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NOVEL NANOCOMPOSITES FOR ACTIVE FOOD PACKAGING APPLICATIONS

Nuevos Nanocompuestos de Interés en Aplicaciones de Envasado Activo Alimentario

Tesis Doctoral

Presentada por María Antonieta Busolo Pons
para optar al grado de Doctor en Química Sostenible

Dirigida por:
Dr. José María Lagarón Cabello

Valencia, abril de 2013

INSTITUTO DE AGROQUÍMICA Y TECNOLOGÍA DE ALIMENTOS
(IATA-CSIC)

GRUPO DE NUEVOS MATERIALES Y NANOTECNOLOGÍA

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El Dr. José María Lagarón Cabello, Investigador Científico del Consejo Superior de Investigaciones Científicas (CSIC) en el Instituto de Agroquímica y Tecnología de Alimentos (IATA) de Valencia, y profesor asociado de Ciencia de Materiales en el Departament de Ingeniería de Diseño Industrial de la Universitat Jaume I de Castellón,

CERTIFICA

Que la presente memoria **NOVEL NANOCOMPOSITES FOR ACTIVE FOOD PACKAGING APPLICATIONS (*Nuevos Nanocompuestos de Interés en Aplicaciones de Envasado Activo Alimentario)*** constituye la tesis doctoral de Dña. María Antonieta Busolo Pons. Asimismo, certifica haber dirigido y supervisado tanto los distintos aspectos del trabajo como su redacción.

Y para que conste a los efectos oportunos, firma la presente en abril de 2013.

Fdo. José María Lagarón Cabello

Para Rodrigo, Ximena y José Luis

… you give me strength to carry on. And you´re always there to lend a hand in everything I do, that´s the wonder of you.
(Baker Knight, performed by Elvis Presley)

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CONTENIDO

LISTADO DE PUBLICACIONES

Este trabajo doctoral comprende las siguientes publicaciones:

1. Busolo, M.A.; Fernandez, P.; Ocio, M.J.; Lagaron, J.M. (2010) Novel silver-based nanoclay as an antimicrobial in polylactic acid food packaging coatings. *Food Additives and Contaminants. Part A: chemistry, analysis, control, exposure and risk assessment.* 27, 11, 1617-26.

2. Busolo, M.A.; Lagaron, J.M. Antimicrobial Biocomposites of Melt-compounded Polylactide Films Containing Silver-based Engineered Clays. *Journal of Plastic Film and Sheeting.* Accepted 2012

3. Busolo, M.A.; Lagaron, J.M. (2012) Oxygen scavenging polyolefin nanocomposite films containing an iron modified kaolinite of interest in active food packaging applications. *Innovative Food Science and Emerging Technologies*, doi:10.1016/j.ifset.2012.06.008

4. Busolo, M.A.; Lagaron, J.M. (2012) Oxygen scavenging polylactide (PLA) films containing a zero-valent iron montmorillonite. *Journal of Plastic Film and Sheeting.* In press, 2013

5. Busolo, M.A.; Lagaron, J.M. (2012) Antioxidant polyethylene films based on a resveratrol containing clay of interest in food packaging applications In press, 2013.

Este trabajo de investigación también ha dado lugar a las siguientes patentes, artículos de revisión, artículos en libros de conferencias y capítulos de libro:

Patentes

- Lagaron, J.M.; Busolo, M.A., Nuñez, M.E. United States Patent US 2011142899 (A1) *Active nanocomposite materials and production method thereof.*

- Lagaron, J.M.; Busolo, M.A., Nuñez, M.E. WO/2009/156975 *Active nanocomposite materials and production method thereof.*

- Lagaron, J.M.; Busolo, M.A., Nuñez, M.E. Patente española ES 2331284 (A1) *Materiales nanocompuestos con actividad antimicrobiana y el procedimiento para su obtención*

- Busolo, M.A.; Lagaron Cabello, J.M. Solicitud de patente español ES 2352626 (B1) *Materiales compuestos con propiedades antioxidantes y procedimientos para su obtención.*

Artículos de revisión

- Busolo, M.A.; Fernández-Saiz, P.; Nuñez, E.; Lagaron, J.M. (2012) Novel nanocomposites to enhance properties and to provide multifuntionality to plastics. *Japan Plastics,* 5, pp. 45-51.

Artículos en libros de conferencias

- Busolo, M.A., Ocio, M.J., Lagaron, J.M (2009) *Development of antimicrobial PLA nanocomposites with silver containing layered nanoclays for packaging and coating applications*. 2009 Annual Technical Conference - ANTEC, Conference Proceedings pp. 236-239.

- Busolo, M.A., Aouad, A.; Lagaron, J.M. (2010) *Development of oxygen scavenging biocomposites based on PLA and oxygen scavenging nanoclays for packaging applications.* 2010 Anual Technical Conference - ANTEC, Conference Proceedings, pp. 2044-2047.

- Busolo, M.A., Lagaron, J.M. (2011) *Multifunctional Active Nanocomposites for Packaging Applications* 2011 EUROTEC Conference, Conference Proceedings.

Capítulos de libros

- Lagarón, J.M.; Busolo. M.A. (2011) Multifunctional nanoclays for food contact applications. In *Multifunctional and nanoreinforced polymers for food packaging*. Lagarón, J.M. Ed. John Wiley & Sons (New Jersey, USA). Pp. 31-42.

- Lagarón, J.M.; Busolo. M.A. (2012) Antimicrobial plastics based on metal-containing nanolayered clays. In *Antimicrobial Polymers.* Lagarón, J.M.; Ocio, M.J.; López-Rubio, A. Eds. John Wiley & Sons (New Jersey, USA). Pp. 317-326.

- Lagarón, J.M.; Busolo. M.A. (2012) Active nanocomposites for food and beverage packaging. In *Emerging food packaging technologies: Principles and practice.* Yam, K.L.; Lee, D.S. Eds. Woodhead Publishing (Cambridge, UK).

RESUMEN

Los nanocompuestos poliméricos, que se obtienen al aditivar polímeros con cargas que tienen al menos una de sus dimensiones en escala nanométrica, ofrecen propiedades físicas superiores con respecto a los polímeros puros y a los propios compuestos tradicionales reforzados con cargas micrométricas. Por esta razón, los nanocompuestos se han estudiado y desarrollado significativamente en las últimas dos décadas, siendo la de envases alimentarios una de las aplicaciones más interesantes por sus potencialidades técnicas y económicas. La acción de los microorganismos y del oxígeno son las principales causas del deterioro de las propiedades nutricionales y organolépticas de los alimentos. El uso de envases activos, particularmente aquellos fabricados a partir de nanocompuestos, es una opción para extender la vida útil de los alimentos.

Este trabajo doctoral contiene la caracterización de nanoarcillas activas dispersas y distribuidas en matrices plásticas que conducen a la formación de nanocompuestos con propiedades antimicrobianas, secuestrantes de oxígeno y antioxidantes de interés en la constitución de envases alimentarios. Se prepararon nanobiocompuestos antimicrobianos incorporando organomontmorillonitas modificadas con plata catiónica y con plata metálica en una polilactida (PLA). Los materiales obtenidos no solamente mostraron efectividad bactericida frente a *Staphylococcus aureus* y *Salmonella spp*, microorganismos patógenos que suelen encontrarse en alimentos contaminados, sino también mejora en la barrera al vapor de agua por la presencia de las láminas de arcilla en la matriz polimérica. Los ensayos de liberación de plata en agua mostraron valores por debajo de los límites recomendados por la agencia europea de seguridad alimentaria.

La montmorillonita y caolinita modificadas con nanopartículas de hierro metálico mostraron ser capaces de absorber hasta 62 mL de O_2/g arcilla, lo que se debe a la reacción de oxidación del hierro en presencia de humedad. Se obtuvieron los correspondientes nanocompuestos activos mediante la incorporación de dichas arcillas en poliolefinas (LLDPE, HDPE) o en PLA. Estos nanocompuestos absorbieron hasta 4.5 mL O_2/g, a 100% HR y 24 ºC. Se demostró que las arcillas secuestrantes actúan como barrera pasiva y activa al paso de oxígeno a través de la matriz. Los resultados de migración global de Fe y Al en simulantes alimentarios mostraron valores inferiores a 60 mg/kg, concentración máxima permitida en la legislación Europea vigente para materiales en contacto con alimentos.

Siendo el resveratrol un polifenol de origen natural con propiedades antioxidantes por su capacidad para captar radicales libres, se evaluó la actividad de montmorillonita modificada con resveratrol y del correspondiente nanocompuesto de LLDPE. Además de capacidad antioxidante, tanto la arcilla modificada con resveratrol como su nanocompuesto, mostraron poseer capacidad antimicrobiana. Ambas propiedades mostraron ser útiles para reducir la oxidación de carne fresca.

Los resultados obtenidos tras la caracterización y evaluación de los nanocompuestos activos obtenidos en este trabajo demuestran que estos materiales basados tanto en polímeros de origen fósil como de fuentes renovables, con probada actividad antimicrobiana, secuestrante de oxígeno y antioxidante, abren nuevas posibilidades en aplicaciones de envases alimentarios, para mantener la calidad y seguridad de los alimentos por más tiempo al evitar la degradación del producto por efecto de microorganismos y oxígeno.

NOVEL NANOCOMPOSITES FOR ACTIVE FOOD PACKAGING APPLICATIONS

ABSTRACT

The polymer nanocomposites, obtained by addition to polymers of nanofillers having at least one dimension in the nanometer scale, provide superior physical properties compared to pure polymer and those composites reinforced with micrometric fillers. The nanocomposites have been significantly studied and developed over the past two decades, being the food packaging one of the most interesting application regarding its technical and economic potential. The action of microorganisms and oxygen are the main causes of deterioration of the nutritional and organoleptic qualities of foods. The use of active packaging made from nanocomposites is an option to extend the shelf life of foods.

The current PhD thesis contains the characterization of active nanoclays and the preparation of their correponding nanocomposites with antimicrobial, oxygen scavenging and antioxidant properties for their potential application in food packaging. Nanobiocomposites were prepared by incorporation of antimicrobial organomontmorillonites modified cationic and metallic silver in polylactide (PLA). The obtained materials not only showed bactericidal effectiveness against *Staphylococcus aureus* and *Salmonella spp*, which pathogenic microorganisms commonly found in contaminated food, but also improving of the water vapour barrier by virtue of the incorporation of the clay platelets. This effect was induced by the dispersion of the clay sheets in the polymeric matrix. The silver release assays in water showed values below the limits allowed by current european legislation.

The modification of kaolinite and montmorillonite with metallic iron nanoparticles allowed to absorb up to 62 mL O_2/g clay, at 24 ºC and 100% RH because of the iron oxidation in the presence of moisture. The corresponding nanocomposites were obtained after incorporation of the iron based clays in polyolefins (LLDPE and HDPE) and PLA. These nanocomposites absorbed up to 4.5 mL O_2/g, at 100% RH and 23 °C. It was demonstrated that oxygen scavenger clays act both as passive and active barrier to the oxygen diffusion through the matrix. The results of overall migration of Fe and Al in food simulants showed values below 60 mg / kg, which is the global maximum migration limit allowed by current legislation for food contact materials.

Resveratrol is a polyphenolic compound of natural accurrence with free radical scavenging ability. The antioxidant activity of the resveratrol modified montmorillonite as well as its corresponding LLDPE nanocomposite was assessed. Besides the antioxidant capacity, both the resveratrol modified montmorillonite and its nanocomposite, also exhibited antimicrobial activity. Both properties were shown to be useful in reducing the oxidation patterns of fresh meat.

The results obtained along this dissertation showed that the obtained active nanocomposites based on fossil and renewable sources, with proven antimicrobial, oxygen scavenger and antioxidant activity, open new possibilities in food packaging applications, to preserve the quality and safety of the foodstuffs and extend their shelf life by avoiding microorganisms spoilage and oxygen adverse effects.

I. OBJETIVOS

El objetivo general de este trabajo consistió en caracterizar morfológicamente y evaluar la actividad de varias nanoarcillas con propiedades antimicrobianas, secuestrantes de oxígeno o antioxidantes, para posteriormente preparar sus correspondientes nanocompuestos en matriz plástica por diferentes técnicas de incorporación. La morfología, propiedades y actividad de estos nanocompuestos, además de la migración de componentes activos a simulantes alimentarios, también fueron evaluadas para establecer la potencialidad de uso como materiales de envasado activo alimentario.

Para alcanzar el objetivo general se fijaron los siguientes objetivos específicos:

- La caracterización química y física de las nanoarcillas activas modificadas con plata, hierro y resveratrol, aplicando técnicas de análisis químico (fluorescencia de rayos X, XRF), difracción de rayos X (WAXS), termogravimetría (TGA) y microscopía de transmisión electrónica (TEM).

- Evaluación de la actividad de las nanoarcillas a través de ensayos de actividad antimicrobiana, absorción de oxígeno y capacidad secuestrante de radicales libres.

- Preparación de nanocompuestos a partir de las nanoarcillas activas y polímeros de origen fósil y biodegradables, aplicando técnicas convencionales de procesado adecuadas según el polímero a utilizar (*solvent casting* o mezclado en fundido).

- Caracterizar morfológicamente los nanocompuestos, determinando el grado de dispersión y distribución de las arcillas por WAXS y TEM, el efecto en las propiedades ópticas (transparencia, color) por la incorporación de las arcillas a las matrices; y evaluar las propiedades barrera y estabilidad térmica, por TGA.

- Evaluar la actividad de los nanocompuestos obtenidos (actividad antimicrobiana, secuestrante de oxígeno o antioxidante) y estimar los niveles de migración de los agentes activos en diferentes simulantes alimentarios.

II. PLANTEAMIENTO Y METODOLOGÍA

i. Antecedentes

Tradicionalmente los envases para alimentos se han usado para contener, proteger, manipular, distribuir y presentar los productos, manteniendo su integridad hasta el momento de su consumo. En este sentido, se considera que un 99.8% de todos los alimentos y bebidas requieren ser envasados, ya que hasta los alimentos frescos como vegetales y frutas necesitan protección durante el transporte y hasta su consumo (Restuccia *et al*., 2010). Los materiales plásticos y complejos son los más empleados en la fabricación de envases alimentarios por su versatilidad y competitividad. Entre sus ventajas están la diversidad en tipos y propiedades mecánicas y de barrera, ligereza, inercia química y facilidad de procesado. Adicionalmente los plásticos son materiales de bajo coste de producción y transporte, y versátiles en el sentido de facilitar el diseño e impresión de los diferentes tipos de envases (botellas, láminas, barquetas, bolsas). La producción mundial de plásticos en 2011 alcanzó los 280 millones de toneladas, de los que un 42% está destinado a la manufactura de envases (Silvestre *et al.*, 2011). En 2008 la producción de envases plásticos se valoró en 46.4 billones de dólares, un 25% más que en 2003, y se estima que aumente a 53.5 billones de dólares en 2013 (Freedonia Group, 2009).

La industria alimentaria, motivada por los cambios en los hábitos y exigencias de los consumidores, está en evolución continua para mejorar la calidad y prolongar su vida útil de sus productos, lo que además repercute en rentabilidad y reducción de desechos. Por otra parte, la demanda de envases técnicos que responda a los requerimientos del mundo desarrollado está fuertemente influenciada por el crecimiento de la población, los patrones de comercialización, los avances en el desarrollo de nuevos materiales, las regulaciones sobre tratamiento de residuos y el aumento de la conciencia ambiental (Fernández Alvarez, 2000; Freedonia Group, 2009). En los últimos diez años se ha producido un cambio sustancial en la concepción del envasado y tratamiento de alimentos y han surgido los materiales activos (Restuccia *et al*, 2010). Estos son materiales y artículos destinados a prolongar la vida útil de los alimentos y mantener sus propiedades nutricionales, al interaccionar con el alimento o el ambiente que le rodea para obtener un efecto determinado. Los envases activos contienen agentes que deliberadamente absorben o liberan sustancias hacia el alimento o el espacio cabeza del mismo, evitando el crecimiento de microorganismos y previniendo la descomposición o pérdida de propiedades nutricionales y organolépticas (Ozdemir y Floros, 2004; Silvestre *et al*., 2011). El envasado activo permite diseñar los envases a medida de las necesidades de utilización de los productos y reducir el contenido de aditivos de los alimentos, incorporándolos directamente en el material de envase. También permite reducir los costes y simplificar procesos, ya que por ejemplo, utilizando un absorbente de oxígeno se reducen los costes derivados de la utilización de equipos de envasado en atmósferas modificadas (Fernández Alvarez, 2000). Ejemplos de materiales activos son los antimicrobianos, secuestrantes de oxígeno, antioxidantes, absorbedores de etileno y absorbedores/emisores de dióxido de carbono, absorbedores de humedad entre otros (Fernández Alvarez, 2000; Brody *et al.*, 2001; Brody *et al.*, 2008; Ozdemir y Floros, 2004; Restuccia *et al.*, 2010; Silvestre *et al.*, 2011). Se estima que la demanda de envases activos aumente un 7.1% anual hasta llegar a una valor de 1.7 billones de dólares en 2013, de los que un 48% corresponderá a absorbentes de oxígeno y etileno (Freedonia Group, 2009).

Los nanocompuestos son materiales que se obtienen por la incorporación de nanopartículas (generalmente en una carga menor al 10%) a matrices poliméricas, siendo una de sus características que exhiben propiedades superiores a las de los polímeros puros. Las nanopartículas para ser consideradas como tales deben tener al menos una de sus dimensiones en escala nanométrica. Las nanoarcillas tienen una sola de sus dimensiones, el espesor, en escala nano. La incorporación de nanopartículas a los polímeros permite mejorar propiedades como rigidez, estabilidad dimensional y resistencia térmica (Okamoto, 2005; Ruiz-Hitzky y Van Meerbeek, 2006; Paul y Robeson, 2008; Pavlidou y Papaspyrides, 2008; De Azeredo, 2009). En este sentido, el uso de nanotecnología no sólo puede mejorar las funciones tradicionales de los envases, sino también proveer otras funciones como la de barrera pasiva o antimicrobiana, lo que permite aumentar la vida útil de los alimentos y garantizar la calidad hasta su consumo. Una consecuencia positiva de la extensión de la vida útil de los alimentos es la reducción del consumo de energía y recursos para la producción, transporte y almacenamiento tanto de alimentos como de envases, lo que a su vez permite reducir el volumen de desechos y de emisiones de CO_2. Actualmente, las nanoarcillas ocupan un 70% del volumen de mercado de nanopartículas en envases alimentarios, particularmente sistemas multicapa para botellas, láminas y bandejas termoconformadas (Silvestre *et al.,* 2011).

Las arcillas en su estado natural presentan una mejor afinidad con polímeros hidrofílicos. El carácter hidrofílico de las arcillas puede alterarse, y con ello lograr su compatibilidad con matrices hidrofóbicas, por intercambio de los iones naturalmente presentes en la estructura (Na^+, Ca^{++}, Mg^{++}, entre otros) por cationes orgánicos, principalmente sales cuaternarias de alquilamonio o alquilfosfonio. La incorporación de las nanoarcillas organomodificadas en los polímeros puede llevarse a cabo por método de solución (*solvent casting*); polimerización en sitio o por mezclado en fundido (Okamoto, 2005; Paul y Robeson, 2008; Pavlidou y Papaspyrides, 2008; De Azeredo, 2009; Duncan, 2011). Para estudiar la morfología de los nanocompuestos normalmente se aplican técnicas de difracción de rayos X (*XRD, X*-Ray Diffraction) y microscopía electrónica de transmisión (*TEM,* Transmission Electron Microscopy).

