bullet}$ by 50%; thus, lower values mean higher antioxidant activity.

Total ascorbic acid (TAA)

TAA was determined by the sum of AA plus L-dehydroascorbic acid (DHA), by using the reducing agent DTT (Sánchez-Mata et al., 2000). One mL of orange juice was diluted to 10 mL with 2.5% (*w/v*) MPA. Two mL of this solution were mixed with 0.4 mL of DTT (20 mg mL^{1}) for 2 h in darkness. Afterwards, the extracts were filtered through a 0.45 µm Millipore filter before being HPLC analyzed.

The HPLC analyses were performed on a Lachrom Elite HPLC (Merck Hitachi, Germany) equipped with a autosampler (Model L-2200), quaternary pump (Model L-2130), column oven (Model L-2300), and diode array detector (Model L-2450). A reversed-phase C18 LiChrospher®100 column (250 x 4 mm, 5 µm-particle, Merck, Darmstadt, Germany) preceded by a precolumn (4 x 4 mm) was used. System conditions were: injection volume 20 µL, oven 25 ºC, detector wavelength 243 nm, flow rate 1 mL min^{-1}. The mobile phase was 2% KH_2PO_4 adjusted to pH 2.3 with H_3PO_4. AA was identified and quantified by comparison of peak areas with external standard and results were expressed as milligrams of AA per 100 mL of juice.

Flavanone glycosides (FGs)

The main FGs identified in citrus fruit (HES, NAT, and DID) were determined by the method described by Cano et al. (2008) slightly modified. Two mL of orange juice were homogenized with 2 mL of DMSO:methanol (1:1 *v/v*) and centrifuged for 30 min at 12,000 rpm and 4 ºC. The supernatant was filtered through one 0.45 μm nylon filter and analyzed by HPLC-DAD using the HPLC equipment described above. System conditions were: injection volume 10 μL, oven 25 ºC, detector wavelength 280 nm, flow rate 1 mL min^{-1}. The column Lichospher 100 RP-18 of 25x0.4 cm was preceded by a precolumn (4x4 mm) with 5 μm particle size (Merck, Darmstadt, Germany). The mobile phase was acetonitrile (A):0.6% acetic acid (B) with initial condition of 10% A for 2 min, reaching 75% A in the following 28 min, then back to the initial condition in 1 min and held for 5 min prior to the next sample injection. The main FGs were identified by matching their respective spectra and retention times with those of commercially obtained standards. NAT, HES and DID contents were calculated by comparing the integrated peak areas of each individual compounds to that of its pure standards. Results were expressed as mg/100 mL.

Total phenolic content (TPC)

The orange juices were analyzed for total phenolics by the Folin-Ciocalteu colorimetric method. 0.3 mL of orange juice was diluted with 1.7 mL of 80% aqueous methanol. Appropriately diluted extract (0.4 mL) was mixed with 2 mL of folin ciocalteau commercial reagent (previously diluted with water 1:10, *v/v*) and incubated for 1 min before 1.6 mL sodium carbonate (7.5% *w/v*) was added. The mixture was incubated for 1 h at room temperature. The absorbance of the resulting blue solution was measured spectrophotometrically at 765 nm (Thermo UV1, Thermo Electron Corporation, UK) and the TPC was expressed as gallic acid equivalents per 100 mL (mg GAE/100 mL).

Total antioxidant capacity, TAA, FGs and TPC were determined in juice from three replicates of 10 fruit each.

Statistical Analysis.

A complete randomized design was used to perform the analysis of the samples. Statistical analysis of the results was performed by one-way analysis of variance (ANOVA) using STATGRAPHICS Plus 4.1 (Manugistics, Inc., Rockville, Maryland, U.S.A.). Significance differences between means was determined by least significant difference test (LSD; $p \leq 0.05$) applied after the analysis of variance (ANOVA). For sensory gloss, specific differences were determined by Friedman test, which is recommended for ranking by the UNE 87 023 (AENOR, 1995). Significance differences were defined at $p \leq 0.05$.

Result and discussion

Physicochemical quality

Weigh losst

Figure 1 shows the weight loss of coated and uncoated oranges stored for 2, 4, 6, 8, and 16 weeks at 5 ºC, followed by 1 week at 20 ºC. Weight loss increased with storage time, increasing to nearly 12% after 16 weeks of storage at 5 ºC plus 1 week at 20 ºC in uncoated samples. No clear tendency was observed on the effect of coating application on weight loss. In general for short storage periods (up to 6 weeks of storage at 5 ºC plus 1 week at 20 ºC), coating application had little effect controlling oranges weight loss. After 8 weeks of storage, the CW (T2) and the HPMC-based coatings containing a BW:shellac ratio 1:3 (T3 and T4) were the most effective treatments controlling weight loss. However, at the end of the storage period the CW did not control fruit weight loss, being T3 the most effective coating controlling weight loss of ‘Valencia’ oranges.

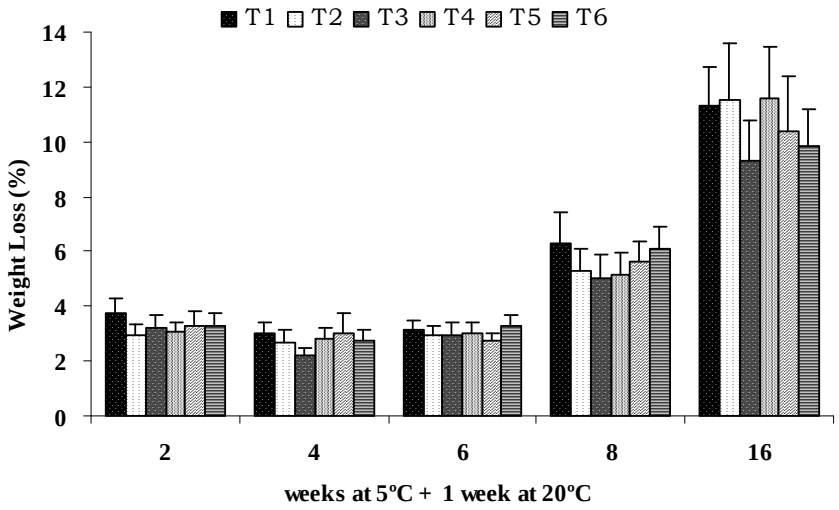

Figure 1. Weight loss of coated and uncoated 'Valencia' oranges during storage.
Error bars indicate standard deviations (n=30).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Application of HPMC-based edible coatings has been reported both with and without significant effects on weight loss of some fruit. For example, Pérez-Gago et al. (2002) reported that HPMC–lipid composite containing different types of lipids reduced weight loss of coated 'Fortune' mandarins. However, HPMC-lipid coatings containing food preservatives did not control weight loss of 'Valencia' oranges after 60 d at 5 ºC followed by 7 d of shelf-life at 20 ºC (Valencia-Chamorro et al., 2009). In 'Angeleno' plums, HPMC–BW coatings containing different types of plasticizers did not reduce weight loss of the fruit as compared with uncoated samples (Navarro-Tarazaga et al., 2008b). Similarly, HPMC coatings containing soybean oil or carnauba wax had minimal effect on water loss of coated cherries or cucumbers (Baldwin et al., 1997).

