Universidad
Politécnica
de Cartagena

**DEPARTAMENTO DE INGENIERÍA DE
ALIMENTOS Y DEL EQUIPAMIENTO
AGRÍCOLA**

**NUEVO ENVASE ACTIVO Y ANTIMICROBIANO
PARA LA CONSERVACIÓN DE PRODUCTOS
VEGETALES**

***A NEW ACTIVE AND ANTIMICROBIAL
PACKAGE FOR PRESERVATION OF
VEGETABLES***

**Amaury Taboada Rodríguez
Cartagena, 2013**

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A NEW ACTIVE AND ANTIMICROBIAL PACKAGE FOR PRESERVATION OF VEGETABLES

Memoria presentada por *Amaury Taboada Rodríguez*, Licenciado en Microbiología, para optar al grado de Doctor por la Universidad Politécnica de Cartagena

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Patente

García García, I. J., **Taboada Rodríguez, A.**, Marín Iniesta, F. y López Gómez, A. 2012. Envase activo para conservación de productos vegetales frescos. Oficina Española de Patentes y Marcas nº 2 393 388.

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Que la referida Tesis Doctoral, ha sido realizada por D. Amaury Taboada Rodríguez, dando mi conformidad para que sea presentada ante la Comisión de Doctorado, para ser autorizado su depósito.

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D. Antonio López Gómez Director de la Tesis doctoral "A new active and antimicrobial package for preservation of vegetables"

INFORMA:

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En reunión de la Comisión Académica de fecha 27 de junio de 2013, visto que la mencionada tesis doctoral tiene acreditados los indicios de calidad, requeridos para el depósito de tesis doctorales, regulados en el artículo 33 del Reglamento de Estudios Oficiales de Máster y Doctorado de la UPCT, y la autorización del Director de la misma, se acordó dar la conformidad para que a dicha tesis le sea autorizado, por la Comisión de Doctorado, su depósito.

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En Cartagena, a 27 de Junio de 2013

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Vistos los informes favorables de los Directores de Tesis y el VºBº de la Comisión Académica para la presentación de la Tesis Doctoral titulada: "*A new active and antimicrobial package for preservation of vegetables*" en la modalidad de "compendio de publicaciones" solicitada por D. Amaury Taboada Rodríguez, la Comisión de Doctorado de la Universidad Politécnica de Cartagena, en reunión celebrada el 27 de junio de 2013, considerando lo dispuesto en el artículo 33 del Reglamento de Estudios Oficiales de Máster y Doctorado de la UPCT, aprobado en Consejo de Gobierno el 13 de abril de 2011 y modificado el 11 de julio de 2012,

ACUERDA

Autorizar la presentación de la Tesis Doctoral a D. Amaury Taboada Rodríguez en la modalidad de compendio de publicaciones.

Contra el presente acuerdo, que no agota la vía administrativa, podrá formular recurso de alzada ante el Sr. Rector-Magnífico de la Universidad Politécnica de Cartagena, en el plazo de un mes a partir de la notificación de la presente.

Cartagena, 27 de junio de 2013
El Presidente de la Comisión de Doctorado

Edo.: Pablo Fernández Escámez

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1 SUMMARY

The food packaging industry is demanding new packages able to improve the quality of the packed products. In this regard some of the problems to be solved are the recyclable and compostable character of the materials, the antimicrobial properties of some substances to be involved in the packaging system and the optimum control of the internal atmosphere in the package. As a solution for these problems a new flow-pack active packaging design was proposed to be developed in this investigation, based on Polylactic acid (PLA)-coated cardboard trays, with some active substances embedded into this coating (plant essential oils and gases scavengers) and the suitable wrapping film.

The antimicrobial effect of this new package was studied using plant essential oils embedded in the inner coating of the cardboard tray. Essential oils are aromatic and volatile liquids extracted from plants. The chemicals in essential oils are secondary metabolites, which play an important role in plant defense, as they often possess antimicrobial properties. The interest in essential oils and their application in food preservation have been amplified in recent years by an increasingly negative consumer perception of synthetic preservatives. Furthermore, food-borne diseases are a growing public health problem worldwide, calling for more effective preservation strategies. The antibacterial properties of essential oils and their constituents have been documented extensively. The main obstacle for using essential oil constituents as food preservatives is that they are most often not powerful enough as single components, and they cause negative sensorial effects when added in sufficient amounts to provide an antimicrobial effect. Exploiting synergies between different essential oils has been suggested as a solution to this problem. The effect of them in antimicrobial packaging systems has been little studied. Because of the latter the proposal for this research was the use of a combination of vanillin, and essential oils of clove, cinnamon and thyme as antimicrobials in the new model of package.

Cardboard trays coated with a film of PLA were studied in order to obtain a new active packaging friendly with the environment. During this investigation the PLA showed active properties in relation with the control of the internal atmosphere of the package used to storage “Cherry” tomatoes. This polymer belongs to the family of aliphatic polyesters commonly made from α -hydroxy acids, which include polyglycolic acid or

polymandelic acid, and are considered biodegradable and compostable. PLA is a thermoplastic, high-strength, high-modulus polymer that can be made from annually renewable resources to yield articles for use in either the industrial packaging field or the biocompatible/bioabsorbable medical device market. It is easily processed on standard plastics equipment to yield molded parts, film, or fibers. In addition, it can be used for food contact and are generally recognized as safe (GRAS). PLA is degraded by simple hydrolysis of the ester bond and does not require the presence of enzymes to catalyze this hydrolysis. The rate of degradation is dependent on the size and shape of the article, the isomer ratio, and the temperature of hydrolysis.

Ethylene has diverse and significant effects on plant physiology. These are usually negative for the quality and shelf life of most horticultural products since this gas accelerates maturation and senescence. Modifications of the atmosphere inside the package can decrease O₂ and increase CO₂ in its interior, which allows the respiration and the production of ethylene by the fruit in the packaging to be reduced. O₂ and CO₂ modified atmosphere packages have been positively used to preserve fresh-cut tomatoes from damages. Also absorbent substances held on various inert support materials are commonly used to remove ethylene in various postharvest situations. Potassium permanganate (KMnO₄) is usually used for this purpose, being added to different absorbent materials such as activated carbon, silica gel, aluminum oxide, and clay minerals like sepiolite, to complement the properties of the active packaging.

For all the above the experimental design of this investigation consisted in the following research lines:

- The study of antimicrobial activity of the plant essential oils, individually and combinations of them, “in vitro” and in a food model system.
- Characterization of physical and water barrier properties, for the PLA-coated cardboard, with or without some gasses scavengers.
- Assesment of the practical application of the latter results in a new active packaging design with ethylene adsorption and antimicrobial characteristics.

This evaluation was performed using as model food “Cherry” tomatoes.

The present work has yielded some interesting results for the packaging industry. The use of vanillin and the essential oils of clove, thyme and cinnamon showed effective antimicrobial activity against *L. monocytogenes* and *E. coli* O157:H7 “in vitro” and in the tested food model system at the concentrations used as food flavouring agent. The

application of these substances in the PLA coating films has shown antimicrobial activity against the target strains studied in this work. Mixtures of plant essential oils of clove, thyme and cinnamon leaves with vanillin also inhibited the growth of *L. monocytogenes* showing a marked synergistic effect. It could be reasonable to use these mixtures to lower the individual sensorial impact on the packed food and to enlarge the spectrum of sensitive microorganisms to this antimicrobial activity.

The physical properties (thickness, weight, smoothness) and water barrier properties of PLA-coated cardboard (Water vapor permeability (WVP), Water absorptivity (WA), wetting energy (WE), and contact angle (CA)) showed an improvement of impermeability. The use of clays additives (Clinoptinolite, Sepiolite, or Sepiolite-Permanganate) reported as gasses scavengers increased the water affinity of PLA-coated paperboard but not as much as the uncoated samples. The embedding of those clays additives in the PLA coating film helps to control the humidity in the internal atmosphere of the package. These results represent an advantage for the manufacture of cardboard trays with improved humidity behavior. The compostable and environmentally friendly nature of PLA amplifies these advantages. The best results were obtained for PLA 2% alone or with Clinoptinolite 1.5% or with 1% of Sepiolite-Permanganate, these conditions were the most effective in increasing the water barrier properties of cardboard. These coatings are suitable for use in cardboard trays for the packaging, storage, and transport of foods such as fresh fruit and vegetables.

The PLA coating on the inside of the cardboard trays has demonstrated a very significant effect on the adsorption of ethylene and water vapour, and can be used in active packaging systems intended to “Cherry” tomatoes to regulate both factors.

When this new active packaging design is applied to the preservation of “Cherry” tomatoes it is shown that in early stages of storage, there is a little production of water vapour and a high production of ethylene, which is adsorbed in the PLA layer. In later stages, ethylene production in tomatoes falls and water vapour production increases, so that ethylene may be exchanged for water vapour in the PLA layer, allowing the progressive exit of small amounts of ethylene through the wall of the thin LDPE wrapping film. Passive modified atmosphere packaging in combination with active packaging using PLA-coated cardboard trays wrapped with thin LDPE film has significantly extended the shelf life of fresh tomatoes. This is a good solution for fresh tomato packaging compared with packaging systems using trays wrapped with macro-perforated films because microbial safety is better, while ethylene concentration inside

the package is controlled at the same time. In addition, with the studied active packaging system, there is a considerable saving of energy during the storage and distribution of fresh tomatoes, as the storage temperature is 20° C, much higher than the storage temperatures usually used with modified atmosphere packaging.

As a result of this thesis work, a new active packaging system has been patented. This system is based on PLA-coated cardboard trays, wrapped and thermo-sealed with LDPE film (20 µm thickness), which allows to prolong the shelf life of fresh vegetables. Some claims of this patent consider the addition of clays additives and plant essential oils to the PLA coating film in order to enhance the preservation of the packed food, acting as gasses scavengers and antimicrobials respectively.

RESUMEN

La industria de envasado de alimentos demanda nuevos envases capaces de mejorar la calidad de los productos y de prolongar su vida útil. Algunos de los problemas a resolver son el carácter reciclable y compostable de los materiales que constituyen el envase, las propiedades antimicrobianas de sustancias que forman parte del sistema de envasado y el control óptimo de la atmósfera interna en el espacio de cabeza. Como solución a estos problemas se ha desarrollado un nuevo diseño de envasado activo a lo largo de este trabajo de investigación. Este envase se basa en bandejas de cartón ondulado recubiertas con una película de ácido poliláctico (PLA), con principios activos incorporados en esta película (aceites esenciales de plantas y secuestrantes de gases) y una película de envoltura adecuada.

Se estudió el efecto antimicrobiano de este nuevo envase con aceites esenciales de plantas inmersos en el recubrimiento interior de PLA de la superficie de la bandeja de cartón. Los aceites esenciales son sustancias aromáticas y volátiles extraídas de las plantas, son metabolitos secundarios, que desempeñan un papel importante en la defensa de la planta, debido en gran parte a sus propiedades antimicrobianas. El interés por los aceites esenciales y su aplicación en la conservación de alimentos se ha incrementado en los últimos años debido a la percepción cada vez más negativa de los consumidores por los conservantes sintéticos. Por otra parte, las enfermedades transmitidas por alimentos son un problema creciente en la salud pública, lo que exige estrategias de conservación más eficaces. Las propiedades antibacterianas de los aceites esenciales y sus componentes se han documentado ampliamente. Los principales obstáculos para el uso aceites esenciales como conservantes de alimentos es que no son suficientemente potentes de manera individual y el efecto sensorial suele ser negativo cuando se añaden en cantidades suficientes para proporcionar un efecto antimicrobiano. La explotación de las sinergias entre los diferentes aceites esenciales ha sido sugerido como una solución a este problema. El efecto de ellos en los sistemas de envasado antimicrobianos ha sido poco estudiado, por lo que este trabajo de investigación se propuso el estudio de un envase activo utilizando como principios activos antimicrobianos una combinación de vainillina, y aceites esenciales de clavo, canela y tomillo.

Las bandejas de cartón recubiertas con una película de PLA se estudiaron con el fin de obtener un nuevo envase activo reciclable y biodegradable. El PLA demostró

propiedades activas en relación con el control de la atmósfera interna de un envase utilizado para almacenamiento de tomates "Cherry". Este polímero pertenece a la familia de los poliésteres alifáticos obtenidos a partir de ácidos α -hidroxiácidos, que incluyen ácido poliglicólico o ácido poli (mandélico), y son considerados biodegradables y compostables. El PLA tiene alta resistencia, es un termoplástico de alto módulo que se puede producir a partir de recursos renovables para producir artículos destinados al envasado industrial, dispositivos médicos, entre otros. Se procesa fácilmente en equipos de plástico estándar para producir piezas moldeadas, películas, o fibras. Además, puede ser utilizado para el contacto con alimentos y generalmente se reconoce como seguro (GRAS). Este plástico se degrada por simple hidrólisis del enlace éster y no requiere la presencia de enzimas para catalizar esta hidrólisis. La velocidad de degradación depende del tamaño y la forma, la relación de isómeros y la temperatura de la hidrólisis.

En un envase destinado a productos frescos, sobre todo si son productos que liberan etileno podemos encontrar efectos diversos y significativos sobre el producto almacenado en dicho envase a lo largo de su vida útil. Estos efectos suelen ser negativos para la calidad de muchos productos frescos ya que este gas acelera la maduración y senescencia. Algunas modificaciones de la atmósfera interna del envase pueden disminuir los niveles O_2 y CO_2 , lo que permite que la respiración y la producción de etileno por el producto envasado sean reducidas. El envasado en atmósfera modificadas de O_2 y CO_2 se ha utilizado de manera positiva para la conservación de vegetales frescos, ensaladas IV gama, frutas cortadas, etc. En este sentido también ha sido estudiado el uso de sustancias absorbentes de etileno, el permanganato potásico ($KMnO_4$) es el más ampliamente conocido al igual que sus aplicaciones prácticas en la industria del envasado. Se utiliza generalmente en materiales de soporte, tales como carbón activado, gel de sílice, óxido de aluminio y minerales de arcilla (ej. sepiolita), para complementar las propiedades de un envasado activo.

Por todo lo anterior, el diseño experimental de esta Tesis doctoral se basó en las siguientes líneas de investigación:

- El estudio de la actividad antimicrobiana de los aceites esenciales de las plantas, de forma individual y combinaciones de ellos, "in vitro" y en un sistema modelo de alimentos.
- Caracterización de las propiedades físicas y barrera al agua para el cartón recubierto con PLA y con absorbentes de gases inmersos en el recubrimiento.
- Evaluación de la aplicación práctica de los últimos resultados en un nuevo diseño de envases activos con adsorción de etileno y características antimicrobianas. Esta evaluación se realizó utilizando como modelo de productos frescos, tomates "Cherry".

El presente trabajo ha dado algunos resultados interesantes para la industria del envasado. El uso de la vainillina y los aceites esenciales de clavo, tomillo y canela mostró actividad antimicrobiana efectiva contra *L. monocytogenes* y *E. coli* O157: H7 "in vitro" y en el sistema modelo de alimento probado con concentraciones habitualmente utilizadas como saborizante. La aplicación de estas sustancias en el recubrimiento de las películas de PLA ha demostrado actividad antimicrobiana frente a las cepas diana estudiadas en este trabajo. Las mezclas de aceites esenciales de plantas de clavo, tomillo y hojas de canela con vainillina también inhibieron sinérgicamente el crecimiento de *L. monocytogenes*. La utilización de estas mezclas puede ser razonable para reducir el impacto sensorial individual en el alimento envasado y para ampliar el espectro de microorganismos sensibles a esta actividad antimicrobiana.

Las propiedades físicas (grosor, el peso, la suavidad) y las propiedades hidrofóbicas del cartón recubierto con PLA (permeabilidad al vapor de agua (PVA), capacidad de absorción de agua (WA), energía de humectación (NOS), y ángulo de contacto (CA)) mostraron una mejora en la impermeabilidad de la superficie interior del envase. El uso de arcillas (Clinoptinolita y sepiolita) y permanganato potásico como absorbentes de gases aumentó la afinidad por el agua de cartón recubierto de PLA, pero mucho menos que las muestras de cartón sin recubrir. La inmersión de estos absorbentes de gases en la película de revestimiento PLA ayudaron a controlar la humedad en la atmósfera interna del envase. Estos resultados representan una ventaja para la fabricación de bandejas de cartón con el comportamiento mejorado de la humedad interna. Las características como material biodegradable y reciclable del PLA incrementa sus ventajas como recubrimiento hidrofóbico. De acuerdo a nuestras observaciones, los mejores resultados fueron obtenidos con PLA 2%, PLA 2% con Clinoptinolita 1,5% y con PLA 2% con sepiolita-permanganato 1%, ya que estas condiciones fueron las más

efectivas en el aumento de las propiedades barrera al agua del cartón. La caracterización hidrofóbica de los cartones con diferentes recubrimientos demostró que pueden ser utilizados para el envasado, almacenamiento y transporte de alimentos, como frutas y hortalizas frescas. Al mismo tiempo el recubrimiento de la superficie interior con PLA de las bandejas de cartón ha demostrado un efecto muy significativo en la adsorción de etileno y vapor de agua, y se puede utilizar en sistemas de envasado activo destinado a tomates "Cherry" para regular la atmósfera interior del envase.

Cuando este nuevo envase activo se utiliza en la conservación de tomates "Cherry" se demuestra que en las primeras etapas de almacenamiento, hay una pequeña producción de vapor de agua y una alta producción de etileno, que es absorbido por la película de recubrimiento de PLA. En etapas posteriores, la producción de etileno en los tomates disminuye y aumenta la producción de vapor de agua, por lo que el etileno puede ser intercambiado por vapor de agua en la película de PLA, permitiendo la salida progresiva de pequeñas cantidades de etileno que difunde al exterior a través de la película de envoltura de polietileno de baja densidad (LDPE). Un envasado en atmósfera modificada pasiva en combinación con el envasado activo utilizando bandejas de cartón recubiertas con película de PLA y termosellados con película de LDPE ha prolongado de manera significativa la vida útil de los tomates envasados. Esta es una buena solución para el envasado de tomates frescos en comparación con los sistemas de envasado que utilizan bandejas envueltas con películas macro-perforadas porque mejora la seguridad microbiana y controla la concentración de etileno en el interior del envase al mismo tiempo. Además, con el sistema de envase activo desarrollado en este trabajo, se produce un ahorro considerable de energía durante el almacenamiento y la distribución de tomates frescos, porque permite que la temperatura de almacenamiento sea de 20° C, mucho más alta que las temperaturas de almacenamiento generalmente utilizadas en productos frescos envasados en atmósfera modificada.

Como resultado de este trabajo de tesis, un nuevo sistema de envasado activo ha sido patentado. Este sistema se basa en bandejas de cartón recubiertos con PLA, envuelto y termo-sellado con película de LDPE (20 micras de espesor), lo que permite prolongar la vida útil de las hortalizas frescas almacenadas en el mismo. Algunas reivindicaciones de esta patente consideran la adición de arcillas y aceites esenciales de plantas a la película de revestimiento de PLA con el fin de mejorar la conservación de los alimentos envasados, en calidad de absorbentes de gases y antimicrobianos, respectivamente.

2 INTRODUCTION

Actually there is a great demand for a better food safety, mainly in meat and dairy products because of their high protein content and pH near neutral. However, this high demand in food safety has been extended in the last decades to vegetables. In this regard it can be mentioned the recent crisis of *E. coli* O104:H4 in Germany, falsely attributed to Spanish cucumbers. Moreover, the trends in the packaging technology are to use natural, recyclable and compostable materials, which not contribute to the greenhouse effect. Regarding antimicrobial active packaging the major problem limiting the use of this type of packaging is migration of toxic compounds to the packaged food, which may be solved by using innocuous levels of natural products such as plant essential oils. The research works contained in this thesis attempt to respond these demands by means of the design and developing of a new active antimicrobial package using PLA-coated corrugated cardboard as biodegradable materials, and plant essential oils as natural antimicrobial. In addition, clays additives embedded in the PLA coating film are used to enhance the preservation of the packed food, acting as ethylene scavengers.

2.1 Antimicrobial activity of essential oils

The so-called emerging technologies for food preservation include the use of antimicrobial substances, which should have effect along the shelf life of the product. Plant extracts, including essential oils, have been used since antiquity usually for their aroma and flavour properties, although recently they have been studied as alternatives to traditional additives because of their potential antimicrobial and antioxidant activities (Burt 2004; Holley and Patel 2005). The most important flavouring agents are cinnamon and vanillin (4-hydroxy-3-methoxybenzaldehyde), the last is a low-molecular weight phenolic molecule that is the principal component of the bean and pod of some plants of *Vanilla* genus with a “generally regarded as safe” status and an acceptable daily intake of 10 mg/kg has been agreed between FAO/WHO and EU (INCHEM 2004). Natural vanilla extracts are expensive due to limited worldwide production and labour-intensive manufacturing methods that are being improving with the introduction of new technologies (Naidu et al. 2009). However, the majority of vanillin currently in use

(90%) is synthetically produced from eugenol, lignin, or guaiacol (Ramachandra and Ravishankar 2000; Sinha et al. 2008). Vanillin is added to a wide range of products, e.g., pastry products, ice cream, soft drinks and baked products (biscuits and cereals) as flavouring. Recently, other useful food applications have been found for vanillin: antioxidant (Burri et al. 1989; Shyamala et al. 2007, Mourtzinis et al. 2009), antimutagenic (Maurya et al. 2007; Shaughnessy et al. 2006; Lirdprapamongkol et al. 2005), antifungal (Cerruti and Alzamora 1996; Fitzgerald et al. 2005; Fitzgerald et al. 2003), and antibacterial as others substances of plant origin (Fitzgerald et al. 2004a; Corte et al. 2004; Moon et al. 2006). Those substances have showed antimicrobial activity in apple juice and fruit purées (Cerruti et al. 1997; Fitzgerald et al. 2004b), in flavoured soy beverage and yogurt (Tipparaju et al. 2004, Penney et al. 2004), fresh apples slices (Rupasinghe et al. 2006), and fresh mango slices (Ngarmsak et al. 2006). They have also been used successfully as antimicrobial agents for edible films (Rojas-Grau et al. 2007) and the combination of essential oils of cinnamon leaves, cinnamon bark and clove with vanillin have increased the antimicrobial effect showing a synergistic behavior (Cava-Roda et al. 2007; Cava-Roda et al., 2010; Ferrante et al. 2007; Char et al. 2009, 2010; Gastélum et al. 2010). But the plant essential oils embedded into packages coatings in order to achieve an antimicrobial effect must be studied more deeply. It should also be related with other factors which could influence on results such as type of product, the film properties for sealing, storage temperatures, etc.