La plata se ha empleado como agente bactericida desde la antigüedad. Su acción se basa en la oxidación y liberación constante de cationes plata bajo condiciones de alta humedad (Martínez-Abad, 2012). Los cationes plata (Ag^+) interfieren en los procesos vitales y en la replicación del ADN de las células bacterianas, además de causarles stress oxidativo (Rai *et al.*, 2009; Duncan, 2011). Se ha reportado la actividad antimicrobiana de arcillas modificadas con plata por intercambio catiónico (Oya *et al.*, 1991; Keller-Besrest *et al.*, 1995; Quintana *et al.*, 2008; Praus *et al.*, 2009). La comparación de actividad antimicrobiana entre agentes inmovilizados en arcillas muestra que la plata catiónica es más activa que la plata metálica (Praus *et al.*, 2009). Los cationes plata son efectivos cuando se liberan de la arcilla, mientras que la plata metálica no presenta en sí misma actividad. La plata metálica, en ambientes húmedos, reacciona con el agua produciendo Ag+, que es la especie activa (Kampmann *et al.*, 2008)

Muchos compuestos polifenólicos de origen natural también han mostrado ser antimicrobianos porque alteran la presión osmótica celular de las bacterias rompiendo así la membrana citoplásmica y provocando fugas de los componentes celulares

(Perumalla y Hettiarachchy, 2011). Sin embargo, una de las capacidades más importantes de los polifenoles es la de captar los radicales libres del oxígeno mediante la donación de uno de sus electrones deslocalizados o protones La función antioxidante de los polifenoles se basa en la interrupción de las reacciones en cadena de los radicales libres con las moléculas insaturadas (Inze y Van Montagu, 1995; Arora *et al.*, 1998). El resveratrol, un polifenol estilbenoide presente en los frutos y hojas de una gran variedad de plantas comestibles, pero en mayor concentración en las uvas y en el helecho japonés (*Polygonum cuspidatum),* se caracteriza por su actividad antioxidante y antimicrobiana (Chan, 2002; Filip *et al.* 2003; Almajano *et al*., 2008, Cravotto *et al.*, 2012), y por los efectos beneficiosos que tienen sus propiedades en la salud, como agente antiviral, cardioprotector, antiinflamatorio, neuroprotector, entre otros (Gülcin, 2010; Li *et al.*, 2012; Cantos-Villar *et al.*, 2012). La incorporación de antioxidantes sintéticos en materiales para envasado, tanto para la protección de los polímeros durante el procesado como para la migración del aditivo a un alimento para mantener éste más fresco y apetecible, es una práctica común. Sin embargo, el uso de antioxidantes sintéticos convencionales como aditivos de alimentos y en la fabricación de materiales para contacto alimentario se ha restringido al demostrarse los efectos tóxicos y cancerígenos de antioxidantes como BHT (butilhidroxitolueno), BHA (butilhidroxianisol) y PG (galato de propilo), entre otros (Kahl y Kappus, 1993; Brody *et al.*, 2001; Miyauchi *et al.*, 2002; Sun Lee, 2005). Una alternativa es el uso de antioxidantes y extractos de origen natural, como por ejemplo, los carotenos, catequinas; y extractos de romero, orégano y té verde (Shibamoto *et al.*, 1995: Mitsumoto, 2000; Grün *et al.*, 2006; Camo *et al.*, 2008; López-de-Di Castillo *et al.* 2011). Aunque los consumidores asocian las sustancias naturales con seguridad y menos toxicidad, estas sustancias suelen tener olores, colores y sabores característicos que posteriormente pueden afectar a las propiedades organolépticas de los materiales de envasado y de los alimentos, y por ende, causar rechazo. Esto, junto con la baja resistencia térmica de muchas de estas sustancias limita la procesabilidad de los materiales poliméricos que se desean aditivar, lo que reduce el uso de antioxidantes naturales en la fabricación de sistemas de envasado de alimentos. El resveratrol puede extraerse con alto rendimiento de las plantas que lo contienen (Hou *et al.*, 2002; Chao, 2004; Arigony-Souto, 2008), es un sólido blanco, inodoro y estable térmicamente (por encima de 275 ºC; Emilio-Mendes *et al.*, 2012), por lo que es una opción como agente antioxidante para materiales de envasado.

Las nanopartículas de hierro metálico soportadas en arcillas se han empleado recientemente en procesos de remediación y tratamiento de aguas (Sun *et al.*, 2006; Hoag et al., 2009; Cho *et al.*, 2005; Frost *et al.*, 2009; Üzüm *et al.*, 2009; Shahwan *et al.*, 2010; Hou *et al.*, 2011; Shi *et al.*, 2011) y en la producción de materiales retardantes de llama (Nawani *et al.*, 2007). El hierro metálico es un potente absorbente de oxígeno, ya que en ambientes húmedos es capaz de absorber hasta 300 mL de oxígeno, formando Fe_2O_3, a través de un proceso redox en el que se cree que el agua adsorbida en la superficie del metal forma soluciones de electrolito que aceleran la reacción (Miltz y Perry, 2005; Mu *et al.*, 2013):

$$Fe \rightarrow Fe^{+2} + 2e^- \qquad (1)$$

$$H_2O + ½\, O_2 + 2e^- \rightarrow 2\, OH^- \qquad (2)$$

$$Fe^{+2} + 2\, OH^- \rightarrow Fe(OH)_2 \qquad (3)$$

$$2\, Fe(OH)_2 + ½\, O_2 + H_2O \rightarrow Fe_2O_3 \cdot 3H_2O \qquad (4)$$

Las nanopartículas de hierro se incorporan directamente a poliolefinas para producir materiales secuestrantes de oxígeno para uso alimentario (Rodgers, 2002; Galotto *et al.*, 2009), pero hasta el momento no se ha descrito la preparación ni actividad de materiales activos que contengan nanopartículas de hierro soportadas en arcillas. El uso de nanoarcillas activas para fabricar materiales poliméricos activos ofrece ventajas como el bajo costo y disponibilidad de materia prima, procesado relativamente simple y fácil dispersión de la carga, además de todas las ventajas tecnológicas asociadas a las nanoarcillas: mejora de barrera por alteración del paso de difusión de los gases, mejora de propiedades mecánicas, adquisición de las propiedades activas de la arcilla, entre otras (Utracki, 2004; Cabedo *et al.*, 2004; Paul y Robeson, 2008; Pavlidou y Papaspyrides, 2008).

ii. Planteamiento y metodología

El presente trabajo de doctorado se basa en la caracterización físico-química y en la evaluación de la actividad antimicrobiana, secuestrante de oxígeno o antioxidante de varias nanoarcillas experimentales que han sido modificadas con plata (metálica o catiónica), hierro metálico o resveratrol. Por extensión, se comprobó que los nanocompuestos obtenidos a partir de estas arcillas presentaron las correspondientes actividades esperadas. Este trabajo incluye adicionalmente la incorporación de las arcillas activas en diferentes matrices poliméricas (poliolefinas y polilactida) a través de métodos tradicionales de procesado, la evaluación de la morfología y actividad de los nanocompuestos activos obtenidos, así como una estimación del potencial de aplicación como materiales de envase para extender la vida útil de los alimentos.

Las actividades de investigación que comprenden este trabajo de doctorado se organizaron en diferentes fases, según se presenta en la Tabla I.

iii*. Aportaciones originales*

Hasta donde se tiene entendido, y de acuerdo a las revisiones de las bases de datos actuales, hasta el momento no se ha reportado la incorporación de nanoarcillas modificadas con plata (metálica o catiónica) y con surfactantes en biopolímeros para la elaboración de nanobiocompuestos para ser empleados como materiales activos.

Se ha descrito ampliamente el uso del hierro metálico como un agente secuestrante en función de su reacción con el oxígeno en presencia de agua. Paralelamente se han reportado procedimientos para síntesis de nanopartículas de hierro en caolinita y montmorillonita para su aplicación en procedimientos de remediación de aguas residuales industriales y materiales retardantes de llama. Sin embargo, no hay publicaciones sobre la incorporación de montmorillonita y caolinita con nanopartículas de hierro en poliolefinas y polilactida para la manufactura de nancompuestos secuestrantes para aplicaciones potenciales en envases activos alimentarios.

Por último, a pesar de que las propiedades antioxidantes del resveratrol se explotan actualmente en la industria nutracéutica, la modificación de montmorillonita con resveratrol para formular arcillas antioxidantes y antimicrobianas, así como la manufactura de nanocompuestos activos a partir de ellas, no han sido reportadas.

Por lo anterior, se afirma que las aportaciones que se presentan en este trabajo de doctorado son originales y novedosas, y que son resultado de un trabajo sistemático de investigación y desarrollo.

Tabla I. Fases del trabajo de investigación

FASE		**ACTIVIDAD**		
		Antimicrobianos	**Secuestrantes de oxígeno**	**Antioxidantes**
Estudio de las arcillas	*Caracterización físico-química*	TEM, WAXS, XRF, TGA		WAXS, TGA
	Evaluación de las propiedades activas	Capacidad antimicrobiana	Absorción de O_2	Capacidad antioxidante
Preparación de nanocompuestos	*Selección de la matriz polimérica*	PLA	PLA LLDPE, HDPE	LLDPE
	Método de procesado	*Solvent casting*; Mezclado en Fundido	Mezclado en fundido; Extrusión	Mezclado en fundido; Extrusión
Caracterización de los nanocompuestos	*Morfología*	Microscopía óptica, TEM, WAXS		Microscopía óptica, WAXS
	Propiedades térmicas	TGA, DSC	TGA	
Evaluación de propiedades de los nanocompuestos	*Propiedades pasivas (barrera)*	H_2O	O_2	-----
	Propiedades activas	Capacidad antimicrobiana	Absorción O_2	Capacidad antioxidante Capacidad antimicrobiana
	Migración	Ag^+ en agua	Fe^{+3}, Al en agua e isooctano	Resveratrol en agua e isooctano
	Ensayos de vida útil	-----	-----	Carne fresca
Redacción de artículos técnicos		2	2	1

III. CONCLUSIONES

En este trabajo doctoral se ha presentado la preparación y caracterización de compuestos activos mediante la adición de arcillas modificadas con plata, con hierro y con resveratrol a poliolefinas y polilactida El uso de las arcillas como vehículo de agentes activos antimicrobianos, secuestrantes de oxígeno y antioxidantes tiene un gran impacto por su potencialidad en la elaboración de materiales activos para envases alimentarios.

De este trabajo doctoral se derivan las siguientes conclusiones:

1) En el caso de los nanobiocompuestos de PLA con arcillas organomodificadas que contienen plata (antimicrobianos):

- Se evaluó por TEM la morfología de los grados experimentales de organomontmorillonitas modificadas con plata, Bactiblock®R1.51 y Bactiblock®T1.51, que permitió distinguir el estado de oxidación en el que se encuentra la plata en la estructura (como catión o como nanopartículas metálicas).

- Las arcillas R1.51 y T1.51 mostraron fuerte efecto antimicrobiano contra *S.aureus*, y la arcilla R1.51 también mostró efecto bactericida contra *Salmonella spp*, con concentración mínima inhibitoria y concentración mínima bactericida por debajo de 1 mg/10 mL.

- Se utilizaron dos técnicas para la incorporación de las organomontmorillonitas modificadas con plata en PLA: *solvent casting* y mezclado en fundido. Estas técnicas permitieron incorporar entre un 1 y un 10% en peso de arcillas, y en todos los casos los materiales mantuvieron un alto grado de transparencia. Sin embargo, los difractogramas no indicaron evidencias claras de intercalación ni exfoliación de la arcilla en los compuestos obtenidos por *solvent casting*, mientras que los compuestos obtenidos por mezclado en fundido sí muestraron intercalación. En todos los casos se observó un alto de grado de distribución de la nanoarcilla en el polímero.

- Se logró mejorar la barrera al agua en los compuestos de PLA hasta en un 40%, dependiendo de la carga de arcilla y del método de incorporación.

- Se observaron diferencias en los resultados de migración de plata y de capacidad antimicrobiana en función de la disponibilidad de la plata catiónica, que es la especie activa. La concentración de plata observada fue de 6-8 ppm, después de 8 días de exposición a un medio acuoso ligeramente acidificado.

- Los compuestos de PLA con arcillas de plata exhibieron propiedades que les permite ser considerados una alternativa sostenible para la manufactura de materiales de envasado antimicrobianos.

2) En el caso de los compuestos basados en arcillas con hierro metálico (secuestrantes de oxígeno):

- Se evaluaron dos grados experimentales de arcillas basadas en hierro: caolinita y montmorillonita. Se observó que el hierro se encuentra en forma de nanopartículas de hierro metálico (Feº), intercaladas entre las láminas y depositadas en los bordes y superficie de las mismas, con un tamaño promedio entre 100 y 150 nm.

- Después de 60 días, la caolinita de hierro mostró un marcado consumo de oxígeno a 100 % HR, de hasta 43 mL de oxígeno por gramo de arcilla, mientras que la montmorillonita de hierro fue capaz de absorber hasta 62 mL de O_2/g arcilla.

- Se prepararon por extrusión los compuestos activos de polietileno de alta y baja densidad (HDPE y LLDPE, respectivamente) con 10% de caolinita de hierro. La arcilla contenida en los compuestos demostró tener dos efectos: como barrera pasiva, entre otros factores por la difusión tortuosa de las moléculas de permeante a través de la matriz; y como barrera activa, por la reacción del oxígeno molecular con las nanopartículas de hierro.

- Los nanocompuestos de poliolefinas con caolinita de hierro absorbieron entre 2.4 y 4.3 mL de oxígeno por gramo de compuesto, dependiendo de la permeabilidad al agua de la matriz. Se comprobó que la humedad y la temperatura son factores determinantes en la cinética de absorción de oxígeno.

- Los ensayos de migración de hierro y aluminio de los compuestos activos a simulantes alimentarios (agua e isooctano) resultaron ser muy bajos o difícilmente detectables, especialmente en isooctano.

- Se obtuvieron compuestos de PLA con 10% de montmorillonita de hierro, FeMMT, aplicando el método de mezclado en fundido. Los compuestos obtenidos fueron capaces de absorber hasta 4.2 mL de oxígeno por gramo de compuesto.

- Mientras que la incorporación de caolinita de hierro en poliolefinas no afectó las propiedades térmicas de las matrices, la estabilidad térmica de los compuestos de PLA y FeMMT disminuyó al menos en 60 ºC, posiblemente porque el hierro contenido en la arcilla cataliza procesos de degradación en el biopolímero. Sin embargo, la permeabilidad al agua del compuesto se mejoró ligeramente debido al efecto barrera inducido por la arcilla.

- A pesar de que tanto los compuestos de PLA y poliolefinas con arcillas de hierro mostraron una marcada actividad secuestrante de oxígeno, los basados en PLA deben descartarse por degradación de la matriz durante el procesado. Sin embargo, los compuestos basados en LLDPE y HDPE mostraron propiedades térmicas y de barrera pasiva adecuadas para ser propuestos como alternativas para la manufactura de materiales de envasado de productos sensibles al oxígeno.

3) En el caso de las arcillas modificadas con resveratrol (antioxidantes):

- El resveratrol, polifenol de origen natural, mostró ser térmicamente más estable y tener mayor capacidad antioxidante que el butilhidroxitolueno, BHT, que es un antioxidante sintético. El resveratrol retuvo su gran capacidad antioxidante una vez fue incorporado en arcillas del tipo montmorillonita.

- Se prepararon compuestos activos por incorporación tanto de resveratrol como de una arcilla modificada con resveratrol a LLDPE, por técnicas convencionales de extrusión, obteniendo materiales translúcidos aunque con ligera coloración parda. Ambos compuestos mostraron no solamente actividad antioxidante, sino también capacidad antimicrobiana contra *S. aureus*.

- Se realizó un ensayo de extensión de vida útil en carne fresca, y se determinó que films del compuesto con arcilla modificada con resveratrol redujeron la oxidación del alimento.

- Los ensayos de migración del compuesto con arcilla modificada con resveratrol, conducidos en agua e isooctano como simulantes alimentarios, demostraron que el resveratrol migra en concentraciones muy bajas (por debajo de 0.01 mg/kg), lo que significa que el antioxidante actúa principalmente por contacto.

- El uso de compuestos que contienen arcillas modificadas con resveratrol puede considerase una opción para la extensión de vida útil de alimentos tales como los envasados en atmosfera modificada con oxígeno.

ABBREVIATIONS AND SYMBOLS

BHA	Butylhydroxyanisol
BHT	Butylhydroxytoluene
DSC	Differencial scanning calorimetry
EVOH	Ethylene vinyl alcohol copolymer
HDPE	High Density Polyethylene
LLDPE	Linear Low Density Polyethylene
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericide Concentration
MMT	Montmorillonite
OMMT	Organomontmorillonite
PG	Propylgallate
PLA	Polylactide
PP	Polypropylene
PSD	Particle Size Distribution
Tg	Glass Transition Temperature
TGA	Thermogravimetric Analysis
TEM	Transmission Electron Microscopy
WAXS	Wide Angle X-Ray Scattering
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence

1. INTRODUCTION

1.1 Active food packaging

Food packaging materials have been used in last decade not only for containing and keeping fresh foodstuffs during shelf-life all the way up to consumption but also for guaranteeing safety by minimizing food losses and preserving quality. Most foods deteriorate during the transport, processing and storage through contamination by growing of microorganisms, chemical reactions and physical changes, being the microbial spoilage and oxidative reactions the greatest impact on limiting the life of perishable products (Brody et al., 2001; Ahvenainen, 2002; Lee *et al.*, 2004). Packaging protects the food goods from environmental influences that cause deterioration such as heat, light, the presence or absence of moisture and oxygen, and so on. Prolonging shelf-life involves application of various strategies such as temperature control, moisture control, and addition of chemicals such as salt, sugar, carbon dioxide, or natural acids, removal of oxygen, or a combination of these with effective packaging (Restuccia *et al.*, 2010). The consumers are constantly demanding better quality and safe, minimally processed, easily prepared and ready-to-eat ‘fresh’ and healthy food products and at the same time they are concerned about environmental issues (Appendini and Hotchkiss, 2002; Ozdemir and Floros, 2004; Emamifar *et al.*, 2011). These demands are strongly influenced by the market trends, and together with globalization of food trade and distribution from centralized processing facilities, are the driving forces for continuous development and upgrade of active packaging systems for food products. Centralization of activities (e.g. preparation of retail packs of fresh meat and sliced vegetables) and the globalization of markets resulting in longer distribution distances, present major challenges to the food-packaging industry to develop packaging concepts that extend shelf-life while maintaining the safety and quality of the packaged food. To provide this shelf-life extension, to improve the quality, safety and integrity of the packaged food, innovative active packaging concepts have to be applied (Emamifar, 2011). Active packaging is defined as a system that involves interactions between package or packaging components and food or internal gas atmosphere, and complies with consumer demands for high quality, fresh-like, and safe products.

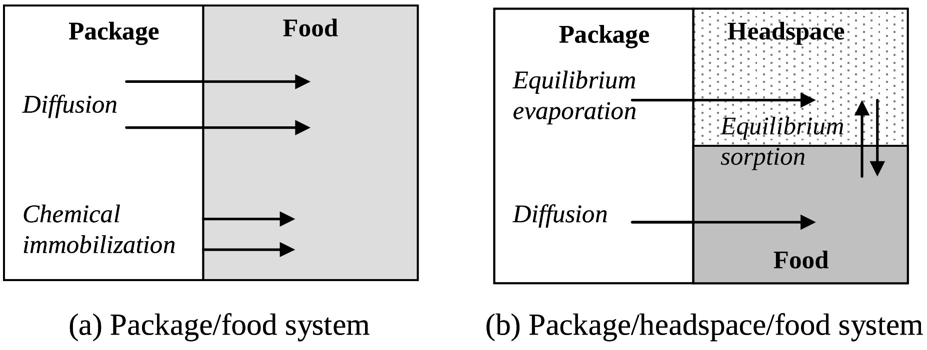
Active packaging involves components that release or absorb substances, from or into the packaged food, or the surrounding environment, thus the shelf life of foods is extended by inhibiting the growth of pathogenic and spoilage microorganisms, preventing and/or indicating the migration of contaminants while maintaining or even improving their nutritional quality and ensuring food safety (Ozdemir and Floros, 2004; Bolumar *et al.*, 2011). In general, active packaging provides several functions that do no exist in conventional packaging systems, such as scavenging of oxygen, moisture or ethylene, antimicrobial activity or free radical trapping, and moisture absorbers, flavor absorbers, among others (Brody *et al.*, 2001; Quintavalla and Vicini, 2002; Lee *et al.*, 2004; Rooney, 2005; Sun Lee, 2005; Brody *et al.*, 2008; Bolumar *et al.*, 2011).

1.1.1 Antimicrobials

In recent years, there has been a slight increase of food-borne diseases that are associated to the development of antibiotic resistance, acquisition of new virulence factors or changes in the ability to survive in adverse conditions of the microbial population, so food-borne diseases caused by microbiological risks is a large growing public health problem (Appendini and Hotchiss, 2002; Duncan, 2011). Food can be contaminated with pathogens carried by farm animals and human beings, specifically by contamination of crops due to proximity or use of infected water bodies as irrigation, or by the improperly use of composted animal manure as fertilizer. The public health implications are especially serious when affected products are those which are consumed fresh, such as vegetables and fruits. The global food distribution seems to be an important factor in the increasing of food-borne illnesses associated with fresh products. The most common infections in the food sector come from *Salmonella*, *Campylobacter*, *S. aureus* and *E. colli*. (Lagaron *et al.,* 2012).