Fruit firmness

Some effect of coating application maintaining fruit firmness was only observed after 4 and 16 weeks of storage at 5 °C plus 1 week at 20 ºC. After these storage periods, HPMC-based coatings reduced firmness loss of 'Valencia' oranges compared to uncoated samples (Figure 2). Even though some significant differences in texture were found among treatments, no tendency was observed between SC or BW:shellac ratio of coating formulations and firmness. The lack of tendency between coating type and fruit texture has also been reported by Rojas et al. (2002) in 'Fortune' mandarins.

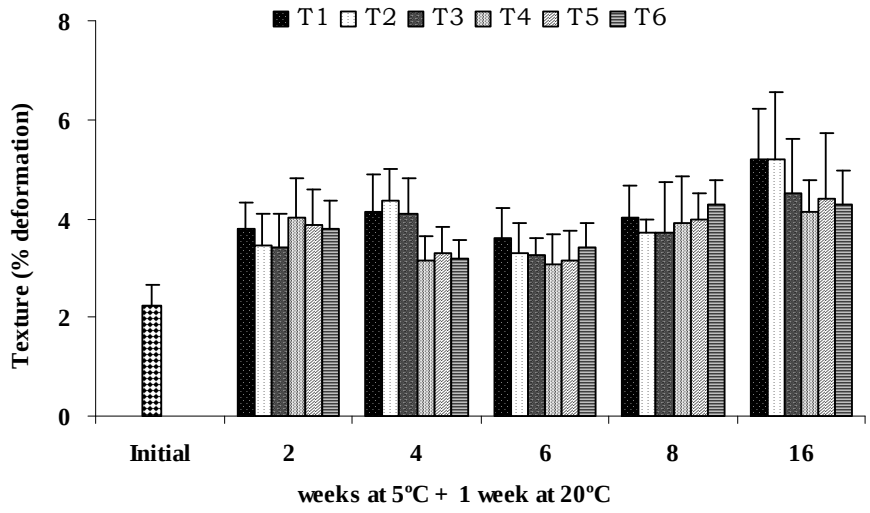

Figure 2. Firmness of coated and uncoated 'Valencia' oranges during storage.
Error bars indicate standard deviations (n=20).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Some investigators have observed a correlation between citrus fruit weight loss and firmness (Ben-Yehoshua, 1985; Pozzan et al., 1993; Navarro-Tarazaga et al., 2008a), whereas in this study and others no correlation has been found (Hagenmaier, 2000; Pérez-Gago et al., 2002). Differences in the results might indicate that in order to see an effect on fruit texture due to coating application, the coatings should provide sufficient weight loss control. Moreover, fruit cultivar and storage conditions could be a factor for the observed differences.

Internal gas concentration

Figure 3 shows the internal CO_2 and O_2 content of coated and uncoated 'Valencia' oranges during storage. All coatings increased the internal CO_2 and decrease the O_2 concentrations of the oranges compared to the control, which indicates that the coatings exerted a barrier to gas exchange. In general, the HPMC-based coatings exerted a higher gas barrier than the CW, although the effect depended on composition of the HPMC-based coatings. As SC of the HPMC-based coating was increased, internal CO_2 level of oranges increased and the O_2 level decreased. Many works have described a direct relation between the internal gas modification of coated fruit and coating thickness, which depends on SC, viscosity, and density of the coating formulation (Banks et al., 1993; Park et al., 1994; Cisneros-Zevallos and Krochta, 2003; Navarro-Tarazaga and Pérez-Gago, 2006).

For similar SC, coatings containing more shellac (BW:shellac ratio 1:3) induced a higher modification of the orange internal atmosphere, which can be explained by the higher gas barrier than shellac provides compared to waxes such as BW (Hagenmaier and Baker, 1994; Hagenmaier, 2000). Therefore, when comparing all the HPMC-based coatings, T4 was the treatment that induced the highest CO_2 and the lower O_2 accumulation in the fruit, since this coating had the highest SC and shellac content (8 % SC and BW:shellac ratio 1:3), whereas, at the end of the storage period oranges coated with T5 (4% SC and BW:shellac ratio 3:1) did not show differences in internal atmosphere with those coated with the CW.

Among the different ingredients incorporated to coating formulations, shellac has been known to reduce gas exchange in a greater extend than waxes, creating in many cases an anaerobic/fermentative environment in the fruit (Baldwin et al., 1995; Hagenmaier, 2000). Although the HPMC-based coatings and the CW contained shellac in their formulation, the

concentration of internal CO_2 and O_2 on coated oranges at the end of storage periods reached values around 7-11 and 5-11%, respectively. In general, these levels of internal O_2 could be considered not low enough to create anaerobic conditions inside the fruit (Baldwin et al., 1997).

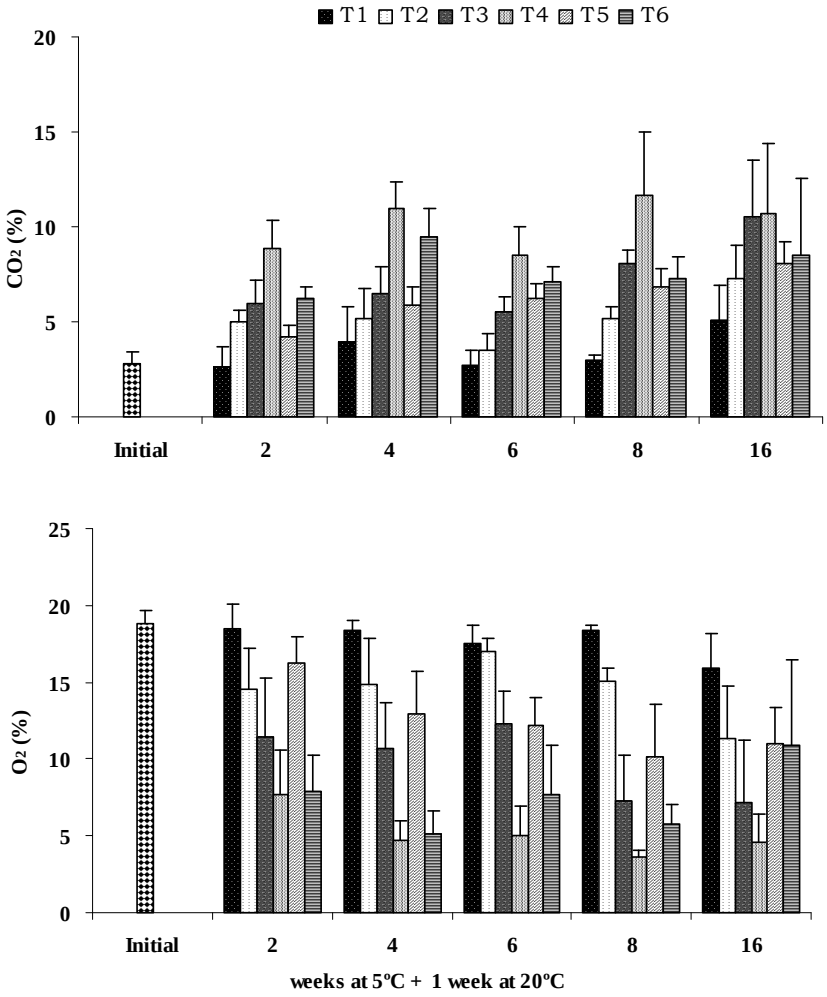

Figure 3. Internal CO_2 and O_2 contents of coated and uncoated 'Valencia' oranges during storage.
Error bars indicate standard deviation values (n=10).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Ethanol and acetaldehyde contents