2.2 Active and hydrophobic properties of cardboard coated with polylactic acid and ethylene scavengers.

Corrugated cardboard is the most widely used of all materials for the manufacturing of paper packaging for foods, and it is recyclable, biodegradable, and compostable. It can serve as a base material to build active packages through the application of coatings with various substances, to achieve enhancement of shelf life of foods and replacement of petroleum-based plastics (Twede and Selke 2005; Lopez-Rubio et al. 2006) The hydrophilic composition of cellulose cardboard makes it likely to absorb water vapor from the environment, especially when it comes in contact with high-moisture products such as fresh agricultural produce (Rhim et al. 2007). A water barrier may be obtained by changing the wettability of the paper surface through coating with hydrophobic materials (Krook et al. 2005). For this purpose, paraffin and polyethylene have been used in a molten form on paper or cardboard to produce a water vapor barrier to

provide a more durable and flexible coating (Robertson 1993). Nevertheless, the characteristics of both packaging materials make it difficult for recycling or composting (Robson 1993). In order to produce more environmentally friendly materials, several renewable and biodegradable biopolymers have been investigated as paper coating and packaging materials (Gällstedt et al. 2005).

Poly(lactic acid) (PLA) has good performance characteristics as a packaging material and has become one of the most promising polymers for this purpose because it is compostable and commercially available (Plackett et al. 2006). New techniques allowing economical production of high molecular weight PLA polymer have also expanded its uses (Datta and Henry, 2006). PLA films are quite resistant to water since they degrade slowly and are associated with water-soluble polymers or water-swollen polymers and show lower water vapor permeability (WVP) than other natural biopolymers (Rhim et al., 2006; Garlotta 2001). They are also compostable, helping with the solid waste disposal problems associated with packaging materials (Lim et al. 2008). The low toxicity of PLA and its environmentally benign characteristics have made it a suitable material for food packaging products (Conn et al. 1995; Webber et al. 2002). The possibilities of covering the corrugated cardboard with hydrophobic biopolymers improve the characteristics of this package material for conservation of horticultural products, which generate moisture as they ripen. The flexible biopolymer films used for packaging vegetables not only provide protection against physical damages, but they also avoid the dehydration and allow for the control of the surrounding environment of the food (Chaves et al. 1998). This possibility has been assessed successfully by the authors in assays included in this thesis and aimed at obtaining an active packaging for cherry tomatoes (García-García et al. 2013).

Ethylene has diverse and significant effects on plant physiology. These are usually negative for the quality and shelf life of most horticultural products since the gas accelerates maturation and senescence (Zagory 1995). Modifications of the atmosphere inside the package can decrease O₂ and increase CO₂ in its interior, which allows the respiration and the production of ethylene by the fruit in the packaging to be reduced (Oms-Oliu et al. 2007). O₂ and CO₂ modified atmosphere packages have been positively used to preserve fresh-cut tomatoes from damages (Aguayo et al. 2004). Combinations of absorbent substances held on various inert support materials are commonly used to remove ethylene in various postharvest situations. Potassium permanganate (KMnO₄) is usually used for this purpose, being added to different

absorbent materials such as activated carbon, silica gel, aluminum oxide, and clay minerals like sepiolite, to complement the properties of the active packaging (DeEll et al. 2006; Vermeiren et al. 1999). The use of co-extruded zeolite–polyethylene film has been found to be acceptable as a packaging material (Aktas et al. 2003). PLA and PLA/zeolite composites can be applied to altering the CO₂/O₂ permeability of the modified atmosphere packaging systems, according to the results obtained on the manufacture of composite materials based on mixtures of PLA and zeolite (Auras et al. 2010). However, the role of the PLA concentration and the combination of substances such as zeolite and others should be studied for use as a specific lining for cardboard destined for fresh food packaging.

2.3 Active packaging for fresh fruits and vegetables

The shelf life of fresh fruits and vegetables, including tomatoes, has become a factor of major importance for satisfying consumer demands in terms of quality and safety (Jagadeesh et al. 2010). Active packaging, a new concept of food packaging, was developed in response to changes in current consumption and market trends and is designed to improve fresh product quality and safety (Gontard and Guillaume 2010).

The main techniques related with active packaging are substance adsorption (e.g. oxygen, ethylene, moisture, carbon dioxide, flavours/odours) and substance release (e.g. carbon dioxide, antimicrobial agents, antioxidants and flavours; Rodríguez-Lafuente et al. 2010). Cardboard, which is one of the major raw materials used for packaging food, could serve as base material for biodegradable active packages, reinforcing their mechanical properties, improving the humidity barrier and minimising microbial contamination. Such active packages could delay the maturity of climacterics fruits by controlling the surrounding environment of the fruits, providing good yield at a relatively low cost (Taechutrakul et al. 2009). New efforts are continuously being made to obtain more environmentally friendly packaging materials, and, in this respect, polylactic acid and cardboard are renewable and biodegradable (Rhim et al. 2007). PLA is a biopolymer that shows a certain resistance to water (Rhim and Kim 2009), so that covering the corrugated cardboard with this hydrophobic biopolymer allows better conservation of vegetable products. Because PLA is compostable and derived from renewable sources, it has been considered as one of the solutions for packaging materials (Rhim et al. 2007).

Ethylene (C_2H_4) accelerates respiration, leading to the ripening, softening and senescence of fruit and vegetables. Many suppliers offer C_2H_4 scavengers based on potassium permanganate ($KMnO_4$), which oxidises the ethylene to acetate and ethanol. However, $KMnO_4$ products cannot be integrated into food-contact materials, so they are only supplied in the form of sachets (Taechutrakul et al. 2009; Sammi and Masud 2009). Another type of ethylene scavenger is activated carbon, which acts as an adsorbent agent (Bailen et al. 2006), although it presents problems for use in primary packaging (Martínez-Romero et al. 2009). Ethylene-adsorbing packaging films incorporating finely dispersed minerals such as zeolite clays have been tested successfully. Nevertheless, they are opaque and not capable of adsorbing ethylene sufficiently (Zagory 1995). However, the ethylene adsorption capacity of materials such as cardboard lined with PLA has been little studied for use in active packaging. Taechutrakul et al. (2009) studied the effect of corrugated cardboard containing palm shell charcoal and activated carbon on the shelf life of Cherry tomatoes, and found that it showed potential as an ethylene absorber to delay fruit ripening. Oliveira et al. (2006) studied the ethylene sorption properties of PLA, finding the Langmuir model applicable, since sorption is closely related to the presence of the free volume in PLA. However, no studies have looked at PLA as ethylene adsorbent for use in the active packaging of fresh fruit and vegetables.

When combined with cold storage, modified atmosphere packaging (MAP) can increase the shelf life of fresh products and reduce economic losses due to damage, making longer transport distances possible and lowering moisture loss during storage, while ensuring a high-quality product (Robertson 2006). MAP technology can retard fresh vegetable respiration rates and extend their shelf life. However, to achieve the required modified atmosphere (RMA) in the package, it is very important to control the permeability properties of the packaging film, the respiration rate of the vegetable, and CO_2 gas and vapour release (Caleb et al. 2011). This is the reason why obtaining the desired steady-state gas composition for some fresh vegetables may require the use of macroperforated films, or a combination of microperforations and suitable permeability characteristics in the films used for wrapping the vegetable trays (Lee and Renault 1998; Rennie and Tavoularis 2009; Gontard and Guillaume 2010).

Most applications of MAP for fresh vegetables use a packaging film that allows restricted gas diffusion (ethylene, CO_2 , O_2 , and vapour) through the film wall. In the packaging design process, the type of material, surface area and thickness of the

packaging film are selected to obtain the desired equilibrium gas composition or RMA. The types of film used include low-density polyethylene (LDPE), poly (vinyl chloride), and oriented polypropylene (Smith et al. 2003; Gontard and Guillaume 2010; Sandhya 2010). But, even after selecting the most suitable film characteristics, it is relatively difficult to achieve the RMA due to the complexity of controlling all the factors affecting the gas composition in the package, which moreover, continue to change during the commercial life of the packaged product. In fact, this is the limitation of both active and passive MAP when attempting to prolong even further the shelf life of packaged fresh fruits and vegetables (Gontard and Guillaume 2010). Active packaging can help to achieve the RMA during the commercial life of fresh fruits and vegetables and thus obtain better quality, increased safety and a longer shelf life (Gontard and Guillaume 2010; Sandhya 2010).

The following work has related the antimicrobial effect of plant essential oils, the hydrophobic properties of the PLA and the friendly character of PLA-coated cardboard with the environment to obtain a new active antimicrobial and compostable package to improve fresh products preservation. The Research Group of Food Biotechnology from the University of Murcia and the Department of Food Engineering and Agricultural Equipment from the Polytechnic University of Cartagena proposed the following aims in order to develop this thesis.

3 Aims

1. Study the “in vitro” antimicrobial activity of vanillin and essential oils of clove, cinnamon bark and cinnamon leaves in trypticase soy broth (TSB) against *L. monocytogenes* and *E. coli* O157:H7 in different conditions of pH and temperatures.
2. Characterization of the hydrophobic properties of PLA coating alone and with zeolite, sepiolite, and sepiolite permanganate to be used in active packaging for fresh fruits and vegetables.
3. To test a new design of active package based on cardboard trays coated with PLA and wrapped with LDPE film for the preservation of fresh Cherry tomatoes.
4. To design a new active package based on PLA coated cardboard, with gases scavengers and plant essential oils embedded into the coating.
5. To study the effect of the ethylene scavengers inside this kind of packages on extending the shelf life of “Cherry” tomatoes stored at 20 °C.
6. To optimize the wrapping film to be used, according to their properties, to enhance the active character of the new active and antimicrobial package.

4 Results and Discussion

This thesis is presented in the form of a compendium of publications.

Articles and Patent comprising the thesis are the followings:

Articles in scientific journals:

Article I: Cava-Roda, R. M., Taboada-Rodríguez, A., Valverde-Franco, M. T., and Marín-Iniesta, F. 2010. Antimicrobial Activity of Vanillin and Mixtures with Cinnamon and Clove Essential Oils in Controlling *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Milk. Food and Bioprocess Technology 5(6), 2120-2131.

Article II: Taboada-Rodríguez, A, García-García, I, Cava-Roda, R, López-Gómez, A, Marín- Iniesta, F. 2013. Hydrophobic properties of cardboard coated with polylactic acid and ethylene scavengers. Journal of Coatings Technology and Research 10(2), doi:10.1007/s11998-013-9493-3

Article III: García-García, I, Taboada-Rodríguez, A, López-Gómez, A, Marín-Iniesta, F. 2013. Active Packaging of Cardboard to Extend the Shelf Life of Tomatoes. Food Bioprocess and Technology 6(3), 754–761.

Patent:

García García, I. J., Taboada Rodríguez, A., Marín Iniesta, F. y López Gómez, A. 2012. Envase activo para conservación de productos vegetales frescos. Solicitud de Patente Española nº 2 393 388, con fecha de publicación: 20/12/2012.

Article I

**Antimicrobial Activity of Vanillin and Mixtures with
Cinnamon and Clove Essential Oils in Controlling *Listeria*
monocytogenes and *Escherichia coli* O157:H7 in Milk**

Antimicrobial Activity of Vanillin and Mixtures with Cinnamon and Clove Essential Oils in Controlling *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Milk

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Abstract The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of vanillin against *Listeria monocytogenes* Scott A and *Escherichia coli* O157:H7 was determined in trypticase soy broth (TSB), pH 7 and 6, incubated at 35 °C/24 h and in semi-skim milk incubated at 35 °C/24 h and 7 °C/14 days. The influence of the fat content of milk on the antimicrobial activity of vanillin was tested in whole and skim milk incubated at 7 °C/14 days. Mixtures of clove and cinnamon with vanillin were also evaluated in semi skim milk incubated at 7 °C. The MICs for *L. monocytogenes* were 3,000 ppm in TSB (pH 7) and 2,800 ppm in TSB (pH 6). The MICs for *E. coli* O157:H7 were 2,800 ppm in TSB (pH 7) and 2,400 ppm in TSB (pH 6). The MBCs in TSB were 8,000 ppm for *L. monocytogenes* and 6,000 ppm for *E. coli* O157:H7. The pH values assayed did not influence significantly the MIC or MBC in TSB. The MICs in semi-skim milk for *L. monocytogenes* and *E. coli* O157:H7 were 4,000 and 3,000 ppm at 35 °C/24 h, and 2,500 and 1,000 ppm at 7 °C/7 days, respectively. The MBCs were 20,000 ppm for *L. monocytogenes* and 11,000 ppm for *E. coli* O157:H7. High incubation temperatures did not affect the MBC but increased the MIC of the vanillin in milk. This effect could be attributed to the increased membrane fluidity and to the membrane perturbing activity of vanillin at low temperatures. The fat in milk reduced significantly the antimicrobial

activity of vanillin, probably due to effect protective of the fat molecules. Mixtures of clove and cinnamon leaves inhibited the growth of *L. monocytogenes* in a similar way that vanillin alone but had a synergistic effect on the *E. coli* O157:H7. Mixtures of cinnamon bark and vanillin had always a synergistic effect and some of the combination assayed showed bactericidal activity on the population of *L. monocytogenes* and *E. coli* O157:H7.

Keywords Natural antimicrobial · Vanillin · *Listeria monocytogenes* · *Escherichia coli* O157:H7

Introduction

The consumption of pasteurized milk has been associated with some outbreaks of listeriosis (Ahrabi et al. 1998; CDC-MMWR 2008). The fact that this foodborne bacterial infection has such a high mortality rate (20–30%) makes the USDA and Food and Drug Administration (FDA) to establish a “zero” tolerance policy for *Listeria monocytogenes* in foods that will be consumed without cooking. According to the risk analysis for *L. monocytogenes*, pasteurized fluid milk has a predicted relative risk of causing listeriosis on a per serving basis. This category includes cow and goat milk, chocolate milk, other flavoured milk, and malted milk (FDA 2003).

It is generally assumed that contamination of pasteurized fluid milk is the result of post-pasteurization recontamination, generally during filling, since normal pasteurization will effectively eliminate the microorganism. Experimental studies have also demonstrated that pasteurized milk will support growth of *L. monocytogenes* to high levels at refrigeration temperatures (Allavi

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et al. 2001). *Escherichia coli* O157:H7 is another pathogen associated with food-borne disease outbreaks all over the world, frequently with fatal results (Rangel et al. 2005). *E. coli* O157:H7 has been responsible of some outbreaks produced by consume of raw milk (CDC-MMWR 2007; Karns et al. 2007; Denny et al. 2008). This fact joined to its ability to grow at temperatures from 7 °C (Park et al. 1999) makes to *E. coli* O157:H7 an emergent pathogen of relevance in pasteurised milk.

Plant extracts, including essential oils, have been used since antiquity usually for their aroma and flavour properties, although recently they have been studied as alternatives to traditional additives because of their potential antimicrobial and antioxidant activities (Burt 2004; Holley and Patel 2005). One of the most important flavouring agents is vanillin (4-hydroxy-3-methoxybenzaldehyde), a low-molecular weight phenolic molecule that is the principal component of the bean and pod of some plants of *Vanilla* genus with a “generally regarded as safe” status and an acceptable daily intake of 10 mg/kg has been agreed between FAO/WHO and EU (INCHEM 2004). Natural vanilla extracts are expensive due to limited worldwide production and labour-intensive manufacturing methods that are being improving with the introduction of new technologies (Naidu et al. 2009). However, the majority of vanillin currently in use (90%) is synthetically produced from eugenol, lignin, or guaiacol (Ramachandra and Ravishankar 2000; Sinha et al. 2008). Vanillin is added to a wide range of products, e.g., pastry products, ice cream, soft drinks and baked products (biscuits and cereals) as flavouring. Recently, other useful food applications have been found for vanillin: antioxidant (Burri et al. 1989; Shyamala et al. 2007; Mourtzinou et al. 2009), antimutagenic (Maurya et al. 2007; Shaughnessy et al. 2006; Lirdprapamongkol et al. 2005), antifungal (Cerruti and Alzamora 1996; Fitzgerald et al. 2005; Fitzgerald et al. 2003), and antibacterial (Fitzgerald et al. 2004a; Corte et al. 2004; Moon et al. 2006). Vanillin has showed antimicrobial activity in apple juice and fruit purées (Cerruti et al. 1997; Fitzgerald et al. 2004b), in flavoured soy beverage and yogurt (Tipparaju et al. 2004; Penney et al. 2004), fresh apples slices (Rupasinghe et al. 2006), and fresh mango slices (Ngarmasak et al. 2006). It has also been used successfully as antimicrobial agent for edible films (Rojas-Grau et al. 2007) and in combination with other treatments (Ferrante et al. 2007; Char et al. 2009, 2010; Gastélum et al. 2010). Since flavoured milk has increased in popularity during recent years and *L. monocytogenes* and *E. coli* O157:H7 are microorganisms that could grow in milk under refrigeration temperatures, the objective of this paper was to study the antimicrobial activity of vanillin in trypticase soy broth (TSB) broth and pasteurized milk against these two emergent pathogens.

Materials and Methods

Microorganisms and Culture Conditions

The *L. monocytogenes* Scott A strain was a human epidemic isolate supplied by the FDA (USA) and the *E. coli* O157:H7 5947 strain (virulence factor detected) was supplied by the Colección Española de Cultivos Tipo, Spain. All strains were kept at −80 °C in Microbank TM vials (Pro-labo Diagnostics, Neston, Wirral, UK). Every 2 months, one of the vials was opened and the stock culture grown in TSB (Cultimed, Barcelona, Spain) for 24 h at 35 °C and streaked onto PALCAM agar plates (Merck, Darmstadt, Germany), which were incubated for 48 h at 35 °C. One colony obtained in agar Palcam was transferred to TSB, and was incubated for 28 h at 35 °C before being stored at −20 °C in a solution of 40% TSB and 60% glycerol until use. The fresh cultures for the experiments were made by incubating one loopful of pure culture in TSB for 24 h at 35 °C. The inocula were standardized in TSB until an optical density (OD) of 0.1 at 595 nm was reached and diluted in TSB to obtain a bacterial concentration of between 2 and 5 × 10⁵ CFU/ml. The bacterial populations were estimated by spreading suitable diluted aliquots onto trypticase soy agar (TSA) plates, followed by incubation at 37 °C for 24 h.

Vanillin Stock Solutions

Following the procedure previously established by Delaquis et al. (2005), vanillin stock solutions were prepared by adding the suitable amount of vanillin (Aldrich, Steinheim, Germany) to TSB previously warmed at 50 °C. The pH was adjusted to 6 and 7 with 5 N HCl or 5 N NaOH, respectively, prior to sterile filtration through 0.22 µm membranes.

Antimicrobial Activity of Vanillin in TSB

The influence of the vanillin concentrations and the pH on the growth of *L. monocytogenes* Scott A and *E. coli* O157:H7 was evaluated by the broth dilution method performed in 96-well microplates. The OD in TSB (pH 6 and 7) amended with 0, 15% agar as suggested by Mann and Markham (1998) using a Multiskan Ascent OD reader was associated with the microbial growth. Five stock solutions of vanillin were prepared in TSB+0.30% agar as indicated above. Five wells of the first column of the microplate received 300 µl of TSB+ 0.30% agar as sterile control and the other five wells received 150 µl TSB+0.30% agar and 150 µl of inoculum as positive control. To the first wells of each remaining column, 300 µl of a vanillin stock solution were added as control and 300 µl of the stock solution were added to the second well of each column as duplicate. All

the other wells received 150 μ l TSB+0.30% agar, while 150 μ l of each stock solution was added to the next well in the appropriate column and mixed, thus obtaining a twofold dilution series. Each well, except the controls, received 150 μ l of the prepared inoculum to provide an initial population of $1.0\text{--}2.5 \times 10^5$ CFU/ml. The microplate was then incubated at 35 °C for 24 h and the OD of each well was registered every hour. The microorganism was considered to have grown if increases in OD of >0.1 were observed. Three replicate experiments were performed with each strain and for each pH. *L. monocytogenes* and *E. coli* O157:H7 growth curves were obtained by representing graphically the OD versus the incubation period. The area under the OD/time curve of the control was compared with the areas of the tests and expressed as percent of growth inhibition (GI%) to determine the antimicrobial activity of each concentration tested. A concentration–effect of the preservatives was observed. Minimal inhibitory concentration (MIC) was considered as the lowest concentration of vanillin that caused 100 GI% (Lambert and Pearson 2000). The partial inhibitory concentration (PIC) was any concentration of vanillin lower than the MIC that produced an inhibition of between 0% and 100%. To determine the minimal bactericidal concentration (MBC), vanillin solutions were prepared as indicated above in test tubes containing TSB. Concentrations of vanillin from 3,000 to

Fig. 1 Growth of *L. monocytogenes* in TSB with different concentrations of vanillin: a TSB (pH 7), b TSB (pH 6)

Fig. 2 Growth of *E. coli* O157:H7 in TSB with different concentrations of vanillin: a TSB (pH 7) and b TSB (pH 6)

10,000 ppm at 500 ppm increments were tested against bacterial populations of $1\text{--}2.5 \times 10^5$ CFU/ml. The test tubes were incubated at 35 °C/24 h and, after incubation, 100 μ l of each tube were plated on TSA plates. The MBC was defined as the lowest concentration at which 99.9% of the initial inoculum in TSB died and no growth was observed when 0.1 ml of the milk sample was plated on TSA and incubated at 35 °C for 24 h.