Antimicrobial packaging is gaining interest from researchers and industry due to its potential to provide quality and safety benefits. The rationale for incorporating antimicrobials into the packaging is to prevent surface growth in foods where a large portion of spoilage and contamination occurs. This approach can reduce the addition of larger quantities of antimicrobials that are usually incorporated into the bulk of the food. A controlled release from packaging films to the food surface has numerous advantages over dipping and spraying. In the latter processes, in fact, antimicrobial activity may be rapidly lost due to inactivation of the antimicrobials by food components or dilution below active concentration due to migration into the bulk food matrix (Sorrentino *et al.,* 2007).

Antimicrobial activity in packaged foods could be achieved by either indirect or direct contact between the active package and the product by using volatiles releasing systems, or by using non-migratory antimicrobial systems, respectively (Guarda *et al.,* 2012). Active antimicrobial materials can be obtained from adding the antimicrobial agents directly into polymers during the extrusion process, coating onto polymer surfaces, immobilization by covalent bonds, or by using inherently antimicrobial polymers (Appendini and Hotchkiss, 2002). The antimicrobial agent incorporated in the packaging material can migrate to the food through diffusion and partitioning when packaging material is in contact, or in case of volatile substances, when they evaporate and distribute across the headspace for further reaching of the food surface. The covalently immobilized active moieties utilize the surface inhibition of microbial growth and no diffusional mass transfer occurs (Quintavalla and Vicini, 2002). The food packaging systems and performance of active substances are shown in Figure 1. The performance of an antimicrobial agent in a packaging is strongly influenced by several parameters such as the physico-chemical characteristics of the food (pH, water activity), storage temperature, and mass transfer coefficients, among others. However, the antimicrobial agent has to be selected taking into account factors such as stability during the polymer processing (temperature) and within the polymer matrix at shelf conditions, solubility in the polymer and release kinetics to avoid biocide concentration depletion below the minimum inhibitory concentration during the food stuffs shelf-life, growth-inhibition, mechanism, biocidal capacity against a broad spectrum of pathogenic microorganisms, minimal impact on quality of food stuffs by altering odor and taste, and cost effectiveness (Han, 2000; Lagaron *et al*., 2012). The antimicrobial activity of the packaging materials can be measured by microbiology experiments. The target microorganisms are inoculated onto the surface of the food or mixed into the food samples, and measuring growth by counting the microorganisms with incubation time can provide the characteristic values of growth rate at the exponential growth phase, maximum growth at the stationary phase, and initial lag period. These values can be compared to conventional packaging or reference samples without packaging (Han, 2000).



(a) Package/food system (b) Package/headspace/food system

Figure 1. Schematic behavior of antimicrobial substances in active food packaging systems (adapted from Han, 2000)

Even though uncontrolled delivery packages intended for food applications are more abundant, the most important desired property of the antimicrobial packaging materials is the controlled release of the antimicrobial agent from the film to the food surface. A rapid release causes fast consumption of the agent in a short period of time, after which the minimum concentration required for the inhibition of microbial growth is not maintained on the food surface. On the other hand, spoilage reactions on the food surface may start if the release rate of the antimicrobial agent from the film is too slow. Thus, the controlled release of the active agent for a long period of time is necessary to extend the shelf life of the packed food. Different controlled release strategies were introduced which are mainly based on changing the structure of the films (Uz and Altinkaya, 2011). Controlled release systems are of industrial relevance also due to their aptitude to prevent sensorial or toxicological problems or inefficiency of the system, caused by a too high or a too low concentration of delivered substance (Mastromatteo *et al.*, 2010). Controlled release strategies including multilayer structures where the antimicrobial agent is contained in an intermediate layer, an outer layer avoid the migration of the active agent to the environments and third layer controls the release to the food; other strategies such as variation of the cross-linking, plasticization, swelling or biodegradation of the polymer matrix, orientation of matrix polymer chains or nanoencapsulation of the active agent in lipids, proteins and saccharide polymers have also been trial (Mastromatteo *et al.*, 2010; Uz and Altinkaya, 2011; Perumalla and Hettiarachchy, 2011).

The antimicrobial agents can be classified as synthetic chemical agents, natural occurring agents and antibiotics. Antimicrobial materials have been prepared from both oil-based and biobased polymers and tried for the preservations of fresh and processed food, as listed in Table 1.

Table 1. Some applications of antimicrobial agents in food packaging

Antimicrobial agent	Active component	Packaging material	Food application	References
Chemical agents (synthetic)	Organic acids: *Acetic acid, benzoic acid, lactic acid, citric acid, malic acid, propionic acid,* *sorbic acid, succinic acid, tartaric acid*	Chitosan, PE, alginate	Apple slices, fresh and frozen strawberries, cut-pear wedges; culture media; cooked ham	Han, 2000; Quintavalla and Vicini, 2002; Takala *et al.*, 2011;
	Organic acid salts: *Potassium sorbate, sodium benzoate, EDTA,* Ethyl Lauroyl Arginate	Cellulose, LDPE, starch/glycerol, methylcellulose, soy protein films, PET	Cheese, dairy products, doughs, chicken breast; broccoli	Quintavalla and Vicini, 2002; Sivarooban *et al.*, 2008; Uz and Altinkaya, 2011; Pezo *et al.*, 2012
	Acid anhydrides: *Sorbic anhydride, benzoic anhydride*	LDPE	Fish fillet, culture media	Weng and Chen, 1997; Han, 2000; Quintavalla and Vicini, 2002
	Metals: *Silver, copper*	LDPE	Orange juice, green asparagus	An *et al.* 2008; Ruparelia *et al.*, 2008; Emamifar *et al.*, 2011.
	Inorganic compounds: *Silver nitrate, silver acetate, silver oxide, titanium oxide, zinc oxide, silver zeolites*	LDPE, HDPE	Orange juice, dressings; short-ripened cheese	Ip *et al.*, 2006; Kampmann *et al.*, 2008; Boschetto *et al.*, 2012; Peressini *et al.*, 2012.
Natural agents	Essential oils: *Clove oil, oregano oil, garlic oil, sage oil, orange oil, mint oil, rosemary oil, cinnamon oil*	Soy protein films; soy protein isolate coated OPP/PE; Gelatin-chitosan; cassava starch	Beef patties, alfalfa and broccoli sprouts; fresh fish; white bread; bakery products	Suppakul *et al.*, 2008; Gamage *et al.*, 2009; Emiroğlu *et al.*, 2010; Kechichian *et al.*, 2010 ; Gamage *et al.*, 2009 ; Gutiérrez, *et al.*, 2009
	Natural extracts: *Green tea, grape seed extract, rosemary extract, citrus extract*	Soy protein films; chitosan, methylcellulose	Extra virgin olive oil, biscuits, tomatoes, broccoli, frankfurters, raw and cooked meat, poultry products, fresh fish	Sivarooban *et al.*, 2008; Perumalla and Hettiarachchy, 2011; Takala *et al.*, 2011, Li *et al.*, 2012b

Table 1. Some applications of antimicrobial agents in food packaging (cont.)

Natural agents	Natural occurrence organic compounds: *Eugenol, cinnamaldehyde, carvacrol, thymol, tannic acid, cytral, geraniol, terpineol, allyl isothiocyanate*	LDPE/EVA PP Starch	Tomatoes, celery, alfalfa seeds, raw chicken, red grouper filet; cheese	Suppakul *et al.*, 2008; Gamage *et al.*, 2009 ; Pyla *et al.*, 2010 ; Ramos *et al.*, 2012
	Enzymes: *Glucose oxidase, lysozyme*	PVOH PA Cellulose Alginate	Fish, culture media	Han, 2000; Buonocuore *et al.*, 2005; Barbirolli *et al.*, 2012
	Bacteriocins : *Nisin, lactocin 3174, lactocin 705, lactocin AL705*	PE/PA PP/PA/PE EVA acetate-ethylene copolymer; chitosan	Sliced cheese, cooked ham; sunflower oil; milk cream	Scanell *et al.*, 2000, Cutter and Miller, 2004 ; Lee *et al*., 2004; Sivarooban *et al.*, 2008; Massani *et al*., 2011
	Peptides : *Lactoferrin*	Cellulose	Carpaccio	Barbirolli *et al.*, 2012
Antibiotics	Benomyl, Imazalil, Natamycin	LDPE	Culture media, peppers	Han, 2000

Silver has been used as an antimicrobial agent in food and beverage storage applications from ancient societies. Historic uses of silver reveal anecdotal reports of early settlers placing silver utility pieces at the bottom of milk and water bottles to prolong shelf life, and of seafaring ships or airliners lining their water tanks with silver to keep water potable for long periods of time. Compared to molecular antimicrobials silver has numerous advantages over other antimicrobial agents, which are generally targeted to specific organisms. Silver is broad spectrum and toxic (to varying degrees) to numerous strains of bacteria, fungi, algae, and possibly some viruses. Being an element, silver is shelf stable for long periods of time (Pal *et al*., 2007; Duncan, 2011). Bulk and macroscopic metallic silver itself has no antibacterial effects but the biocidal properties of silver rely on the sustained oxidation and release of very small quantities of silver cations (Ag^+) to an aqueous or moisture environment (Pal *et al*., 2007; Kampmann *et al*., 2008; Martínez-Abad, 2012). Thus, the oxidized cations are the ones behind the historic antimicrobial activity of silver. The mechanism of cationic silver activity is not properly understood, but is based on at least one of the following mechanisms as inferred from structural and morphological changes: (a) Interference with vital cellular processes by binding to sulfhydryl, thiol or disulfide functional groups on the surfaces of membrane proteins and other enzymes; (b) disruption of DNA replication when silver ions penetrate inside the bacterial cell; and (c) oxidative stress through the catalysis of reactive oxygen species (ROS) formation (Rai *et al*., 2009; Duncan, 2011). Bacterial sensibility has been found to differ highly among different strains of a single species. Gram-negative bacteria, containing a negatively charged lipopolysaccharide which attracts the silver cations, are more sensitive to the presence of silver than gram-positive bacteria. These ones, in change, possess a thick peptidoglycan in their cell that acts as protective shell against ions (Martínez-Abad, 2012). Even though macroscopic silver has no antimicrobial properties, silver nanoparticles less than 100 nm exhibit similar effects on bacteria than silver ions (Li *et al.*, 2010). The antimicrobial activity of silver nanoparticles may be related to several mechanisms including, induction of oxidative stress due to generation of reactive oxygen species (ROS) which may cause the degradation of the membrane structure of the cell and release of ions from the surface of nanoparticles (Rai *et al.*, 2009). Of all the antimicrobials, silver substituted zeolites are the most widely used as polymer additives for food application (Boschetto *et al.*, 2012). Sodium ions present in zeolites are substituted by silver ions, which are antimicrobial against a wide range of bacteria and molds. These substituted zeolites are incorporated into polymers like polyethylene, polypropylene, nylon and butadiene styrene at levels of 1-3% (Brody et al., 2001). The bactericidal action of silver zeolite is based on the action of the silver ion itself released from zeolite and of reactive oxygen species generated from silver in the matrix (Matsumura *et al.,* 2003).

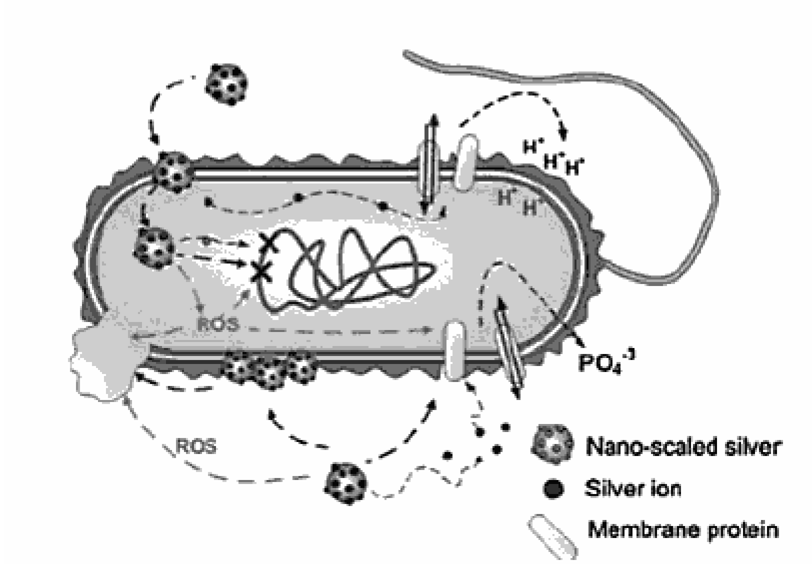


Figure 2. Diagram summarizing nano-scaled silver and silver cations interaction with bacterial cells (taken from Durán y Marcato, 2012)

Out of the activity of silver its application on industrial scale can be limited due to the raw materials high costs. Therefore, other inorganic nanoscaled particles compounds like ZnO, TiO_2 are being studied and tested as antibacterial agents to prevent bacterial growth. The mechanism of antibacterial action of nano ZnO has already been reported in the literature. It is believed that nano ZnO produce reactive oxygen species in moisture environments. Since these species are negatively charged they cannot penetrate into the cell membrane and remain in direct contact with the outer surface of bacteria, causing severe damage to proteins, lipids, and DNA (Tankhiwale and Bajpai, 2012).

Because the essential oils contain a number of components, their antimicrobial action cannot be confirmed by the effect of a single compound. Generally, the essential oils possessing the strongest antibacterial properties against food-borne pathogens contain higher concentrations of phenolic compounds such as carvacrol, eugenol (2-methoxy-4-(2-propenyl) phenol) and thymol (Burt, 2004). These compounds exhibit a wide range of biological effects including antioxidant and antimicrobial properties. The volatile compounds of essential oils have been found to exhibit a complete inhibitory action on some of the pathogens, however, these volatiles could not be able to exert antibacterial efficacy when applied individually (Bajpai *et al.*, 2012). Besides, different essential oils or their different components show antimicrobial mode of action not only at a particular location but also at different cell sites. The functions of cell components including nucleus can be reduced by the effect of natural organic agents due to the permeability changes that occurred in the membrane of bacterial cell. The hydrophobic nature of the essential oils or extracts components makes them to interact with lipid membrane of bacterial pathogens, resulting in the leakage of the inner cell components of the cell as well as affecting potassium ion reflux, and eventually leading to cell death. Volatiles compounds of essential oils can bind to protein structures of the cell and inhibit the enzymatic proteins in some bacterial pathogens. On the other hand, terpenoid compounds have been well known to affect the bacterial cell membrane, producing changes of cell functions, leakage of cell components as well as making starving conditions to survive the cell or cell components (Bajpai *et al.,* 2012). Volatile components of essential oils use to be not thermally resistant, thus antimicrobial packaging material made of essential oils are edible films, gels or coatings based on low melt temperature or film forming biobased materials such as starch, cellulose, chitosan/chitin, proteins and lipids (animal, plant-derived).

Extracts from green tea as well grape seeds have demonstrated inhibitory effects on Gram-positive as well as Gram-negative bacteria. These extracts are constituted by polyphenols (catechins, flavonoids), volatile oils, alkaloids, aminoacid among other uncharacterized components. The catechins have deteriorating effect on the lipid bilayer membrane that results in the loss of cell structure and function eventually leading to cell death (Sivarooban *et al*., 2008) On the other hand, the functional hydroxyl groups and conjugated double bonds in the reactive groups of natural plant extracts may be involved in their binding to the bacteria cell wall components (usually proteins). The polyphenols are able of bactericidal activity by inhibition of DNA and RNA synthesis in the bacterial cells and by inhibition of cytoplasmic membrane function of bacteria (Almajano *et al.,* 2008; Siripatrawan and Noipha, 2012). Major phenolic constituents like epicatechin, caffeic acid and benzoic acid may alter the cell morphology by influencing the osmotic pressure of the cell, thus disrupting the cytoplasmic membrane and causing leakage of cell constituents (Perumalla and Hettiarachchy, 2011). The antimicrobial effects of the natural extracts due to polyphenolic compounds are enhanced by the presence of volatile oils (Li *et al.,* 2012b)

The bacteriocins are produced by many species of lactic acid bacteria, and nowadays extensively studied for their food preservation properties in a variety of food products of animal and vegetable origin, against a range of food-borne pathogenic and spoilage bacteria. Particularly the enterocins of Classes I and II are considered most suitable alternatives to chemical preservatives because they are non-toxic and natural substances that inhibit not only closely related species but also Gram positive food-spoilage and food-borne pathogens. Besides the enterocins are harmless to eukaryotic cells, they are usually pH and heat tolerant and are readily digested (Brody et al., 2001; Khan *et al.*, 2010).

Other polymeric systems that constitute a valid alternative for solving the problem of contamination and spoilage by microorganisms in food packaging are those that exhibit antimicrobial activity by themselves and those whose biocidal activity is conferred through their chemical modification (Fernandez-Saiz, 2012). Some inconveniences such as release control of the low molecular weight biocides through the polymeric matrix, low thermal stability of antimicrobial agents, impact on odor and flavor, among others, are avoided. Besides, these antimicrobial polymers usually present longer-term activity. The mechanism of the bactericidal action of the polycationic biocides involves destructive interaction with the cell wall and/or cytoplasmic membranes. Macromolecules may interact more effectively with the cell of Gram-positive bacteria as their polyglycane outer layer is sufficiently loosely packed to facilitate deep penetration of the polymer chain inside the cell to interact with the cytoplasmic membrane. The Gram-negative bacterial cell has an additional membrane which protects the inner cytoplasmic membrane to a greater degree against the adverse action of the polymeric biocide (Muñoz-Bonilla and Fernandez-Garcia, 2012). Even though there is no evidence of trials or applications of these synthetic polymers in food packaging models, many efforts are being done in that direction. Classification of synthetic antimicrobial polymers regarding their chemical nature or the procedure for obtaining them is listed in Table 2.

Some cationic biopolymers are inherently antimicrobial, such as chitosan, alginates and poly-L-lysine, and they have been used in films and coatings (Appendini and Hotchkiss, 2002). Chitosan is the most known and investigated material showing a diversified range of applications. It is a cationic polysaccharide derived from shells of crustaceans and found to be nontoxic, biodegradable, biofunctional and biocompatible, in addition to having antimicrobial characteristics (Fernandez-Saiz, 2012). The antimicrobial character of chitosan is explained by the interaction of its positively charged amino group with the negatively-charged residues of the macromolecules at the bacterial cell surface, competition with calcium for electronegative sites on the membrane compromise its integrity, thus causing leakage of intracellular material and leading to bacterial cell death (Cooksey, 2001; Dutta *et al.*, 2009; Fernandez-Saiz, 2012). Solution casting using water as solvent is the most popular method to prepare chitosan films and coatings for food packaging applications. However, recent techniques include the preparation of chitosan/starch films by using supercritical carbon dioxide, microwave or irradiation treatments, or combinations with essential oils, amilose or fatty acids. Chitosan is a versatile material; in addition to serving as antimicrobial packaging material itself, chitosan can be additivated with traditional flavoring and coloring additives, with antioxidant and antimicrobial agents to potentiate the active characteristics of the packaging systems (Dutta *et al.*, 2009). It can also be combined with other preservations techniques such as modified atmosphere packaging (MAP) (Xing *et al.*, 2010; Duan *et al.*, 2010). Chitosan can be complexed with Ag$^+$, Zn^{2+} and Zr^{2+} (Higazy *et al.*, 2010); essential oils, natural extracts, peptides and chemical additives have been added to chitosan or chitosan blends for obtaining potent antimicrobial food packaging materials (Pranoto *et al.*, 2005; Kanatt *et al.*, 2012; Abdollahi *et al.*, 2012). Chitosan also have been blended with other biobased materials such as wey protein (Di Pierro *et al.*, 2011) and PLA (Sébastien *et al.*, 2006) to obtain enhanced gas barrier properties for specific food packaging final uses.