Coatings induce an increase in the amount of some internal volatiles associated with anaerobic conditions. Ethanol has been found to be the volatile component undergoing the greatest change occurring in citrus during storage (Baldwin et al., 1995). Figure 4 shows the ethanol levels in juice for coated and uncoated oranges during storage. The results confirm the creation of a modified atmosphere, as can be seen by the lower ethanol accumulation during storage in uncoated fruit than in coated fruit.

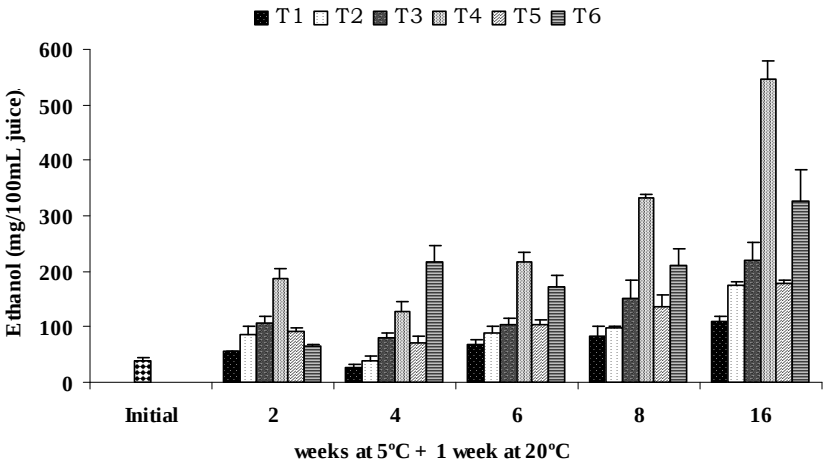

Figure 4: Ethanol Content of coated and uncoated 'Valencia' oranges during storage. Error bars indicate standard deviation values (n=3).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

As observed in the fruit internal atmosphere, the CW showed a moderate increase in ethanol level compared to some HPMC-based coatings. Comparing HPMC-based coatings, an increase in SC significantly increased the ethanol level in the fruit, which correlated with the higher gas barrier that these coatings offered to the fruit. Citrus fruit coated with shellac-based

coatings generally have been reported as having higher ethanol content than those treated with wax-based coatings (Hagenmaier and Baker, 1994; Baldwin et al., 1995; Hagenmaier, 2000). In our experiment, we found that in coatings with 4% SC, an increase in shellac content did not affect the ethanol level of 'Valencia' oranges; whereas, at 8% SC an increase in shellac content significantly increased the ethanol level. At the end of storage, coatings containing 4% SC showed ethanol levels close to the CW. Levels of ethanol were kept fairly constant during the 6 first weeks of storage at 5 °C followed by 1 week at 20 ºC. However, an important increased in ethanol level was observed after prolonged storage for 16 weeks at 5 °C plus 1 week at 20 °C, reaching values between 1780 and 5470 mg L^{-1} juice. Different workers have reported higher levels of ethanol on coated fruit after prolonged cold storage of citrus fruit. For instance, 'Fortune' mandarins coated with HPMC:lipid (20% lipid content, db) reached ethanol values between 3000 and 4000 mg L^{-1} after 30 days at 9 ºC plus 7 days at 20 ºC (Pérez-Gago et al., 2002). In another study with 'Ortanique' mandarins coated with HPMC:BW, the ethanol content was higher than 4000 mg L^{-1} after 45 days at 5 ºC plus 7 days at 20 ºC (Navarro-Tarazaga et al., 2008a). At the end of the storage period, acetaldehyde values ranged between 9.0 and 14.0 mg L^{-1} juice, and differences among treatments were not observed (data not shown).

External disorders

The physiological disorders considered were stem-end-rind-breakdown (SERB) and chilling injury. SERB is generally imputed to be an imbalance in nutrition, besides water loss between picking and packaging, but its influence in storage at low temperature has also been reported as a cause.

SERB was not observed during all the storage period. Slight chilling injury (stained) was observed after 16 weeks of storage at 5 ºC plus 1 week at 20 °C, probably due to water loss in the fruit, but values could be considered insignificant. 'Valencia' orange is a chill-sensitive fruit and storage temperatures below 2 ºC are reported to be the limit to avoid chilling injury (Martínez-Jávega et al., 1999). In our case, oranges were stored above this limit avoiding the appearance of this disorder.

Waxing has been demonstrated to reduce chilling injury in grapefruit, oranges, pineapples, and cucumbers. The effectiveness of waxing in

alleviating chilling injury is related by many authors to reducing moisture loss and modifying internal atmosphere (Wang, 2000).

Internal quality parameters

Table 3 shows the effect of coating application on SSC, TA and MI of 'Valencia' oranges after 16 weeks of storage at 5 ºC plus 1 week at 20 ºC. SSC of 'Valencia' oranges were not affected by application of the coatings or storage time. Whereas, TA was reduced after 16 weeks of storage at 5 ºC plus 1 week at 20 ºC, except for those oranges coated with 8% SC coatings that maintained initial TA values. This translated in higher MI for uncoated samples and those coated with the CW and HPMC-based coatings at 4% SC. Some authors have found no differences in these parameters after coating application on different citrus cultivars (Baldwin et al., 1995; Obenland et al., 2008); whereas others have found a decrease in SSC and TA losses compared to uncoated fruits, which was always related to a decrease in weight loss and respiration rate (Togrul and Arslan, 2004). Since, the application of the coatings in the present work had no effect on fruit weight loss, the results obtained could be related to the higher effect on the HPMC-based coatings with 8% SC on the orange internal atmosphere.

Table 3. Soluble solid content, titratable acidity and maturity index of coated and uncoated 'Valencia' oranges stored 16 weeks at 5 ºC followed by 1 week at 20 ºC.