Antimicrobial Activity of Vanillin in Semiskim Milk

Pasteurized semi-skim milk (El Prado Cervera S.L., Valencia, Spain; 2.5% fat) was used to prepare samples.

Table 1 MIC and MBC of *L. monocytogenes* and *E. coli* O157:H7 in TSB at 35 °C

	TSB			
	MIC (ppm)		MBC (ppm)	
	pH 7	pH 6	pH 7	pH 6
<i>L. monocytogenes</i>	3,000	2,800	8,000	8,000
<i>E. coli</i> O157:H7	2,800	2,400	6,000	6,000

All values are the mean of triplicate trials

Fig. 3 Growth curves of *L. monocytogenes* in: **a** semi-skim milk incubated at 35 °C, **b** semi-skim milk incubated at 7 °C, and **c** whole and skim milk incubated at 7 °C

The milk was purchased the day before the experiment was performed and 5–6 days before its use-by date. Test tubes with 10 ml of milk were exposed in an autoclave to steam for 30 min and cooled immediately in cold water for sterilization, which was confirmed by plating on TSA. The sterile milk samples were used to prepare the different concentrations of vanillin to be assayed and the inoculums. Based on the MIC previously found in TSB, the following vanillin concentrations (ppm) were assayed in milk: 4,000, 3,500, 2,500, 2,000, and 1,500 for *L. monocytogenes* and 3,000, 2,000, 1,500, 1,000, and 500 for *E. coli* O157:H7. The solutions were prepared by adding aseptically the vanillin to the test tubes containing sterile milk, which were previously heated to 50 °C to ensure dissolution and shaken vigorously. After inoculation to obtain a bacterial population of $1\text{--}3 \times 10^4$ CFU/ml, a group of tubes were incubated at 7 °C for 14 days and another group was incubated at 35 °C for 24 h; the experiments were carried out in triplicate. Positive and negative controls were

always performed. The surviving population was enumerated by plating 100 μ l on TSA plates at 0, 2, 4, 6, 8, 11, and 14 days for the tubes incubated at 7 °C and each hour for the tubes incubated at 35 °C. The growth curves were determined by representing graphically the log CFU/ml versus the incubation period. The area under the curve was used again to calculate GI%. The MIC was the lowest concentration of vanillin that produced 100 GI%. The PIC was any concentration of vanillin lower than the MIC that produced an inhibition between 0% and 100% (Cava et al. 2007). The experimental procedure used to determine the MBC in milk was the same as described above for determining the MBC in TSB. The assayed concentrations of vanillin ranged between 8,000 and 30,000 ppm.

Antimicrobial Activity of Vanillin in Whole and Skim Milk

The antimicrobial activity of vanillin was compared in skim (0.3% fat) and whole milk (3.9% fat) following the same

Fig. 4 Growth/survival curves of *E. coli* O157:H7 with different concentration of vanillin in: **a** semi-skim milk incubated at 35 °C, **b** semi-skim milk incubated at 7 °C, and **c** whole and skim milk incubated at 7 °C

procedure as used in semi-skim milk. The vanillin concentrations tested were: 2,500, 2,000, and 1,500 ppm for *L. monocytogenes* and 1,000 and 500 for *E. coli* O157:H7.

Statistical Analysis

The GI% reached at the end of the incubation periods for each vanillin concentration assayed were compared by analysis of variance and Duncan's test ($P < 0.05$) with the Statgraphic Plus, version 5.0 (StatPoint, Inc., Herndon, VA, USA).

Antimicrobial Activity of Mixtures of Vanillin and Essential Oils of Clove, Cinnamon Leaves, and Cinnamon Bark in Semi-Skim Milk

The mixtures evaluated were combinations of vanillin and cinnamon bark, cinnamon leaves and clove in concentrations 1, 1/2, and 1/4, above and under its MIC. These essential oils have also been studied as antimicrobials in milk and their CMI for *L. monocytogenes* and *E. coli* O157:H7 previously determined (Cava et al. 2007). The

experiments were performed for *L. monocytogenes* and *E. coli* O157:H7 in microplates by triplicate and every combination was performed twice. The microplates were incubated for 14 days at 7 °C and every 2 days the microbial growth was evaluated through plate count agar. Interactions between antimicrobials may lead to additive, synergistic, or antagonistic effects. The mixture was considered synergistic when its antimicrobial activity was greater than any of the individual components alone, additive when it was equal and antagonistic when was less (Davidson and Parrish 1989).

Results

Antimicrobial Activity of the Vanillin in TSB

The growth curves of *L. monocytogenes* and *E. coli* O157:H7 in TSB (pH 6 and 7) supplemented with different concentrations of vanillin and incubated for 24 h at 35 °C are shown in Figs. 1 and 2. Table 1 shows the MICs obtained.

Table 2 MIC and MBC of *L. monocytogenes* and *E. coli* O157:H7 in semi-skim milk

	Semi -skim milk			
	MIC (ppm)		MBC (ppm)	
	35 °C	7 °C	35 °C	7 °C
<i>L. monocytogenes</i>	4,000	2,500	20,000	20,000
<i>E. coli</i> O157:H7	3,000	1,000	11,000	11,000

All values are the mean of triplicate trials

Antimicrobial Activity of Vanillin in Semi-Skim Milk

The *L. monocytogenes* and *E. coli* O157:H7 growth curves in semi-skim milk supplemented with different concentrations of vanillin after 14 days of incubation at 7 °C or for 24 h at 35 °C are shown in Figs. 3 and 4. The MICs and the GI% are shown in Tables 2, 3, and 4. For the incubation conditions of 7 °C/14 days, 2,500 ppm of vanillin (MIC) were necessary to inhibit the *L. monocytogenes* growth, while concentrations of 2,000 and 1,500 ppm could be considered PICs, with inhibition percentages of 83.8% and 52.33%, respectively. Concentrations of 4,000 and 3,500 ppm produced inhibition, without bactericidal effect. Under the same

Table 3 Growth inhibition (%) of *L. monocytogenes* in TSB at 35 °C/24 h

Vanillin (ppm)	pH 7	pH 6
3,000	100.00±0.00	100.00±0.00
2,800	93.18±5.00 m	100.00±0.00 n
1,600	86.03±3.25 k	83.37±0.99 k
1,500	83.37±0.99 k	77.19±1.83 l
1,400	64.90±0.41 j	57.37±3.66 j
1,000	42.16±2.70 h	45.61±3.66 h
800	43.86±4.70 h	34.90±2.24 g
700	30.04±6.40 f, g	29.90±2.20 f
600	28.67±2.70 f	19.26±1.52 d
500	18.30±0.82 d,e	18.26±2.00 d
400	15.51±1.57 d,c	14.79±0.49 c
300	14.77±1.38 c	10.92±0.61 c
200	9.72±0.12 b	9.54±1.58 b
100	8.46±0.10 b	7.76±0.43 b
50	2.94±0.62 a	1.94±0.12 a
50	1.62±0.53 a	1.38±1.19 a
Control	0.00±0.00	0.00±0.00

Different letters denote significant differences ($P<0.05$)

All values are the mean of triplicate trials

Table 4 Growth inhibition (%) of *E. coli* in TSB at 35 °C/24 h

Vanillin (ppm)	pH 7	pH 6
2,800	100.00±0.00	100.00±0.00
2,400	87.99±0.92 o	100.00±0.00 p
2,000	79.01±1.72 k,i	81.92±0.00 i
1,600	74.08±0.98 k	79.65±2.16 k
1,500	60.63±1.41 l	67.88±1.38 m
1,400	55.25±1.59 j	53.46±4.72 j,n
1,200	56.06±2.80 l	61.76±8.76 l
1,000	55.98±3.11 h	53.50±1.49 h,n
900	39.43±3.22 g	34.37±4.00 g
800	33.06±2.62 e,f	33.88±2.65 e
600	28.38±3.29 d,e	26.53±4.28 d
500	23.58±1.31 d	23.69±2.59 d
400	16.99±1.50 c	16.21±1.33 c
200	8.58±2.42 b	10.17±2.67 b
100	2.51±1.45 a	2.36±0.80 a
50	1.73±0.58 a	1.86±0.80 a
Control	0.00±0.00	0.00±0.00

Different letters denote significant differences ($P<0.05$). All values are the means of triplicate trials

conditions 1,000 ppm of vanillin acted as MIC for *E. coli* O157:H7, 500 ppm as PIC (with 65% inhibition) and 1,500, 2,000, and 3,000 ppm produced a negative area under the curve in relation to the control (GI% >100). When milk was incubated at 35 °C for 24 h, a concentration of 4,000 ppm of vanillin was MIC for *L. monocytogenes*, while 3,500, 3,000, 2,500, 2,000, and 1,500 ppm were PICs, with percentages of inhibition of 78%, 61%, 45%, 42%, and 23%, respectively. Under same conditions, a concentration of 3,000 ppm was MIC for *E. coli* O157:H7 and 2,000, 1,500 ppm were PICs, with percentages of inhibition of 91% and 64%, respectively. Concentrations of 4,000 and 3,500 ppm led to complete inhibition, with no bactericidal effect.

Antimicrobial Activity of Vanillin in Whole and Skim Milk

Figures 3 and 4 show the *L. monocytogenes* and *E. coli* O157:H7 growth curves obtained in skim and whole milk with the vanillin inhibitory concentrations indicated. Tables 5 and 6 show the MICs and the percentages of inhibition, respectively. Significant differences ($P<0.05$) between whole and skim milk were found for the inhibition of both microbial strains using the same

Table 5 Growth inhibition (%) of *L. monocytogenes* in milk

Vanillin (ppm)	7 °C		35 °C	
	Semi-skimmed milk	Whole milk	Skimmed milk	Semi-skimmed milk
4,000	100,00 g	ND	ND	100,00 g
3,500	100,00 g	ND	ND	78.34±1.40 d
3,000	100,00 g	ND	ND	61.45±9.20 a
2,500	100,00 g	100,00 g	100,00 g	45.98±3.36 b
2,000	81.69±1.92 d	80.15±0.91 d	100 f	42.44±11.70 b
1,500	52.33±2.51 c	56.92±1.15 c	65.33±0.88 a	23.80±19.26 d

Different letters denote significant differences between data. All values are the mean of triplicate trials
ND Not determined

vanillin concentrations; however, no significant differences were found between whole and semi-skim milk ($p<0.05$). For *L. monocytogenes*, a concentration of 2,000 ppm was inhibitory in skim milk and PIC in whole milk (with 81% inhibition). The 1,500 ppm concentration was PIC for both milk types, although the % GI for skim milk (65%) was significantly higher ($p<0.05$) than that for whole milk (56%). The 1,500 ppm concentration was PIC for both milk types, although the percentage of inhibition for skim milk (65%) was significantly higher ($p<0.05$) than that for whole milk (56%). For *E. coli* O157:H7, a concentration of 500 ppm was inhibitory in skim milk and PIC in whole milk (with 54% GI). The concentration of 1,000 ppm was inhibitory in skim and whole milk. Significant differences ($P<0.05$) between whole and skim milk were found for the inhibition of both microbial strains using the same vanillin concentrations. For *E. coli* O157:H7, a concentration of 500 ppm was inhibitory in skim milk and PIC in whole milk (with 54% GI). The concentration of 1,000 ppm was inhibitory in skim and whole milk.

MBC of Vanillin in TSB and Semi-Skim Milk Incubated at 7 and 35 °C

The MBCs obtained in TSB (pH 6 and 7) at 35 °C were 8,000 ppm for *L. monocytogenes* and 6,000 ppm for *E. coli* O157:H7 (Table 1). In skim milk, the MBC was obtained with concentrations of 20,000 and 11,000 ppm for *L. monocytogenes* and *E. coli*, respectively (Table 2). The MBC of vanillin was not influenced by the pH or storage temperature.

Antimicrobial Activity of Mixtures of Vanillin and Some Essential Oils (Clove, Cinnamon Leaves, and Cinnamon Bark) in Semi-Skim Milk Incubated at 7 °C

Combinations of 1/2 CMI of clove and 1/2 CMI cinnamon leaves with 1/2 CMI vanillin inhibited the growth of *L. monocytogenes* during the 14 of incubation and showed additive effect. Combination of 1/4 CMI of cinnamon bark with 1/4 of vanillin inhibited the growth of *L. monocytogenes* during 7 days of storage but not for 14 days showed some synergistic effect. Combinations of 1/2 CMI of cinnamon bark and 1/2 CMI of vanillin gave a partial bactericidal effect, reducing the bacterial population one log cycle (Fig. 5). The growth of *E. coli* O157: H7 was inhibited with combinations of 1/4 CMI of clove and 1/4 CMI of cinnamon leaves with 1/4 CMI of vanillin during 14 days of incubation and therefore showed also synergistic effect. Combinations of 1/2 CMI of clove and 1/2 CMI of cinnamon leaves with 1/2 CMI of vanillin gave a bactericidal effect and the final population was reduced one log cycle. Combination of 1 CMI of clove with 1 CMI of cinnamon leaves with 1CMI of vanillin had a bactericidal effect, reducing the population two log cycles. All the assayed combination of cinnamon bark with vanillin gave bactericidal effect reducing the population of *E. coli* O157: H7 between one and two log cycles (Fig. 6).

Discussion

The range in effective MICs of vanillin in TSB broth for both microorganisms was narrow and only slightly influ-

Table 6 Growth inhibition (%) of *E. coli* O157:H7 in milk

Vanillin (ppm)	7 °C		35 °C	
	Semi-skimmed milk	Whole milk	Skim milk	Semi-skimmed milk
3,000	100,00 e	ND	ND	100,00 e
2,000	100,00 e	ND	ND	91.45±9.20 a
1,500	100,00 e	100,00 e	100,00 e	64.98±3.36 b
1,000	100,00 e	97.02±0.77 g	100,00 e	ND
500	65.95±0.68 a	54.55±1.15 f	100,00 e	ND

Different letters denote significant differences between data ($p<0.05$). All values are the mean of triplicate trials
ND not determined

Fig. 5 Growth of *Listeria monocytogenes* in semi-skim milk with mixtures of vanillin with: a clove, b cinnamon leaves, and c cinnamon bark

enced by the pH (Table 1). The GI% was also the same for most of the assayed vanillin concentrations at both pHs ($p < 0.05$; Table 3). The non-effect of pH in the antimicrobial activity of vanillin in the range assayed was also observed by Delaquis et al. (2005) for different *Listeria* species. The MIC obtained by these authors for four *L. monocytogenes* strains ranged from 3,000 to 5,000 ppm (pH 6 and 7), close to the results obtained in this study for *L. monocytogenes* Scott A in TSB.

Colony formation on agar plates inoculated with microbial cells from cultures exposed to MIC levels of vanillin and the differences found between MICs and MBCs (Tables 1 and 2) indicate that the inhibitory action of vanillin was bacteriostatic rather than bactericidal. This effect was also evident in the growth curves obtained in milk where most of the vanillin concentrations assayed were bacteriostatic (Fig. 4). The antimicrobial activity of a phenolic compound depends mainly on its chemical structure and concentration (Davidson and Naidu 2000). In the present study, the vanillin antimicrobial effect was also dependent on time, temperature, culture medium, and the microorganism target. Fitzgerald et al. (2005) investigated the relationship between the structure of vanillin and its antimicrobial activity and found that the aldehyde group in vanillin is the main responsible for its antimicrobial

effect despite the influence of the type and position of some of the lateral groups in the benzene ring. Due to their hydrophobic nature, the vanillin antimicrobial mechanism is mainly based on its ability to destroy the plasmatic membrane of the microbial cells through interaction with the lipids or proteins, or with both structures, with a subsequent loss of the ionic gradient and inhibition of the respiratory activity. However, Fitzgerald et al. (2004) found that the addition of 50–70 mmol of vanillin to bacterial cell suspensions of *Listeria innocua* and *E. coli* O157:H7 led to an increase in the uptake of the nucleic acid stain propidium iodide only in a small part of the inhibited microbial population, and therefore a significant proportion of cells still remained unstained indicating their cytoplasmic membranes were largely intact and the extent of damage caused by vanillin was minor. This fact provides an explanation for the bacteriostatic activity of vanillin.

In all the experiments performed in this study, *E. coli* O157:H7 was always more susceptible than *L. monocytogenes* to the inhibitory effect of vanillin. Fitzgerald et al. (2004b), Moon et al. (2006) and Corte et al. (2004) also found that *E. coli* was more susceptible to vanillin than some *Listeria* strains. However, antimicrobials are usually less effective against Gram-negative than Gram-positive bacteria due to its more complex double membrane (Chao

Fig. 6 Growth curves of *E. coli* O157:H7 in semi-skim milk incubated at 7 °C with mixtures of vanillin with a clove, **b** cinnamon leaves, and **c** cinnamon bark

et al. 2000; Helander et al. 1998). The low resistance of *E. coli* O157:H7 could be explained by the presence of certain proteins in the external membrane, which create channels for the penetration of low molecular weight compounds (Evrendilek 2007). Vanillin showed higher MICs in TSB against *L. monocytogenes* and *E. coli* O157:H7 than found by other authors against other bacterial species. Ngarmasak et al. (2006) found MICs for vanillin of between 2,000 and 1,500 ppm against *Pantoea agglomerans*, *Aeromonas enteropelogenes*, *Micrococcus lylae*, and *Sphingobacterium spiritovorun*. Jay and Rivers (1984) reported MICs of 1,000 ppm in the case of *Staphylococcus aureus* 196E and *E. coli*, 125 ppm for *Torulopsis candida*, and 250 ppm for *Aspergillus niger*. However, the wide variation in the MIC values for vanillin found in the literature is probably related with the inoculum size, species and strain of microorganism involved.

The MIC of vanillin against *L. monocytogenes* and *E. coli* O157:H7 was approximately 1.25 times and three times, respectively, higher in milk incubated at 7 °C/14 days than that observed in TSB broth and very similar in milk incubated at 35 °C/24 h. Some authors (Karatzas et al. 2001; Burt 2004) report that the MICs of some essential oils in milk are practically double that observed in TSB. The MICs obtained in pasteurized milk are higher than those obtained for the same microorganisms in acidic

products like juice or fruit purées. Thus, for example, Moon et al. (2006) found that vanillin concentrations of 6,000 ppm exerted lethal effect over populations of *L. monocytogenes* and *E. coli* O157:H7 (10^5 CFU/ml) in apple juice at pH 4.0. Corte et al. (2004) and Char et al. (2010) found that vanillin concentrations of 3,000 ppm diminished *L. innocua* counts by between 4 and 5 log cycles after 4–8 h of incubation at 30 °C in apple juice and that it was more efficient at pH 3 than at pH 3.8. Tipparaju et al. (2004) reported that the presence of vanillin in yoghurt reduced a population of 10^7 CFU/ml of *L. monocytogenes* by 5 log cycles during 31 days at 8 °C, while in neutral pH soy beverage the vanillin was not able to inhibit the growth of this microorganism. Penney et al. (2004) found that the presence of 2,000 ppm vanillin resulted in suppression of initial microbial load (yeasts and bacterial cells) in fresh fruit yogurt containing wild blueberries over a 3-week period of storage at 4 °C. The higher antimicrobial activity of vanillin in fruit juices than in milk can be attributed to the influence of pH and to the higher lipid and protein content of milk. Fitzgerald et al. (2004a) and Moon et al. (2006) found more antimicrobial vanillin in apple juice with diminished pH. At a low pH, the antimicrobial molecules bind better to the hydrophobic zone of the membrane, where they are diluted in lipid phase, improving

their antimicrobial activity (Juven et al. 1994). Several reports have detailed the joining between vanillin and proteins that interacts with the active groups of the vanillin molecules and decrease its antimicrobial activity (Ng et al. 1989; Li et al. 2000; Chobpattana et al. 2002). Studies performed by Cava et al. (2007) with cinnamon and clove essential oils in milk and Smith-Palmer et al. (2001) in cheese also found that fat diminished the antilisterial activity of vanillin. It has been suggested that fat may form a protective film around the bacterial cells, which prevents the antimicrobial actions (Mejlholm and Dalgaard 2002) or allow the migration of the antimicrobial to the food fat phase, leaving the water phase free of antimicrobial agents and therefore a lower ability to inhibit microbial growth (Tassou et al. 1995). The decrease in incubation temperature from 35 °C to 7 °C enhanced vanillin antimicrobial activity against *L. monocytogenes* and *E. coli*. This phenomenon is attributable to the higher insaturation degree of the cellular membrane lipids in microbial cells grown at low temperature, which would favour the disruption of the membrane and consequently increase the antimicrobial effect of the vanillin. Other authors (Cava et al. 2007; Fitzgerald et al. 2004) have also mentioned this enhancing effect of low temperatures on antimicrobials.

Vanillin is usually added to foods such as ice creams, beverages, and candies in concentrations between 150 and 4,000 ppm (Hocking 1997), depending on the product type. For that reason, the MICs found in this study represent the doses frequently used in the food industry as flavouring in some food types. However, due to the antimicrobial activity showed by the mixtures evaluated in this study, it would also be possible to use vanillin at the PIC doses in combination with essential oils of clove, cinnamon leaves and cinnamon bark, to lower the sensorial impact in the milk and to make it more acceptable to consumers. This effect would be more efficient with mixtures of vanillin and cinnamon bark, due to the synergy obtained in this study with these two natural antimicrobials against the assayed *E. coli* and *L. monocytogenes* strains. However, organoleptic quality in the application of these mixtures in milk still needs to be examined. The antimicrobial activity of mixtures of vanillin with other preservatives has also been studied in fungus (Lopez-Malo et al. 2006) and in *L. inocua* (Char et al. 2010). Vanillin can undergo several types of reaction, amongst them, oxidation with changes in its antimicrobial capacity (Delaquis et al. 2005; Mourtzinou et al. 2009). In dairy products, vanillin may be oxidised to vanillic acid during milk processing through the action of the enzymes present in the milk, as well as a result of heat during pasteurization. Furthermore, in milk and dairy products, vanillin may be oxidised to di-vanillin in the presence of peroxidase (Anklam et al. 1997). Thus, further experiments are needed to determine the antimicrobial

activity of vanillin that remains when it is added to milk before thermal treatment and the vanillin/vanillic acid relation in the resulting pasteurized milk.