Table 2. Synthetic antimicrobial polymers (adapted from Muñoz-Bonilla and Fernandez-Garcia, 2012)

Polymers with inherent antimicrobial activity	Polymers with quaternary nitrogen atoms	Polymers containing aromatic or heterocyclic structures	Quaternized Poly(4-vinylpyridine)(P4VP); imidazole derivatives; cationic quaternary poly(diallyldialkylammonium) salts; insoluble crosslinked quaternized polystyrenes
		Acrylic and methacrylic polymers	Poly(methyl methacrylate) PMMA; derivatives from 2-(dimethylamino)ethyl methacrylate (DMAEMA)
		Cationic conjugated polyelectrolytes	Poly(phenylene ethynylene) (PPE)-based cationic conjugated polyelectrolytes; anionic conjugated polythiophene with cationic porphyrin
		Polysiloxanes	Polysiloxanes with pendant biocidal N,N_-dialkylimidazolium salt groups; polysiloxanes bearing quaternary ammonium salt (QAS) groups; Polysilsesquioxanes containing QAS and secondary namylammonium salt groups
		Hyperbranched and dendritic polymers	Quaternized polyethyleneimine (BPEI); branched PEI where the primary amine groups are functionalized with quaternary ammonium groups, alkyl chains of different length, allylic and benzylic groups; poly(ethyleneglycol)diacrylate (PEGDA) based dendrimers, copolymerized with EDGMA
		Polymers with quaternary ammonium end groups	Polyoxazolines
		Polymers with quaternary nitrogen atoms within the main chain	Amphiphilic quaternary dimethylammonium compounds; N,N-dodecyl,methyl-polyethylenimine

Table 2. Synthetic antimicrobial polymers (adapted from Muñoz-Bonilla and Fernandez-Garcia, 2012) (cont.)

Polymers with inherent antimicrobial activity	Guanidine containing polymers	---	Polyguanidines and polybiguanides
	Polymers mimic natural peptides	Synthetic peptides	Peptides composed of leucyl and lysyl residues (LK peptides); histidine–lysine (HK) polymers
		Arylamide and phenylene ethynylene backbone polymers	Amphiphilic arylamide oligomers and polymers
		Polynorbornene derivatives	Homopolymers and random copolymers of modular norbornene derivatives with guanidine and amine groups
	Halogen polymers	Fluorine-containing polymers	Polyurethanes (PU) containing antibiotics that bear fluorine atoms; polymer containing quinolone moieties and fluorine atoms; Poly(4-[3-(2,4-dichloro-5-fluorophenyl)-3-oxoprop-1-en-1-yl] phenyl acrylate)
		Chlorine-containing phenyl methacrylate polymers	Polymers from acrylic monomers based on 2,4-dichloro and 4-chloro-3-methylphenol; Poly(2,4-dichlorophenyl methacrylate), 2,4,4-trichloro-2_-hydroxydiphenylether.
		Polymeric N-halamines	Copolymers of 1-acryloyl-2,2,5,5-tetramethylimidazolidin-4-one, a cyclic-amine monomer with VA, acrylonitrile (AN) and MMA; copolymers of 2-vinyl-4,6-diamino-1,3,5-triazine monomer and styrene
	Polymers containing phospho and sulfo derivatives	----	Copolymer NIPAAm and methacryloyloxyethyl trialkyl phosphonium chlorides; poly(sodium 4-styrene sulfonate) (PSS); Zwitterionic carboxybetaine polymethacrylate; copolymer of poly(2-hydroxypropyl methacrylate) (PHPMA) poly(2-(methacryloyloxy)ethyl phosphorylcholine) (PMPC)
	Phenol and benzoic acid derivative polymers	----	p-acryloyloxybenzaldehyde and copolymers with MMA were prepared; copolymers of ferulic acid; mandelic acid condensation polymer (SAMMA)

Table 2. Synthetic antimicrobial polymers (adapted from Muñoz-Bonilla and Fernandez-Garcia, 2012) (cont.)

Polymers chemically modified to achieve antimicrobial activity	Organometallic polymers	----	Copolymers of organotin monomers (N-tri-n-butyltin) maleimide (TBTM) and m-acryloylamino-(tri-n-butyltin benzoate) (ATBTB) and styrene; silver(I) polymeric complexes
	Other molecular structures	----	Polyvinylamine (PVAm), poly(allylamine); poly(propyleneoxide) amines; dendritic structures based on polyglycerol (PGL) and O-carboxymethylated chitosan
	Covalent incorporation of low molecular weight antimicrobial compounds	----	Cyclic N-chloramine moieties (hydantoins, oxazolidinones, and imidazolidinones) into polyester fabrics; cyclic halamine precursors poly(m-phenylene isophthalamide), poly(aromatic imide-amide) and polybenzimidazole/poly(p-phenylene terephthalamide); levofloxacin attached to poly(dimethylsiloxane)
	Coupling of antimicrobial peptides	----	Nisin chemically bonded to PEO-b-PPO-b-PEO triblock copolymers
	Grafting other antimicrobial polymers	----	Grafting of protoporphyrin IX and zinc protoporphirin IX to nylon fibers; chitosan (poly-_(1–4)-d-glucosamine) immobilized onto PP; lysozyme anchored to poly(vinyl alcohol)

1.1.2 Oxygen scavengers

Oxygen is one of the detrimental elements that many packages are designed to guard its contents against. The concept of removing oxygen from inside a package is nothing new. Traditionally, packages have been flushed with an inert gas (N2 or CO2) to remove as much oxygen from the inside as possible (Rodgers, 2002). Oxygen which permeates through the packaging film (even those manufactured from high barrier packaging materials) or that is trapped inside the food cannot be efficiently removed by the use of MAP or vacuum packaging. The oxygen scavengers can absorb the residual oxygen present in the headspace of packaging below 0.03%, thus the oxidation of products is reduced and proliferation of molds, aerobic bacteria and insects (Ahvenainen, 2002). The development of oxygen-scavenging systems has followed two lines, depending upon whether the oxidizable substance was designed to be a part of the package (incorporation of scavenging agent into the packaging film) or to be inserted into it with the food. The sachet concept is simply to introduce an oxygen scavenging material into a barrier package via a small semipermeable pouch. This pouch or sachet, which is typically made from a non-woven material, contains a small quantity of the oxygen-scavenging compound, such as iron. Oxygen readily permeates the pouch material and reacts with the scavenging compound (Yoshikawa *et al.*, 1978; Rodgers, 2002). Packaging inserts are commonly based on the oxidation of iron-based compositions but they also can be based on sulfur compounds, oxydase enzymes, catechols, glycols, ascorbic acid, photosensitive dyes (Rooney, 2005; Kerry *et al.*, 2006; Brody *et al.*, 2008; De Azeredo, 2009; Restuccia *et al.*, 2010). The insert approach includes self-adhesive labels, adhesive devices or free sachets included with the food. Even though the performance of oxygen-absorbing sachets is satisfactory for a wide range of food storage conditions, important limitations restrict their use. The esthetics of inserts (that causes low consumer acceptance), the food product distortion, the risks of possible ingestion or rupture, as well as their unsuitability for use with beverages, drove researchers to seek package-based solutions. The most promising way to overcome these issues is the introduction of active oxygen scavenger directly into the polymeric matrix even though the design and the production of a functional and efficient active package can be difficult to carry out because of the complexity of the systems (Ahvenainen, 2002; Rooney, 2005; Kerry *et al.*, 2006; Galdi *et al*., 2008).

Modified oxygen-scavenging packaging materials includes monolayer and multilayer materials, as well as reactive closure liners for bottles and jars, and the scavenging active agents consist of inorganic, organic or polymeric materials. The active agent proper selection depends on the matrix selection, on the food characteristics and on the shelf-life required (Galdi *et al*., 2008). These active products can be melt extruded with other polymers to form a film or coating and used to produce the barrier/active package all as one structure (Rodgers, 2002). The structure of a typical multi-layer oxygen scavenging system is shown in Figure 3. An oxygen absorbing substance imbedded in a layer very permeable to oxygen is used to absorb the oxygen present in the interior package. Oxygen ingress from the outside environment to the oxygen absorbing layer is limited by a barrier layer, which is highly impermeable to oxygen. An inner layer, or a control layer, next to the oxygen absorbing layer may be used to minimize any migration of the oxygen absorbing substance into the food. In addition to the elimination of food product distortion that may occur when a sachet contacts the food, the incorporation of active systems into the packaging materials also allows the potential use with retort packaging and cost savings due to increased production efficiency and convenience (Ozdemir and Floros, 2004).

The Table 3 displays the current oxygen scavenger technologies and the patent number which protects them. The most developed oxygen scavenger system is that based on iron. They consist on the oxidation of iron into Fe_2O_3 and require water for their action at ambient or chilled temperatures, according to the theoretical equation which 1g of iron is capable to absorb 300 ml of oxygen (Miltz and Perry, 2005). Recently, the iron scavenging reactions have been described as electrochemical process in which the water adsorbed on the surface of iron forms electrolyte solutions that accelerate the electrochemical reaction (Mu *et al*., 2013):

$$Fe \rightarrow Fe^{+2} + 2e^- \qquad \text{(anodic reaction)}$$
$$H_2O + \tfrac{1}{2} O_2 + 2e^- \rightarrow 2\,OH^- \qquad \text{(catodic reaction)}$$
$$Fe^{+2} + 2\,OH^- \rightarrow Fe(OH)_2$$
$$2\,Fe(OH)_2 + \tfrac{1}{2} O_2 + H_2O \rightarrow Fe_2O_3 \cdot 3H_2O$$

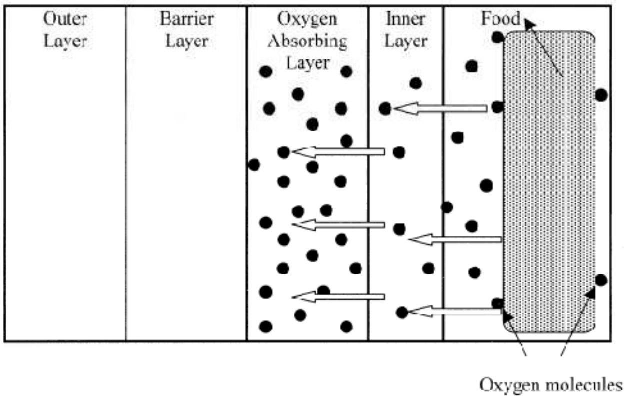


Figure 3. Schematic of typical oxygen absorbing multi-layer active film (taken from Ozdemir and Floros, 2004)

Free flowing pellets containing iron powder blended with low density polyethylene as carrier resin are commercially available, with the convenience of co-extrusion possibilities with EVOH and LDPE (Galotto *et al.,* 2009). Although films containing iron powder are strongly effective oxygen scavengers, they may impart undesirable flavor to foods with which are in contact (Ozdemir and Floros, 2004). In addition, the black colored iron powder alters the color and transparency of packaging materials, thus the application range in food packaging may be limited. Another systems described in Table 3, unlike to those iron-based, do not require water activity to be activated, and since oxygen absorbing material is virtually invisible in the films, these films do not alter the look of the package.

Some patents claim the manufacture of non-moisture dependent polymeric oxygen scavenger in conjunction with a patented UV triggering system, in which the polymer serves as the scavenger itself, and there are commercial products based on this principle (Schmidt *et al.*, 2003; Speer *et al*., 2006). However, these UV activation steps can reduce packaging line speeds, hence, profits. In addition, there is a significant cost increases for oxygen scavenging films production due to the high costs of photoinitiators, as well as operation and maintenance of the UV machinery (Byun *et al.,* 2011). The concept of using transition metals as catalysts for polymer oxidation processes was introduced in the 1980's and many polymers, such as MXD6 and polybutadiene, can be used as an oxygen scavenger (Speer *et al.,* 1994; Speer *et al.,* 1995; Murali *et al.,* 2002, Akkapedi *et al.,* 2002; Deshpande *et al.,* 2010). The problem with many of the oxidizable polymers that can be used for flexible packaging is that once the oxidation process starts it continues until the base polymer has degraded into small migratory compounds. Migration of oxidation products coming from scavenging action of the materials can be also migrate from systems based on other organics oxidizable compounds, as seen in Table 3. Because flexible packages only rely upon thin multiple layers of polymers to provide all the properties necessary for package performance, more complex structures have to be developed to prevent any degradation compounds from migrating into the food within the package. This challenge has been difficult to overcome for flexible packaging until now.

Table 3. Examples of the current oxygen scavenger technologies

Composition	**Mechanism**	**Patent Nº**	**Reference**
Iron zeolites *(sachets)*	Fe → Fe^{+2} + 2e^- H_2O + ½ O_2 + 2e^- → 2 OH^- Fe^{+2} + 2 OH^- → $Fe(OH)_2$ 2 $Fe(OH)_2$ + ½ O_2 + H_2O → $Fe_2O_3 \cdot 3H_2O$	US2825651	Yoshikawa *et al.*, 1978
Iron particles		US7,435,704B2 EP1640408 B1	Hekal, 2008; Harish and Tammaji, 2008
Iron modified bentonites		US 2011142899 (A1)	Lagarón *et al.*, 2011
Oxidizable organic compounds	Oxidation of organic cations interchanged in the nanoclays	US6,610,772B1	Clauberg *et al.*, 2003
	Oxidation of benzophenone derivatives by action of a photoinitiator	US2005/0085577A1	Ching *et al.*, 2005
	Oxidation of unsaturated carboxilic acids, anhydrides in presence of catalyst	US5,641,825	Bacskai *et al.*, 1997
	Oxidation of cyclic olefin, allylic and benzylic groups, in presence of catalysts	US5,627,239 WO9948963	Ching *et al.*, 1997; Ching *et al.*, 2010
Reducible organic compounds (quinones)	Quinone reduction (by UV light, heat, corona discharge) followed by reaction with molecular oxygen	US2005/0098765A1	Rooney, 2005b
Poliamides /transition metal catalyst	Passive barrier and polyamide oxidation	US6,410,156 B1 US7,691,290	Akkapedi *et al.*, 2002; Deshpande *et al.*, 2010
	Nanocomposites with enhanced barrier properties and polyamide oxidation	US2005/0159526	Bernard *et al.*, 2005
Polybutadienes or polyether/metal transition catalysts	Polibutadiene or polyether oxidation	US5,350,622 US5,425,896 US 6,423,776	Speer *et al.*, 1994; Speer *et al.*, 1995 ; Murali *et al.*, 2002
Hydrogen precursor/ Pd as catalyst	$NaBH_4$ + 2H_2O → $NaBO_2$ + 4 H_2 ½ O_2 + H_2 →H_2O	US2010/0028499	Rule *et al.*, 2010
H_2 atmosphere and catalyst	½ O_2 + H_2 →H_2O	P201230904	Lagaron and Busolo, 2012
Sulfites and bisulfites	$NaHSO_3$ + ½ O2 → $NaHSO_4$	US5,885,481	Venkateshwaran *et al.*, 1999

1.1.3 Antioxidants

The reactive oxygen species (ROS) is a collective term to include oxygen radicals and non-radical derivatives of oxygen. The oxygen radicals are superoxide anion ($O2•^{-}$), hydroxy (HO•), peroxy (ROO•), alkoxy (RO•), and hydroperoxy (HOO•) radicals. Non-radical derivatives are hydrogen peroxide (H_2O_2), ozone (O_3), and singlet oxygen ($^1O_2$). ROS are formed enzymatically, chemically, photochemically, and by irradiation of food. It is also formed by the decomposition and the inter-reactions of ROS. Formation of ROS in foods during storage and processing is also closely interrelated among ROS. They are mainly responsible for the initiation of oxidation reactions in foods. They react with lipids, proteins, sugars and vitamins; produce undesiderable volatile compounds; destroy essential fatty acids, aminoacids, and vitamins, and produce carcinogens. ROS change functionalities of proteins, lipids and carbohydrates by forming oxidized dimers and trimers (Choe and Min, 2005). ROS effects on the food products causeless acceptance or unacceptable to consumers, thus it is extremely important to control the formation of free radicals in foods to mantain food quality and extend shelf life.

The most important ROS are hydroxy radical and singlet oxygen. Hydrogen peroxide and superoxide anion are important precursors for hydroxy radical and singlet oxygen formation. Metal ions, heat, and particularly the ultraviolet light activate oxygen to produce singlet oxygen. Singlet oxygen is most often produced by photosensitizers reactions in the presence of UV light. Photosensitizers are dyes such as curcumin, pigments such as chlorophyll, aromatic hydrocarbons and, vitamins such as riboflavin, and metals such as iron (Choe and Min, 2005; Byun *et al.*, 2011). Thus, the high reactive singlet oxygen can be formed inside the food packaging systems, when headspaces contains oxygen, in presence of physical factors such as UV light and sensitizers as chemical factors, which can be found in the composition of foods.

The lipid oxidation is a free radical chain process consisting of chain initiation, propagation and termination steps (see Fig. 4). The key event in initiation is the formation of a lipid radical, R• (Chaijan, 2008). This can occur by homolytic cleavage of an R-H bond, or by hydrogen atom abstraction from an unsaturated molecule by an initiator free radical (In•), where RH is any unsaturated fatty acid. The hydroperoxides come from the reaction between unsaturated fatty acids and molecular oxygen; they can combine with fats to form additional reactive products during the propagation step. The volatile secondary oxidation products such as aldehydes, ketones, alkenes are formed at the termination step and are responsible of either loss of smell or rancid odors (Pereira de Abreu *et al*., 2011). Lipid oxidation can be also influenced by the concentration of pro-oxidants, endogenous ferrous iron (that plays as catalyst), enzymes, pH, temperature, ionic strength and fatty acid composition.

Proteins and aminoacids are oxidized mostly by hydroxy radical and singlet oxygen. Singlet oxygen reacts with proteins containing an electron-donating double bond activated by amino or alkoxy groups to produce dioxetanes. The dioxetanes are unstable to room temperature and are easily decomposed and produce carbonyl compounds. Hydroxyl radical reacts with proteins for producing crosslinked products and carbonyl compounds. In the case of muscle foods it has also been demonstrated that both lipid and myoglobin oxidation are coupled and capable of influencing each other. Myoglobin is an iron (II) protoporphyrin-IX complex found in the muscle of meat-producing animals. Depending upon the concentration and the oxidation state of iron and the structure of the complex, color changes in muscle can undergo and consequently affect the consumers acceptance of meat (Figure 5). The Fenton reaction, which is the principal mechanism for myoglobin oxidation, involves the oxidation of ferrous-oxymyoglobin to ferric-metmyoglobin, causing the undesiderable brown color of meat discoloration during its storage. At the same time, ferrous iron (Fe^{2+}) reacts with molecular oxygen to produce superoxide anion ($O_2•^-$) with concomitant oxidation to ferric iron (Fe^{3+}). Hydrogen peroxide (H_2O_2), which may be produced by dismutation of $O_2•$, can react with Fe^{2+} to produce highly reactive and unstable hydroxyl radical (OH•), which could initiate lipid peroxidation (Chaijan, 2008).

Initiation
$In• + RH \rightarrow InH + R•$

Propagation
$R• + O2 \rightarrow ROO•$
$ROO• + RH \rightarrow R• + ROOH$

Termination
$2\ ROO• \rightarrow ROOOOR \rightarrow$ non radical products, O_2

Figure 4. Mechanism of lipid oxidation

Traditionally, antioxidants have been mixed into food formulations or alternatively, food is dipped into solutions containing these additives. However, these approaches are limited by the inactivation of additives and lack of selectivity in those systems where microbial and oxygen spoilage also occurs. The antioxidant packaging materials have emerged as an alternative method to traditional preservations technologies such as chilling, freezing, direct addition of chemical preservatives or natural additives to food and vacuum or modified atmosphere (Zhou *et al.*, 2010). Antioxidants can be incorporated or coated onto food packaging materials, remain stable there for a required time, and finally be released from the packaging surface in a controlled way to inhibit the oxidation of oxidizable components, pigments and vitamins (Lee *et al*., 2004; Lee, 2005). Those molecules which contain in their structure at least one phenolic moiety with specific arrange of hydroxyl groups and double bond conjunction that allows electron delocalization can act as radical scavenger (Michalak, 2006). It has demonstrated that polyphenolic compounds can scavenge free radical from oxygen by donating one of their delocalized electrons or hydrogen atoms due to both their acidic nature and ability to transfer electrons while remaining relatively stable (Inzé and Van Montagu, 1995; Arora, Nair and Strasburg, 1998).

Flexible and thermoformable plastic packaging materials containing *t*-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), propyl gallate (PG), butylated hydroxyanisole (BHA), among others, have been developed and commercially applied. However, toxic and carcinogenic effects of BHA, TBHQ, PG, BHT have been demonstrated (Kahl and Kappus, 1993; Miyauchi *et al.*, 2002) and their use in food packaging have to be restricted, as others synthetics antioxidants that have been long used. Because the growing concern about the use of food chemicals due to the possibility of migration of additives and their degradation products into food that may affect the sensory properties or even lead to toxicity, the replacement of synthetic and toxic free radical scavengers by natural compounds may provide added benefits and positive consumer acceptation because they are perceived as safer, healthier and much less toxic than synthetic compounds (Lee, 2005; Pezo *et al.*, 2007; Arrua *et al.*, 2010; López-de-Di Castillo *et al.*, 2010; Gülcin, 2010). In this sense, the compounds that are already known by their beneficial effects for health, like tocopherols, flavonoids, catechins, stilbenoids, and natural extracts containing them (rosemary, green tea, oregano, etc.), have been considered for developing antioxidant active packaging materials to improve food protection (Lee, 2004; Lee, 2005; Camo *et al.*, 2008; Bolumar *et al.*, 2011; López-de-Di Castillo *et al.*, 2011).