Treatment	SSC (ºBrix)	*TA* (g citric acid / 100 ml)	MI
Initial (at harvest)	11.33±0.28	1.31±0.07	8.69±0.38
T1	11.60±0.18 a	0.82±0.04 ab	14.15±0.68 bc
T2	11.65±0.22 a	0.84±0.03 ab	13.95±0.08 bc
T3	11.72±0.26 a	0.87±0.03 bc	13.47±0.21 ab
T4	11.57±0.36 a	0.92±0.06 c	12.56±0.51 a
T5	11.27±0.23 a	0.78±0.04 a	14.90±0.68 c
T6	11.88±0.40 a	0.92±0.06 c	12.90±0.68 a

SSC= Soluble solid content; TA= titratable acidity; MI=Maturity index.
Mean values±standard deviations (n=3).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Sensory quality

Sensory quality of 'Valencia' oranges was evaluated within the range of acceptability after 16 weeks of storage at 5 ºC plus 1 week at 20 ºC, with values around 4 and no differences were found among treatments (Table 4). Under this storage conditions, coated and uncoated oranges were evaluated as having very slight or slight off-flavor. Several works showed that the contribution to off-flavor of volatile content depends on citrus cultivar. Ke and Kader (1990) established the minimum ethanol content associated with off-flavor in 'Valencia' oranges to be 2000 mg L^{-1}; whereas, Pérez-Gago et al. (2002) found flavor degradation in 'Fortune' mandarin at an ethanol content above 3000 mg L^{-1} and Navarro-Tarazaga and Pérez-Gago (2006) found that ethanol content of 1000 mg L^{-1} reduced flavor quality of 'Clemenules' mandarins. In this work, the ethanol level found at the end of the storage period (5470 mg L^{-1}) was well above the limit shown by other authors associated with off-flavor development.

The general coating appearance of the orange was evaluated as acceptable throughout all the storage time (data not shown). One of the aims of coating applications, together with the control of the weight loss, is the enhancement of external citrus appearance by conferring gloss. Panelists were asked to rank the five treatments on the basis of perceived gloss (1=the most glossy and 6=the least glossy) and the sum of the rank values was calculated (Figure 5). Therefore treatments with low scores represent shinier oranges. Among all the coatings, treatment T5 was not effective providing gloss during storage.

The experimental coating that provided the highest gloss was T4, being similar to that of the CW-coating during storage. This could be related to its higher SC and shellac content. It has been reported that shellac and other resins provide higher gloss to fruit than waxes, this being the main reason for their incorporation into many coating formulations (Baldwin et al., 1997; Hagenmaier and Baker, 1994).

Table 4. Flavor and off-flavor of coated and uncoated 'Valencia' oranges after storage.

Treatments	Initial		2wk 5 ºC + 1wk 20 ºC		4wk 5 ºC + 1wk 20 ºC		6wk 5 ºC + 1wk 20 ºC		8wk 5 ºC + 1wk 20 ºC		16wk 5 ºC + 1wk 20 ºC	
	Off-flavor (0-5)	flavor (1-9)	Off-flavor (0-5)	flavor (1-9)	Off-flavor (0-5)	flavor (1-9)	Off-flavor (0-5)	flavor (1-9)	Off-flavor (0-5)	flavor (1-9)	Off-flavor (0-5)	flavor (1-9)
Initial (At harvest)	0.08	7.08										
T1			1.05 a	5.24 a	0.67 b	5.88 a	0.65 bc	5.55 ab	0.48 c	5.19 a	1.41 a	4.41 a
T2			0.76 a	5.24 a	0.92 b	5.79 ab	0.45 c	6.35 a	1.19 bc	4.81 ab	1.18 a	4.71 a
T3			0.86 a	5.48 a	1.13 b	5.29 abc	0.65 bc	6.30 a	1.90 ab	3.90 bc	2.06 a	4.35 a
T4			1.48 a	4.29 a	2.00 a	4.50 c	2.05 a	4.15 c	2.24 a	4.05 bc	1.82 a	3.82 a
T5			0.52 a	5.62 a	1.13 b	5.63 ab	1.35 ab	5.25 b	1.52 ab	4.43 abc	1.24 a	4.53 a
T6			1.14 a	4.95 a	1.46 ab	4.92 bc	1.95 a	3.90 c	2.33 a	3.57 c	1.24 a	4.24 a

Means within each storage with the same letter are not different ($p \leq 0.05$).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content

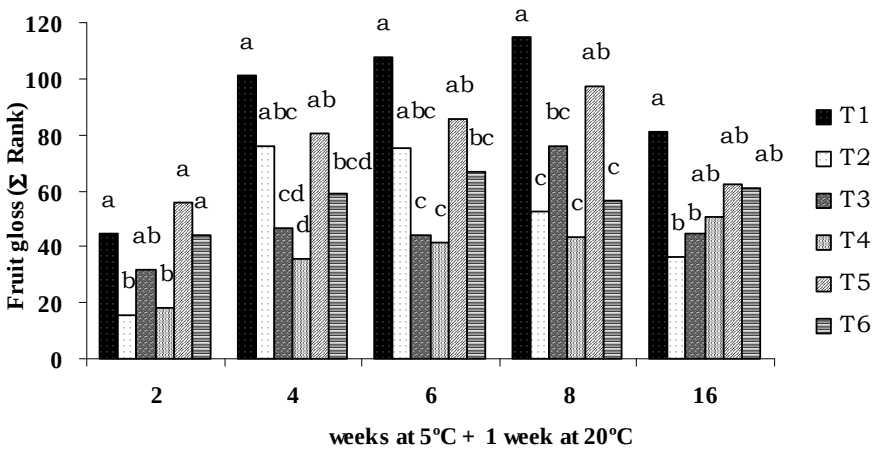

Figure 5: Gloss visual rank of coated and uncoated 'Valencia' oranges during storage.
Panelists ranked visually the treatments from highest (1) to lowest gloss (6) and the sum of the rank is presented. Within each storage period, bars with different letter are significantly different at $p \leq 0.05$.
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content

Nutritional quality

Total antioxidant capacity (EC_{50})

Table 5 shows the EC_{50} values of coated and uncoated 'Valencia' oranges stored at 5 ºC for 2, 4, 6, 8 and 16 weeks plus 1 week at 20 ºC. As mentioned earlier, the $DPPH^{\bullet}$ radical decreases by reacting with antioxidants present in the sample; therefore, a higher EC_{50} value indicates a lower total antioxidant capacity of the sample. In this work, no effect was observed by coating application in the total antioxidant capacity of the 'Valencia' oranges. Only, a decrease in the total antioxidant capacity was observed after 2 weeks of storage at 5 ºC plus 1 week at 20 ºC; however, a

further increase in storage time did not affect the total antioxidant capacity. Artés-Hernández et al. (2007) found that the total antioxidant capacity in fresh-cut 'Lisbon' lemon products stored at different temperatures (0, 2, 5 or 10 ºC) remained constant during 12 days.

Total ascorbic acid (TAA)

The TAA of the oranges was not affected by coating application or the storage length (Table 5). Togrul and Arslan (2004), however, reported that AA loss after storage was delayed when mandarins were coated with carboxymethyl cellulose. This result was explained by the gas barrier of the coatings which decreased the potential autoxidation of AA in the presence of oxygen. In our work, although HPMC-coatings and the CW reduced the level of internal O_2 (Figure 3), these levels could be not low enough to affect the TAA of the orange.