Conclusions

Vanillin showed effective antimicrobial activity against *L. monocytogenes* and *E. coli* O157:H7 in TSB and fluid milk at the concentrations used as food flavouring agent. Therefore, the use of vanillin can also be considered a tool for increasing the safety of pasteurized milk, due to especially because refrigerated storage does not totally ensure the control of microorganisms such as *L. monocytogenes* or *E. coli* O157: H7. Mixtures of clove and cinnamon leaves with vanillin also inhibited the growth of *L. monocytogenes* and may be reasonable to use mixtures of these essential oils to lower the individual sensorial impact

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Article II

Hydrophobic properties of cardboard coated with polylactic acid and ethylene scavengers

Hydrophobic properties of cardboard coated with polylactic acid and ethylene scavengers

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Abstract The corrugated cardboard used in food packaging readily absorbs moisture from the environment, especially when stored under high humidity conditions. In this research, the water barrier of cardboard was modified by changing the wettability of the cardboard surface, applying hydrophobic coatings of polylactic acid (PLA), alone or in combination with other substances that absorb gases (ethylene scavengers) such as clinoptilolite (C), sepiolite (S), and sepiolite permanganate (SP). The water barrier properties tested as water vapor permeability (WVP), water absorptiveness (WA), and water drop contact angle (CA) showed that the water resistance of the resulting cardboard was improved by the coating. The best results of these properties were obtained by using a coating of PLA 2% alone. In addition, PLA 2% with C 1.5% also showed good results, but the WA results were higher. The use of PLA coating produces a greater uniformity and smoothness of the coated cardboard surface due to the best filling of the pores or spaces between cellulose fibers. Our findings and the environmentally friendly nature of PLA strengthen the advantages of using these kinds of coatings on cardboard trays for the packaging, storage, and transport of foods such as fresh fruit and vegetables.

Keywords PLA coating, Ethylene scavengers, Cardboard, Water barrier properties

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Introduction

Corrugated cardboard is the most widely used of all materials for the manufacturing of paper packaging for foods, and it is recyclable, biodegradable, and compostable. It can serve as a base material to build active packages through the application of coatings with various substances, to achieve enhancement of shelf life of foods and replacement of petroleum-based plastics.^{1,2} The hydrophilic composition of cellulose cardboard makes it likely to absorb water vapor from the environment, especially when it comes in contact with high-moisture products such as fresh agricultural produce.³ A water barrier may be obtained by changing the wettability of the paper surface through coating with hydrophobic materials.⁴ For this purpose, paraffin and polyethylene have been used in a molten form on paper or cardboard to produce a water vapor barrier to provide a more durable and flexible coating.⁵ Nevertheless, the characteristics of both packaging materials make it difficult for recycling or composting.⁶ In order to produce more environmentally friendly materials, several renewable and biodegradable biopolymers have been investigated as paper coating and packaging materials.⁷

Poly(lactic acid) (PLA) has good performance characteristics as a packaging material and has become one of the most promising polymers for this purpose because it is compostable and commercially available.⁸ New techniques allowing economical production of high molecular weight PLA polymer have also expanded its uses.⁹ PLA films are quite resistant to water since they degrade slowly and are associated with water-soluble polymers or water-swollen polymers and show lower water vapor permeability (WVP) than other natural biopolymers.^{10,11} They are also compostable, helping with the solid waste disposal problems associated with packaging materials.¹² The low toxicity of PLA and its environmentally benign

characteristics have made it a suitable material for food packaging products.^{13,14} The possibilities of covering the corrugated cardboard with hydrophobic biopolymers improve the characteristics of this package material for conservation of horticultural products, which generate moisture as they ripen. The flexible biopolymer films used for packaging vegetables not only provide protection against physical damages, but they also avoid the dehydration and allow for the control of the surrounding environment of the food.¹⁵ This possibility has been assessed successfully by the authors in previous assays aimed at obtaining an active packaging for cherry tomatoes.¹⁶ Ethylene has diverse and significant effects on plant physiology. These are usually negative for the quality and shelf life of most horticultural products since the gas accelerates maturation and senescence.¹⁷ Modifications of the atmosphere inside the package can decrease O₂ and increase CO₂ in its interior, which allows the respiration and the production of ethylene by the fruit in the packaging to be reduced.¹⁸ O₂ and CO₂ modified atmosphere packages have been positively used to preserve fresh-cut tomatoes from damages.¹⁹ Combinations of absorbent substances held on various inert support materials are commonly used to remove ethylene in various postharvest situations. Potassium permanganate (KMnO₄) is usually used for this purpose, being added to different absorbent materials such as activated carbon, silica gel, aluminum oxide, and clay minerals like sepiolite, to complement the properties of the active packaging.^{20,21} The use of co-extruded zeolite-polyethylene film has been found to be acceptable as a packaging material.²² PLA and PLA/zeolite composites can be applied to altering the CO₂/O₂ permeability of the modified atmosphere packaging systems, according to the results obtained on the manufacture of composite materials based on mixtures of PLA and zeolite.²³ However, the role of the PLA concentration and the combination of substances such as zeolite and others should be studied for use as a specific lining for cardboard destined for fresh food packaging.

The aim of this study was to characterize the hydrophobic properties of PLA coating alone and with zeolite, sepiolite, and sepiolite permanganate to be used in active packaging for fresh fruits and vegetables.

Materials and methods

Materials

Poly(lactic acid (L-lactic) (Terramac Unitika Plastics, Valencia, Spain). Analytical grade chloroform (Merck KgaA 64271 Darmstadt, Germany).

Clinoptilolite (C) 92–96% purity, natural zeolite (<150 µm) (Zeo Cat, Barcelona, Spain). Apparent density 0.64 g/mL, specific surface 50 m²/g, water adsorption 13–100%.^{24–28}

Sepiolite (S) 96% purity (Bioconservación Inc., Barcelona, Spain). Apparent density 0.75 g/mL specific surface 400 m²/g, water absorption 75–250%.^{29–31}

Sepiolite permanganate (SP) (2–4 mm) Bi-On. Retarder® (Bioconservación Inc., Barcelona, Spain). Sepiolite 88% and KMnO₄ 12%, apparent density 0.88 g/mL, specific surface 400 m²/g.

PLA coating with C, S, or SP

The C, S, and SP pellets were milled by means of a balls mill, and then were sieved using a couple of wire mesh sieves (Filtro Vibración S.L., Barcelona, Spain) with opening sizes of 63 and 38 µm. The particles remaining between both screens were used to prepare the different coatings. In order to measure the final size of particles applied with the coating, the trinocular stereo-microscope and scanning electron microscopy (SEM) were used.

The PLA coating solution was prepared by dissolving 2 g of PLA in 100 mL of chloroform, stirring with a magnetic stirrer for 2 h.³² The C (1.5%), the S (1.5%), and the SP (1, 1.5, and 2% w/v) were added slowly to the solutions of PLA with continuous stirring. The microcorrugate cardboard (using pieces of 25 cm × 12 cm) was coated using calibrated gages and a metal bar coater no. 20 (SA-203 wire bar coaters, Tester Sangyo Co. Ltd, Japan). The cardboard was mounted on a flat glass plate by applying masking tape around the paper edges to prevent wrinkling of the paper during coating.¹⁰ The pieces were stored in a dry chamber at 20 ± 0.5°C and 55 ± 2% RH for 24 h prior to use. The thickness of coated cardboard samples was measured by two ways, using a handheld micrometer (dial thickness gage Nr. 7301, Mitutoyo Co. Ltd, Tokyo, Japan) with 0.005 mm accuracy and using optical microscopy. The thickness was determined at five positions along the length of the strip and mean values were reported.³³

Coating weights were determined by subtracting the weight of the uncoated cardboard from the weight of the coated cardboard, determined using an analytical balance accurate to 0.0001 g (GRAM accuracy Series SV, Taiwan).

Scanning electron microscopy (SEM) images and stereo-microscope micrographs

SEM images were used to gather information on the clays distribution and smoothness uniformity on the surface of the PLA coated microcorrugated cardboard. The uncoated and coated microcorrugated cardboard samples were observed without previous treatment in a Hitachi S-3500N Scanning Electron Microscope provided with a detector of secondary electrons, using the back-scattered electrons (BSE) signal, a working distance (WD) of 15 mm, and a voltage of 15 kV.

Micrographs of the uncoated and coated samples of cardboard were obtained in an Olympus SZ61-TR Trinocular Stereo-microscope, provided with a Olympus Altra 20 High-Resolution Digital Camera.

Water vapor permeability

WVP was measured by gravimetry according to the ASTM method described and modified by Gennadios et al.³⁴ Cardboard samples were mounted on methylacrylate cups (46 mm inside diameter and 27 mm depth), exposing the coated face to the outside. The WVP cups were filled with 18 mL of distilled water and placed in an environmental chamber (model 317332, Hotpack Corp., Pennsylvania, USA) set at 25°C and 50–60% RH. The air flow (speed 198 m/min) inside the chamber was generated by a fan. The weight of the cups was monitored every hour for a period of 8 h and the water vapor transmission rate (WVTR) was calculated by linear regression of the steady state portion of weight loss vs time curve. The WVP ($\text{g m/m}^2 \text{ s Pa}$) was determined by multiplying the WVTR by the thickness (m) of the cardboard and then dividing by the water vapor partial pressure difference across the two sides of the cardboard.³⁴

Water absorptivity

The WA of the cardboard samples was determined using the method and apparatus described by Han and Krochta.³⁵ A coated cardboard sample ($7 \text{ cm} \times 7 \text{ cm}$) was placed with the coated face up between the base and the ring of the apparatus and firmly clamped with a screw nut. Five milliliters of water was added to the surface at $25 \pm 2^\circ\text{C}$ to allow water absorption during a period of 2 min. The excess surface water on the paper was removed by wiping with tissue paper. To avoid the edge effect, the cardboard was cut into $3 \text{ cm} \times 3 \text{ cm}$ squares. The WA (g water/m^2) was determined as $\text{WA} = (W_2 - W_1)/S$, where W_1 and W_2 are the weights of the paper before and after water absorption, respectively, and S is the surface area of the cardboard

sample (0.0009 m^2). In the determination of WA, the calculated value of the initial weight of cardboard (W_1) was used, which was calculated by multiplying the weight of the initial paper sample ($7 \times 7 \text{ cm}^2$) by the area ratio of two samples, i.e., $(3 \times 3 \text{ cm}^2)/(7 \times 7 \text{ cm}^2)$.

Contact angle

The CA of water in air on the cardboard surface was measured after dropping $10 \mu\text{L}$ of bidistilled water over the coated cardboard (using pieces of $3 \text{ cm} \times 10 \text{ cm}$) placed horizontally on a glass surface. Dynamic CA variations were measured by recording the changes in a water drop during 300 s at 25°C and $50 \pm 5\%$ RH, using a video camera. The contact angles on both sides of the drop were determined from the pictures. The wetting energy of the cardboard samples was determined by the product of $\text{ST} \times \cos \theta$, where ST is the surface tension of the probe liquid (72.8 mJ/m^2 for water) and θ is the average CA measured.

Statistical analysis

All the measurements of each sample were made in triplicate on individually prepared coated or noncoated paperboard in replicate units. The data was processed statistically and the mean values determined ($p < 0.05$) using Duncan's multiple range test, from the Statgraphic package version 5.0 (Stat Point, Inc. Herndon, VA).

Results and discussion

Coated cardboard

Coatings increased the thickness and weight of the cardboard (Table 1; Fig. 1). According to our findings, the PLA film thicknesses were between 23.03 and $65.79 \mu\text{m}$ and weight from 13.08 to 23.71 g/m^2 depending on the composition of the coating material

Table 1: Physical properties of uncoated and coated paperboard with PLA 2% and PLA 2% with C, S, and SP

Paperboards	Paperboard thickness (μm)	Film thickness (μm)	Coating weight (g/m^2)
Uncoated	1184.2 ± 10.7^a	—	—
PLA 2%	1207.2 ± 7.5^b	23.03 ± 13.06^a	13.08 ± 2.91^a
PLA 2% + S 1.5%	1235.2 ± 5.7^d	50.99 ± 10.9^{bcd}	19.54 ± 3.56^{bcd}
PLA 2% + SP 1%	1226.9 ± 6.6^{cd}	42.8 ± 12.6^{bc}	16.97 ± 3.06^{abc}
PLA 2% + SP 1.5%	1236.8 ± 10.7^{de}	52.6 ± 10.7^{cd}	20.66 ± 2.59^{cd}
PLA 2% + SP 2%	1250.0 ± 12.6^e	65.79 ± 12.6^d	23.71 ± 2.11^d
PLA 2% + C 1.5%	1217.9 ± 6.8^{bc}	33.72 ± 11.51^{ab}	15.14 ± 4.56^{ab}

The shown data is means of three replicates $\pm$ standard deviation

Any two means in the same column followed by the same letter are not significantly different ($p > 0.05$) by Duncan's multiple range test. All the percentages in coating composition are in w/v

(Table 1). The particle size of C and S were 41.3 ± 4.05 and 58.5 ± 4.17 μm , respectively. This fact and the particle concentration in the coating solution increased thickness and weight of coated paperboard. Other authors have reported that the particle size of clays also should influence water barrier properties because the distribution of particles is different depending on their size and concentration in the coating.³⁶ Our findings are consistent with Han and Krochta³⁵: when the solids content increased in the coating solutions, the coating thickness increased also and that dependence was not always proportional. For the alginate and soy protein isolate (SPI) coatings it was found that the thickness also depended on the smoothness and homogeneity of the mixtures that filled the pores of the cardboard, which was more uniform than the uncoated samples.¹⁰ The results of the present work showed that the concentration of C, S, and SP influenced the coating thickness. It is interesting to note that biopolymer coatings induced a more homogeneous surface on paperboards. Figure 2 shows that the application of PLA coating to paperboard increased the smoothness of the surface. This observation has been reported by other authors³⁵ and it is

Fig. 1: Micrograph of the three layers of paper forming the microcorrugated cardboard with PLA coating and 2% sepiolite permanganate. The coated paper is on top and thickness can be observed as a white layer in the top paper. The sepiolite permanganate particles are shown on the surface of the top layer of paper

Fig. 2: Micrograph of surface of uncoated cardboard (control) (left), and surface of the PLA coated cardboard (right)

mainly due to the fact that the pores between cellulose fibers in the surface are filled with PLA.

No significant differences in thickness were observed between PLA 2% alone and PLA 2% with C 1.5%; however, the same concentration of S and even lower concentration of SP (1%) were significantly different from PLA 2%. This finding should be due to the higher particle size of S (Fig. 3).

Water vapor permeability (WVP)

The WVP of the uncoated cardboard was 4.31×10^{-9} g m/m² s-Pa. Significant reduction ($p < 0.05$) of this parameter was found for coated paperboards (Table 2). The coating with PLA 2% decreased almost tenfold the WVP of the cardboard, which is in the

Fig. 3: SEM images of surface of the sepiolite permanganate 2% PLA coated cardboard (top), showing the surface distribution of particles, and surface of uncoated cardboard (control) (down)

range of values reported by other authors for coating solutions of PLA 2%. This observed increase in water vapor barrier properties was attributed to the hydrophobicity of PLA.^{3,37} This possibility is very interesting for food packaging applications, because moisture is one of the most significant factors which affects quality of food products.

Significant differences of WVP were not found between PLA 2% alone, with S 1.5%, with C 1.5%, and with SP 1%, obtaining the values of 0.46, 0.69, 0.52, and 0.7×10^{-9} g m/m² sPa, respectively. The PLA 2% alone showed the lowest WVP and the observed increase in WVP when the concentration of SP was higher in the coating could be due to the hydrophilic properties of sepiolite.^{29–31}

In this work, the WVP of PLA coatings with the same percentage of clays (C 1.5% and SP 1.5%) showed a difference between them. PLA coating with 1.5% of SP became more hydrophilic than when the same percentage of C was applied. Although the apparent density of SP is higher, this difference is attributed to the channel structure of sepiolite, which improves the water absorption properties of coating and/or the movement of water vapor through these channels.^{38,39}

Films retaining water and preserving humidity are desirable, preventing the loss of weight of food products while keeping them fresh and without microorganism contamination. Although the addition of SP increased the WVP, the use of this additive in coatings of PLA 2% will depend on the product to be packaged and the desired adsorption of water vapor and ethylene.

Water absorptivity (WA)

The WA property provides important information on water resistance of the cardboard as it indicates how much the cardboard can absorb water when it directly contacts it. PLA coating reduced drastically the WA, and it decreases to 18.4 times in comparison to the uncoated samples. This result is consistent with that

obtained for WVP (Table 2). Rhim et al.³⁷ also found similar WA values for coated paperboard with PLA 2% and reported a decrease of WA when the PLA concentration was higher. According to the present result, Han and Krochta³⁵ and Rhim et al.³ reported that WA values of paper or cardboard increased when coated with hydrophilic biopolymers. Rhim et al.³ suggested that by choosing appropriate biopolymer materials or proper modification methods, the water resistance of paperboards could be improved. This indicates that coating with hydrophilic biopolymers is not as effective as PLA coating to increase the water resistance of paperboard.

The addition of C, S, or SP particles to the PLA 2% coating significantly increased its WA, but to a lesser extent than the uncoated paperboard (Table 2). This increase is related to the water affinity of clay additives used (C and S). Significant differences were not observed for the same concentration of C, S, and SP. The higher WA was detected in coated paperboard when more SP was applied. This fact is attributed to the water affinity of sepiolite based on the channels structure, the highly specific surface and the water absorption capacity of this material.

García-García et al.¹⁶ have already reported about the benefits of coated paperboard with PLA 3% to extend the shelf life of tomatoes. The present results showed differences between PLA 2% alone and the other coatings regarding the water absorptivity values. Therefore, the use of C 1.5%, S 1.5%, and SP 1% in these PLA-based coatings could be interesting due to the added role of controlling the water vapor or ethylene absorption.

Contact angle (CA)

The initial CA values were determined 1–2 s after water was dropped onto the coated and uncoated cardboard surfaces, and they are shown in Table 2. No significant variations were observed between the CA of coated cardboard (ranging from 73.67° to 78.33°), while the values varied significantly ($p < 0.05$) compared to

Table 2: Water vapor barrier and water resistance properties of uncoated and coated paperboard with PLA 2% and PLA 2% with C, S, and SP

Paperboards	Water vapor permeability $\times 10^{-9}$ (g m/m ² sPa)	Wetting energy (mJ/m ²)	Water absorptivity (g/m ²)	Contact angle (t_0) (°)
Uncoated	4.31 $\pm$ 0.25 ^a	29.55 $\pm$ 5.34 ^a	238.87 $\pm$ 14.61 ^a	66.00 $\pm$ 4.58 ^a
PLA 2%	0.46 $\pm$ 0.05 ^b	16.34 $\pm$ 5.65 ^b	12.97 $\pm$ 3.40 ^b	76.67 $\pm$ 4.16 ^b
PLA 2% + S 1.5%	0.69 $\pm$ 0.06 ^{bc}	17.16 $\pm$ 6.35 ^b	38.08 $\pm$ 4.56 ^{cd}	76.33 $\pm$ 5.13 ^b
PLA 2% + SP 1%	0.7 $\pm$ 0.05 ^{bc}	17.17 $\pm$ 4.97 ^b	32.15 $\pm$ 5.83 ^c	76.33 $\pm$ 4.04 ^b
PLA 2% + SP 1.5%	0.82 $\pm$ 0.12 ^{cd}	17.61 $\pm$ 1.23 ^b	43.67 $\pm$ 5.38 ^{cd}	76.00 $\pm$ 1.00 ^b
PLA 2% + SP 2%	1.05 $\pm$ 0.24 ^d	20.45 $\pm$ 4.27 ^b	48.92 $\pm$ 4.12 ^d	73.67 $\pm$ 3.51 ^b
PLA 2% + C 1.5%	0.52 $\pm$ 0.06 ^b	14.71 $\pm$ 2.59 ^b	36.13 $\pm$ 5.97 ^c	78.33 $\pm$ 2.08 ^b

The shown data is means of three replicates $\pm$ standard deviation

Any two means in the same column followed by the same letter are not significantly different ($p > 0.05$) by Duncan's multiple range test. All the percentages in coating composition are in w/v

uncoated samples (66°). PLA films, with or without added C, S, or SP, provided a lower wetting energy in the cardboard, leading to greater water resistance. These results, which are consistent with those obtained for WVP and WA, point to the reliability of the water barrier properties of these films.

The CA changes are shown in Fig. 4. The values for coated paperboards decreased at a lower rate than the uncoated. The rate of CA change on the uncoated surfaces was 23° after 5 min, while for the PLA 2% with C 1.5% and PLA 2% alone was 6° and 3°, respectively. Following the uncoated cardboard, the highest rates were observed for the PLA with SP films, and the decrease of the CA was faster when the concentration of this additive was higher. These results are according to the WVP and WA observed values and could be explained by the hydrophilic properties of sepiolite.^{29–31}

The hydrophobic performance of the coated cardboard is attributed to the water barrier properties of these coatings, which are mainly due to the hydrophobicity of PLA.^{20,37} There are two distinctively different factors, which contribute to a higher hydrophobicity, the low surface energy and a high surface roughness. In fact the low surface energy is the major factor and roughness only serves to enhance it. The latter explains why rougher surfaces (Fig. 2) (where clays additives were used) were more hydrophilic than the coating with PLA 2% because of the water affinity of clays. The same is observed in coatings with different concentrations of SP, where rougher surfaces were more hydrophilic. Other authors have reported that the surface tension of PLA 2% is due to the surface activity of the nonpolar segments in the polymer chain during film formation. Also it was observed that when the ratio of polar component increased in the film

composition, the hydrophobic properties of film surface decreased.⁴⁰

Cardboard coatings without SP, PLA 2% alone, and PLA 2% with C 1.5 showed an almost constant dynamic behavior. The positive results of the PLA coating film on the studied hydrophobic properties point out a significant increase in the water resistance of the cardboard.