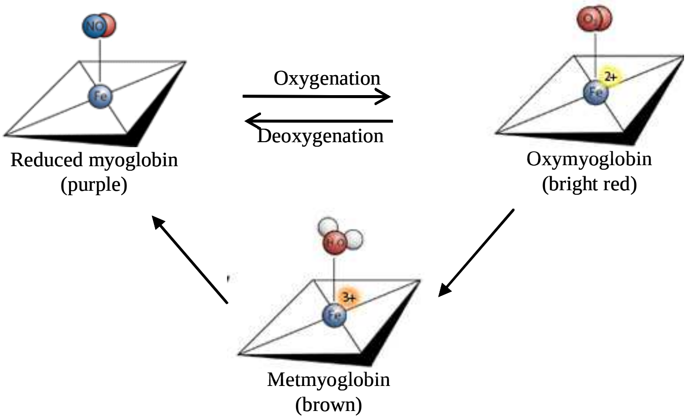


Figure 5. Schematic redox process of myoglobin

1.1.4 Other active systems

The removal of ethylene gas from the package headspace slows senescence, slows the rate of chlorophyll degradation and prolongs shelf life of climacteric fruits and vegetables. The most well-known, inexpensive, and extensively used ethylene absorbing system consists of potassium permanganate imbedded in silica. The silica absorbs ethylene, and potassium permanganate oxidizes it to ethylene glycol. These systems are usually prepared in sachet format due to the toxicity of permanganate. Another systems available to absorb ethylene are those based impregnated- potassium permanganate- quaternary ammonium salts- zeolites , bromine activated charcoal, $PdCl_2$ as catalyst supported on active charcoal and tetrazine (Brody *et al.*, 2001; Ozdemir and Floros, 2004; Freedonia Group, 2009).

Moisture scavengers are other systems that have been conveniently developed in recent years for controlling moisture excess in food packages for avoiding microbial growth and prevent foggy film formation, mainly in low water permeability packaging where water accumulation is more pronounced. Moisture scavengers, such as silica gel, molecular sieves, natural clays, calcium oxide, calcium chloride and modified starch, or other moisture absorbing substances, can be incorporated into a blanket or a pad of desiccant, prepared in sachet format or incorporated into a polymeric laminate. Depending on the final application one or another format has to be used. In packaged foods, flavor and odor absorbers take in unwanted gaseous molecules such as volatile package ingredients, chemical metabolites of foods, microbial metabolites, respiration products, or off-flavors in raw foods. Flavor and odor absorbers are usually placed inside packages or combined with other flavor permeable materials. Although scavenging of malodorous constituents is recommended to improve the quality of packaged foods, this technology should not be used to mask off-odors produced by hazardous microorganisms that could place consumers at risk.

1.2 Nanoclays

1.2.1 Structure and features of layered silicates

Layered silicates commonly used for nanocomposites preparation are natural or synthetic minerals, consisting of very thin layers that are usually bound together with counter-ions. They are constituted by tetrahedral sheets in which silicon is surrounded by four oxygen atoms, and octahedral sheets in which a metal like aluminum is surrounded by eight oxygen atoms (see Figure 6). The crystal lattice of 2:1 layered silicates consists of two-dimensional layers where a central octahedral sheet of alumina is fused to two external silica tetrahedra by the tip, so that the oxygen ions of the octahedral sheet also belong to the tetrahedral sheets (Ray and Okamoto, 2003; Okamoto, 2005; Pavlidou and Papaspyrides, 2008). The general formula for dioctahedral smectites is $(M_{x+y}{}^{+}\cdot nH_2O)(R_{2-y}{}^{3+})(Si_{4-x}{}^{4+}\ Al_x{}^{3+})O_{10}(OH)_2$, where x and y indicate the layer charge resulting from substitutions in tetrahedral and octahedral sites, respectively; R^{2+} refers to a generic divalent octahedral cation; M^{+} refers to a generic monovalent interlayer. A wide range of cations can occupy tetrahedral, octahedral and interlayer positions. Commonly Si^{4+}, Al^{3+} and Fe^{3+} are found in tetrahedral sites. The montmorillonite, one of the dioctahedral smectites, has the formula $(M_y{}^{+}\cdot nH_2O)(Al_{2-y}{}^{3+}Mg_y{}^{2+})Si_4{}^{4+}O_{10}(OH)_2$, and consists of platelets with an inner octahedral layer sandwiched between two silicate tetrahedral layers. The difference in valences of Al and Mg creates negative charges distributed within the plane of the platelets that are balanced by positive counter ions, typically sodium ions. In its natural state, this clay exists as stacks of many platelets. Hydration of the sodium ions causes the galleries to expand and the clay to swell; indeed, these platelets can be fully dispersed in water (Paul and Robeson, 2008).

Most of the technological uses of smectite are related to reactions that take place in the interlayer space. Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+}, which balance the negative 2:1 layer charge, are commonly hydrated and exchangeable (Brigatti *et al*., 2006).

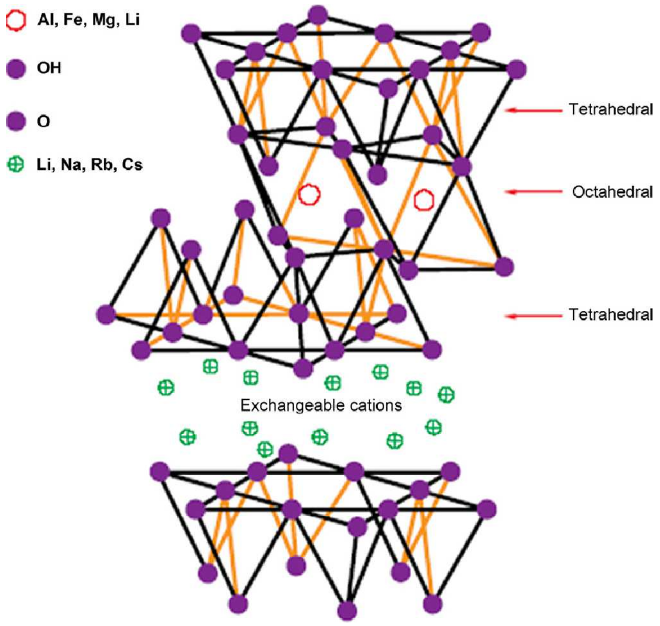


Figure 6. Structure of a 2:1 layered silicate (taken from Pavlidou and Papaspyrides, 2008)

1.2.2 Organomodified layered silicates

In their pristine state layered silicates are only miscible with hydrophilic polymers, such as poly(ethylene oxide) or poly(vinyl alcohol) (PVA). To render layered silicates miscible with other polymer matrices the normally hydrophilic silicate surface has to be converted to an organophilic one, making the intercalation of many engineering polymers possible (Ray and Okamoto, 2003; De Azeredo, 2009). The exchangeable ions can be substituted by organic cations to form an organoclay. Alkylammonium ions are mostly used, although other “onium” salts can be used, such as sulfonium and phosphonium (Pavlidou and Papaspyrides, 2008). The ammonium cation may have hydrocarbon tails and other groups attached and is referred to as a ‘‘surfactant’’ owing to its amphiphilic nature. The extent of the negative charge of the clay is characterized by the cation exchange capacity (CEC) (Paul and Robeson, 2008). The characteristic clay mineral–organic reactions of kaolinites and 2:1 clay minerals (i.e. montmorillonites) allow the modification of the surface character of clay mineral particles and the colloidal behavior of clay dispersions in industrial applications. Polar molecules form intercalation complexes with smectites, while neutral molecules can be adsorbed on by chemical interactions (hydrogen bonds, ion–dipole interaction, co-ordination bonds, acid–base reactions, charge-transfer, and van der Waals forces).

Organic modification of clay minerals is currently directed to the tailoring of clay minerals for different applications such as thickening agents, nanocomposites preparation and catalysts, among others. The interlayer cations of smectites can be exchanged in solution by organic and organometallic cations and in the solid state. Commonly, cation exchange reactions are performed by mixing aqueous dispersions of clay mineral and a solution of cationic surfactants including primary, secondary, tertiary, and quaternary alkylammonium or alkylphosphonium cations. These result in expansion of the interlayer spacing which leads to an increase in the basal spacing. As a result, the wettability and the thermodynamical interactions increase significantly. The arrangement of the intercalated surfactant cations depends on the layer charge and the alkyl chain length. While short chain of alkylammonium ions are arranged in monolayers, longer chain alkylammonium ions in bi- or three-layered structures with the alkyl chain axes parallel to the silicate layers (Figure 7). The monolayer has a basal spacing about 1.4 nm, the bilayer about 1.8nm; and multilayer up to 2.2 nm (Lagaly *et al*., 2006; Ruiz-Hitzky and Van Meerbeek, 2006; Ray and Okamoto, 2008).

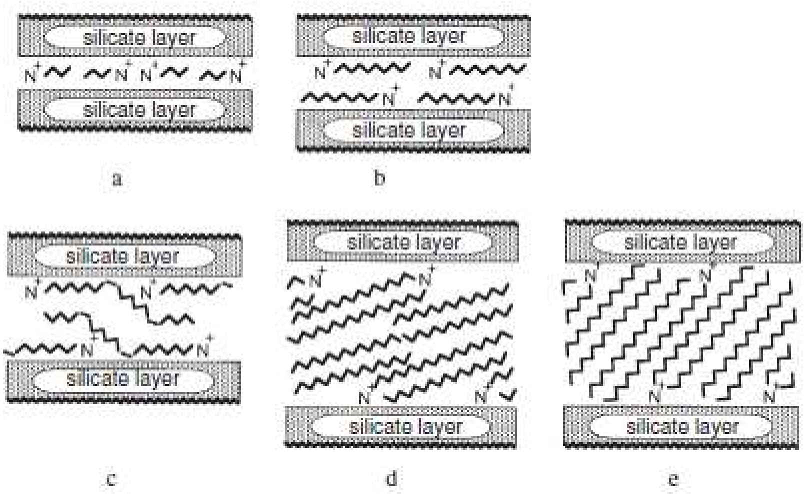


Figure 7. Alkylamonnium cations arrangement in the interlayer space of smectites (monolayers (a); bilayers (b); *pseudo*-trimolecular layers (c); paraffin-type arrangements of dialkylammonium ions (d,e)). (Taken from Lagaly *et al*., 2006)

1.3 Nanocomposites

Composite materials are solids resulting from the combination of two or more simple materials characterized by a continuous phase and a dispersed phase (fillers such as glass fibers, carbon particles, silica powder, clay minerals, among others). In addition they have properties that are essentially different from the components taken separately. The dispersed particles in the nanocomposites have at least one dimension in the nanometer range, thus they are typically named nanofillers. The dispersion process that entails the transition from microparticles to nanoparticles and a good distribution of these nanoparticles in the matrix yields dramatic changes in the physical properties of composites because nanoscaled materials have a large surface area for a given volume (Hussain *et al*., 2006). Since many important chemical and physical interactions are governed by surfaces and surface properties, a nanostructured material can have substantially different properties from a larger-dimensional material of the same composition. Typical nanomaterials currently under investigation include nanoparticles, nanotubes, nanofibers, fullerenes and nanowires. Clay-polymer nanocomposites could be considered as ‘‘one-nano-dimensional’’ material because the clay filler has one dimension at the nanometer scale, although the clay filler has two microscaled dimensions (Ruiz-Hitzky and Van Meerbeek, 2006).

The properties of a nanocomposite are greatly influenced by the size scale of its component phases and the degree of mixing between the two phases. Depending on the nature of the components used (layered silicate or nanofiber, cation exchange capacity and polymer matrix) and the method of preparation, significant differences in composite properties may be obtained. In general, layered silicates have layer thicknesses on the order of 1 nm and a very high aspect ratio (e.g. 10–1000). A few weight percent of layered silicates that are properly distributed throughout the polymer matrix thus create much higher surface area for polymer/filler interactions as compared to conventional composites (Ray and Okamoto, 2008). The physical mixture of a polymer and layered silicate may not form nanocomposites; thus previous organomodification of the silicate may be required for compatibilization between the filler and the matrix as explained above.

1.3.1 Preparation and morphology of nanocomposites

The preparative methods of nanocomposites are divided into three main groups according to the starting materials and processing techniques: solution method, *in situ* polymerization technique and melt processing (Paul and Robeson, 2008; De Azeredo, 2009; Duncan, 2011)

In the solution method, the nanoclay is swollen in a solvent before being added to a polymer solution, then polymer molecules are allowed to extend between the layers of the filler. Finally, the solvent is evaporated. The driving force for the polymer intercalation into layered silicates from solution is the entropy gained by desorption of solvent molecules, which compensates for the decreased entropy of the confined, intercalated chains. This method is good for the intercalation of polymers with little or no polarity into layered structures, and facilitates production of thin films with polymer-oriented clay intercalated layers (Ray and Okamoto, 2003).

The *in situ* polymerization process involves the swelling of the nanofillers by absorption of a liquid monomer. After the monomer has penetrated between the layers of silicates, polymerization is initiated by heat, radiation or catalysts.

The melt method is the most commonly used method due to the lack of solvents and because this is generally considered more economical, environmentally friendly, more flexible for formulation, and involves compounding and fabrication facilities commonly used in commercial practice. In melt processing, the filler is incorporated into a molten polymer and then formed into the final material (Brody *et al*., 2008; Paul and Robeson, 2008). The melt method process involves annealing a mixture of the polymer and OMMT above the softening point of the polymer. The polymer chains diffuse from the bulk polymer melt into the galleries between the silicate layers. The polymer intercalation depends critically on silicate functionalization and constituent interactions. The greater the polarizability or hydrophilicity of the polymer, the shorter the functional groups in the OMLS should be in order to minimize unfavorable interactions between the aliphatic chains and the polymer (Ray and Okamoto, 2003).

The types of polymer–layered silicate nanocomposites that can be obtained depending on the preparation method as well as the nature of the polymer matrix, the layered silicate and organic cation are immiscible (conventional or microcomposite), intercalated, and miscible or exfoliated (see Figure 8) (Pavlidou and Papaspyrides, 2008; Bordes *et al*., 2009; De Azeredo, 2009; Silvestre *et al*., 2011).

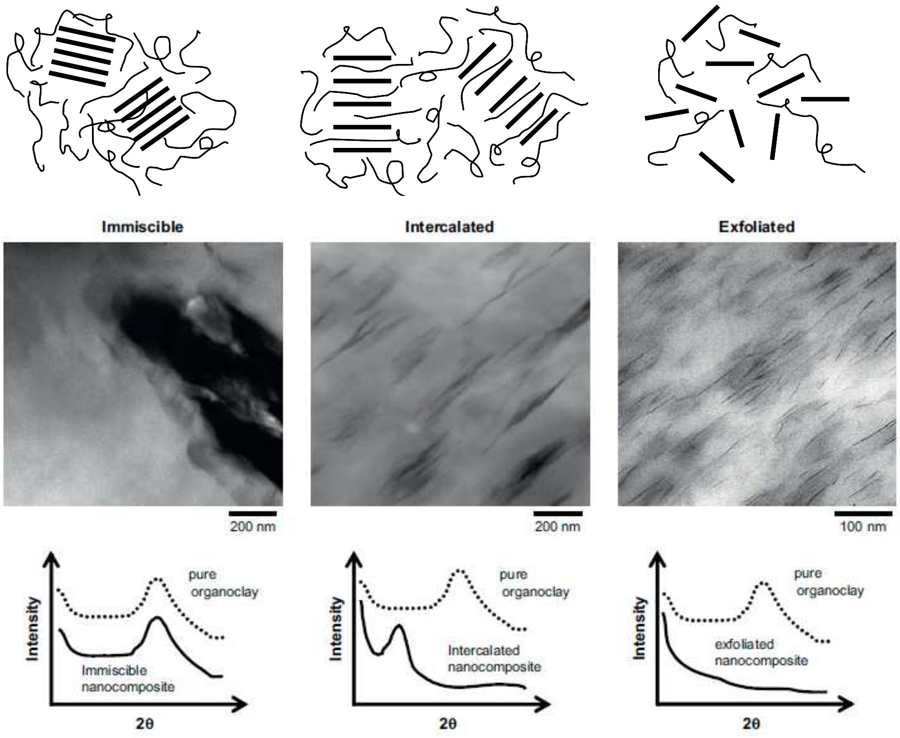


Figure 8. Classification of nanocomposites according to dispersion degree of the organoclay with the corresponding schematic WAXS and TEM (adapted from Ray and Okamoto, 2003; Paul and Robeson, 2008)

In the case called “immiscible”, microcomposites, the organoclay platelets are aggregated due to the poor physical attraction between polymer and filler; they exist in particles comprised of tactoids or aggregates of tactoids more or less as they were in the organoclay powder. Particle agglomeration tends to reduce strength and produce weaker materials (Pavlidou and Papaspyrides, 2008; Paul and Robeson, 2008). The intercalated structures are formed when the extended polymer chains are intercalated between the silicate layers. The result is a well ordered multilayer structure of alternating polymeric and inorganic layers, with a repeat distance between them. However, silicate layers are sometimes flocculated due to hydroxylated edge–edge interaction of the silicate layers (Ray and Okamoto, 2003). The exfoliated structures involve the complete separation of platelets and dispersed individually in the polymer matrix. Even though the goal of the formation process and the desired situation for most purposes is the complete exfoliation of the clay platelets, this ideal morphology is most often not achieved, but varying degrees of dispersion are more common (Paul and Robeson, 2008).

The homogeneous dispersion of most clays in organic polymers is not easy due to the hydrophilicity of their surface. Compatibilization between organic and inorganic phases in nanocomposites can be achieved either by organomodification of the nanoclays or blending with a functionalized polymer. The organophilization of clays reduces the energy of the clay and improves its compatibility with organic polymers, thus a proper organophilization is a key step for successful exfoliation of clay particles in most polymeric matrices. (De Azeredo, 2009). Organomontmorillonite (OMMT) has been systematically produced by exchanging inorganic cations of the pristine montmorillonite (MMT) with organic ammonium ions, commonly quaternary ammonium cations, to increase the affinity between the hydrophilic aluminosilicate clay and the organophilic polymer matrix, leading to a more regular organization of the layers in the structures, and decreasing the water uptake by the nanocomposite. Maleic anhydride grafted polymers and functionalized waxes are also often used as compatibilizer for increasing the matrix polarity and for improving platelets exfoliation.

X-ray diffraction (XRD) and transmission electron microscopy (TEM) are two complementary techniques generally used to characterize the structures of nanocomposites (Ray and Okamoto, 2003; Ray and Bousmina, 2005; Pavlidou and Papaspyrides, 2008; Silvestre *et al.*, 2011). Due to its ease of use and availability, XRD is most commonly used to probe the nanocomposite structure and polymer intercalation degrees. The spaces between structural layers of the silicate are determined by applying the Bragg's law: $\sin \theta = n\lambda/2d$, where λ corresponds to the wave length of the X-ray radiation used in the diffraction experiment, d the spacing between diffractional lattice planes and θ is the measured diffraction angle or glancing angle. By monitoring the position, shape, and intensity of the basal reflections from the distributed silicate layers, the nanocomposite structure (intercalated or exfoliated) may be identified (see Figure 8). The structure of the silicate is not affected in immiscible polymer/OMMT mixtures, thus, the characteristics of the OMMT basal reflections do not change. Interlayer spacing of the organoclay increases with the intercalation of the polymer, leading to a shift of the diffraction peak towards lower angle, according to Bragg's law. In an exfoliated nanocomposite, the extensive layer separation associated with the delamination of the original silicate layers in the polymer matrix results in the eventual disappearance of any coherent X-ray pattern from the distributed silicate layers. In some cases an opposite shift may also occur, that is usually attributed to loss of unbound surfactant from the gallery or to surfactant degradation. In addition, the influence of polymer intercalation on the order of the OMMT layers may be monitored by changes in the full width-at-half-maximum (FWHM) and intensity of the basal reflections. An increase in the degree of coherent layer stacking (i.e. a more ordered system) results in a relative decrease in the FWHM of the basal reflections upon hybrid formation. On the other hand, a decrease in the degree of coherent layer stacking (i.e. a more disordered system) results in peak broadening and intensity loss (Pavlidou and Papaspyrides, 2008).