Flavanone Glycosides (FGs)

In citrus the major FGs are NAT, HES and DID. FGs contents in 'Valencia' oranges were in the range of those reported for citrus fruit (Table 5), being HES the most abundant flavanoid followed by NAT and DID (Dhuique-Mayer et al., 2005; Nogata et al., 2006). The content of the different flavonoids, were not affected by storage length. Similarly, these FGs were not affected after 3 months of storage at 5 ºC in 'Fortune' mandarin (Palma et al., 2005) or 24 days of storage at cold-quarantine temperature at 1 ºC in 'Valencia' oranges (Contreras-Oliva et al., 2010a). In general, coating application had not an important effect on the level of the different flavonoids, although some significant differences were found among treatments for NAT after 16 weeks of storage at 5 ºC plus 1 week at 20 ºC. Application of a chitosan coating at different SC did not affect the FGs contents of 'Oronules' mandarins during storage at 5 ºC (Contreras-Oliva et al., 2010b).

Table 5. Antioxidant activity (EC_{50}), total ascorbic acid (TAA), flavonoids and total phenolics contents of coated and uncoated 'Valencia' oranges after storage.

		EC_{50} (L juice/Kg DPPH)	TAA (mg/100 mL juice)	Narirutin (mg/100 mL juice)	Hesperidin (mg/100 mL juice)	Didymin (mg/100 mL juice)	Total phenolics (mg/100 mL juice)
Initial		232.9 ± 14.2	33.7 ± 1.7	2.8 ± 0.2	21.7 ± 1.1	0.9 ± 0.0	74.3 ± 8.0
2 wk 5 ºC + 1 wk 20 ºC	T1	371.6 ± 13.3 a	44.5 ± 1.7 a	3.8 ± 0.1 a	28.0 ± 0.5 b	1.2 ± 0.1 a	82.6 ± 4.1 a
	T2	345.6 ± 20.6 a	46.1 ± 0.1 a	3.4 ± 0.1 a	26.4 ± 0.4 a	1.1 ± 0.0 a	77.0 ± 4.8 a
	T3	334.4 ± 11.7 a	46.2 ± 2.9 a	3.6 ± 0.2 a	27.8 ± 0.4 b	1.1 ± 0.0 a	68.2 ± 8.8 a
	T4	337.4 ± 18.5 a	44.2 ± 3.3 a	3.7 ± 0.3 a	26.5 ± 0.7 a	1.1 ± 0.0 a	82.1 ± 3.1 a
	T5	341.1 ± 4.0 a	47.3 ± 1.8 a	3.6 ± 0.2 a	26.7 ± 0.5 a	1.0 ± 0.1 a	74.0 ± 10.6 a
	T6	331.2 ± 21.2 a	47.8 ± 0.9 a	3.6 ± 0.2 a	26.2 ± 0.7 a	1.1 ± 0.0 a	80.6 ± 1.7 a
4 wk 5 ºC + 1 wk 20 ºC	T1	337.8 ± 25.2 a	45.3 ± 2.8 a	3.8 ± 0.0 a	26.8 ± 0.4 a	1.1 ± 0.0 a	62.5 ± 2.1 a
	T2	344.8 ± 22.1 a	44.5 ± 3.2 a	3.4 ± 0.4 a	25.3 ± 0.4 a	1.0 ± 0.1 a	78.7 ± 2.3 b
	T3	339.1 ± 22.4 a	46.3 ± 2.1 a	3.5 ± 0.2 a	27.0 ± 1.7 a	1.2 ± 0.1 a	81.1 ± 3.2 bc
	T4	360.2 ± 23.0 a	42.0 ± 3.0 a	3.7 ± 0.1 a	25.0 ± 1.1 a	1.1 ± 0.0 a	78.4 ± 3.3 b
	T5	354.0 ± 22.5 a	43.4 ± 2.4 a	3.6 ± 0.2 a	28.8 ± 2.3 a	1.1 ± 0.0 a	78.3 ± 2.2 b
	T6	349.9 ± 18.0 a	41.7 ± 2.1 a	3.4 ± 0.2 a	27.6 ± 1.7 a	1.1 ± 0.0 a	83.5 ± 2.2 c
6 wk 5 ºC + 1 wk 20 ºC	T1	367.6 ± 35.7 a	42.2 ± 3.9 bc	3.7 ± 0.4 a	28.0 ± 2.2 a	1.1 ± 0.0 a	80.3 ± 1.2 a
	T2	353.7 ± 7.4 a	42.0 ± 0.8 bc	3.5 ± 0.4 a	25.5 ± 1.3 a	1.0 ± 0.0 a	82.1 ± 1.6 a
	T3	358.6 ± 7.1 a	42.1 ± 1.1 bc	3.3 ± 0.4 a	27.5 ± 2.0 a	1.2 ± 0.1 a	82.1 ± 3.0 a
	T4	345.4 ± 8.1 a	43.3 ± 4.4 c	3.4 ± 0.2 a	27.7 ± 2.1 a	1.2 ± 0.0 a	80.6 ± 1.3 a
	T5	360.0 ± 14.9 a	37.6 ± 1.8 ab	3.1 ± 0.1 a	28.9 ± 1.9 a	1.0 ± 0.1 a	82.4 ± 2.6 a
	T6	382.4 ± 24.5 a	34.0 ± 2.7 a	3.6 ± 0.0 a	29.0 ± 0.5 a	1.1 ± 0.0 a	86.0 ± 3.9 a
8 wk 5 ºC + 1 wk 20 ºC	T1	367.7 ± 15.0 a	36.6 ± 2.4 a	3.7 ± 0.1 a	22.0 ± 7.5 a	1.0 ± 0.0 a	86.6 ± 1.4 d
	T2	363.3 ± 25.4 a	34.2 ± 1.8 a	3.9 ± 0.1 a	26.2 ± 1.1 a	1.0 ± 0.0 a	79.4 ± 0.8 ab
	T3	341.7 ± 16.9 a	35.8 ± 1.5 a	3.7 ± 0.2 a	26.3 ± 0.6 a	0.9 ± 0.1 a	76.8 ± 2.7 a
	T4	338.1 ± 2.4 a	35.5 ± 0.7 a	3.7 ± 0.4 a	27.4 ± 0.4 a	1.0 ± 0.0 a	83.8 ± 2.9 cd
	T5	325.0 ± 31.4 a	37.9 ± 1.3 a	3.8 ± 0.1 a	27.0 ± 1.4 a	1.0 ± 0.0 a	82.7 ± 3.0 bcd
	T6	364.8 ± 15.2 a	35.5 ± 1.9 a	3.9 ± 0.2 a	27.1 ± 1.2 a	1.0 ± 0.0 a	82.4 ± 1.1 bc
16 wk 5 ºC + 1 wk 20 ºC	T1	385.8 ± 16.3 a	34.2 ± 2.7 a	4.6 ± 0.3 b	30.3 ± 1.6 a	1.2 ± 0.1 a	84.4 ± 0.4 a
	T2	368.8 ± 25.8 a	36.0 ± 1.9 a	4.4 ± 0.2 b	29.6 ± 1.2 a	1.1 ± 0.1 a	83.7 ± 3.8 a
	T3	383.3 ± 11.5 a	34.8 ± 1.0 a	3.9 ± 0.0 a	30.0 ± 0.9 a	1.0 ± 0.0 a	86.3 ± 1.4 a
	T4	345.1 ± 26.4 a	38.1 ± 3.3 a	3.8 ± 0.2 a	29.3 ± 0.9 a	1.0 ± 0.0 a	88.7 ± 1.3 a
	T5	378.6 ± 20.8 a	35.2 ± 2.6 a	4.4 ± 0.3 b	32.3 ± 0.7 a	1.1 ± 0.1 a	83.4 ± 1.2 a
	T6	352.0 ± 6.2 a	35.7 ± 1.4 a	3.9 ± 0.2 a	29.3 ± 2.2 a	1.0 ± 0.0 a	85.1 ± 2.1 a