Conclusions

The physical properties (thickness, weight) and water barrier properties of PLA coated cardboard (WVP, WA, and CA) showed an improvement of impermeability. PLA 2% alone or with C 1.5% or with 1% of SP were the most effective in increasing the water barrier properties of cardboard. Also, these coatings increased less the thickness and weight of coated paperboard. The use of C, S, or SP increased the water absorption of PLA-coated paperboard. These results represent an advantage for the manufacture of cardboard trays with improved humidity behavior. The compostable and environmentally friendly nature of PLA amplifies these advantages. In general, the coating of PLA 2% alone, or with C 1.5% or with SP 1%, is suitable for use in cardboard trays for the packaging, storage, and transport of foods such as fresh fruit and vegetables.

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Fig. 4: Changes in CA of the water drop on surfaces of coated cardboard with PLA 2% with addition of clinoptilolite (C), sepiolite (S), and sepiolite permanganate (SP). Filled circle—uncoated, empty triangle—PLA 2%, filled rhombus—PLA 2% + S 1%, empty square—PLA 2% + SP 1%, filled square—PLA 2% + SP 1.5%, empty circle—PLA 2% + SP 2%, filled triangle—PLA 2% + Z 1.5%

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Article III

Active Packaging of Cardboard to Extend the Shelf Life of Tomatoes

Active Packaging of Cardboard to Extend the Shelf Life of Tomatoes

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Abstract In order to extend the shelf life of fresh tomatoes using biodegradable materials, an active microcorrugated cardboard tray package was tested for use with fresh Cherry tomatoes (*Lycopersicon esculentum* var. *cerasiforme*). Active packaging characteristics were achieved by coating the interior surface of the cardboard tray with polylactic acid (PLA; 3% w/v) and, after filling with tomatoes, the tray was wrapped with a low-density polyethylene (LDPE) film of 70 or 20 μm thickness and hermetically heat sealed. Uncoated trays were used as control. The trays of tomatoes were stored at 20 ± 0.5 °C and $55 \pm 2\%$ relative humidity, for 30 days, and the ethylene concentration in the package atmosphere was measured periodically as an indicator of ripeness. The following fruit quality parameters were also studied: weight loss, surface colour, microbial count, firmness, soluble solids and pH. It was demonstrated that the capacity of PLA to adsorb ethylene and water vapour and the thickness of the LDPE film were decisive for controlling the ethylene concentration of the package atmosphere and for preventing condensation of water vapour on the fruit and film. The active packaging configuration and storage conditions used preserved fruit quality for a month, thus extending the useful life of the tomatoes and saving refrigeration costs.

Keywords Cardboard · Active packaging · Tomato shelf life · PLA · LDPE

Introduction

The shelf life of fresh fruits and vegetables, including tomatoes, has become a factor of major importance for satisfying consumer demands in terms of quality and safety (Jagadeesh et al. 2010). Active packaging, a new concept of food packaging, was developed in response to changes in current consumption and market trends and is designed to improve fresh product quality and safety (Gontard and Guillaume 2010).

The main techniques related with active packaging are substance adsorption (e.g. oxygen, ethylene, moisture, carbon dioxide, flavours/odours) and substance release (e.g. carbon dioxide, antimicrobial agents, antioxidants and flavours; Rodríguez-Lafuente et al. 2010). Cardboard, which is one of the major raw materials used for packaging food, could serve as base material for biodegradable active packages, reinforcing their mechanical properties, improving the humidity barrier and minimising microbial contamination. Such active packages could delay the maturity of climacterics fruits by controlling the surrounding environment of the fruits, providing good yield at a relatively low cost (Taechutrakul et al. 2009). New efforts are continuously being made to obtain more environmentally friendly packaging materials, and, in this respect, polylactic acid (PLA) and cardboard are renewable and biodegradable (Rhim et al. 2007). PLA is a biopolymer that shows a certain resistance to water (Rhim and Kim 2009), so that covering the corrugated cardboard with this hydrophobic biopolymer allows better conservation of vegetable products. Because PLA is compostable and derived from renewable sources, it has

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been considered as one of the solutions for packaging materials (Rhim et al. 2007).

Ethylene (C_2H_4) accelerates respiration, leading to the ripening, softening and senescence of fruit and vegetables. Many suppliers offer C_2H_4 scavengers based on potassium permanganate ($KMnO_4$), which oxidises the ethylene to acetate and ethanol. However, $KMnO_4$ products cannot be integrated into food-contact materials, so they are only supplied in the form of sachets (Taechutrakul et al. 2009; Sammi and Masud 2009). Another type of ethylene scavenger is activated carbon, which acts as an adsorbent agent (Bailen et al. 2006), although it presents problems for use in primary packaging (Martinez-Romero et al. 2009). Ethylene-adsorbing packaging films incorporating finely dispersed minerals such as zeolite clays have been tested successfully. Nevertheless, they are opaque and not capable of adsorbing ethylene sufficiently (Zagory 1995). However, the ethylene adsorption capacity of materials such as cardboard lined with PLA has been little studied for use in active packaging. Taechutrakul et al. (2009) studied the effect of corrugated cardboard containing palm shell charcoal and activated carbon on the shelf life of Cherry tomatoes, and found that it showed potential as an ethylene absorber to delay fruit ripening. Oliveira et al. (2006) studied the ethylene sorption properties of PLA, finding the Langmuir model applicable, since sorption is closely related to the presence of the free volume in PLA. However, no studies have looked at PLA as ethylene adsorbent for use in the active packaging of fresh fruit and vegetables.

When combined with cold storage, modified atmosphere packaging (MAP) can increase the shelf life of fresh products and reduce economic losses due to damage, making longer transport distances possible and lowering moisture loss during storage, while ensuring a high-quality product (Robertson 2006). MAP technology can retard fresh vegetable respiration rates and extend their shelf life. However, to achieve the required modified atmosphere (RMA) in the package, it is very important to control the permeability properties of the packaging film, the respiration rate of the vegetable, and CO_2 gas and vapour release (Caleb et al. 2011). This is the reason why obtaining the desired steady-state gas composition for some fresh vegetables may require the use of macroperforated films, or a combination of micro-perforations and suitable permeability characteristics in the films used for wrapping the vegetable trays (Lee and Renault 1998; Rennie and Tavoularis 2009; Gontard and Guillaume 2010).

Most applications of MAP for fresh vegetables use a packaging film that allows restricted gas diffusion (ethylene, CO_2 , O_2 , and vapour) through the film wall. In the packaging design process, the type of material, surface area and thickness of the packaging film are selected to obtain the desired equilibrium gas composition or RMA. The types of

film used include low-density polyethylene (LDPE), poly (vinyl chloride), and oriented polypropylene (Smith et al. 2003; Gontard and Guillaume 2010; Sandhya 2010). But, even after selecting the most suitable film characteristics, it is relatively difficult to achieve the RMA due to the complexity of controlling all the factors affecting the gas composition in the package, which moreover, continue to change during the commercial life of the packaged product. In fact, this is the limitation of both active and passive MAP when attempting to prolong even further the shelf life of packaged fresh fruits and vegetables (Gontard and Guillaume 2010). Active packaging can help achieve the RMA during the commercial life of fresh fruits and vegetables and thus obtain better quality, increased safety and a longer shelf life (Gontard and Guillaume 2010; Sandhya 2010).

The main objective of this study was to test active packaging based on cardboard trays coated with PLA and wrapped with LDPE film for the preservation of fresh Cherry tomatoes. The reduction of the ethylene content inside the package and its effect on extending the shelf life of tomatoes at storage temperature of 20 °C were analysed.

Materials and Methods

Experimental Design

Healthy Cherry tomato fruits (*Lycopersicon esculentum* var *cerasiforme*), with no physical damage and harvested the day before, were supplied by SAT Duran (Mazarrón, Murcia, Spain). The fruits were selected by size and colour (colour 3 of the colour chart of Difrusa Export SA; Artés and Gómez 2003) and transported directly to the laboratory for direct packaging.

The active package consisted of cardboard trays lined with PLA (3% w/v) of 80 µm thickness and wrapped with LDPE films of 20 and 70 µm thickness (20 trays for each LDPE thickness). Twenty cardboard trays without PLA lining were used as control for each thickness of the LDPE wrapping film.

The lining PLA solution was prepared by mixing 3 g of PLA in 100 mL of chloroform, using a magnetic stirrer for 2 h. Using calibrated gauges and a metal bar, 2.8 mL of this PLA solution was applied to the surface of the inner wall of the microcorrugated cardboard (area of 25×12 cm). The coated cardboard was dried in an oven at 100 °C for 24 h and cooled to room temperature in desiccators at 20±0.5 °C until use. The thickness and homogeneity of the PLA coating on the cardboard samples were measured using a hand-held micrometre (dial thickness gauge Nr 7301, Mitutoyo Co. Ltd., Kawasaki, Japan) with 0.005 mm accuracy. The thickness was determined (after drying) in five positions of

the sample along the length of the sample strip, taking the mean values (Rhim and Kim 2009).

Tomatoes were distributed randomly in two layers within each cardboard tray, to reach a weight of 250 ± 10 g. The trays containing the tomatoes were then wrapped with LDPE film of 20 or 70 μm thickness and thermosealed to achieve an airtight closure.

Using a factorial design (2×2), with the factors PLA coating (with and without) and wrapping LDPE film thickness (20 and 70 μm), 80 trays of tomatoes were prepared, with 20 packages for each treatment. All containers were placed in a thermostatic chamber at 20 ± 0.5 °C and $55 \pm 2\%$ relative humidity (RH). Samples were taken periodically from three trays per treatment from days 0 to 30 of storage. Analyses were performed in triplicate, determining: ethylene, weight loss, colour, firmness, soluble solids, pH and microbiological count of bacteria, yeasts and moulds in each tray.

Ethylene Determination

The ethylene composition of the package atmosphere was measured using a gas chromatograph (GC), with a Thermo Trace Gas 2000 FID detector (Thermo Finnigan, Milan, Italy) and ethylene GS-Q capillary column (J&W Scientific, Agilent Technologies Inc., USA). For this, 1 mL of gas was extracted from the package atmosphere with a syringe through a silicone septum attached to the wall of the wrapping film of the tray, and 0.5 mL was injected into the GC. The gases used were helium, hydrogen, nitrogen and air at a pressure of 4 bar. The flow rate of hydrogen was 35 mL/min, air flow was 350 mL/min and nitrogen flow 30 mL/min, at a working temperature of 250 °C. Helium was used as carrier gas (16 mL/min; Serrano et al. 2005; Bailen et al. 2006). The standard of ethylene was a mixture of four components: CO_2 ($2 \pm 0.04\%$), O_2 ($10 \pm 0.1\%$), C_2H_4 (1 ± 0 $\mu\text{L/L}$), and N_2 (the rest). The results (mean $\pm$ SE) are expressed as microlitre per litre (ethylene/interior atmosphere). After the ethylene gas analysis, the packages were opened and the tomatoes were used for physical, chemical and microbiological tests.

Weight Loss, Count of Microorganisms, Colour, Firmness, pH and Total Soluble Solids

Weight loss percentage was determined as the difference between the initial weight of the containers with tomatoes and the corresponding weight after the period of storage ($n=4$), using a balance with an accuracy of ± 0.1 g (Gram Precision Series BH, Taiwan).

For quantification of the microorganisms, approximately 25 g of tomatoes were homogenised in 250 mL of sterile saline in a Masticator (IUL Instruments, Spain) to prepare

decimal dilutions for plating. To count total mesophilic microflora, dilutions of the homogenate were plated by the pouring method in plate count agar (Cultimar, Spain). To count moulds and yeasts, the same medium was used, adding 0.1 g/L of chloramphenicol ($n=9$). The plates for counting the total microflora were incubated at 31 ± 1 °C for 72 h and the plates for yeast and mould counts were incubated at 25 °C for 5–7 days. The results are expressed as the logarithms of colony-forming unit per gramme of tomatoes (Log CFU/g; Serrano et al. 2005; Rodríguez-Lafuente et al. 2010).

The surface colour of each of the tomatoes was measured with a colorimeter (Konica Minolta Co. Ltd., Japan) using a D65 light source. Three readings were made at five equidistant points ($n=15$) for each replica, obtaining the parameters: *L* lightness, *a* the balance between green and red, and *b* the balance between yellow and blue as described in Bailen et al. (2006). The *a/b* ratio was calculated as an index of maturation.

Firmness was measured with an FT 011, 0–11 lb, penetrometer (Facchini, Alfonsine, Italy), to determine the resistance of the fruit to penetration with a rod of 8.0 mm diameter for soft fruits. The rod was placed perpendicularly on the tomato and the fruit was pressed to cause a gradual visible cut, at which point the measurement was recorded (OECD 2009).

To determine pH and soluble solids, small portions of juice were obtained by squeezing the tomatoes, using a pH metre Crison GLP 21 (Crison Instruments, SA, Barcelona, Spain) and a Digital Hand-held “Pocket” Refractometer PAL-1 (Atago Co., Ltd., Tokyo, Japan). The values are expressed as °Brix.

Statistical analysis

All measurements were made for each sample in triplicate. Sources of variation were the time of storage and treatments. Means comparison was performed ($p < 0.05$) by Duncan's multiple range test and by analysis of variance. All analyses were performed using the Statgraphic Plus package version 5.1 (Statpoint Technologies, Inc., Warrenton, VA).

Results and Discussion

Behaviour of Ethylene in the Package

In all cases, ethylene showed maximum concentration inside the packages of tomatoes after the first day of storage. This behaviour is consistent with the results obtained by other authors (Bailen et al. 2006) and it is due to the fact that the greatest release of ethylene in tomatoes occurs shortly after separation from the plant, when fruits contain a high internal concentration of ethylene. It has been demonstrated that

tomato skin is an effective barrier to ethylene and is about 1,000 times less permeable to gas exchange than cracked skin. When the tomato is harvested, the ethylene is quickly released from inside the fruit through the scar area of union with the plant (Cameron and Yang 1982). As Fig. 1 shows, the concentration of ethylene in the packages decreased after the first day and reached a minimum value at day 14. This decrease was probably due to the adsorption of ethylene by the PLA coating of the cardboard tray, since PLA-lined packages (wrapped with LDPE film of 70 μm) showed a significantly ($P<0.05$) lower ethylene concentration (0.543 $\mu\text{L/L}$) than the corresponding uncoated trays used as control (1.127 $\mu\text{L/L}$), during the storage period. In all the trays coated with PLA and wrapped with the 70 μm LDPE film, a significant ($P<0.05$) increase in ethylene concentration was observed in the third week of storage, when the value reached (1.245 $\mu\text{L/L}$) was more than double that reached the previous week. This ethylene increase can be explained by the adsorption of water vapour by the PLA coating, leading to the release of ethylene adsorbed in the same PLA coating (Oliveira et al. 2006). At the same time, ethylene-producing microorganisms may cause the supplementary production of ethylene, as might the tomatoes due to microbial stress, making ethylene release through the thick LDPE film wall more difficult. The lowest value detected for ethylene concentration in the PLA-coated trays wrapped with thick LDPE film was 0.54 $\mu\text{L/L}$ in the second week of storage, when the condensation of water vapour was not perceptible. Nevertheless, it may be necessary to further reduce ethylene to increase the shelf life since there is biological action on tomato at concentrations of only 0.1 $\mu\text{L/L}$ (Wills and Warton 2000). In the case of PLA-coated cardboard trays wrapped with thin LDPE film (20 μm), the ethylene concentration was always five-

sevenfold lower than in trays wrapped with thick LDPE film throughout the storage time. However, no great differences were found between the trays wrapped with thin LDPE film, whether or not they were coated with PLA. These results underline the great influence of the thickness of the LDPE film on the rate of ethylene and water vapour release through the film wall for achieving the RMA during the shelf life of fresh tomatoes and at a storage temperature of 20 °C.

The capacity of the PLA coating to absorb ethylene and water is largely responsible for the positive results obtained for tomato quality preservation as demonstrated primarily by colour evolution during storage. Tomatoes packaged in PLA-coated cardboard trays with both types of wrapping films showed higher fruit quality than the fruit stored in the corresponding uncoated packages used as control.

Tomato Fruit Quality Parameters

Figure 2 indicates the evolution of weight loss for tomatoes during the 30 days of storage at 20 ± 0.5 °C and $55\pm2\%$ RH. In all cases, weight loss was directly proportional to the storage time, and less than 3.5% at the end of the storage period. The weight loss in tomatoes was greatest for uncoated cardboard trays wrapped with thin LDPE film, and lowest for the PLA-coated trays wrapped with thick LDPE film. In studies of different systems for preserving fresh tomatoes, other authors (Artés et al. 1998; Bailen et al. 2006) have found weight losses of 1–4%. The observed loss of water from the tomato fruit did not affect quality, which remained optimal throughout the storage time, with tomatoes maintaining their good appearance of freshness and firmness, and a low microbial count. These quality results suggest that the loss of water in tomatoes was sufficiently controlled by keeping both RH and microbial growth within the package low.

The trays wrapped in thick LDPE film showed greater retention of water vapour from the third week of storage onwards (Fig. 2), resulting in lower weight losses in the

Fig. 1 Evolution of the ethylene concentration within the package of cherry tomatoes based on cardboard trays with and without PLA coating and wrapped in thick LDPE film of 70 μm thickness (left axis) or thin LDPE film of 20 μm thickness (right axis), during storage at 20 ± 0.5 °C and $55\pm2\%$ RH. Empty circle uncoated and thick LDPE film, empty square uncoated and thin LDPE film, filled circle PLA coated and thick LDPE film, filled square PLA coated and thin LDPE film

Fig. 2 Weight loss in cherry tomatoes packaged in cardboard trays, with or without PLA coating and wrapped in thin and thick (20 and 70 μm) LDPE film, during storage at 20 ± 0.5 °C and $55\pm2\%$ RH. Empty circle uncoated and thick LDPE film, empty square uncoated and thin LDPE film, filled circle PLA coated and thick LDPE film, filled square PLA coated and thin LDPE film

tomatoes. However, the higher RH within the package caused an increase in microbial growth on the tomato fruits packaged in uncoated trays. This situation led to the rejection (due to the poor appearance of the fruit) of 5% of the tomatoes in trays coated with PLA, and 20% in uncoated trays due to alterations of a microbial origin (Table 1). Packages wrapped with thin LDPE film caused no rejection of fruit as a result of bacterial growth or moisture condensation in any trays during storage, probably due to the better release of water vapour through the thin film wall.

Figures 3 and 4 show the development of yeasts, moulds and bacteria on tomatoes during storage at 20 ± 0.5 °C and $55 \pm 2\%$ RH. Microbial proliferation was significantly lower ($P < 0.05$) in tomatoes packaged in trays wrapped with thin LDPE film regardless of the coating, a result that was closely related to the moisture content of the container. Nevertheless, all the counts were below 10^4 CFU/g, meeting the standards set for the sanitary conditions of tomatoes (Rodríguez-Lafuente et al. 2010). These results obtained for microbial contamination and quality in tomatoes packaged in PLA-lined cardboard trays after storage for 30 days at 20 °C point to the advantage of using this kind of active packaging. However this packaging system is also very interesting from an energy-saving point of view, because it is possible to obtain a longer shelf life by maintaining the required quality at a temperature much higher than the 8–12 °C storage temperatures recommended for tomatoes (Artés et al. 1998). Some authors, like Siripatrawan and Assatarakul (2009), propose an even lower storage temperature of 5 °C. But postharvest environmental conditions need to be considered carefully when evaluating particular bioactive compounds in fresh tomatoes, because room temperature stored tomatoes can show a significant increase in their lycopene content (Javanmardia and Kubota 2006).

These findings contrast with those obtained by Rodríguez-Lafuente et al. (2010), who evaluated a new active paraffin coating for paper and cardboard for antimicrobial protection (using cinnamon bark and oregano essential oil as antimicrobial agents) and decay retardation in the active packaging of Cherry tomatoes. However, their studied storage time was only 10 days, and ethylene control was not considered.

The variation of parameter a/b , indicating colour changes in tomato skin during storage, is shown in Fig. 5. Up to the

Table 1 Defective fruit rejected because of microbial damage in cherry tomatoes packaged in cardboard trays with and without PLA coating and wrapped in LDPE film of 70 µm thickness

Coating	7 days	14 days	21 days	Total
Uncoated	—	2	2	4
PLA 3%	—	0	1	1

Fig. 3 Growth of bacteria in cherry tomatoes packaged in cardboard trays, with or without PLA coating and wrapped in thin and thick (20 and 70 µm) LDPE film, during storage at 20 ± 0.5 °C and $55 \pm 2\%$ RH. Light grey uncoated and LDPE thick, white uncoated and LDPE thin, dark grey PLA 3% coated and LDPE thick, black PLA 3% coated and LDPE thin

end of the first 2 weeks of storage, there was a significant increase in the a/b ratio in all cases due to the increase in a , although the ratio decreased significantly during the second fortnight due to an increase in the values of b . The red colour of tomatoes is due to the replacement of degraded chlorophylls by carotenoids (orange and red) indicating increasing ripening (Artés and Gómez 2003). In general, the peak of ripeness was reached after 2 weeks of storage, although the tomatoes in trays wrapped with thick LDPE film had significantly ($P < 0.05$) higher a/b ratios than those wrapped in thin LDPE film, pointing to faster ripening. In this report, the best behaviour was presented by the tomatoes stored in PLA-coated cardboard trays wrapped in thin LDPE film. So, there was a significant influence of the PLA coating on the value of the colour parameter a/b , especially in the trays wrapped with thin LDPE film, where the PLA lining absorbed part of the ethylene, contributing to delay maturation. Despite these changes in the a/b ratio, the tomatoes retained an acceptable red colour between levels 3 and 9 of the Tomato Colour Chart from Difrusa (Artés and Gómez 2003), in the active packaging and storage conditions studied.