On the other hand, TEM allows a qualitative understanding of the internal structure and can directly provide information in real space, in a localized area, on morphology and defect structures. Use of TEM is often criticized because it reveals the morphology in such a small region. However, this can be overcome by taking images at different magnifications and from different locations and orientations until a representative picture of the morphology is established. Fortunately, the elemental composition of the clay compared to that of the polymer matrix is such that no staining is required (Paul and Robeson, 2008). Since the silicate layers are composed of heavier elements (Al, Si and O) than the interlayer and surrounding matrix (C, H and N), they appear darker in bright-field images. Exfoliation is generally never complete, but in the cases when it is essentially complete, particles consisting of two, three or more platelets about 1 nm thick can be seen as dark lines when the microtome cut is perpendicular to them (Silvestre *et al*., 2011).

Besides XRD and TEM techniques, other powerful techniques have been employed to understand and evaluate the structure of nanocomposites. Among these, small angle X-ray scattering (SAXS), solid-state nuclear magnetic resonance (NMR, ^{1}H and ^{13}C), FTIR (Fourier Transform Infrared Spectroscopy), Raman and neutron scattering, among others, are tools for gaining greater insight about the morphology, surface chemistry, and to a very limited extent, the dynamics of exfoliated polymer clay nanocomposites. However, these techniques are not widespread or widely used except in a few laboratories probably because lacking of facilities or experience in interpreting the results (Ray and Okamoto, 2003; Paul and Robeson, 2008; Okamoto, 2008).

1.3.2 Properties of nanocomposites

1.3.2.1 Gas barrier properties

Low gas permeation of nanocomposites due to the tortuous path imposed by the nanofiller was long considered as one of their most important technological advantages. The barrier properties of polymers can be significantly altered by inclusion of inorganic platelets with large length-to-width (aspect) ratio that create a maze or tortuous path that retards the diffusion of gas molecules through the polymer matrix. Because the filler materials are essentially impermeable inorganic crystals, gas molecules must diffuse around them rather than taking a (mean) straight line path that lies perpendicular to the film surface. The result is a longer mean path for gas diffusion through the film in the presence of fillers (Figure 9) (Pavlidou and Papaspyrides, 2008; Duncan, 2011). The degree of enhancement in the barrier properties depends on the degree of tortuosity created by clay layers in the diffusion way of molecules trough the polymer film. The tortuous factor is determined by the ratio of actual distance which diffusive molecule is walked to the shortest distance to diffuse (polymer film thickness). This factor is affected by the aspect ratio of clay dispersed in the matrix.

Contrary to what one may think total exfoliation with random orientation of the individual clay mineral particles may not be favorable to low permeability but the morphology of intercalated compounds. In these, the particles are well oriented and remain parallel to each other (Ruiz-Hitzky and Van Meerbeek, 2006). To take full advantage of the reinforcement or tortuosity clay platelets or particles can provide to mechanical and thermal or barrier properties of nanocomposites, they must be oriented in the appropriate direction and not curled or curved. While the mixing process influences the degree of dispersion or exfoliation, the type of processing used to form the test specimen (extrusion, injection molding, etc.) strongly affects the alignment of particles (Paul and Robeson, 2008).

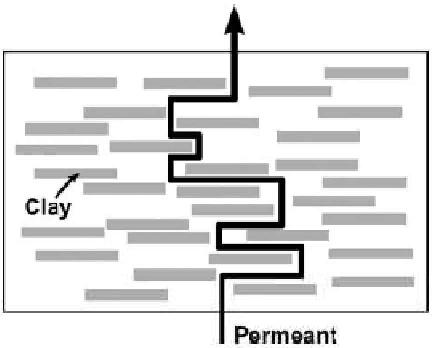


Figure 9. Scheme of barrier to permeation imposed by nanoparticles imbedded in a polymeric matrix (taken from De Azeredo, 2009)

1.3.2.2 Optical transparency

Microsized particles used as reinforcing agents scatter light, thus reducing light transmittance and optical clarity. On the other hand, layered silicate platelets, albeit their micron lateral size, are just 1nm thick. Thus, when single layers are dispersed in a polymer matrix, the resulting nanocomposite is optically clear in the visible region, whereas, there is a loss of intensity in the UV region (for < 250 nm) mostly due to scattering by the clay particles. (Ray and Okamoto, 2003; Pavlidou and Papaspyrides, 2008).

1.3.2.3 Mechanical properties

Properly dispersed and aligned clay platelets have proven to be very effective for increasing stiffness. Regardless of preparation method or type of clay, the modulus and strength of the nanocomposites are remarkably similar, and proportional to the content of clay mineral. The data suggest that the organoclay is much more efficient than other mineral fillers in increasing the mechanical properties of the polymer (Ruiz-Hitzky and Van Meerbeek, 2006). Nanocomposites require less mass of filler than traditional filler reinforced composites, thus nanocomposites exhibit superior weight advantages. Furthermore, if the platelets are aligned in the plane of the sample, the same reinforcement should be seen in all directions within the plane, whereas traditional fillers reinforce only in the direction of their alignment (Paul and Robeson, 2008).

1.3.2.4 Other properties

The increased flammability resistance of nanocomposites can be done by the formation of a stable carbon/nanoplatelet or nanofiber surface that exhibits analogous characteristics to intumescent coatings whereby the resultant ‘‘char’’ provides protection to the interior of the specimen by preventing continual surface regeneration of available fuel to continue the combustion process. The primary advantage noted with nanofiller incorporation is the reduction in the maximum heat release rate (commonly determined by cone calorimetry). In other cases, the incorporation of nanofillers reduces the flammability by increase of the melt viscosity that prevents dripping (Paul and Robeson, 2008). The onset of the degradation temperature can increase in some organoclay based nanocomposites. Even though this is not an indication of an improvement in the resistance to ageing or oxidation of the nanocomposite, this is generally regarded as a consequence of the low permeability of the nanocomposites preventing oxygen to reach the polymer, and preventing the volatiles generated during the decomposition of the polymer to leave the nanocomposites (Ruiz-Hitzky and Van Meerbeek, 2006).

Crystal formation includes nucleation and crystal growth. However, although it is well established that nanometer sized clay platelets are effective nucleating agents, different effects have been reported on the linear growth rate and the overall crystallization rate, depending on the type of polymer (Pavlidou and Papaspyrides, 2008).

1.3.3 Food packaging applications of nanocomposites

The purpose of conventional food packaging systems is just to protect the food, acting as a barrier between the food and the surrounding environment. The active food packaging not only acts as a passive container of the food avoiding the contact with the environment but also interacts with the food in specific ways for keeping or even improving safety and quality. Polymer nanotechnology is actually developed mainly to improve barrier performance pertaining to gases. It is proved also to enhance the barrier performance to UV light, as well as to add strength, stiffness, dimensional stability and heat resistance. The use of polymer nanotechnology can in fact extend and implement all the principal functions of the package (containment, protection and preservation, marketing and communication) (Silvestre *et al*., 2011).

Moreover, several nanoparticles can provide active and/or ‘‘smart” properties to food packaging materials, such as antimicrobial properties, oxygen scavenging ability, enzyme immobilization, or indication of the degree of exposure to some degradation related factor. So, nanocomposites can not only passively protect the food against environmental factors, but also incorporate properties to the packaging material so it may actually enhance stability of foods, or at least to indicate their eventual inadequation to be consumed. At the moment, nanocomposites for food packaging have been mainly developed for antimicrobial applications, but other main promising applications comprise oxygen scavengers, ethylene removers and carbon dioxide absorbers/emitters, among others (Ozdemir and Floros, 2004; De Azeredo *et al*., 20; Silvestre *et al*., 2011).

The market leader in active packaging is Japan, with the 45% of the current market, followed by the United States and Australia. In Europe, however, industrial applications are coming slow because of legislation restrictions regarding nanotechnology, and the consumers low acceptance and distrust derived from the lack of knowledge (Silvestre *et al*., 2011). Consequently, most packaging incorporating nanoparticles is currently receiving attention at the research stage rather than in commercial applications. This great opportunity for advancement will continue to be overlooked by the commercial packaging industry until the cost of manufacture becomes more affordable. Fortunately, nanoclays present the advantage of exhibiting low costs and have therefore, reached already industrial and commercial implementation in the food packaging sector (Lagaron, 2011).

1.3.4 Nanobiocomposites and sustainability

The use of plastics in food packaging has been increasing at accelerated rates in the last decades driven by the market trends and consumers requirements. These demand reduction in the cost of packaging materials and versatility to fulfill the technical approaches related to the active and intelligent food packaging. The use of non-degradable plastic materials for these purposes has a significant environmental impact due to the persistence of plastic wastes and the polluting character of the disposal methods such as incineration or landfills. Incineration of plastic wastes not only produces a large amount of carbon dioxide that contributes to global warming but also toxic gases and residues. Landfills constitute a dubious option because destroy the ecosystem of the place where they are located; in addition they contaminate the soil and groundwaters by leakage, generate methane and cause problems like dust, bad odors, vermin and noise pollution. In the developing countries where the landfills are improperly operated, the mentioned problems are magnified with terrible consequences for the environments and the public health. Other disadvantages of non-degradable plastics are the limited fossil origin resources and the generation of green house gases during the manufacturing process. Taking into account this background, there is a need to develop renewable, source-based, environmentally benign plastic materials (biopolymers), especially for use in short-term packaging and disposable applications. The manufacture of these biomaterials would not involve the use of toxic or noxious components, and could allow for composting into naturally occurring degradation products.

Currently, there is a growing tendency to use environmentally friendly or “green” materials for replacing nondegradable materials and reducing the environmental pollution caused by large amounts of plastic waste. Environmentally friendly materials with applications in agriculture, the building and food industries, or biomedicine are the main objective of many research groups. Biodegradable natural polymers extracted from renewable resources (i.e. PLA, starch, cellulose, PHB, among others) are replacing the oil-based non degradable polymers plastics mostly in the production of biomaterials for packaging applications (Darder *et al.,* 2007). The use of nanocomposites based on biodegradable polymers for food packaging (nanobiocomposites) not only protects the food and increases its shelf life but can also be considered a more environmentally friendly solution because it reduces the requirement of oil-based plastics as packaging materials.

Current alternative biodegradable materials exhibit poor barrier and mechanical properties and these properties need to be improved considerably before they replace traditional plastics and thus help to manage the world waste problem. Biopolymer materials are excellent vehicles for incorporating a wide variety of additives and modified inorganic particles to enhance the inherent properties of the material as well as to introduce other functionalities, such as antioxidants, antifungal agents, antimicrobials, among others (De Azeredo, 2009). Biodegradability of a packaging material can also be enhanced with the introduction of organoclay nanoparticles into the biopolymeric matrix and can also be controlled with surfactants

(Sozer and Kokini, 2009). The innovations in the development of materials from biodegradable polymers, the preservation of fossil-based raw materials, complete biological degradability, the reduction in the volume of garbage and compostability in the natural cycle, protection of the climate through the reduction of carbon dioxide released, as well as the application possibilities of agriculture resources for the production of green materials are some of the reasons why polymers from renewable sources have attracted the academic and industrial interest (Ray and Bousmina, 2005).

The *Green Chemistry* is a concept developed in early 90´s that involves the design of environmentally benign products and processes by addressing the hazards related to global issues such as climate change, energy production, availability of a safe and adequate water supply, food production, and the presence of toxic substances in the environment (Anastas and Kirchhoff, 2002). The *Green Chemistry* is governed by twelve principles that can be applied to all those areas in which chemistry and chemical processes are involved, including nanocomposites. Some principles of the *Green Chemistry* that have been adapted to the procedures of nanobiocomposites preparation are:

- Use of biopolymers from renewable sources instead of oil-based polymers. In this sense, PLA is by far the most experienced and applied biopolymer in nanobiocomposites preparation, but others matrixes such as cellulose, chitosan and PHA are being deeply evaluated (Okamoto, 2005)

- Preparative procedures that do not involve the use of solvents (melt intercalation) (Paul and Robeson, 2008; Duncan, 2011; De Azeredo, 2011); or use of water and ionic liquids as solvents (solution *in situ* polymerization and solution method) (Jia *et al*., 2011)
- Solvent recycling

However, the discovery and application of more environmentally benign technologies at the research stage does not guarantee that they will be adopted on an industrial scale (Anastas and Kirchhoff, 2002). Adoption of environmentally benign processes in nanocomposites preparation may be facilitated by flexibility in regulations, tax incentives for implementing cleaner technologies, research programs to facilitate technology transfer, and patent life extensions for cleaner process optimization.

1.3.4.1 Biopolymers

Biopolymers have been widely investigated over the last two decades because they can be a viable solution to the waste disposal of foods plastic packaging materials. They are defined as those that undergo microbial induced chain scission leading to the mineralization. Specific conditions in terms of pH, humidity, oxygenation and the presence of some metals are required to ensure the biodegradation of such polymers. Most biodegradable polymers have excellent properties comparable to many petroleum-based plastics, thus biodegradable polymers have great commercial potential to constitute plastic articles, but some of their properties such as brittleness, low heat distortion temperature, high gas permeability, low melt viscosity for further processing etc., restrict their use in a wide-range of applications. Besides the modification of the biodegradable polymers, the nanoreinforcement of pristine polymers to prepare nanobiocomposites has already proven to be an effective way to improve these properties (Ray and Bousmina, 2005, Lagaron, 2011).

Biopolymers can be classified as presented in Figure 10. Renewable sources-based biodegradable polymers so far used for the preparation of nanocomposites are polylactides (PLA), poly(3-hydroxy butyrate) (PHB) and its copolymers, thermoplastic starch, cellulose, gelatine and chitosan.

On the other hand, unlike other petrochemical-based resins, a broad range of synthetic biodegradable resins based on aliphatic polyesters and their co-polymers break down rapidly into carbon dioxide, water and humus in appropriate conditions of composting. The most used oil-based biodegradable for the preparation of nanocomposites are poly(butylene succinate), aliphatic polyesters, poly(e-caprolactone), and poly(vinyl alcohol).

Polylactides (PLA) are biodegradable polymers, made primarily from renewable agricultural resources such as corn starch. PLA can be totally amorphous or up to 40 % crystalline. As a consequence, PLA polymers can display a broad range of mechanical properties (exhibits tensile strength comparable to other commercially available polymers), their glass transition temperature may range from 50 °C to 80 °C, while their melting point can vary from 130 °C to 180 °C. In addition to its strength, biodegradability, and compostability, PLA polymers exhibit good processability on standard plastic equipment (extrusion, injection, and film and fiber forming), also are resistant to oil-based products, are sealable at lower temperatures, and can act as flavor or odor barriers for foodstuffs. PLA can be modified in a controlled way by changing its stereochemical structure (during the polymerization stage) and/or by blending it with selected polymers or filling it with inorganic microparticles or nanoparticles (Marras *et al.*, 2007).

1.3.4.2 Properties of nanobiocomposites

The problems associated with biodegradable polymers are performance, processing and cost. Although these factors are somewhat interrelated, problems due to performance and processing are common to all biodegradable polymers in spite of their origin. In particular, low heat distortion temperature, high gas and vapour permeability, poor resistance to protracted processing operations have strongly limited their applications. The application of nanotechnology to these polymers may open new possibilities for improving not only the properties but also at the same time the cost-price-efficiency (Sorrentino *et al*., 2007).

Incorporation of nanoclays in biopolymers allows several advantages in functionality:

(a) Improved mechanical properties both in solid and melt states compared to conventional filler composites, because reinforcement in nanocomposites occurs in two dimensional rather than one dimensional, and no special processing is required to laminate the composites.

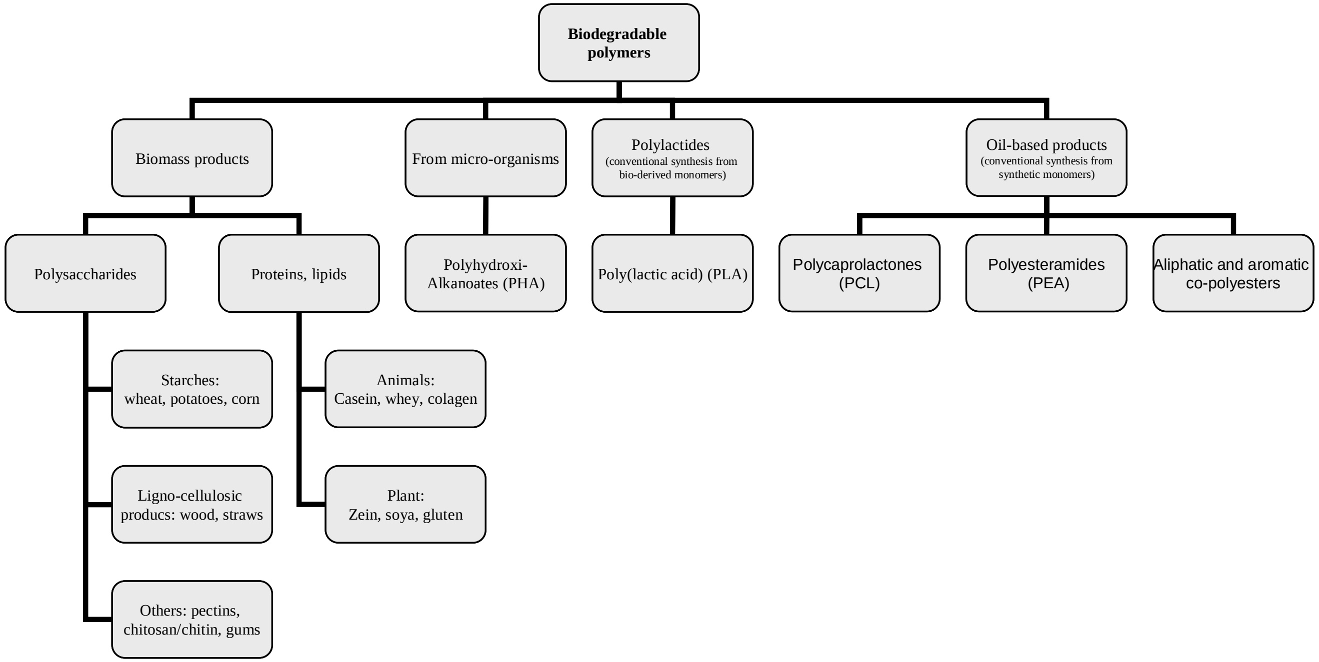


Figure 10. Classification of biodegradable polymers
(adapted from Cha and Chinnan, 2004; and Ray and Bousmina, 2005)

(b) They show improved barrier properties to small gases because of the formation, among other factors, of a more tortuous path in the presence of exfoliated layered silicates.

(c) Thermal stability of biodegradable polymers also increases after nanocomposites preparation, because the clay acts as a heat barrier, which enhances the overall thermal stability of the system, as well as assist in the formation of char after thermal decomposition.

(d) The compost degradation rate of some biodegradable polymers is significantly enhanced after nanocomposite preparation with organically modified layered silicate and we can control the rate of biodegradability by judicious choice of clay.

(e) Melt viscosity of pure polymer increases after nanocomposites formation. This will help us for further processing.

Typically, plasticizers such as glycerol, or vegetable oils are added to biopolymers with a melting temperature close to their decomposition temperature to overcome degradation problems, transforming them into thermoplastic polymers. These compounds also contribute to a better dispersion of the nanofiller in the biopolymer matrix, resulting in an enhancement of the mechanical properties (Darder et al., 2007).

PLA–clay nanocomposites have special importance because of the promising properties of polylactides and the possibility of high property enhancements by filling with nanoparticles. PLA/layered silicate nanocomposites using organomodified clays (MMT, saponite, mica, monetita, among others) have been prepared by several methods such as melt extrusion and solution casting (Pluta *et al*., 2002; Okamoto *et al.*, 2005; Cava *et al*., 2006). Exfoliated and intercalated morphologies are obtained, as well as improved thermal stability and mechanical properties. The nanoclays act as nucleating agents facilitating the polymer crystallization. On the other hand, it was found that the addition of the natural to PLA does not have any significant effect on both thermal due to the poor interaction between the untreated clay and the polymer matrix (Pluta *et al*., 2002; Marras *et al*., 2007).

The nanobiocomposites may be soon competitive with the existing oil based polymers. However, current production costs are still high and restrict the use of biopolymers in a wide range of applications. Thus, the most important factors to the formation of a biodegradable polymer based industries include cost reduction of biodegradable polymers as well as public and political acceptance. Finally, to achieve the required characteristics that meet wide specific applications, nanobiocomposites formulation must be further researched and modified so that mechanical and other properties can be easily enhanced depending on end-user requirements.