Means within each storage with the same letter are not different ($p \leq 0.05$).
T1=uncoated, T2=CW, T3= 1:3 BW:Sh-4% SC, T4= 1:3 BW:Sh-8% SC, T5= 3:1 BW:Sh-4% SC, T6= 3:1 BW:Sh-8% SC.
CW=commercial wax, BW=beeswax; Sh=shellac; SC=solid content.

Total phenolic content (TPC)

The citrus fruit in addition to flavanones also contains other phenolic compounds, such as flavones and hydroxycinnamic acids (represented by ferulic, caffeic, synapic, and *p*-coumaric acids) that, although present in a lower concentration, contribute to the total phenolic concentration (Rapisarda et al., 1999; Gil-Izquierdo et al., 2002). TPC of 'Valencia' oranges ranged from 62.5±2.1 to 88.7±1.3 mg/100 mL juice (GAE) (Table 5). These results are in agreement with those found in orange juice (87.8 mg/100 mL) and pulp (71.7 mg/100 mL) following domestic and commercial squeezing (Gil-Izquierdo et al., 2002) and in commercial orange juices (from 50.4±1.0 to 75.5±1.8 mg/100 mL) (Gardner et al., 2000).

TPC of 'Valencia' oranges was not affected by storage time at 5 ºC. Other works have shown that cold storage at quarantine temperatures at 1 ºC increased TPC of 'Valencia' oranges (Contreras-Oliva et al., 2010a). However, Rapisarda et al. (2008) found a decrease of TPC in 'Valencia' oranges after 40 days of storage at 6 ºC attributed to senescence phenomena during storage. Other works have shown either an increase during storage, attributed to an increase in the PAL activity during low temperature storage of citrus fruit (Patil et al., 2004) or no effect, such as in 'Fortune' mandarins after 90 d of storage at 5 ºC (Palma et al., 2005). Although some significant differences were found among treatments after 4 and 8 weeks of storage at 5 ºC, no tendency was found due to coating application, which makes difficult to withdraw any conclusion regarding the effect of coating composition. Contreras-Oliva et al. (2010b) found an increase in the TPC of 'Oronules' mandarins coated with chitosan at 1.2% SC after 4 weeks of storage at 5 ºC plus 1 week at 20 ºC. This effect was attributed to a possible action of chitosan acting as plant exogenous elicitor in plant tissue inducing the biosynthesis of phenolic compounds, however, these authors suggest the need to confirm the results for other storage periods.

Conclusion

Coating application had little effect controlling weight loss of 'Valencia' oranges. However, at the end of the storage period T3 (4% SC and 1:3 BW:shellac ratio) was the most effective coating controlling weight loss, even better than the CW. Whereas, SC and the BW:shellac ratio had no

clear effect on fruit weight loss, this factor affected the internal orange atmosphere and the ethanol content during storage. Increasing SC and shellac content in the formulation had a greater effect in fruit internal atmosphere and significantly increased the level of ethanol, showing the importance of controlling this parameters when selecting coating formulation. In general, sensory quality, coating appearance and the nutritional quality was not affect by the application of the different coatings.

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CONCLUSIONES GENERALES

1. Los tratamientos innovadores de cuarentena aplicados, atmósferas insecticidas (AI) (95% de CO_2 a 20 ó 25 ºC) o irradiación por rayos X (RX) a dosis bajas (0, 30, 54 y 164 Gy), combinados con períodos cortos de almacenamiento en frío no afectaron negativamente a la capacidad antioxidante total ni al ácido ascórbico total de las mandarinas 'Clemenules'. Sin embargo, los glucósidos de flavanona y el contenido de fenoles totales fueron ligeramente modificados. La aplicación de la AI a 20 ºC indujo una mayor inhibición en la síntesis de glucósidos de flavanonas que las AI a 25 ºC. Cuando la irradiación por RX se aplicó sin previa cuarentena por frío, la síntesis de glucósidos de flavanona aumentó al aumentar la dosis de irradiación.

2. La aplicación en naranjas 'Valencia' de una AI con 95% CO_2 a 23, 28 ó 33 ºC durante 20 horas no afectó negativamente a su calidad poscosecha, por lo que podría ser utilizada como tratamiento insecticida o, combinado con temperaturas de curado, para el control de las podredumbres de naranjas 'Valencia'.

3. Entre las condiciones ensayadas, la exposición de las naranjas 'Valencia' a la AI de 95% CO_2 a 28 ºC redujo la pérdida de peso y firmeza comparado con la fruta control. Aunque el contenido de etanol aumentó en las naranjas 'Valencia' expuestas a 95% de CO_2 a 28 y 33 ºC comparado con la fruta control, estos niveles no afectaron a la calidad sensorial de las naranjas.

4. La combinación de las AI (95% CO_2 a 23, 28 ó 33 ºC) y periodos de cuarentena de 8 ó 16 días a 1 ºC no afectó al contenido en ácido ascórbico de las naranjas 'Valencia'. Sin embargo, cuando el periodo de cuarentena se incrementó a 24 días, los frutos expuestos a esta AI presentaron el contenido más bajo de ácido ascórbico total con respecto a las frutas control.

5. La aplicación de un recubrimiento comercial a base de quitosano a distintos contenidos en sólidos (CS) (0,6, 1,2 y 1,8%) no fue efectiva en el control de la pérdida de peso de las mandarinas 'Oronules' y naranjas 'Valencia' durante el almacenamiento en frío, mientras que la aplicación de una cera comercial a base de polietileno y goma laca disminuyó la pérdida de peso de la fruta en comparación con el control. Por tanto, con el fin de mejorar las propiedades barrera al agua del recubrimiento de quitosano sería necesario añadir componentes hidrofóbicos a la formulación.