Fig. 4 Growth of moulds and yeasts in cherry tomatoes packaged in cardboard trays, with or without PLA coating and wrapped in thin and thick (20 and 70 µm) LDPE film, during storage at 20 ± 0.5 °C and $55 \pm 2\%$ RH. Light grey uncoated and LDPE thick, white uncoated and LDPE thin, dark grey PLA 3% coated and LDPE thick, black PLA 3% coated and LDPE thin

Fig. 5 Changes in colour of cherry tomatoes packaged in cardboard trays, with or without PLA coating and wrapped in thin and thick (20 and 70 μm) LDPE film, during storage at 20 ± 0.5 °C and $55\pm2\%$ RH. Empty circle uncoated and thick LDPE film, empty square uncoated and thin LDPE film, filled circle PLA coated and thick LDPE film, filled square PLA coated and thin LDPE film

The firmness of Cherry tomatoes was significantly lower ($P<0.05$) after 30 days of storage at 20 ± 0.5 °C and $55\pm2\%$ RH (Fig. 6), except for those packaged in PLA-coated trays wrapped in thin LDPE film, which presented no significant differences. The firmness of tomatoes was significantly higher when they were packaged in trays wrapped with thin LDPE film, compared to tomatoes wrapped with thick LDPE film ($P<0.05$) for the same type of packing tray. These results demonstrate the positive effect of low concentrations of ethylene and water vapour on the firmness and other quality parameters of tomato during storage.

Figures 7 and 8 show the evolution of pH and soluble solids in tomatoes during storage in the different packaging systems studied. In general, the change in pH during storage was very small but significant ($p<0.05$), ranging from 4.20 to 4.45 between the different packaging systems. Tomatoes packaged in PLA-coated trays showed less variation in pH (between 4.35 and 4.43) than those packaged in uncoated trays (between 4.20 and 4.37). It is interesting to note the small variation observed in the pH of tomatoes packed in

Fig. 6 Changes in the firmness of cherry tomatoes packaged in cardboard trays, with or without PLA coating and wrapped in thin and thick (20 and 70 μm) LDPE film during storage at 20 ± 0.5 °C and $55\pm2\%$ RH. Light grey uncoated and LDPE thick, white uncoated and LDPE thin, dark grey PLA 3% coated and LDPE thick, black PLA 3% coated and LDPE thin

Fig. 7 Changes in pH of cherry tomatoes packaged in cardboard trays, with or without PLA coating and wrapped in thin and thick (20 and 70 μm) LDPE film during storage at 20 ± 0.5 °C and $55\pm2\%$ RH. Empty circle uncoated and thick LDPE film, empty square uncoated and thin LDPE film, filled circle PLA coated and thick LDPE film, filled square PLA coated and thin LDPE film

trays wrapped with thin LDPE film, with values ranging between 4.37 and 4.43.

The values of soluble solids in tomatoes packaged in cardboard trays wrapped with thin LDPE film presented no significant changes during the storage time (between 7.40 and 7.45). This observation confirms the interest of this kind of active packaging using thin LDPE film for maintaining the quality of tomatoes during storage. These results agree with those obtained by Srinivasa et al. (2006) in a study of tomatoes in cardboard trays. However, they found some degree of deterioration in tomatoes wrapped with LDPE film of 25 μm after 21 days of storage (at 27 °C) related with shrivelling, blemishes and shine, but there were no significant changes in pH and soluble solids compared with cardboard trays wrapped in chitosan film. These authors did not consider the use of PLA coating.

Fig. 8 Changes in soluble solids of cherry tomatoes packaged in cardboard trays, with or without PLA coating, and wrapped in thin and thick (20 and 70 μm) LDPE film, during storage at 20 ± 0.5 °C and $55\pm2\%$ RH. Empty circle uncoated and thick LDPE film, empty square uncoated and thin LDPE film, filled circle PLA coated and thick LDPE film, filled square PLA coated and thin LDPE film

According to Artés et al. (1998), tomato fruits held at a constant 20 °C or 9 °C, or intermittently warmed, increase pectolytic enzyme activity, accompanied by fall in respiration rates and ethylene production. These authors concluded that intermittent warming is more beneficial than intermittent cooling because of pitting development at 2 °C in intermittently cooled fruit. They therefore recommended low storage temperatures (between 9 °C and 12 °C) and intermittent warming, but did not consider active packaging systems.

This work highlights the interest of using active packaging systems as ethylene scavengers for tomatoes, in this case cardboard trays coated with PLA, and an appropriate selection of wrapping film (material and thickness) to maintain the RMA throughout the shelf life of the product.

Conclusions

This study has shown that the PLA coating on the inside of the cardboard tray has a very significant effect on the adsorption of ethylene and water vapour, and can be used in active packaging systems to regulate both factors. In the early stages of storage, there is a little production of water vapour and a high production of ethylene, which is adsorbed in the PLA layer. In later stages, ethylene production in tomatoes falls and water vapour production increases, so that ethylene may be exchanged for water vapour in the PLA layer, allowing the progressive exit of small amounts of ethylene through the wall of the thin LDPE film.

In conclusion, passive modified atmosphere packaging in combination with active packaging using PLA-coated cardboard trays wrapped with thin LDPE film significantly extends the shelf life of fresh tomatoes. This is a good solution for fresh tomato packaging compared with packaging systems using trays wrapped with perforated films because the active package is closed and microbial recontamination by pathogens is avoided. Therefore, microbial safety is better, while ethylene concentration inside the package is controlled at the same time. In addition, with the studied active packaging system, there is a considerable saving of energy during the storage and distribution of fresh tomatoes, as the storage temperature is 20 °C, much higher than the storage temperatures usually used with MAP.

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Patent

**Active Packaging for Preserving Fresh
Vegetables**

Object of the invention

In general, this invention is in relation with active packaging technology for fresh fruit and vegetables. The package involved in this invention is characterized by having adsorption properties of ethylene and water vapor, so that interacts with the internal atmosphere of the container filled with the fresh product (removing a part of the ethylene and water vapor), and thus achieves a better preservation of horticultural products, extending significantly its shelf life. Also, the active container object of this invention has antimicrobial properties because it allows control of the growth of pathogenic and spoilage microorganisms of the packed product.

Background of the invention

The quality of fresh products, such as fruits and vegetables, is affected by different factors of packaging, such as, among others, the relative humidity inside the container, the microbial contamination of the product, and the atmosphere inside the container which is composed by different gases (mainly O₂ and ethylene) which accelerate ripening and spoilage of the product. For example, fresh tomato quality is primarily determined by the color, firmness, the presence of microorganisms, and other sensory characteristics such as taste and odor. The color changes, loss of firmness and acidity are caused and regulated by the tomatoes ethylene production as has been reported by Carrari and Fernie (2006). Thus, the concentration of gases, mainly produced during respiration and ripening of the vegetable product, such as O₂, CO₂, ethylene and water vapor, has been treated in various ways to control the atmosphere inside the package. An example has been described in the patent ES 2246307 T3, where different active packages with adsorbent properties are used. The microbial growth control has been attempted by the inclusion of antimicrobial substances to the container (WO 2007/144444). Plant essential oils have been assessed in this sense to avoid microbial proliferation.

Many attempts have been developed to obtain an active package with antimicrobial and adsorptive properties, using polymers and plant essential oils (WO 2007/144444; ES 2144761; US 7387205 B1 and US 7128210 B2). The main problems of these approaches are the non-recyclable character of the materials used, and the control of the more important gases inside the package. Actually the packages used commercially are

wrapped with macro-perforated films to avoid the increase of ethylene inside, but other problems arise because of the packaging is relatively open to the outside.

Cardboard is one of the main materials used in packaging for food and non-food products because it is biodegradable. This achieves desirable characteristics in the package, such as lightness (low weight high capacity container), stiffness, and the availability of a suitable surface for coating, which could enhance its waterproof, allow easy printing and all at a relatively low cost. However, due to the hydrophilic nature of cellulose, cardboard is likely to absorb water vapor from the surrounding air, especially when it is used as packaging material for fresh products. Moisture absorption lowers the mechanical strength, causing package deterioration, and loss of the functionality.

Paraffins and polyethylene, applied on the cardboard surface can get a good moisture barrier. However, coatings with these materials, which are not biodegradable, cause difficulty for recycling, due to the impossibility of separating the board after use. This can be solved with the use of polylactic acid (PLA), which is a biodegradable biopolymer obtained from corn and can be recycled together with the carton,

Active packages with antimicrobial and adsorptive properties intended for fruits and vegetables, based on PLA coating as adsorptive material or as carrier for antimicrobial substances, have not been found in the consulted literature.

The water vapor permeability of PLA films are known to be lower than most natural biopolymers. Therefore, with the recent cost reduction in large-scale production, the application of PLA to packaging has widely extended. In fact, according to Rhim et al. (2007) the cardboard with PLA coating improves the water impermeability, increasing the hydrophobicity of the surface. From these investigations resulted that the optimal concentration of the PLA solution for coating the paperboard surface is 3% (weight/volume, i.e. PLA 3 g per 100 mL of solution) to obtain the best impermeability results.

PLA presents adsorption properties of gases such as CO₂, ethylene, and water vapor. According to Oliveira et al. (2006) the adsorption of CO₂ and ethylene into the PLA, at temperatures between 10 and 40 °C, and atmospheric pressure, follows a Langmuir-type mechanism while the water vapor adsorption occurs following the Henry's law.

So, would be useful to have a package combining these properties of gasses adsorption (CO₂, ethylene and water vapor). At the same time, it must contain antimicrobial substances to control the growth of microorganisms that normally spoiled these products, especially if these substances were also of natural origin. Such a package

would be particularly interesting if, in addition, all the elements that compose the container for containing plant products were biodegradable, thereby facilitating their destruction or recycling.

The present invention provides an application to this problem.

Description of the invention

This invention describes an active primary packaging, which has properties of water vapor and ethylene adsorption, and also it carries antimicrobial substances for preservation of fresh vegetables.

What is claimed is:

1 – A container for packaging fresh vegetables, comprising a body of biodegradable material, wherein the inner surface of the container, intended to be in contact with the product being packaged, is provided with a coating film of polylactic acid (PLA), this layer containing plant essential oils with antimicrobial activity.

2 – The container of claim 1 wherein the body is of a recyclable material, for example cardboard.

3 – The container of claim(s) 1 and/or 2, wherein the coating layer of the inner surface is polylactide acid (PLA). The use of PLA is especially advantageous when the container body is cardboard because this polymer provides a barrier against moisture and consequently reduces deterioration of cardboard.

4 – Another claim of the present invention refers to an active packaging intended for fruits and vegetables. The container of claim 3 is wrapped with a plastic material not perforated. The film wraps the package containing the fresh vegetables inside and gives it properties of active packaging.

5 – This invention also refers to the use of the package of claim 4 intended for horticultural products, mushrooms or cut flowers. The horticultural products could be, for example, fresh vegetables and fruits.

6 – Finally this invention refers to a method for manufacturing the container of claim 4, which comprises the steps:

- a) The fabrication of the PLA film with antimicrobial substances, which could be carried out through the extrusion of the mixture according to already known industrial procedures.
- b) The fabrication of microcorrugated cardboard sheets, with the application of the PLA coating obtained as the previous step explains according to already known industrial procedures.
- c) Cutting procedure of the aforementioned cardboard sheets to allow them to be folded for the formation of the corresponding trays, with application of the joining elements (such as adhesives or other elements).
- d) Folding and formation of trays.
- e) This step could comprise the application of a plastic material for heat sealing a wrapping film to the tray.

As used in this patent application, the container further comprises the body of the same, a layer or coating covering the inner surface composed of a polymeric material able of absorbing water vapor and ethylene, which also acts as support for antimicrobial substances.

The term "active packaging", refers to the set of the container and wrapping film which closes the package. The film covers completely the upper opening of the container once the vegetables have been introduced in it, so the stored product has no contact with the outside. That container is considered active because of the properties conferred by the components of the coating film on the inner surface of the container. First, the presence of antimicrobial substances into the coating, which inhibit the microbial spoilage and improve the preservations of the products packaged en the conatiner. Second, the presence of PLA or another compostable polymer with adsorptive properties to remove ethylene and water vapor, leads to a modification of the atmosphere inside the container. Because of that this coating reduces the amount of ethylene present, and acts as a barrier against water or water vapor and thereby helping to prolong the shelf life of vegetables contained therein, particularly the most sensitive to ethylene (ex. climateric fruits)

One embodiment of the active packaging object of the invention, not limiting, is described in Figures 1, 2 and 3, and consists of a microcorrugated cardboard tray opened at the top, with a coating application of PLA on the inner surface and wrapped with a film of low density polyethylene (LDPE) of 20 microns thickness and no perforated. The wrapping film is sealed trough heat and must be tied to the cardboard tray. The coating is forming by a thin film (40 to 70 microns thickness) of PLA, which must be previously prepared in a solution at 3% in a solvent, giving to an additional weight to the cardboard between 10-25 g/m² after production process. The plant essential oils (for example cinnamon and/or oregano) are also prepared together with the coating solution at a concentration between 2-6% (4% in the embodiment shown). The invention of the active package described here, determines a gas composition (CO₂, O₂, ethylene, and water vapor) in the atmosphere inside the container, which lengthens the shelf life of tomatoes or other vegetables packed. This invention also achieves a long-term storage, even at room temperature, of products which usually need refrigeration temperatures during storage. For example, in experiments carried out by the authors on the conservation of fresh tomato (variety Cherry) in containers such as those described in this patent, have been achieved storage times of a month, and at 20° C.

Essential oils incorporated to the coating film can be selected from the group of essential oils of cinnamon, thyme or oregano. Oils of thyme and oregano as oils extracted from wild mustard (*Lepidum country*) and bergamot, contain carvacrol, which is responsible of the typical oregano smell. That compound inhibits the bacterial growth of some microorganisms, which are habitual contaminations of different vegetables such as *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa*. Thus, the plant essential oils could be any which contain carvacrol or even pure carvacrol. In fact, any essential oil from natural extracts could be used, if it inhibits the growth of spoilage microflora, typical in vegetables, as bacteria (Gram+ and Gram -), molds ad yeasts. Particularly if it is able to inhibit the growth of at least one of the microorganism that appears on fresh vegetables and fruits, for example: *Escherichia coli*, *Listeria monocytogenes*, *Penicillium* sp., and *Rhizopus* sp.

The PLA coating improves the water barrier and the sorption properties of the cardboard. The coating adsorbs the ethylene and water vapor produced by the fruit or vegetable, achieving by this way a decreasing of the maturation rate and spoilage of quality. Vapors from the mentioned essential oils, which at the same time are embedded into the PLA coating, allow the control of microbial growth and avoid the spoilage of

the packed vegetable. The wrapping film (LDPE) has a low permeability to gases and vapors, but it allow the evacuation of the water vapor generated inside the container, and remove some of the ethylene generated. This film also obtains better conditions for the preservation of fruit, the delay of the ripening process, achieving an ideal relative humidity (RH) inside the package, and avoiding the dehydration of the vegetable packed, and finally the humidity level is insufficient for microbial growth. Also the LDPE film (preferably greater than 15 microns, 20 microns being specifically in the illustrated embodiment) prevents communication of the container with the environment becoming an additional barrier to microbial recontamination. The transparency of wrapping film allows the fruit display and to appreciate its quality by the consumer.

Most of the polymer films commercially used as wrappers are low density polyethylene (LDPE), polypropylene (PP) and polyvinyl chloride (PVC) film. Any of them could be used for wrapping the active packaging of the invention and also other polymeric materials. The CO₂ permeability of these films is usually three to six times higher than that for oxygen. The use of these films as packaging is suitable for fruits and vegetables, because it achieves an appropriate balance between the levels of O₂ and CO₂.

The water vapor permeability (WVP) is the main indicator in the evaluation of the water barrier properties of coating films applied to paper or paperboard. According to data obtained by the authors of this patent, in uncoated samples the WVP values are between 3.20 – 4.31 ng·m/m²·s·Pa, whereas in PLA coated cardboard are between 0.46 - 2.49 ng·m/m²·s·Pa. Those results confirm that this coating has significant properties of adsorption and retention of water vapor.

This allows a suitable RH within the container, but not excessive, which obtains, as previously indicated, to keep the hydration and freshness of the packaged product without stimulating the growth of spoilage microorganisms. The water absorption of PLA is 9.3 times smaller than the cardboard, which is consistent with WVP values obtained for these materials, as the PLA is less permeable. Also, the PLA film decreases the wetting energy of the cardboard and increases the water resistance (evaluated by contact angle of a water droplet on a smooth surface, which is measured over time).

Figure 1.

Figure 2.

Figure 3.

5 Conclusions

1. Vanillin and the essential oils of clove, thyme and cinnamon showed effective antimicrobial activity against *Listeria monocytogenes* and *Escherichia coli* O157:H7 “in vitro” and in fluid milk at the concentrations used as food flavouring agent.
2. The use of these substances can also be considered as a tool for increasing the safety of some food products, due especially to refrigerated storage does not totally ensure the control of microorganisms such as the pathogen target strains studied in this work
3. Mixtures of essential oils from clove and cinnamon leaves with vanillin also inhibited the growth of *L. monocytogenes* showing a marked synergistic effect, which may be reasonable to use these mixtures to lower the individual sensorial impact.
4. The physical properties (thickness, weight) and water barrier properties of PLA coated cardboard (WVP, WA, and CA) showed an improvement of impermeability.
5. The use of Clinoptinolite, Sepiolite, or Sepiolite-Permanganate increased the water absorption of PLA-coated paperboard. These results represent an advantage for the manufacture of cardboard trays with regulated humidity behavior. The compostable and environmentally friendly nature of PLA enhances these advantages.
6. PLA 2% alone or with Clinoptinolite 1.5% or with 1% of Sepiolite-Permanganate were the most effective in increasing the water barrier properties of cardboard. These coatings are suitable for use in cardboard trays for the packaging, storage, and transport of foods such as fresh fruit and vegetables.

7. PLA coating on the inner surface of the cardboard trays has a very significant effect on the adsorption of ethylene and water vapour, and can be used in active packaging systems intended to “Cherry” tomatoes to regulate the concentration of both gases.
8. In the early stages of “Cherry” tomatoes storage, there is a little production of water vapour and a high production of ethylene, which is adsorbed in the PLA layer. In later stages, ethylene production in tomatoes falls and water vapour production increases, so that ethylene may be exchanged for water vapour in the PLA layer, allowing the progressive exit of small amounts of ethylene through the wall of the thin LDPE film.
9. Passive modified atmosphere packaging in combination with active packaging using PLA-coated cardboard trays wrapped with thin LDPE film significantly extends the shelf life of fresh tomatoes. This is a good solution for fresh tomato packaging compared with packaging systems using trays wrapped with perforated films because microbial safety is better, while ethylene concentration inside the package is controlled at the same time.
10. In addition, with the studied active packaging system, there is a considerable saving of energy during the storage and distribution of fresh tomatoes, as the storage temperature is 20 °C, much higher than the storage temperatures usually used with MAP.
11. An active packaging system has been patented after finishing this experimental work. This system is based on PLA-coated cardboard trays and thermo-sealed with LDPE film (20 µm thickness), which allows to prolong the shelf life of fresh vegetables. Some claims of this patent also refer to the addition of clays additives and plant essential oils to the PLA coating in order to enhance a better preservation of the packed food.

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ANNEX I. Copy of the Patent

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DE ELZABURU MÁRQUEZ, Alberto54 Título: **ENVASE ACTIVO PARA CONSERVACIÓN DE PRODUCTOS VEGETALES FRESCOS.**

57 Resumen:

Envase activo para conservación de productos vegetales frescos. Se describe un recipiente de envasado, y un envase activo que lo comprende, con propiedades de adsorción del etileno, control del vapor de agua y capacidad antimicrobiana, de aplicación a productos vegetales frescos, en especial hortofrutícolas, con el que se modifica la atmósfera en el interior del envase, se controla la humedad y que actúa de barrera frente a los microorganismos, extendiendo la vida útil de los productos envasados, sin necesitar temperaturas bajas de conservación. Este envase activo consta de un recipiente de material biodegradable como el cartón, recubierto en su superficie interna de una lámina de un polímero con capacidad de absorción del etileno y vapor de agua, tal como PLA, en la que se incorporan aceites esenciales naturales con acción antimicrobiana, y de una película de envoltura que se aplica y termosella tras llenar el envase de producto.

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DESCRIPCIÓN

ENVASE ACTIVO PARA CONSERVACIÓN DE PRODUCTOS VEGETALES
FRESCOS

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OBJETO DE LA INVENCION

Esta invención está relacionada, en general, con la tecnología de envases activos para productos frescos hortofrutícolas. El envase objeto de esta invención se caracteriza por tener propiedades adsorbentes del etileno y del vapor de agua, por lo que interacciona con la atmósfera reinante en el interior del envase lleno de producto fresco (eliminando parte del etileno y del vapor de agua), y de esta manera consigue una mejor conservación del producto hortofrutícola que contiene, alargando de forma importante su vida útil comercial. También, el envase activo objeto de esta invención posee propiedades antimicrobianas, porque permite el control de la proliferación de microorganismos patógenos y de deterioro del producto hortofrutícola que se envasa.

ANTECEDENTES DE LA INVENCION

La calidad de los productos vegetales frescos, como las frutas y hortalizas, se ve afectada por distintos factores de envasado, como son, entre otros, la humedad relativa dentro del envase, la contaminación microbiana del propio producto y del envase, y la presencia en la atmosfera dentro del envase de diferentes gases que aceleran la maduración y el deterioro (como el O₂ y el etileno) del producto en cuestión. Por ejemplo, la calidad del tomate fresco está determinada principalmente por el color, la firmeza, la presencia de microorganismos, y otras características sensoriales como el sabor y olor; y estos cambios de color, firmeza y pérdida de acidez en tomates

son desencadenados y regulados por la producción de etileno del fruto según demuestran las investigaciones de los autores Carrari y Fernie (Carrari, F., Fernie, A.R.; Metabolic regulation underlying toamto fruit development. 5 *J. Exp. Bot.* 57: 1883-1987. 2006).