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2. OBJECTIVES

The overall objective of this dissertation was the morphological characterization and evaluation of the active antimicrobial, oxygen scavenger and antioxidant properties of several active nanoclays, and further preparation of their corresponding nanocomposites by applying common incorporation techniques in polymers. The morphology, active properties and migration behavior of these nanocomposites were also assessed in order to establish their potentialfor food contact use.

To achieve the overall objective, the following concrete objectives were set:

- Chemical and physical characterization of the active nanoclays modified with silver, iron and resveratrol, by using chemical analysis techniques (X-ray fluorescence, XRF), X-ray diffraction (WAXS), thermogravimetry (TGA) and transmission electron microscopy (TEM).

- Evaluation of the active properties of nanoclays through antimicrobial activity, oxygen depletion and free radical scavenging assays.

- Nanocomposites preparation from active nanoclays and biodegradable and oil-based polymers, using appropriate processing techniques depending on the polymeric matrix (solvent casting or melt mixing).

- Morphological characterization of nanocomposites to ascertain the dispersion and distributionof the clays in the polymeric matrix, by WAXS and TEM; determination of the changes in color and transparency caused by the incorporation of clays and; and assessment of barrier properties and thermal stability (by thermogravimetric analysis).

- Evaluation of the active properties of the nanocomposites (antimicrobial, oxygen scavenger and free radical scavenger)

- Detection and quantification of migrants from active composites to food simulants (migration tests) to determine their feasibility of use to constitute food contact materials.

3. NOVEL SILVER-BASED NANOCLAY AS AN ANTIMICROBIAL IN POLYLACTIDE FOOD PACKAGING COATINGS

Abstract

This paper presents a complete performance study of polylactide (PLA) biocomposites obtained by solvent casting, containing a novel silver-based antimicrobial layered silicate additive of interest in active food biopackaging applications. The silver-based clay showed an strong antimicrobial effectiveness against Gram-negative *Salmonella spp*. Despite the fact that no complete exfoliation of the active clay in PLA as was suggested by TEM and WAXS, the additive is dispersed very nicely throughout the PLA matrix to a so-called nanoscale, hence yielding nanobiocomposites. They resulted in transparent films with enhanced water barrier and strong biocide capacity. Silver migration to a slightly acidified water medium, that may be considered as a highly aggressive food simulant, was also measured by voltamperometry. Silver migration was found to increase with exposure time due to plasticization and/or possible partial hydrolisis of the PLA matrix. The study suggests that very low migration levels of silver, i.e. within the specific migration levels for silver ions as defined by European Food Safety Agency (EFSA), can lead to sufficiently strong antimicrobial films, hence supporting the application of this antimicrobial additive in active food packaging applications for maintaining food quality and safety.

Keywords: Silver, PLA, antimicrobials, food packaging, migration

1. Introduction

Antimicrobial materials and surfaces are becoming increasingly important in different areas such as healthcare, home and personal hygiene, foods, active packaging, automotive and textiles among other application industries. Antimicrobial technologies are being investigated and developed to prevent the outbreak of harmful microorganisms. However, these systems need to combine desirable attributes such as strong antibacterial efficacy, environmental safety, low toxicity, cost effectiveness and ease of fabrication.

It is well-known that silver is a powerful antimicrobial agent that has been used since ancient times. Recent technical innovations and findings facilitate the availability and incorporation of silver products in a wide range of materials at the manufacturing stage, providing novel antimicrobial formulations. Currently, a specific form of silver does not exist for every application, procedure or matrix. In this sense, nanotechnology is being a key factor due to the capability of modulating metals, compounds and materials into the nanosize, which often changes their chemical, physical and optical properties, as well of these of the matrixes in which are incorporated. Stable silver nanoparticles can be obtained by way of using soluble starch as both the reducing and stabilizing agent (Vigneshwaran *et al*, 2006), or being synthesized via the regular borohydride reduction of Ag^+ ions (Lok *et al*, 2007; Oh *et al*, 2004). Silver nanoparticles can also be synthesized in the interlamellar space of a pretreated kaolinite with DMSO by UV radiation or chemical induced reduction (Dékány *et al*., 2003; Dékány *et al*., 2004), in layered laponite suspensions via photoreduction (Huang and Yang, 2007), or supported on micro and mesoporous structures after ion exchange followed by *in situ* reduction (Yang *et al*., 2008; Lv *et al*., 2009).

Silver (I) nitrate adducts with diverse electronic and steric characteristics can be synthesized with *N* and *P*-donor ligands (Pettinari *et al.*, 2007). Thus, Ag/SiO_2 coating solutions have been prepared to serve as antimicrobial refinement of temperature sensitive materials like fabrics or wood (Mahltig *et al*., 2009). Moreover, a suspension of silver nitrate in an ammonium salt medium has been reported as a precursor of stable nanoscale AgBr particles (Zhang *et al*., 2007).

On another line of work, many efforts have also been carried out for the development of inorganic materials, such as zeolites, for supporting Ag^+ ions due to their capability to incorporate and release ionic species. Coleman *et al. (*Coleman *et al.*, 2009) prepared Ag^+ and Zn^+ exchanged tobermorites and demonstrated that they have marked bacteriostatic effect and they can be potentially used as antimicrobial materials for *in situ* bone tissue regeneration. Thermal stability of Ag^+ supported clinoptilolite and possible applications were tested by Akdeniz and Ülkü (Akdeniz and Ülkü, 2008).

Reports based on silver modified clays by a cation exchange method have been published before. Oya *et al*. (Oya *et al*., 1991) reported the antimicrobial properties of Ag^+-exchanged montmorillonite on 1991. Keller-Besrest *et al.* (Keller-Besrest *et al*., 1995) prepared a silver loaded montmorillonite for possible topic uses in the treatment of burns. They demonstrated the coexistence of both silver metal particles and Ag^+ ions, and they also observed significant differences on the final silver content in clays using exchange resin procedure (up to 10 wt%) with regard to regular CEC exchange in solution (up to 1 wt%). Quintana *et al.* (Quintana *et al.*, 2008) studied the effects of calcination and mechanical grinding on silver exchanged Na-MMT and its antimicrobial performance. They reported metallic silver nanoparticles on the clay aggregates, and pointed out that the antibacterial performance is affected by the availability of the ionic silver to be in contact with the bacteria. Praus *et al.* (Praus *et al.,* 2009) compared the antimicrobial activities of cetylpyridinium, cetyltrimethylammonium, silver ions and metallic silver immobilised on montmorillonite. They demonstrated that antibacterial compounds are effective just when they are released from the inorganic carrier, and they concluded that intercalated silver ions are the most effective antibacterial elements while metallic silver does not show any antibacterial effects.

Applications wise, one of the most important potential uses of antimicrobial materials is currently in active packaging of foods (López-Rubio *et al.*, 2008). In the food packaging field there is also an ongoing trend to substitute petroleum-based plastics by renewable plastic packaging with potential lesser impact in the carbon-foot print of food packaging operations. Within the renewable family of polymers, poly(lactic acid) is a very promising alternative material due to its excellent mechanical properties, transparency and commercial availability. However, some of its properties such as gas and water permeability and heat distorsion temperature are considered to be too low for widespread applications (Avérous *et al.,* 2009). In this context, the incorporation of nanolayered additives in polymer formulations is thought to lead to enhanced barrier due to synergistic tortuosity, crystal nucleation and chain immobilization effects (Cabedo *et al.,* 2004; Lagaron *et al.,* 2005). Various organoclay systems based on montmorillonite (MMT) and different organomodifiers have been reported in the formulation of novel PLA/clay nanobiocomposites by solvent intercalation, melt intercalation or masterbatch formulation (Avérous *et al*. 2009). As an example, PLA based composite films containing natural and organomodified MMT commercial grades were prepared by Rhim *et al. (*Rhim *et al.*, 2009), and the authors reported enhanced barrier and antimicrobial properties in the materials.

This study reports on the formulation of novel nanobiocomposites of PLA containing a new commercial food contact compliant silver-based clay additive. Morphology, barrier, silver migration and biocide performance are also reported. The implementation of such active clay-based technology in plastics and bioplastics can potentially lead to the formulation of novel antimicrobial films and coatings with synergistic enhancement in physical properties.

2. Experimental

2.1 Materials

An experimental patent pending organosilver-based MMT layered silicate with general market denomination Bactiblock®, was supplied by Nanobiomatters S.L. (Paterna, Spain) as a white powder with particle size D50 of 4.5 microns. This nanoclay is reported by the manufacturer to be compliant with the current food contact legislation in its complete formulation and to provide active antimicrobial performance. Elemental analysis of the received sample was performed by energy dispersive X-ray fluorescence, EDXRF (PW2400, Panalytical) and was reported to yield 3.4% of silver content in the sample. Further details about sample preparation or composition were not disclosed by the manufacturer.

Tryptone soy broth, tryptone soy agar and Mueller Hinton broth, for antimicrobial analysis, were purchased from Conda Laboratories (Madrid, Spain).

A chloroform synthetic grade and nitric acid 68% were purchased from Scharlab (Barcelona, Spain). A semicrystalline polylactide (PLA) film extrusion grade produced by NatureWorks^{TM} (US) (with a D-isomer content of approximately 2%) was used. The molecular weight had a number-average molecular weight (M_n) of ca. 130,000 g/mol, and the weight-average molecular weight (M_w) was ca. 150,000 g/mol.

2.2 Antimicrobial test

A pathogenic microorganism of food origin, *Salmonella spp*. (CECT 554, from Spanish Type Culture Collection, Valencia, Spain) was used to determine the antimicrobial activity of silver-based clay. The strain was stored in Tryptone Soy Broth (TSB) with 20% glycerol at -80ºC until needed. For experimental use, the stock cultures were maintained by regular subculture on agar Tryptone Soy Agar (TSA) slants at 4ºC and transferred monthly. A loopful of bacteria was removed to 10 mL of TSB and incubated at 37ºC overnight. A 100 µl aliquote from the overnight culture was again transferred to TSB and grown at 37ºC to the mid-exponential phase of growth. This culture served as the inoculum in the susceptibility study, starting with approximately 10^5 CFU/mL in the tests tubes. These CFU counts were accurately and reproducibly obtained by inoculation of 0.1 mL of the culture having an absorbance value of 0.2, as determined by optical density at 600 nm by ultraviolet visible (UV) spectroscopy.

To perform the antimicrobial tests, 0.25 to 10.00 mg of silver-based clay was added to the 10 mL Mueller Hinton Broth (MHB). After 5 h, the clay sample was inoculated with 0.1 mL of *Salmonella spp*. culture. Control experiments without clay and natural unmodified clay were performed under same conditions. Samples and control were incubated to 37 ºC for 24 hours. Afterwards, 0.1 mL of each one was sowed in agar-triptone-soybean (TSA) plates and incubated for 24 h at 37ºC. Finally, viable cells were counted.

2.3 Preparation of films

Nanobiocomposites of PLA with 1, 5 and 10 wt.-% of nanoclay were prepared by a solution casting method. Initially, a solution of PLA in chloroform was prepared by stirring on a hot plate at 40ºC until complete dissolution of the pellets occurred. A dispersion of clay in chloroform was promoted by applying 1 min of homogenization (Ultra-turrax T25), then this was added to a 5wt.-% PLA solution in chloroform. The resulting mixture was stirred for 10 min at room temperature and cast onto Petri dishes to obtain ca. 100 microns thick films after solvent evaporation at room temperature. Pure PLA films were also prepared by the same procedure.

2.4 Morphology **evaluation**

The structure and dispersion of the silver-based montmorillonite (MMT) clay in the PLA matrix were evaluated by wide angle X-ray scattering (WAXS) as well as by transmission electron microscopy (TEM). WAXS Diffractograms were acquired using a Bruker Advance D8 diffractometer, equipped with Cu Kα filament (0.1546 nm, 45 KV, 45 mA), time step of 8s at a scanning rate of 0.5 º/s. Transmission electron micrographs (TEM) were made in a JEOL1010 transmission electron microscope equipped with a digital Bioscan image acquisition system on microtomed layers of the samples. The accelerating voltage was 90 KV.

2.5 Silver migration

Samples in duplicate of 0.75 g of PLA containing 1, 5 and 10 wt.-% of nanoclay content films were cut and placed in test tubes of 30 ml. An aliquote of 20 ml of HNO_3 $2.0\text{x}10^{-3}$ M was added to each tube and mixed in an orbital shaker (JP Selecta Rotatherm 3000435) at 150 r.p.m. and room temperature for time intervals ranging from 1 to 8 days. Once the test time was accomplished the films were removed and the solutions analysed by voltamperometry. The samples showed after six days a strong silver ion signal and the testing was stopped after 8 days.

Voltamperometric analysis of the samples were performed in a μAutolab instrument (Eco Chemie B.V., Netherlands), equipped with a working electrode, a reference electrode and an auxiliary electrode. The applied method was adapted from Radheshkumar and Münstedt (Radheshkumar and Münstedt , 2006). An aliquote of 1 ml of each sample solution was added into 24 ml of KNO_3 0.1N contained in the electrolytic cell. Voltamperograms were recorded between 0 and 0.5V (10 scans, scan rate 0.1) in normal differential pulse method. Previously, Ag^+ standard solutions 0.5-50 mg kg^{-1} in acidic media were measured to establish a calibration curve.

2.6 DSC analysis

The thermal properties of PLA-silver-based clay nanobiocomposites were analyzed by differential scanning calorimetry (DSC). The tests were performed on samples of approximately 5 mg in a Perkin Elmer DSC7. The thermal program consisted of a first heating scan from 30ºC up to 190ºC, at a scanning speed of 10ºC/min under N_2 atmosphere. The samples were then cooled back to 30ºC in order to eliminate the thermal history and reheated to 190ºC at a heating rate of 10ºC/min. The thermograms were subtracted similar runs of an empty pan.

The crystallinity (X_c%) of the PLA composites was estimated using the equation 1:

$$\%X_c = \frac{\Delta H_f}{\Delta H_f^0} x100 \qquad \text{(eq. 1)}$$

where ΔH_f is the enthalpy of fusion of the studied specimen, and ΔH_f° of 91 J/g is the estimated enthalpy of fusion of a 100% crystalline PLA-L at the equilibrium melting temperature (Pyda *et al.*, 2004).

2.7 Water vapor permeability (WVP)

The water vapour permeability of the films was determined according to the ASTM method E96, using aluminium cells with internal and external diameters of 3.5 and 6 cm, respectively. Films samples were previously dried in a vacuum oven, at 50ºC during 4 h in order to remove solvent residues. Each sample film was sealed to a permeation cell containing liquid water, and then the permeation cells were placed under controlled environmental conditions (40ºC and 0% relative humidity) and weighed regularly until steady-state was reached. The water vapour transmission rate was easily determined from the slope of the cell weight loss vs. time plot following equation 2.

$$WVP = (G * L) / (A * \Delta p) \qquad \text{(eq. 2)}$$

where G is the slope of weigh loss vs. time straight line, L is the film thickness, and Δp is the vapour partial pressure differential across the film. All tests were conducted in duplicate, and aluminium films were used as blanks to evaluate water vapour loss through the sealing.

2.8 Antimicrobial tests on films

Salmonella spp. CECT 554 was used to evaluate the antimicrobial activity of PLA nanobiocomposites. Bacterial strain and growth conditions were indicated above.

The antimicrobial activity of PLA nanobiocomposites was determined according to the *Standard test method for determining the antimicrobial activity of immobilized antimicrobial agents under dynamic contact conditions* (ASTM E2149), with some modifications. Films samples were previously dried in a vacuum oven, at

70ºC during 12-24 h in order to remove chloroform residues. Specimens of 0.75 and 1.5 g were introduced in test tubes containing 20 mL of phosphate buffer solution pH 7.4. Subsequently, tubes were inoculated with 10^5 cells/mL of each microorganism in mid-exponential phase and incubated in a wrist action shaker (150 rpm) at 25ºC for 24h. Then, a 0.1 mL aliquote of buffer solution from each tube was sub-cultivated on TSA plates. Finally, plates were read after overnight incubation at 37ºC. Three replicate experiments per condition were performed. The results were expressed as CFU/mL and the percent reduction was calculated based on the decrease of final counts from appropriate untreated controls.

3. Results and discussion

3.1 Characterization of the silver-based nanoclay

A typical TEM image of the silver-based nanoclay used is shown in Figure 1. Silver nanoparticles can be very easily spotted as spherical dark nanodots of various diameters rising up to 20 nm, which seem unevenly distributed in size and dispersion across the platelets surface of the clay. It is very likely that the silver either in the form of nanoparticles are randomly distributed across the whole morphology of the clay, but the observable particles appear to have grown bigger in some of the platelets.

Figure 2 shows the antimicrobial evaluation tests carried out against *Salmonella spp*. on increasing clay weights. In the presence of 1 and 10 mg of the active nanoclay, and after 24 h incubation, the number of bacterial colonies decreased significantly, by up to eight orders of magnitude with regard to the control, suggesting a strong antibacterial performance for the nanoadditive. As the quantity of clay decreased, i.e. for the 0.5 and 0.25 mg samples, the silver-based system was found to be less efficient. Thus, by careful observation of Figure 2, it is suggested that the minimum inhibitory and biocide concentrations of the material against *Salmonella spp* lay in fact between 0.5 and 1 mg of the silver-based clay under the current testing conditions.

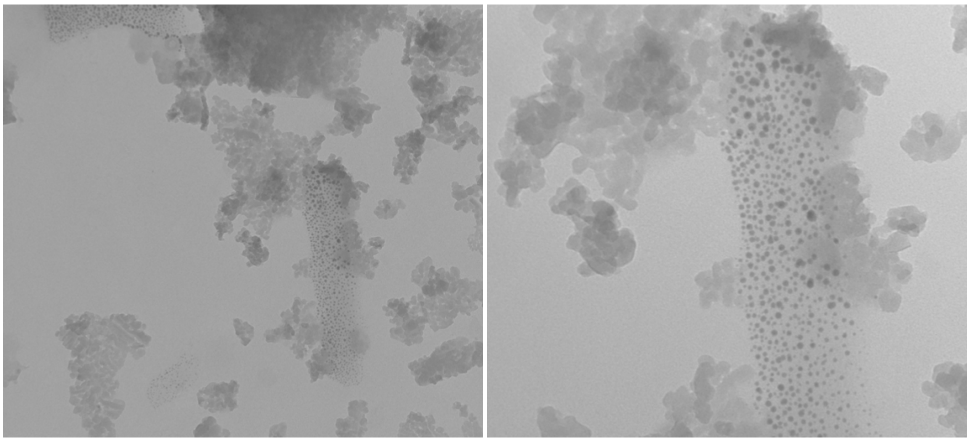


Figure 1. Typical TEM images of the silver-based nanoclay. The scale marker is 0.2 microns for the left picture and 0.1 microns for the right picture, which is a magnification of the former.

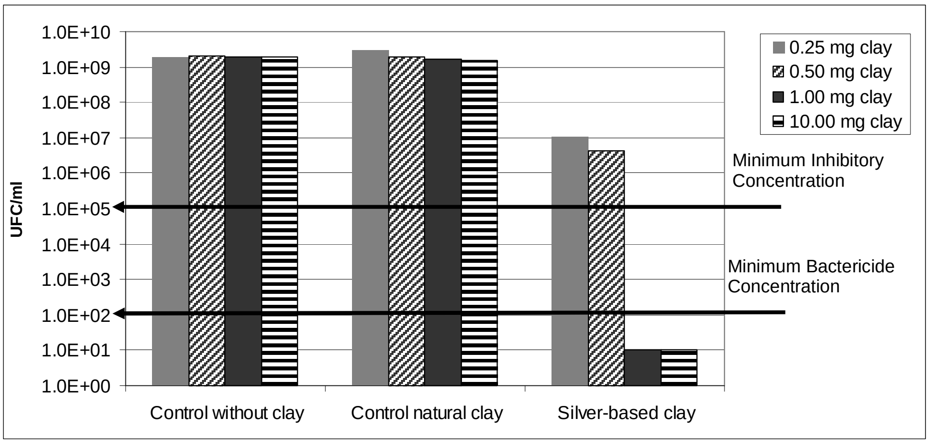


Figure 2. Antimicrobial activity of the natural clay and of the silver-based nanoclay vs. content against *Salmonella spp*. determined after incubation (37°C, 24 h).

3.2 Characterization of the PLA nanobiocomposite films

After obtaining nanobiocomposites of PLA with various active filler contents, the obtained cast films retained the transparency and apparent optical properties of the PLA to a large extent. The active clay seemed well dispersed across the matrix as judged by naked eye observation (see Figure 3). From this Figure, films of 5 and 10 wt.-% of filler showed a whitish aspect (wich may be related to partial crystallization of matrix), and only the sample with 10 wt.-% filler content shows comparatively lower transparency.