6. Las mandarinas ‘Oronules’ recubiertas con quitosano a 1,8 de % CS presentaron menor porcentaje de deformación que las mandarinas control después del almacenamiento poscosecha. Sin embargo, no se observó ningún efecto de los recubrimientos de quitosano manteniendo la firmeza de naranjas ‘Valencia’. Los resultados indican que factores como el tipo de recubrimiento, condiciones de almacenamiento, o el cultivar influyen de manera significativa en la firmeza de las frutas recubiertas.

7. La aplicación del recubrimiento a base de quitosano restringió el intercambio gaseoso y modificó la atmósfera interna de las mandarinas ‘Oronules’ y naranjas ‘Valencia’ en comparación con las frutas control, aumentando el contenido de etanol y acetaldehído en zumo. Estos parámetros de calidad aumentaron al aumentar el CS de la formulación de quitosano. Sin embargo, los niveles alcanzados de compuestos volátiles se pueden considerar bajos, indicando que la restricción en el intercambio gaseoso provocada por los recubrimientos no fue lo suficientemente alta para crear condiciones anaeróbicas dentro del fruto y afectar negativamente a la calidad sensorial de las mandarinas ‘Oronules’ y naranjas ‘Valencia’.

8. En general, ni la aplicación del recubrimiento de quitosano a distinto CS, ni el periodo de almacenamiento tuvo un efecto importante en la calidad interna o en el contenido de los compuestos bioactivos de las mandarinas ‘Oronules’. Mientras que en naranjas ‘Valencia’ se observó un aumento durante el almacenamiento del contenido de los flavonoides mayoritarios, sin detectar un efecto importante debido al recubrimiento aplicado (quitosano o cera comercial).

9. La aplicación de recubrimientos comestibles a base de HPMC-lípido resultaron efectivos controlando la pérdida de peso de mandarinas ‘Oronules’ durante la frigoconservación, mientras que en naranjas ‘Valencia’ presentaron menor efectividad. El recubrimiento de HPMC-lípido con una relación cera de abeja:goma laca de 1:3 y aplicado al 8% CS (T4), aunque menos efectivo que la cera comercial, fue el recubrimiento experimental más efectivo controlando la pérdida de peso de las mandarinas ‘Oronules’ tras un almacenamiento prolongado. En naranjas ‘Valencia’, sin embargo, el recubrimiento de HPMC-lípido con una relación cera de abeja:goma laca de 1:3 y aplicado al 4% CS (T3) resultó el más efectivo controlando la pérdida de peso al final del almacenamiento, siendo más efectivo que la cera comercial.

10. El CS de los recubrimientos de HPMC-lípido y la relación cera de abeja:goma laca no tuvieron un efecto claro en la pérdida de peso o de firmeza de las mandarinas ‘Oronules’ y naranjas ‘Valencia’. Sin embargo, si que tuvieron un efecto en la atmósfera interna de la fruta recubierta.

11. Al aumentar el CS y el ratio de goma laca de los recubrimientos de HPMC-lípido aumentó el nivel de CO_2 y disminuyó el nivel de O_2 interno de las mandarinas ‘Oronules’ y naranjas ‘Valencia’, aumentando el contenido en etanol durante su almacenamiento poscosecha. En mandarinas ‘Oronules’, estos parámetros de calidad se vieron más afectados por el CS de las formulaciones que por el ratio de goma laca, dando lugar a la aparición de malos sabores cuando los recubrimientos se aplicaron a un 8% de CS. En naranjas ‘Valencia’, aunque un aumento en el CS y el ratio de goma laca en la formulación aumentó el nivel de etanol en zumo, la calidad organoléptica no se vio afectada por la aplicación de los recubrimientos.

12. En general, la calidad nutricional de mandarinas ‘Oronules’ y naranjas ‘Valencia’ no se afectó de manera significativa tras la aplicación de los recubrimientos (HPMC-lípido o cera comercial).

ANEXOS

Foto 1. Planta de irradiación Beta Gamma Service, BGM (Bruchsal, Alemania).

Foto 2. Cabinas utilizadas para la aplicación de atmósferas insecticidas (Centro de Tecnología Poscosecha, IVIA, Valencia, España).

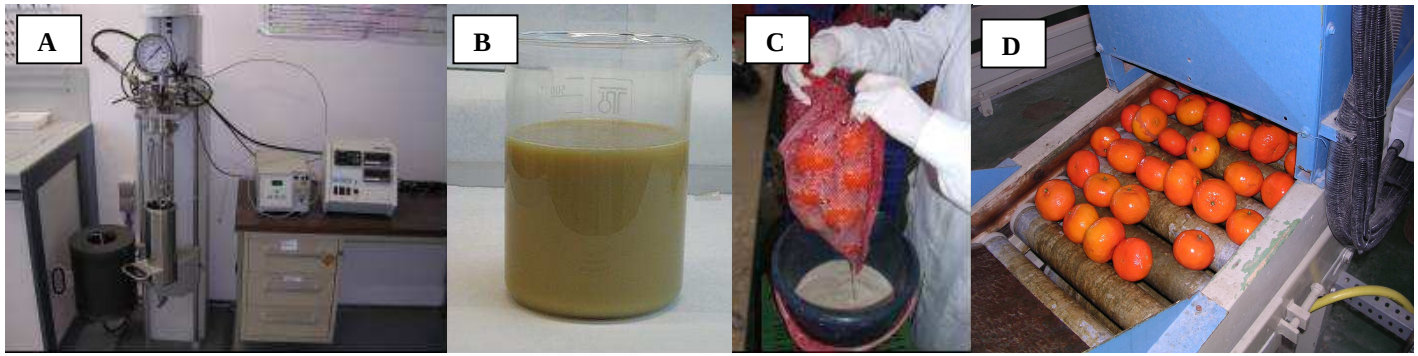

Foto 3. Preparación de recubrimientos comestibles: (**A**) reactor a presión, (**B**) formulación tipo, (**C**) aplicación por inmersión y (**D**) secado en tunel.

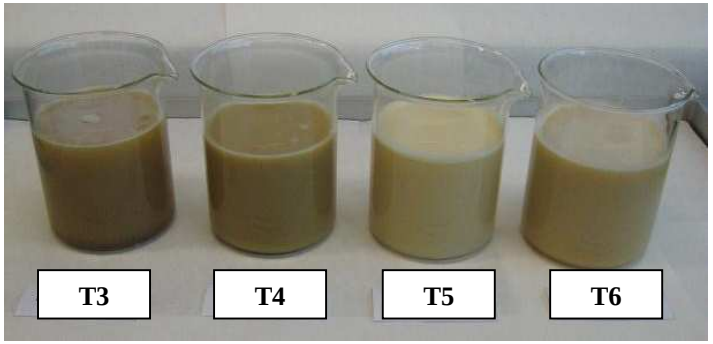

Foto 4. Formulaciones de recubrimientos comestibles compuestos a base de HPMC-lipido (**T3**=1:3 BW:Sh-4% SC, **T4**=1:3 BW:Sh-8% SC, **T5**=3:1 BW:Sh-4% SC, **T6**=3:1 BW:Sh-8% SC).