Así, la concentración de gases y vapores, producidos principalmente durante la respiración y maduración del producto vegetal, como el O₂, el CO₂, el etileno y el vapor de agua, se ha tratado de controlar de varias maneras en la 10 atmósfera del interior del envase, por medio del uso de diferentes envases activos con propiedades adsorbentes, como los que se describen, por ejemplo, en la patente ES 2246 307 T3. En esta última patente se utiliza como material de construcción del envase una lámina de 15 poliestireno (PS) expandido que contiene una sustancia orgánica o inorgánica que puede adsorber productos volátiles que deterioran la calidad del producto, tales como etileno, vapor de agua, acetaldehído y etanol. Pero, este material PS no es biodegradable. Otra solicitud de 20 patente, como la WO 2007/144444, se caracteriza por incluir sustancias naturales antimicrobianas en el material del envase a través de parafinas como vehículo, la cual se utiliza entonces como recubrimiento en papel o en cartón, para conseguir un control de la proliferación de 25 microorganismos de deterioro de los productos envasados y cierta permeabilidad.

Los aceites esenciales son sustancias naturales, extraídas de plantas, que presentan actividad antimicrobiana. Por ello, se pueden incorporar al material 30 del envase para conseguir un envase activo antimicrobiano, que permite el control de la proliferación de microorganismos patógenos y alterantes en el producto envasado, tal como se expone en la citada solicitud de patente WO 2007/144444, que utiliza los aceites esenciales 35 de tomillo y canela como agentes antimicrobianos; y en la

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patente española ES 2144761, que describe un envase activo antimicrobiano que incorpora aceite de romero, de tomillo o su combinación.

Otras patentes, como las patentes US 7,387,205 B1 y US 5 7,128,210 B2, se refieren a sistemas de envasado que utilizan determinados materiales plásticos con distinto grado de permeabilidad a los gases y al vapor de agua, y permiten así controlar hasta cierto punto la composición gaseosa en la atmosfera del interior del envase. Pero, se 10 ha demostrado que estos envases no son adecuados para ciertas hortalizas como el tomate, que tiene una gran producción de etileno. Esta es la razón de que actualmente solo se utilicen comercialmente envases con envolturas macroperforadas para dejar salir el etileno producido por 15 el tomate. Pero, con esta solución no se controla la composición de O₂, CO₂ y la humedad, en la citada atmosfera, ni es posible el control microbiano en el producto, porque el envase está relativamente abierto al exterior.

El cartón es uno de los principales materiales 20 utilizados en los envases para productos alimentarios y no alimentarios, ya que, además de ser biodegradable, consigue una serie de características muy apreciadas en el envase, como son la ligereza (poco peso en un envase de gran 25 capacidad), la rigidez y la disponibilidad de una superficie apropiada para la aplicación de recubrimientos que mejoran sus características de impermeabilidad, y permiten una fácil impresión, todo ello a un coste relativamente bajo. Sin embargo, debido a la naturaleza 30 hidrofílica de su composición de celulosa, los cartones son propensos a absorber vapor de agua del aire que le rodea, especialmente cuando se utilizan como material de envasado de productos hortofrutícolas frescos. La absorción de 35 humedad reduce la resistencia mecánica de los cartones, causando su deterioro, y la pérdida de la funcionalidad

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principal del envase como es la de continente.

Las parafinas y el polietileno, aplicados sobre la superficie del cartón pueden conseguir una buena barrera a la humedad. Sin embargo, los recubrimientos con estos
5 materiales, que no son biodegradables, provocan dificultades a la hora del reciclaje del cartón, por la imposibilidad de separarlos del cartón después de su uso. Esto puede resolverse con el uso del ácido poliláctico (PLA), que es un biopolímero biodegradable que se obtiene
10 del maíz, que se puede reciclar junto al cartón, y que permite la mejora de la impermeabilidad del cartón recubierto con el mismo.

En ninguna de las patentes encontradas sobre envases activos adsorbentes y antimicrobianos, para frutas y
15 hortalizas, se utiliza el PLA como material adsorbente, o de soporte de sustancias antimicrobianas, en la construcción del envase correspondiente, sea de cartón o de otro material.

Los valores de permeabilidad al vapor de agua de la
20 película de PLA son conocidos por ser dos órdenes de magnitud inferiores a las de la mayoría de los biopolímeros naturales. Por ello, con la reciente reducción de costes en su producción a gran escala, se ha extendido la aplicación de PLA a los envases. En efecto, según el artículo de RMI y
25 colaboradores (J.W. Rhim, J.H. Lee, y S.I. Hong (2007). "Increase in water resistance of paperboard by coating with poly (lactide)". Packaging Technology and Science 20: 393-402), el recubrimiento de cartones con PLA mejora la impermeabilidad al agua, ya que aumenta la hidrofobicidad
30 de su superficie. De estas investigaciones se deduce que la concentración óptima de la solución de PLA para el revestimiento de la superficie del cartón es del 3 % (en peso / volumen, es decir 3 g de PLA por cada 100 mL de solución), para obtener los mejores resultados de
35 impermeabilidad.

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El PLA tiene propiedades de adsorción de los gases y vapores, como el CO₂, el etileno, y el vapor de agua. En efecto, según el artículo de los autores Oliveira y colaboradores (Oliveira, N.S., Gonzalves, C.M., Coutinho, J.A.P., Ferreira, A., Dorgan, J., Marrucho, I.M., 2006. Carbon dioxide, ethylene and water vapor sorption in poly(lactic acid). Fluid Phase Equilibria. 250, 116-124), la adsorción del CO₂ y del etileno en el PLA, a temperaturas entre 10 y 40°C, y a presión atmosférica, presenta un mecanismo tipo Langmuir, mientras que la adsorción del vapor de agua se ajusta a la ley de Henry.

Sería interesante disponer de un envase que combinara las propiedades de capacidad de adsorción de gases y vapores que influyen en la calidad y el tiempo de conservación de los productos vegetales envasados, tales como el CO₂, el etileno y el vapor de agua, con la ventaja de contener sustancias antimicrobianas capaces de controlar el crecimiento de los microorganismos que normalmente atacan a estos productos, especialmente si dichas sustancias fueran también de origen natural, en particular de origen vegetal. Dicho envase sería especialmente interesante si, además, todos los elementos que compusieran el recipiente destinado a contener los productos vegetales fueran biodegradables, facilitando con ello su destrucción o su reciclado.

La presente invención proporciona una solicitud a ese problema.

DESCRIPCION DE LA INVENCION

En esta invención se propone el uso del biopolímero biodegradable PLA, o de cualquier otro biopolímero con capacidad de adsorción del etileno y del vapor de agua, como material de recubrimiento de recipientes que forman parte de envases activos para el envasado de productos vegetales frescos, para conseguir que el recipiente, y el

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envase formado a partir del mismo, posean propiedades de adsorción del etileno y de soporte de sustancias antimicrobianas y, al mismo tiempo, mejorar la resistencia a la humedad de dicho recipiente, ventaja que es especialmente interesante como el material de base del que está formado el cuerpo del recipiente es un material biodegradable propenso a adsorber vapor de agua del aire que le rodea, como sucede con el cartón.

En esta invención se describe un envase primario activo con propiedades de adsorción del vapor de agua y del etileno, y de soporte de sustancias antimicrobianas, para su aplicación al envasado y conservación de productos vegetales. También se describe el recipiente que forma parte de dicho envase activo, que es el receptáculo destinado a contener los productos vegetales, cuyo cuerpo se recubre con una lámina de PLA u otro polímero biodegradable con propiedades similares de adsorción del vapor de agua y del etileno, lámina que actúa de soporte de sustancias antimicrobianas de origen vegetal. El recipiente como tal, por tanto, posee por sí mismo capacidad de adsorción del etileno producido por los productos que contenga y propiedades de barrera frente a los microorganismos y frente a la humedad. Las condiciones adecuadas de utilización del mismo se consiguen al cubrirlo con una envoltura una vez depositado el producto sobre el mismo, obteniéndose el envase activo de la invención, con el cual puede conseguirse la modificación de la composición de la atmósfera en el interior del envase, reduciendo la cantidad de etileno presente, permitiendo además el control de la humedad dentro del mismo y actuando como barrera a los microorganismos. Todo ello permite extender la vida útil de los productos envasados, sin necesidad de utilizar bajas temperaturas de conservación.

Así, en un primer aspecto, la invención se refiere a un recipiente para el envasado de productos vegetales, que

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comprende un cuerpo (2) de material biodegradable, caracterizado porque la superficie interna del recipiente, destinada a estar en contacto con el producto que se envasa, está provista de una lámina de recubrimiento (1) de
5 ácido poliláctico (PLA) o de cualquier otro biopolímero con capacidad de adsorción del etileno y del vapor de agua, lámina que contiene extractos naturales de aceites esenciales con actividad antimicrobiana.

En una realización preferida de este aspecto de la
10 invención, el material biodegradable que forma el sustrato del cuerpo del recipiente es cartón microcorrugado. En otra realización, que puede o no combinarse con la anterior, la lámina de recubrimiento de la superficie interna del recipiente es de ácido poliláctico (PLA). La presencia de
15 PLA resulta especialmente ventajosa cuando el cuerpo del recipiente es de cartón microrrugado, porque el PLA proporciona al cartón una barrera frente a la humedad y disminuye su deterioro.

En un segundo aspecto, la invención se refiere a un
20 envase activo para el envasado de productos vegetales, que comprende el recipiente de envasado de productos vegetales de la invención y una película de envoltura de material plástico sin perforar, que envuelve o cierra el lote compuesto por el recipiente de envasado y los productos
25 vegetales contenidos en el mismo.

En otro aspecto, la invención se refiere al uso del recipiente de envasado de la invención para el envasado de productos vegetales hortofrutícolas, setas o flores cortadas. Los productos hortofrutícolas pueden ser, por
30 ejemplo, verduras, hortalizas o frutas. En una realización preferida de este aspecto de la invención, el recipiente de envasado se envuelve, una vez introducidos los productos vegetales, con una película de envoltura de un material plástico sin perforar, de manera que el conjunto formado
35 por el recipiente de envasado y su contenido queda cerrado.

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Finalmente, en un aspecto más, la invención se refiere a un método para la fabricación de un recipiente de la invención, que comprende las etapas de:

a) fabricación de la película de PLA con agentes antimicrobianos, que se puede llevar a cabo mediante extrusión de la mezcla, según procedimientos industriales ya establecidos; b) fabricación de planchas de cartón microcorrugado, con la aplicación del film de PLA obtenido en el paso anterior, según procedimientos industriales establecidos; c) realización del corte de las citadas planchas que permita su plegado para la formación de las bandejas correspondientes, con aplicación de los elementos de unión (como adhesivos u otros elementos) necesarios entre los laterales y el fondo de la bandeja; y d) plegado o formado. La etapa c) puede comprender la aplicación sobre los bordes de las paredes de la bandeja de una laca de material plástico a utilizar para termosellar a la bandeja una película de envoltura.

Así, tal como se utiliza en la invención, el término "recipiente para el envasado de productos vegetales" se refiere a un recipiente abierto por su parte superior, que consta de un cuerpo abierto por su parte superior, con una forma que le permite contener los productos vegetales que han de situarse en el mismo. Dicho cuerpo puede adoptar distintas formas, que habitualmente comprenderán una base esencialmente plana y una o más paredes laterales que circundan su perímetro, que determinan una superficie interior del cuerpo del recipiente, destinada a estar en contacto con los vegetales a contener, y una superficie exterior, que estará en contacto con el medio exterior. Tal es la disposición, por ejemplo, del cuerpo en forma de bandeja que muestra el recipiente utilizado en los Ejemplos que se muestran más adelante en la presente solicitud. Tal como se utiliza en la presente solicitud, el recipiente comprende, adicionalmente al cuerpo del mismo, una lámina

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que recubre su superficie interior, compuesta por un material polimérico con capacidad de absorción de vapor de agua y etileno, que actúa como soporte de sustancias con actividad microbiana.

5 El término "envase activo", por su parte, se refiere al conjunto formado por el recipiente de envasado y una película de envoltura con la que se cierra el mismo, que cubre completamente la abertura superior del recipiente una vez que los vegetales se han introducido en él, de forma
10 que los vegetales no tienen contacto con el exterior. Dicho envase se considera activo por las propiedades conferidas por los componentes de la lámina de recubrimiento de la superficie interior del recipiente: por una parte, las sustancias antimicrobianas inhiben el crecimiento de los
15 microorganismos que aparecen en los productos hortofrutícolas frescos, ayudando a su conservación; por otra parte, la presencia del PLA u otro polímero biodegradable con capacidad de absorción de etileno y vapor de agua, da lugar a una modificación de la atmósfera
20 reinante en el interior del envase, disminuyendo la cantidad de etileno presente, actuando de barrera frente a la humedad y ayudando con ello a alargar el tiempo de vida útil de los productos vegetales en ellos contenidos, especialmente los más sensibles al etileno.

25 Una realización, no limitante, del envase activo de la invención, se describe en las **Figuras 1, 2 y 3**, y consiste en un envase de cartón microcorrugado, en forma de bandeja abierta por la parte superior, que tiene una aplicación de un recubrimiento **1** de una lámina de PLA sobre el papel
30 interno **2** de la pared de cartón microcorrugado **3**, y que tiene una envoltura **4** de lámina de polietileno de baja densidad (LDPE) de 20 µm de espesor, y sin perforar, que se cierra mediante termosellado de sus lados **5**, y que ha de estar ceñida a la bandeja de cartón. Esa lámina **1** está
35 formada, por una película fina (preferiblemente, de 40 a 70

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µm de espesor) de PLA (aplicada, preferiblemente, según una solución al 3%, en peso / volumen de disolvente, dando lugar a un gramaje comprendido entre 10 y 25 g/m²) que contiene aceites esenciales (por ejemplo, de canela u
5 orégano), en una proporción comprendida entre el 2% y el 6% en peso, que es concretamente del 4% en la realización representada. Esta solución de recipiente de envasado **3** y envoltura **4** determina una composición gaseosa (concentración de CO₂, de O₂, de etileno, y de vapor de
10 agua) en la atmósfera **6** del interior del envase, que permite alargar la vida útil de los tomates **7**, u otro producto hortofrutícola envasado, consiguiendo además, una conservación de larga duración y en condiciones de temperatura ambiente, sin necesidad de conservar a baja
15 temperatura. Por ejemplo, en experimentos llevados a cabo por los autores sobre conservación de tomate fresco (variedad Cherry), en envases como los que se describen en esta patente, se han conseguido tiempos de conservación de un mes, y a una temperatura de 20°C.

20 Los aceites esenciales incorporados en la lámina de recubrimiento se pueden seleccionar del grupo de aceites esenciales de canela, tomillo u orégano. Los aceites de tomillo y orégano en particular, al igual que los aceites extraídos de la mostaza silvestre (*Lepidum campestre*) y los
25 de bergamota, contienen, entre otros, el compuesto denominado carvacrol que, además de ser responsable del olor característico a orégano, tiene capacidad de inhibir el crecimiento de distintas bacterias que son contaminantes habituales de distintos alimentos, entre ellos los de
30 origen vegetal, tales como *Escherichia coli*, *Bacillus cereus* y *Pseudomonas aeruginosa*, por lo que, además de los indicados, el aceite esencial puede ser otro cualquier aceite esencial procedente extractos naturales que contenga carvacrol o, incluso, carvacrol puro. En general, puede

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utilizarse cualquier aceite esencial de extractos naturales, siempre que dé lugar a la inhibición del crecimiento de microorganismos que aparecen en productos hortofrutícolas frescos, tales como bacterias Gram
5 negativas, Gram positivas, mohos y levaduras, particularmente si son capaces de inhibir el crecimiento de al menos un microorganismo que aparece en los productos hortofrutícolas frescos que se selecciona entre *Escherichia coli*, *Listeria monocytogenes*, *Penicillium sp.* y *Rhizopus*
10 *sp.*

El revestimiento o lámina de recubrimiento **1** de PLA le confiere al cartón **3** propiedades hidrofóbicas y la función de absorción del etileno y del vapor de agua, que produce el fruto, consiguiendo así la disminución de la velocidad
15 de maduración y deterioro de la calidad del mismo; desde el recubrimiento **1** de PLA se liberan los vapores de los citados aceites esenciales que permiten mantener el control del desarrollo microbiano y evitar el deterioro microbiano del fruto; la envoltura **4** de lámina de LDPE (con cierta
20 permeabilidad a los gases y los vapores) de la bandeja de cartón **3** permite la evacuación de parte del vapor de agua que se genera en el interior del envase y la evacuación de parte del etileno generado, obteniendo unas condiciones más adecuadas para la conservación del fruto, ya que retrasa el
25 proceso de maduración y consigue una humedad relativa (HR) idónea, para que no se deshidrate el fruto y, a la vez, que sea insuficiente para el desarrollo microbiano. La envoltura en forma de película **4** evita la comunicación con el ambiente exterior del envase, convirtiéndose en una
30 barrera adicional a la recontaminación microbiana.

La película de envoltura **4** de LDPE, con el espesor seleccionado (preferiblemente superior a 15 μm , siendo concretamente de 20 μm en la realización representada), permite controlar el intercambio de gases y vapores entre

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el interior del envase y el aire exterior que lo rodea. Esto logra que se mantenga la atmósfera deseada en el interior del envase, y permite mantener durante su conservación las características organolépticas deseadas en el producto hortofrutícola. La transparencia de la película de envoltura **4** permite visualizar los frutos y apreciar su calidad por parte del consumidor.

Las películas poliméricas más usadas comercialmente como envolturas son polietileno de baja densidad (LDPE), polipropileno (PP) y cloruro de polivinilo (PVC). Para la película de envoltura del envase activo de la invención puede usarse cualquiera de ellas, u otro material polimérico. La permeabilidad al CO₂ de estas películas es usualmente de tres a seis veces mayor que para el oxígeno. La utilización de estas películas como envoltorios es adecuada para frutas y hortalizas, porque se logra un equilibrio adecuado entre los niveles de O₂ y CO₂.

La permeabilidad al vapor de agua (PVA) es de los principales indicadores en la evaluación de las cualidades hidrofóbicas y/o hidrofílicas de las películas aplicadas como revestimiento del papel o cartón. Según datos obtenidos por los autores de esta patente, en el papel no recubierto los valores de PVA son de 3,20±0,21 ng.m/m².s.Pa, mientras que cuando está recubierto con una lámina de PLA son de 2.49±0.22 ng.m/m².s.Pa, poniéndose de manifiesto que este recubrimiento posee propiedades significativas de adsorción y retención del vapor de agua. Esto permite que haya una HR adecuada dentro del envase, pero no excesiva, lo que consigue, tal como se ha indicado anteriormente, el mantenimiento de la hidratación y frescura del producto envasado sin estimular la proliferación de microorganismos de deterioro de la calidad del mismo.

La absorción de agua del PLA es hasta 16 veces menor que la del papel solo, lo cual es concordante con los

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valores obtenidos de PVA para estos materiales, ya que el PLA es menos permeable. Asimismo, la película de PLA confiere a la lámina de papel una inferior energía de humectación y un menor trabajo de adherencia a su superficie, lo que implica una mayor resistencia al agua (evaluada a través del ángulo de contacto de una gota de agua sobre una superficie lisa, que se mide a lo largo del tiempo.

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BREVE DESCRIPCION DE LOS DIBUJOS

La **Figura 1** se muestra una vista general del envase activo objeto de esta invención y sus componentes principales: la bandeja **3** de cartón microcorrugado, y la envoltura plástica **4** del mismo, que encierran al producto **7** envasado.

La **Figura 2** muestra una sección de este envase activo que permite distinguir la pared de la bandeja **3** de cartón microcorrugado, y la pared de la envoltura plástica **4** que está ceñida a la parte superior e inferior de la bandeja **3**.

La **Figura 3** muestra el detalle de un corte de la pared de la bandeja **3** de cartón microcorrugado, cuya capa interna **2** (en contacto con el producto que se envasa), que es de papel, está recubierta por una capa **1** de PLA.

La **Figura 4** muestra un gráfico en el que se representa la evolución a lo largo del tiempo, expresado en días, de la concentración de etileno, en partes por millón (ppm, equivalentes a miligramos por litro) en el interior de envases activos de cartón microcorrugado con tomates *Cherry*, almacenados a 20°C y 55% de humedad relativa, en envases con o sin recubrimiento de PLA y con o sin lámina de envoltura de LPDE según se indica en las leyendas.

La **Figura 5** muestra la evolución del porcentaje de pérdida de peso a lo largo del tiempo, expresado en días,

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de los tomates almacenados en las mismas condiciones que en el ensayo de la Figura 4.

La **Figura 6** muestra un gráfico en el que se representa la evolución a lo largo del tiempo, expresado en días, de la concentración de bacterias, expresada como el logaritmo en base 10 de las unidades formadoras de colonia (UFC) detectadas por gramo (UFC/g), de los tomates almacenados en las mismas condiciones que en el ensayo de la Figura 4.

La **Figura 7** muestra un gráfico en el que se representa la evolución a lo largo del tiempo, expresado en días, de la concentración de hongos y levaduras, expresada como el logaritmo en base 10 de las unidades formadoras de colonia (UFC) detectadas por gramo (UFC/g), de los tomates almacenados en las mismas condiciones que en el ensayo de la Figura 4.

La **Figura 8** muestra un gráfico en el que se representa la evolución a lo largo del tiempo, expresado en días, del color, expresada como la relación entre los parámetros a (proporción rojo/verde) y b (proporción amarillo/azul) del color, de los tomates almacenados en las mismas condiciones que en el ensayo de la Figura 4.

La **Figura 9** muestra un gráfico en el que se representa la evolución a lo largo del tiempo, expresado en días, de la firmeza de los tomates, expresada como la fuerza en Kg necesaria para provocar un corte con un penetrómetro), de los tomates almacenados en las mismas condiciones que en el ensayo de la Figura 4.

DESCRIPCIÓN DE UN EJEMPLO PRÁCTICO DE REALIZACIÓN DE
LA INVENCION.