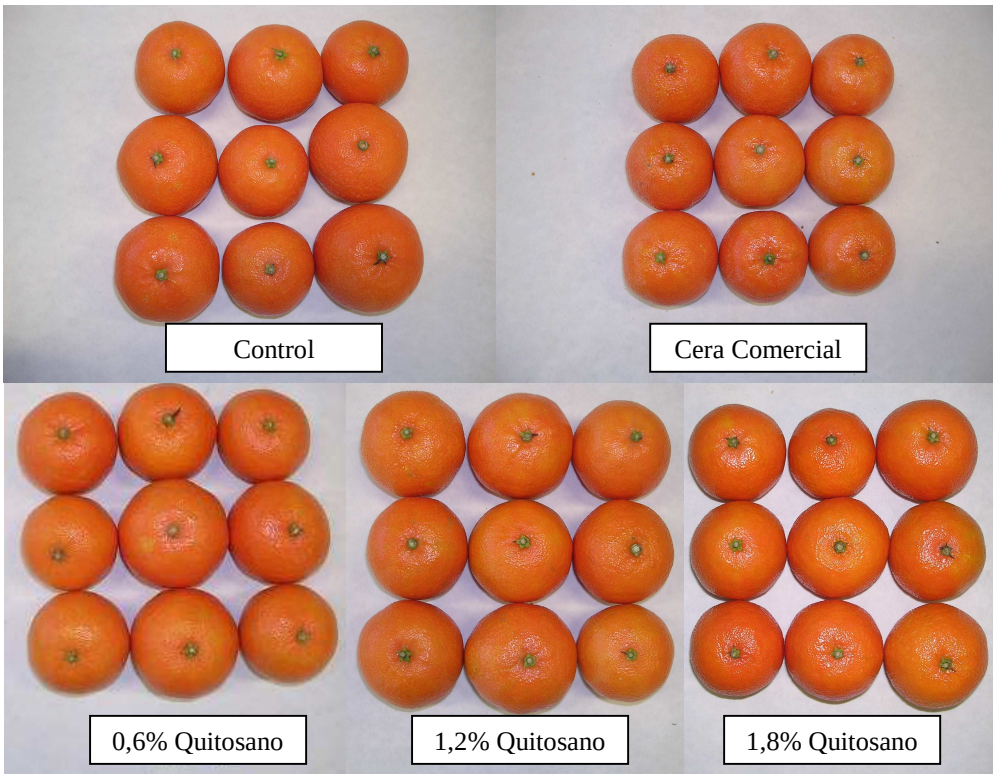

Foto 5. Mandarinas ‘Oronules’ sin recubrir, recubiertas con cera comercial de polietileno/goma laca al 10% CS, y con quitosano al 0,6%, 1,2% y 1,8% de CS tras 4 semanas de almacenamiento a 5 ºC más 1 semana a 20 ºC.

Foto 6. Evaluación sensorial de cítricos: sabor y aspecto externo.

Nombre: **Fecha:**
Experiencia:

Evalúa en las muestras que te presentamos los malos sabores y el flavor (sabor+aroma).

Ten en cuenta que:

- *si la muestra tiene 2 de malos sabores no puede ser de calidad excelente, si tiene 3 de malos sabores no puede ser de calidad aceptable.*
- *si una muestra la califiques 'mala calidad' (1, 2, 3), justifica en observaciones porque la consideras no comercial.*

MALOS SABORES

0	Ausencia
1	Muy ligeramente perceptibles
2	Medianamente perceptibles
3	Bastante perceptibles
4	Muy perceptibles
5	Presencia acusada

FLAVOR (sabor+aroma)

9	Calidad excelente
8	
7	
6	Calidad aceptable (satisfactorio)
5	
4	
3	Mala calidad (no comercial)
2	
1	

Código	Malos sabores	Flavor: sabor+aroma	Observaciones

Otras observaciones:

Figura 1. Hoja de cata para la evaluación sensorial de cítricos.

Nombre: **Fecha:**
Experiencia:

ASPECTO DEL RECUBRIMIENTO
Homogeneidad,manchas,grietas..

3	**Bueno**
2	**Aceptable**
1	**Malo**

Código	Aspecto del recubrimiento (en fruto)	Observaciones

BRILLO (ordena de izquierda a derecha los códigos de + a – brillo en las siguientes casillas)

+ Brillo ⟶ **- Brillo**

Figura 2. Ficha de cata para la evaluación del aspecto externo y brillo de cítricos recubiertos.

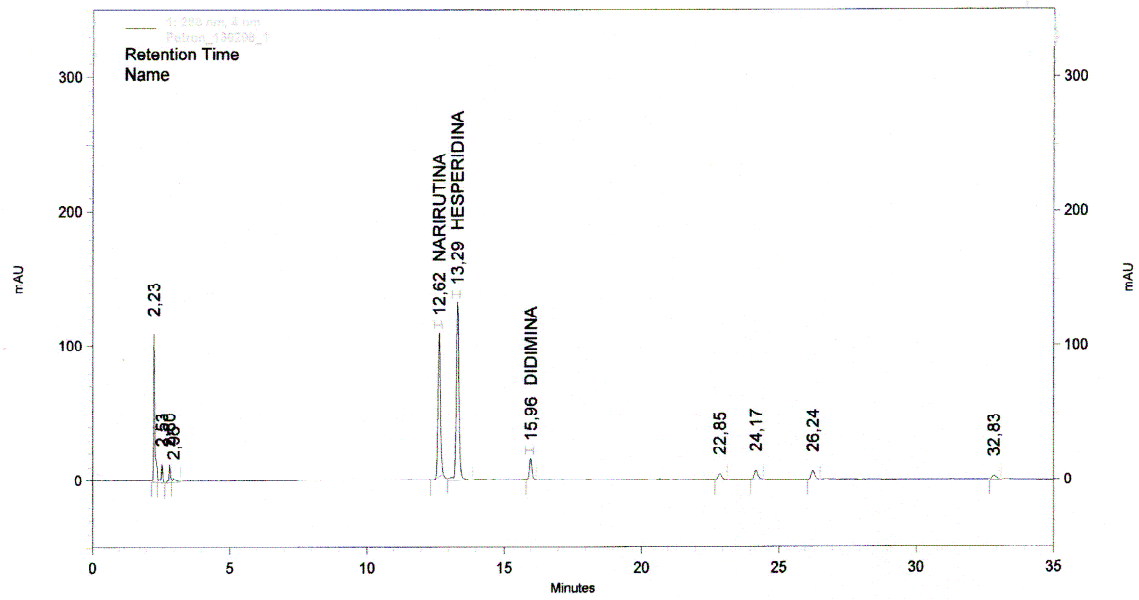

Figura 3. Cromatograma de los glucósidos de flavanonas mayoritarios determinados por HPLC en zumo de mandarinas ‘Oronules’.