Un modo no exclusivo, de realización y aplicación del envase activo objeto de esta invención, a partir de cartón microcorrugado recubierto con una capa **1** de PLA que contiene aceites esenciales, implicaría llevar a cabo

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distintas etapas, que incluirían las de fabricación del propio envase activo, es decir:

- 5 a) Fabricación del cartón microcorrugado: Se toma una plancha de cartón microcorrugado, fabricada según procedimientos industriales ya establecidos. Se realiza el corte de la misma que permita el plegado para la formación de la bandeja correspondiente, con aplicación de los elementos de unión (como adhesivos u otros
- 10 elementos) necesarios entre los laterales y el fondo de la bandeja; aquí también se incluiría, en su caso, la aplicación de laca del material plástico a utilizar para termosellar esta bandeja, que se realizaría sobre los bordes de
- 15 las paredes de esta bandeja.
- b) Preparación en laboratorio de la mezcla de PLA con aceites esenciales antimicrobianos: El PLA se puede preparar según una disolución en cloroformo (al 3% peso/volumen, es decir 3 g de PLA en 100
- 20 mL de cloroformo; siguiendo el procedimiento establecido por el citado artículo de los autores J.W. Rhim, J.H. Lee, y S.I. Hong, (J.W. Rhim, J.H. Lee, y S.I. Hong (2007). "Increase in water
- 25 resistance of paperboard by coating with poly (lactide)". Packaging Technology and Science 20: 393-402) y se adiciona previo al PLA, el aceite esencial natural, con agitación magnética y según la proporción deseada (en este caso, según una concentración del 4% peso / volumen).
- 30 c) Aplicación del recubrimiento **1** de PLA con aceites esenciales a la superficie de la capa **2** de papel del cartón microcorrugado que constituye la cara interna de la bandeja **3** de cartón microcorrugado: Se hace para conseguir un espesor deseado de 40 a
- 35 70 micras (μm) sobre la capa **2** del cartón. Para

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ello, se puede utilizar una máquina automática de aplicación de este recubrimiento, consiguiendo un espesor de capa muy homogéneo. El gramaje del recubrimiento (que será de 15 a 20 g/m²) se controla por pesada. En laboratorio, los cartones con el recubrimiento aplicado de PLA se secan en estufa a 100° C durante 24 h. Se sacan de la estufa y se dejan en desecador (a 50% de HR y 25°C) hasta su uso en la formación de la bandeja **3** correspondiente. El gramaje del recubrimiento aplicado se obtiene por diferencia de peso del cartón con y sin recubrimiento, mediante pesada en balanza analítica. También, se mide el espesor de la película **1**, formada sobre la capa interna de papel **2** del cartón, con un micrómetro manual con una precisión de 0.005 mm.

El análisis de la capacidad antimicrobiana del citado recubrimiento de PLA con aceites esenciales se comprueba mediante ensayos sobre placas Petri, donde se adiciona el medio de cultivo específico para el crecimiento de cada tipo de microorganismo (se han ensayado microorganismos Gram (+), Gram (-), mohos y levaduras, con una concentración de inóculo de 10⁴ UFC/mL), y se coloca la muestra de cartón (un disco de cartón microcorrugado con una capa de papel revestida con el PLA y aceite esencial, tal como se ha indicado anteriormente) pegada a la placa Petri, y con la superficie de la capa de papel recubierta de PLA orientada hacia el espacio libre de la placa. Las placas tapadas son selladas con una cinta de parafilm para evitar la salida de los vapores de aceite esencial. La placa Petri, así dispuesta, se incuba en una estufa en las condiciones de crecimiento microbiano. Para el control, se sigue igual procedimiento, pero en lugar de utilizar cartón con aceite esencial, se coloca cartón con revestimiento de

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PLA sin aceite esencial, y cartón sin revestimiento, y, además, una placa Petri sin cartón en la tapa de la placa. Se utiliza el método del recuento de células viables para cuantificar el crecimiento microbiano. La mayor disminución
5 de colonias equivale a una mayor actividad antimicrobiana del cartón con la capa de papel recubierta con PLA y aceite esencial. La diferencia observada con los ensayos control ha permitido poner de manifiesto de una manera significativa la capacidad antimicrobiana del vapor de los
10 aceites esenciales liberados desde el recubrimiento con PLA del papel.

Así, los autores de esta patente han podido demostrar que los aceites esenciales de orégano, canela, tomillo y carvacrol, aplicados a la superficie del cartón
15 microcorrugado, en el citado recubrimiento de PLA, según una concentración del 4% (peso / volumen), provocan una inhibición total de las bacterias y hongos evaluados.

d) Llenado de las bandejas anteriormente fabricadas (con recubrimiento de PLA y aceites esenciales) con tomates *Cherry*, y
20 envoltura de estas bandejas con láminas de LDPE: Se utilizan tomates (*Lycopersicum esculentum* Mill.) tipo *Cherry* recolectados cuando tenían una calidad comercial estándar, y se prepara un lote de 20
25 bandejas de tomates sin defectos. Cada una de estas bandejas se llenan con dos capas de tomates, consiguiendo un peso total por bandeja de 250±15 g. Para todos los frutos
30 de cada bandeja, se determina su peso, diámetro, color (parámetros L*: luminosidad, a*: proporción rojo/verde, b*: proporción amarillo azul), firmeza (según la metodología del Instituto di
35 Coltivazioni arborea, Milán, Italia que se

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detalla más adelante). Cada una de las bandejas se envuelven con lámina de polietileno de baja densidad (LDPE), de 20 µm de espesor, y con cierre hermético termosellado (según la **Figura 1**), y se almacenan a 20 °C y 55±5 % HR.

A lo largo de la vida útil de estos tomates así conservados, en envases activos de cartón microcorrugado, con propiedades adsorbentes del etileno y capacidad antimicrobiana, se han determinado los siguientes parámetros de calidad: Pérdida de peso en % respecto del peso inicial; Recuento microbiano; Color; Índice de madurez, y Firmeza.

La pérdida de peso se determina semanalmente por diferencia respecto del peso inicial de los envases conteniendo los tomates, y se mide en balanza con exactitud de ±0,1 g (por ejemplo, de la marca GRAM Precisión, serie BH, de Taiwan).

Para la realización del recuento microbiano en los tomates así conservados, se toman aproximadamente 25 g de tomate y se homogeneizan en 250 mL de suero fisiológico estéril en un equipo, por ejemplo, de la marca Masticator IUL Instruments (de España). Se siembran en placas Petri diferentes cantidades de este homogeneizado, en medios específicos según el tipo de microorganismo a evaluar, y se llevan a estufa para incubación (se analizan Bacterias, Hongos o Levaduras).

El color superficial de cada uno de los tomates se mide con colorímetro, por ejemplo, de la marca Konica (de Minolta Co Ltd., Japón), y una fuente de luz D65. Se miden los parámetros L, a, y b. El cociente a/b es empleado como índice de madurez para el caso de los tomates.

La firmeza se mide con un penetrómetro, por ejemplo, de la marca FT 011 (de 0 a 11 lb, según metodología del Instituto di Coltivazioni arborea, de Milán, Italia),

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utilizando una punta de 8.0 mm de diámetro, de aplicación a frutos blandos. Esta punta se coloca perpendicularmente sobre el tomate y se presiona sobre el fruto hasta provocar un corte visible, momento en que se registra la medida.

5 Del análisis de los resultados de evolución de los distintos parámetros de calidad del tomate estudiado, a lo largo de su conservación, se ha podido deducir que las mejores condiciones de conservación de tomates *Cherry* se obtuvieron utilizando envases activos de cartón
10 microcorrugado recubiertos en su cara interior con PLA al 3% (peso/volumen), con aceite esencial de canela u orégano, aplicado al 4% (del peso del recubrimiento de PLA), y envueltos con películas de LDPE de 20 µm de espesor. Se ha
15 podido comprobar que en estas condiciones se mantiene la calidad comercial del tomate hasta 30 días.

En efecto, en la **Figura 4** se muestra la evolución de la concentración de etileno en el interior de los envases activos de cartón microcorrugado con tomates *Cherry*, con y sin recubrimientos de PLA, y envueltos en lámina de LDPE de
20 70 µm (grueso, eje de la izquierda) y 20 µm (delgado, eje de la derecha), en almacenamiento a 20 °C y con 55% HR. Se comprueba que los menores niveles de etileno se mantienen en el caso de bandejas recubiertas con PLA 3%, y envueltas con película LDPE fina (de 20 µm de espesor). Esto hace que
25 los tomates se conserven mejor que en las demás condiciones. En este tipo de bandejas también tienen lugar las menores pérdidas de peso en los tomates (ver **Figura 5**), a lo largo de los citados 30 días; y se tiene un menor desarrollo de bacterias (ver **Figura 6**), y de hongos y
30 levaduras (ver **Figura 7**). Con el envase activo objeto de esta patente, se ha obtenido una menor variación del color (ver **Figura 8**), y de firmeza (ver **Figura 9**).

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APLICACIONES INDUSTRIALES

El envase activo con propiedades de adsorción de etileno, control de la humedad y capacidad antimicrobiana, que se describe en esta invención, sería de aplicación al envasado y conservación de productos hortofrutícolas frescos con producción significativa de etileno, cuya vida útil dependa del contacto que mantengan con atmósferas relativamente ricas en etileno, como es el caso de los tomates, los plátanos, los albaricoques, etc.

Este envase activo tiene gran interés en su aplicación al transporte de productos hortofrutícolas frescos a grandes distancias, no haciendo necesaria la refrigeración a bajas temperaturas. El transporte a unas temperaturas entorno a 20°C podrá repercutir positivamente en los costes energéticos de transporte de este tipo de productos.

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REIVINDICACIONES

1. Recipiente para el envasado de productos vegetales,
5 que comprende un cuerpo (2) de material biodegradable,
caracterizado porque la superficie interna del recipiente,
destinada a estar en contacto con el producto que se
envasa, está provista de una lámina de recubrimiento (1) de
ácido poliláctico (PLA) o de cualquier otro biopolímero con
10 capacidad de adsorción del etileno y del vapor de agua,
lámina que contiene extractos naturales de aceites
esenciales con actividad antimicrobiana.

2. Recipiente según la reivindicación 1, en el que el
15 cuerpo (2) de material biodegradable es de cartón
microcorrugado.

3. Recipiente según la reivindicación 1 ó 2, en el que
la lámina de recubrimiento (1) es de ácido poliláctico
20 (PLA).

4. Recipiente según la reivindicación 3, en el que la
lámina de recubrimiento (1) de ácido poliláctico tiene un
espesor de 40 a 70 micrómetros (μm).
25

5. Recipiente según la reivindicación 3 ó 4, en el que
el gramaje de la lámina de recubrimiento (1) de ácido
poliláctico está comprendido entre 10 y 25 g/m^2 .

30 6. Recipiente según una cualquiera de las
reivindicaciones anteriores, en el que los agentes
antimicrobianos están incorporados a la lámina de
recubrimiento (1) en una proporción comprendida entre el 2%
y el 6% del peso total de la lámina de recubrimiento.

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7. Recipiente según una cualquiera de las reivindicaciones anteriores, en el que los extractos naturales de aceites esenciales se seleccionan del grupo de
5 aceites esenciales de canela, tomillo u orégano, aceites esenciales que contengan carvacrol, o combinaciones de los mismos.

8. Recipiente según una cualquiera de las
10 reivindicaciones anteriores, en el que los aceites esenciales incorporados a la lámina de recubrimiento (1) son capaces de inhibir el crecimiento de los microorganismos que aparecen en los productos hortofrutícolas frescos, tales como bacterias Gram
15 negativas, bacterias Gram positivas, mohos y levaduras.

9. Recipiente según la reivindicación 8, en el que los aceites esenciales incorporados a la lámina de recubrimiento (1) son capaces de inhibir el crecimiento de
20 al menos un microorganismo que aparece en los productos hortofrutícolas frescos que se selecciona entre *Escherichia coli*, *Listeria monocytogenes*, *Penicillium sp.* y *Rhizopus sp.*

25 10. Recipiente según una cualquiera de las reivindicaciones anteriores, que tiene forma de bandeja.

11. Recipiente en forma de bandeja según la reivindicación 10, en el que el sustrato (2) de embalaje es
30 de cartón corrugado, la lámina de recubrimiento (1) es de ácido poliláctico (PLA) y tiene un espesor de 40 a 70 micrómetros (μm), un gramaje comprendido entre 10 y 25 g/m^2 y presenta agentes antimicrobianos incorporados a la lámina de recubrimiento (1) en una proporción comprendida entre el

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2% y el 6% del peso total de dicha lámina de recubrimiento.

12. Envase activo para el envasado de productos vegetales, que comprende un recipiente de envasado de una
5 cualquiera de las reivindicaciones 1 a 10 y una película de envoltura de material plástico sin perforar, que envuelve o cierra el lote compuesto por el recipiente de envasado y los productos vegetales contenidos en el mismo.

10 13. Envase activo según la reivindicación 12, en el que la película de envoltura es de un material plástico que se elige entre polietileno de baja densidad (LDPE), polipropileno (PP), cloruro de polivinilo (PVC) u otro material polimérico.

15 14. Envase activo según la reivindicación 13, en el que la película de envoltura cierra el lote compuesto por el recipiente de envasado y los productos vegetales contenidos en el mismo por estar termosellada sobre la
20 superficie exterior del recipiente de envasado o por cubrir la totalidad de la superficie exterior del recipiente de envasado estando termosellados los extremos de la propia película.

25 15. Envase activo según una cualquiera de las reivindicaciones 12 a 14, en el que el espesor de la película de envoltura es tal que permite una adecuada densidad de flujo másico a su través de los gases etileno, vapor de agua, CO₂ y O₂.

30 16. Envase activo según la reivindicación 15, en el que la película de envoltura es de polietileno de baja densidad (LDPE) y el espesor de la película es superior a 15 µm.

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17. Uso de un recipiente de envasado de una cualquiera de las reivindicaciones 1 a 11 para el envasado de productos vegetales hortofrutícolas, setas o flores cortadas.

5

18. Uso según la reivindicación 17 para el envasado de productos vegetales hortofrutícolas que se seleccionan entre verduras, hortalizas o frutas.

10

19. Uso según la reivindicación 17 ó 18, en el que el recipiente de envasado se envuelve con una película de envoltura de material plástico sin perforar una vez introducidos los productos vegetales, de manera que el conjunto del recipiente de envasado y su contenido queda

15

20. Uso según la reivindicación 19, en el que la película de envoltura es de un material plástico que se elige entre polietileno de baja densidad (LDPE), polipropileno (PP), cloruro de polivinilo (PVC) u otro material polimérico.

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21. Uso según la reivindicación 19 ó 20, en el que la película de envoltura se termosella, bien directamente sobre la superficie exterior del recipiente de envasado o bien termosellando los extremos de la propia película de envoltura, una vez que se han introducido los productos vegetales.

25

22. Uso según la reivindicación 19 ó 20, en el que la película de envoltura tiene una porción de su perímetro exterior termosellada sobre la superficie exterior del recipiente de envasado previamente a la introducción de los productos vegetales, termosellándose la parte libre de dicho perímetro exterior una vez que los productos

30
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vegetales han sido introducidos en el recipiente.

23. Método para la fabricación industrial de un
recipiente de la reivindicación 11, que comprende los
5 siguientes pasos:

- a) fabricación de la película de PLA con agentes
antimicrobianos, que se puede llevar a cabo
mediante extrusión de la mezcla, según
procedimientos industriales ya establecidos;
- 10 b) fabricación de planchas de cartón microcorrugado,
con la aplicación del film de PLA obtenido en el
paso anterior, según procedimientos industriales
establecidos;
- 15 c) realización del corte de las citadas planchas que
permita su plegado para la formación de las
bandejas correspondientes, con aplicación de los
elementos de unión (como adhesivos u otros
elementos) necesarios entre los laterales y el
fondo de la bandeja; y d) plegado o formado.

20

24. Método según la reivindicación 23, en el que la
etapa c) comprende la aplicación sobre los bordes de las
paredes de la bandeja de una laca de material plástico a
utilizar para termosellar a la bandeja una película de
25 envoltura.

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FIGURA 1

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FIGURA 2

FIGURA 3

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FIGURA 4

FIGURA 5

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FIGURA 6

FIGURA 7

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FIGURA 8

FIGURA 9

OFICINA ESPAÑOLA
DE PATENTES Y MARCAS

ESPAÑA

21 N.º solicitud: 201031030

22 Fecha de presentación de la solicitud: 03.07.2010

32 Fecha de prioridad:

INFORME SOBRE EL ESTADO DE LA TÉCNICA

5 Int. cl.: **B65D81/28** (2006.01)

DOCUMENTOS RELEVANTES

Categoría	56 Documentos citados	Reivindicaciones afectadas
X	WO 2010057658 A2 (UNI. DEGLI STUDI DI FOGGIA [IT]; CONSIGLIO NAZIONALE RICERCHE [IT]) 27.05.2010, páginas 4,5,11,13.	1,3,6-8,10,17,18
A	OLIVEIRA, N.S. et al. Carbon dioxide, ethylene and water vapor sorption in poly (lactic acid). 2006. <i>Fluid Phase Equilibria</i> . Vol. 250, páginas 116-124.	1-24
<p>Categoría de los documentos citados</p> <p>X: de particular relevancia</p> <p>Y: de particular relevancia combinado con otro/s de la misma categoría</p> <p>A: refleja el estado de la técnica</p> <p>O: referido a divulgación no escrita</p> <p>P: publicado entre la fecha de prioridad y la de presentación de la solicitud</p> <p>E: documento anterior, pero publicado después de la fecha de presentación de la solicitud</p>		
<p>El presente informe ha sido realizado</p> <p>☒ para todas las reivindicaciones</p> <p>☐ para las reivindicaciones nº:</p>		
Fecha de realización del informe 14.11.2012	Examinador I. Rueda Molíns	Página 1/4

INFORME DEL ESTADO DE LA TÉCNICA

Nº de solicitud: 201031030

Documentación mínima buscada (sistema de clasificación seguido de los símbolos de clasificación)

B65D

Bases de datos electrónicas consultadas durante la búsqueda (nombre de la base de datos y, si es posible, términos de búsqueda utilizados)

INVENES, EPODOC, WPI, TXT

OPINIÓN ESCRITA

Nº de solicitud: 201031030

Fecha de Realización de la Opinión Escrita: 14.11.2012

Declaración**Novedad (Art. 6.1 LP 11/1986)**Reivindicaciones 1-24
Reivindicaciones**SI**
NO**Actividad inventiva (Art. 8.1 LP11/1986)**Reivindicaciones 2, 4, 5, 9, 11-16, 19-24
Reivindicaciones 1, 3, 6-8, 10, 17 y 18**SI**
NO

Se considera que la solicitud cumple con el requisito de aplicación industrial. Este requisito fue evaluado durante la fase de examen formal y técnico de la solicitud (Artículo 31.2 Ley 11/1986).

Base de la Opinión.-

La presente opinión se ha realizado sobre la base de la solicitud de patente tal y como se publica.

OPINIÓN ESCRITA

Nº de solicitud: 201031030

1. Documentos considerados.-

A continuación se relacionan los documentos pertenecientes al estado de la técnica tomados en consideración para la realización de esta opinión.

Documento	Número Publicación o Identificación	Fecha Publicación
D01	WO 2010057658 A2 (UNI. DEGLI STUDI DI FOGGIA [IT]; CONSIGLIO NAZIONALE RICERCHE [IT]).	27.05.2010
D02	OLIVEIRA, N.S. et al. Carbon dioxide, ethylene and water vapor sorption in poly (lactic acid). <i>Fluid Phase Equilibria</i> . Vol. 250, páginas 116-124.	2006

2. Declaración motivada según los artículos 29.6 y 29.7 del Reglamento de ejecución de la Ley 11/1986, de 20 de marzo, de Patentes sobre la novedad y la actividad inventiva; citas y explicaciones en apoyo de esta declaración

En las reivindicaciones 1, 3, 6-8, 10, 17 y 18, de la solicitud de patente se reivindica un recipiente, que tiene forma de bandeja, para el envasado de productos vegetales, seleccionados de entre verduras, hortalizas, frutas, setas o flores cortadas, que comprende un cuerpo de material biodegradable, caracterizado porque la superficie interna del recipiente, destinada a estar en contacto con el producto que se envasa, está provista de una lámina de recubrimiento de ácido poliláctico o de cualquier otro biopolímero con capacidad de adsorción del etileno y del vapor de agua, lámina que contiene extractos naturales de aceites esenciales, seleccionados del grupo de aceites esenciales de canela, tomillo u orégano, aceites esenciales que contengan carvacrol, o combinaciones de los mismos, con actividad antimicrobiana, en una proporción comprendida entre el 2% y el 6% del peso total de la lámina de recubrimiento. Dichos aceites esenciales, incorporados a la lámina de recubrimiento son capaces de inhibir el crecimiento de bacterias Gram positivas, bacterias Gram negativas, mohos y levaduras.

El documento D01, que es el que refleja el estado de la técnica más cercano, divulga en las reivindicaciones 9 y 10, un recipiente, como por ejemplo una bandeja (líneas 3-5, página 5) para alimentos hecho de un polímero biodegradable, como el ácido poliláctico y de una sustancia con actividad antimicrobiana, frente a *Pseudomonas* spp. (líneas 4-8, página 11) como puede ser el aceite esencial de tomillo, en una proporción comprendida entre el 1% y el 20% del peso total, tal y como se indica en las líneas 5-8, de la página 4, del documento D01.

Es ampliamente conocido en el estado de la técnica, tal y como muestra el documento D02, como el ácido poliláctico presenta propiedades de adsorción de gases y vapores como el etileno y el vapor del agua.

Por tanto, teniendo en cuenta la información divulgada en el documento D01, las reivindicaciones 1, 3, 6-8, 10, 17 y 18 presentan novedad, pero no actividad inventiva, según lo establecido en los Artículos 6 y 8 de la Ley de patentes 11/1986.