In order to check the additive dispersion to a submicron scale, TEM images were taken in the microtomed specimens of the various samples (see Figure 4). This Figure suggests that a homogeneous dispersion but with no complete exfoliation of the clay particles in the matrix was attained by the casting method. Conventional casting processes involving homogenization and stirring in organic solvents are not usually yielding high levels of exfoliation in PLA. Nevertheless, in all cases most platelets exhibit sizes in the thickness dimension which are below 100 nm. Albeit, some authors consider 300 nm as the upper lever for a nanotech, 100 nm is more widely recognized as the upper limit for a nanomaterial. In spite of this, some bigger tactoids are observed with increasing filler loading, particularly for the 10 wt.-% filler sample.

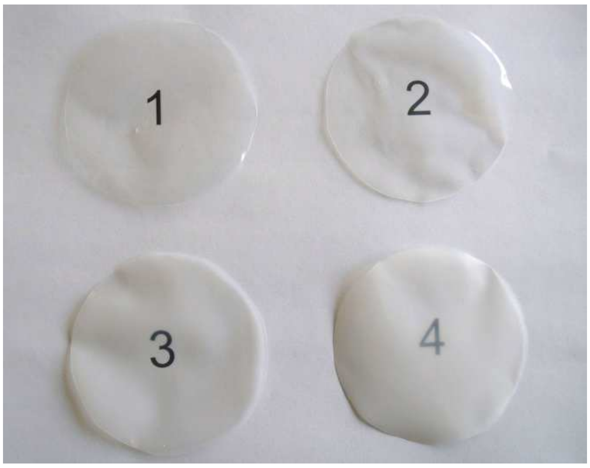
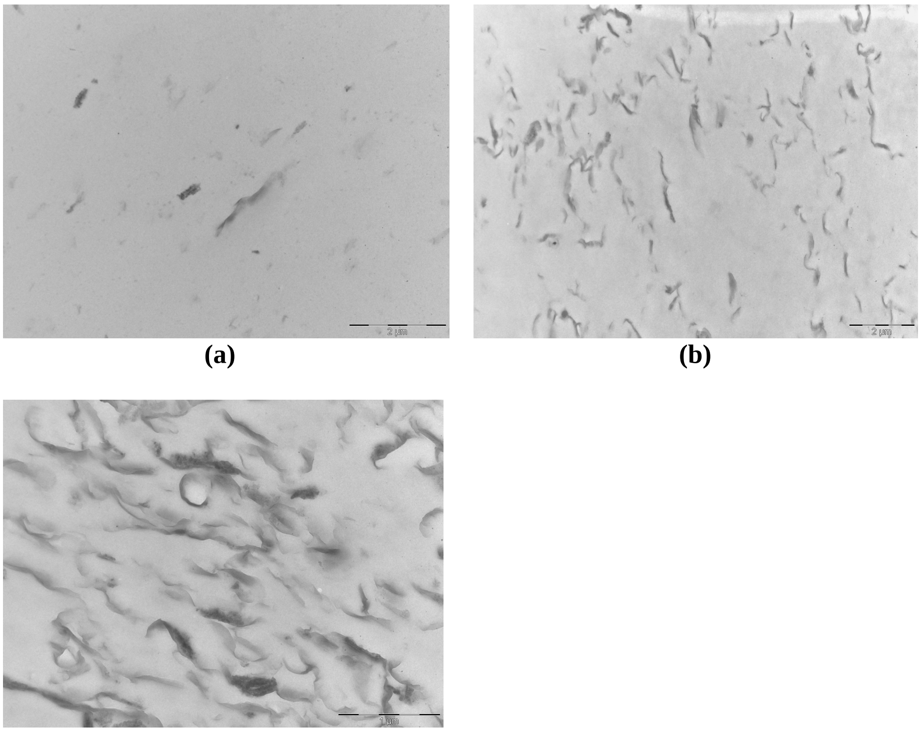


Figure 3. Optical microscopy pictures of cast specimens of PLA nanobiocomposites taken in (1) Neat PLA, (2) PLA containing 1 wt.-% nanofiller, (3) PLA containing 5 wt.-% nanofiller and (4) PLA containing 10 wt.-% nanofiller.

In many studies WAXS is often used, together with TEM, to support the discussion regarding dispersion of the nanoclays in terms of intercalation and/or exfoliation. Figure 5 shows the WAXS diffractograms of the nanoclay, the neat PLA and the various biocomposites. The silver-based MMT clay has a very intense clay basal peak at 2.4 (2θ), a less intense second one associated to the second order at 4.8 (2θ), and an even smaller third peak at 7.0 (2θ) which is associated to the third order. This is in good agreement with a previous characterization work (Sanchez-Garcia *et al.,* 2008*)*. It is clear that a long intercalating molecule has been used in the formulation of this clay, since the unmodified MMT shows the basal peak at much higher angles, i.e. typically from 6.9 to 8.4 (2θ). Curiously, the second order peak at 4.8 shows a double peak maximum, which in agreement with previous work suggests that there may be two different populations of very similarly intercalated clay platelets in the material, due to most likely the uneven distribution of the silver particles formation and size in the clay (Dékány *et al*., 2008).



(a) (b)

(c)

Figure 4. TEM images of PLA nanobiocomposites containing: (a) 1 wt.-% nanofiller, (b) 5 wt.-% nanofiller, and (c) 10 wt.-% nanofiller. Scale markers are 2 microns for (a) and (b) and 1 micron for (c)

The unfilled PLA seems completely amorphous as only the broad amorphous halo is observed in the diffractogram. On the other hand, the biocomposites show broad small clay peaks at 2.4 and 4.6 (2θ) due to the presence of the modified MMT clay, which support that there is a significant fraction of the clay not intercalated nor exfoliated in the solvent cast biopolymer, in agreement with the TEM observations of Figure 3. It is curious to observe how the clay peaks seem comparatively much more intense for the 10 wt.-% filled composite, suggesting that a bigger proportion of the composite is less dispersed than for the 1 or 5 wt.-% filled biocomposites. As a result, most of the clay additive has in fact undergone only tactoic size reduction and no extensive intercalation or exfoliation. From previous work, melt compounding, rather than solving casting, is a more favorable processing route to disperse this type of nanoclays in biopolyesters since a higher degree is shear can be established during melt processing (Sanchez-Garcia *et al.*, 2008). Interestingly, a strong reflection does also appear at 16.7 (2θ) and smaller ones at 14.5 (2θ) and 19.20 (2θ) in the clay containing samples, which seem weaker in the 10 wt.-% filled biocomposite. These features are attributed to some filler-induced crystallization in the biocomposites (Yasuniwa *et al.*, 200*8).*

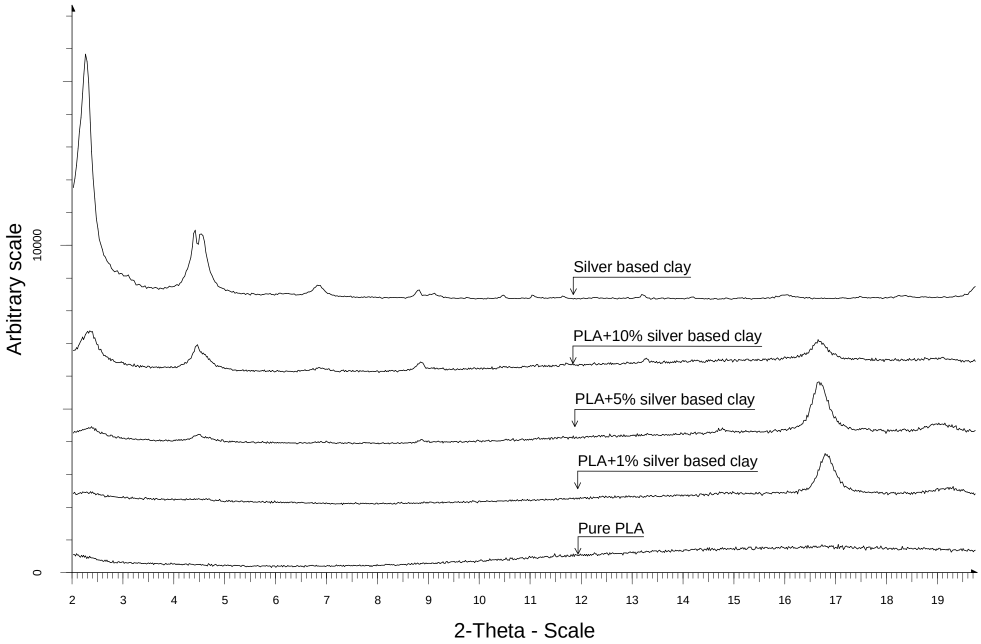


Figure 5. WAXS diffractograms overposition, from the bottom to the top, of the neat PLA, PLA containing 1 wt.-% nanoclay, PLA containing 5 wt.-% nanoclay, PLA containing 10 wt.-% nanoclay and silver-based nanoclay.

3.3 Thermal analysis

The thermal properties of the neat PLA and of the nanobiocomposites were determined by DSC during the second heating run. It should be noticed, that the DSC experiments were intended to determine (after elimination of the thermal history) the effect of the filler in the thermal properties of the composites and, therefore, correlation cannot be directly established with the obtained barrier properties or the WAXS results, which were measured in the as formed samples. Table 1 shows the melting peak (T_m), the cold crystallization peak (T_c), the glass transition temperature (T_g) and the cristallinity (%) of the samples of the second heat endotherm. From this table, the T_g and the T_m were seen to remain rather unchanged with increasing filler content. Crystallinity, was only seen to increase slightly in the biocomposites with increasing filler content in excess of 1 wt.-% filler loading. However, the cold crystallization temperature increased with filler loading suggesting that the presence of the filler hinders to some extent the cold crystallization process during heating.

Table 1. Thermal parameters obtained from the heating thermograms for PLA-Bactiblock®101T1.51 composites

Sample	Tg (ºC)	Tc (ºC)	Tm (ºC)	ΔHf (J/g)	% Xc
PLA	59.115 ± 0.219	124.620 ± 1.379	151.370 ± 0.001	4.743	5.21
PLA+1% Bactiblock®101T1.51	59.090 ± 0.099	126.785 ± 1.498	151.70 ± 0.467	3.676	4.04
PLA+5% Bactiblock®101T1.51	59.915 ± 0.304	131.450 ± 0.234	152.790 ± 1.075	6.699	7.36
PLA+10% Bactiblock®101T1.51	60.240± 1.061	130.700 ± 0.144	152.700 ± 0.467	7.147	7.85

3.4 Water vapour permeability

Table 2 shows the lowest water permeability values measured for the films as a function of filler content. In permeability of non-industrial samples, the lowest permeability value is often the one selected to represent each sample. Nevertheless, the permeability variations between specimens of different samples were for these particular specimens below 3%. From Table 2, it can be seen that all composites reduced water vapour permeability. The permeability reduction was, however, seen slightly smaller with increasing filler content, most likely due to filler agglomeration and the higher likelihood for the creation of preferential paths for diffusion. The water barrier improvement is here attributed to the presence of the nanoclays but also to some crystallinity as observed by WAXS in the biocomposites.

Table 2. Water vapour permeability of PLA and its biocomposites

Sample	P_{H2O} [(m*g)/(m^2*s*Pa)]	Reduction (%)
Unfilled PLA	1.62E-11 ± 6.01 E-13	--
PLA + 1 wt.-% silver-based clay	9.72E-12± 5.66 E-13	40.01
PLA + 5 wt.-% silver-based clay	1.26E-11± 5.16 E-13	22.30
PLA + 10 wt.-% silver-based clay	1.30E-11± 1.96 E-13	19.87

3.5 Silver migration from PLA nanocomposites

According to the norm UNE-ENV 13130-2 (AENOR, 2005), which is a procedure for the evaluation of the specific migration of plastic substances that can come into contact with foods, water is considered as a food simulant for a wide range of food products such as bread, fresh fruits and vegetables, meat and fish, among others. The use of slightly acidified water solution (containing in the norm 3% acetic acid) is also used as an even more aggressive food simulant. In our case, acidified water solutions about 0.03% of HNO_3 is the necessary media for avoiding the oxidation of

Ag^+ cations once they migrate from composite to water, thus a proper voltamperometric evaluation of silver ions in the liquid phase can be carried out.
The European Food Safety Authority (EFSA) has recently evaluated the use of several silver-based substances intended to come into contact with foods, and defines a general specific migration limit of 0.05 mg of silver per kg of food (EFSA Journal, 2004; EFSA Journal, 2005; EFSA Journal, 2006; EFSA Journal, 2007). In this study, this particular value and units were adopted as reference for assessing migration from the films.

The peak corresponding to silver oxidation is located around 0.35V in the voltamperogram. The corresponding calibration curve used to correlate the latter peak height with the actual silver content in the solution is presented in Figure 6. Using this calibration, the peak height (see Figure 7 as an example) was used to determine silver migration from the films into slightly acidified water medium. Using the described methodology, Figure 8 shows the migration curves of silver ions with increasing exposure time for the various biocomposites. From the Figure 8, an undetectable silver migration, which is below 0.05 mg per kg of food simulant, is observed after one day of exposure for the 1 and 5 wt.-% filler loaded composites. With increasing exposure time beyond one day, migration becomes detectable and rather constant, i.e. below 0.5 mg/kg. However, at longer times, i.e. after six days, the migration profile seems to accelerate in a rather exponential fashion. Curiously, the 1 and 10 wt.-% loading samples exhibit lower migration in the accelerated regime. The overall results suggest that plasticization of the PLA matrix by the slightly acidified aqueous simulant may trigger the migration of the noble active metal from inside the sample. By the other hand, a partial hydrolysis of PLA would have occurred and facilitate the migration process. The protons supplied by nitric acid would have contributed to hydrolysis catalysis, however it has been considered that specific acid strength is not enough to catalyze a severe hydrolytic degradation into few days.

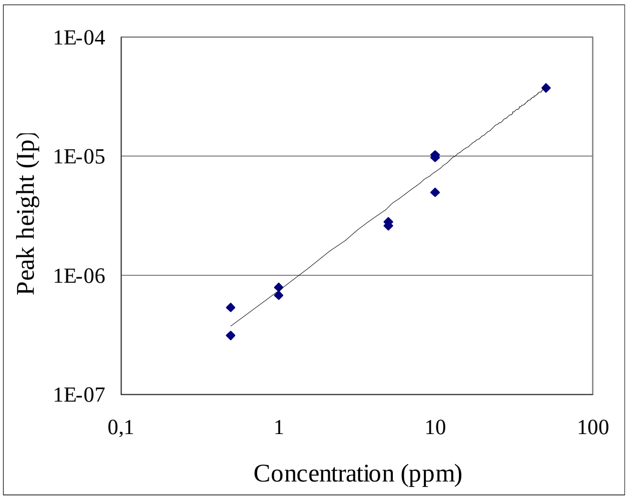


Figure 6. Calibration curve for silver content using voltamperometric analysis

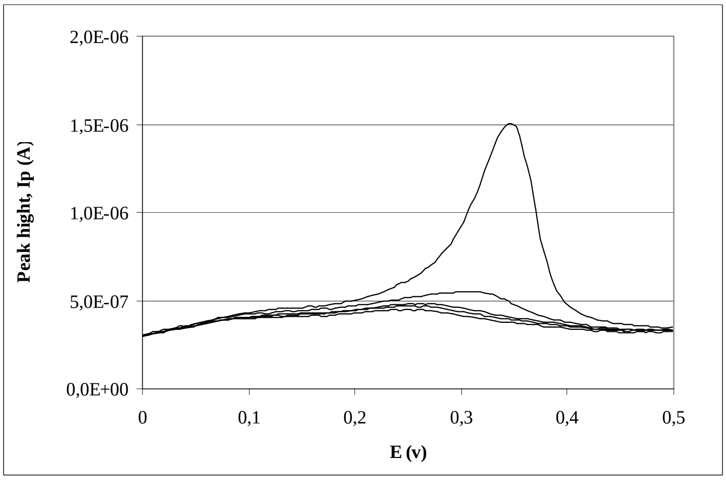


Figure 7. Voltammograms showing the silver release from PLA-1 wt% silver-based nanoclay

In general, PLA degradation can take several months (Agarwal *et al.,* 1998). It has been reported that the relative hydrophilicity of the clay layers play a key role in the hydrolytic degradation of the polymer chains of PLA nanocomposites with organomodified montmorillonites (Dubois *et al.,* 2004). In the case of this study, the clay filler would have had an effect on the partial degradation of PLA. However, further tests have to be performed in order to study this effect.

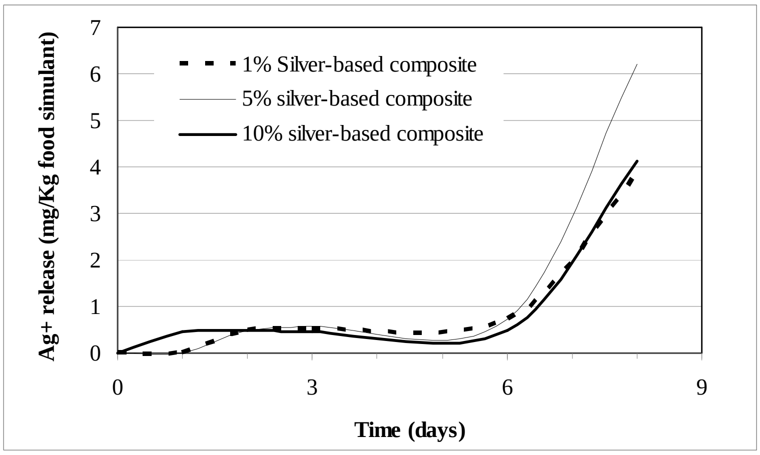


Figure 8. Silver release (ppm) vs. time from silver-based nanoclay composites

As a summary, it should be noted that the maximum migration levels detected for the solvent cast biocomposite films could become 10 fold higher beyond one day of exposure, but over 100 fold higher in the accelerated regime than allowed by the EFSA restriction limit. This indicates that for this particular material, when applied as a solvent coating, lower quantities of filler might be recommended. Nevertheless, it is clear that PLA is a hydrolysable and not water resistant material which may not be the polymer of choice for direct food contact packaging in high humidity applications.

3.6 Antimicrobial activity of the nanobiocomposites

Table 3 gathers the antimicrobial tests carried out in 1.5 and 0.75 g of the nanobiocomposites. For the 1.5 g samples, all biocomposites exhibit a very strong biocide capacity (99.99% CFU reduction) against *Salmonella spp*. suggesting the expected strong antimicrobial power of the biocomposites. Lower sample quantities were also tested, i.e. 0.75 g, and all the samples proved again to show a strong biocide performance, except the 1 wt.-% filled specimen, which showed bacteriostatic performance. It is therefore interesting to observe that after 24 hours of incubation, which from observation of Figure 8 leads to a non detectable silver migration for 1 and 5 wt.-% filled samples, the samples still present antimicrobial properties strongly suggesting that it is feasible to use silver as an active biocide agent in food packaging applications while complying with the existing legislation.

Table 3. Antimicrobial activity of 0.75g and 1.5g of nanobiocomposites PLA+1 wt.-% 101T1.51 samples against *Salmonella spp.*

	Salmonella spp.	
	Sample weight: 1.5 g	**Sample weight: 0.75 g**
Sample	**FinalCFU/ml**	**FinalCFU/ml**
Control	1.05E07	2.44E07
Unfilled PLA	1.72E07	4.64E07
PLA+1%101 T1.51	0	5.24E04
PLA+5%101 T1.51	0	Not evaluated
PLA+10%101 T1.51	0	Not evaluated

4. Conclusions

A complete evaluation of a novel food contact complying experimental silver-based MMT grade, traded as Bactiblock®, was carried out in PLA. The silver-based clay showed a strong antimicrobial effectiveness against Gram-negative *Salmonella spp*. with minimum inhibitory concentration and minimum bactericide concentration below 1mg $10ml^{-1}$. Nanobiocomposites with different filler loadings were obtained by casting from chloroform solutions and despite the good optical properties exhibited by the films and the apparent dispersion of the active nanoadditive, no clear intercalation or exfoliation of the clay was achieved as suggested by WAXS. In any case, a nanodispersion of the clay was still attained by fractionation and high dispersion of the original tactoids across the PLA matrix as suggested by TEM. An improved barrier to water was measured for the biocomposites, which also exhibited strong antimicrobial performance. Regarding silver migration to an slightly acidic aqueous medium, it was observed that after six days of contact, migration of silver was accelerated suggesting that plasticization and/or possible partial hydrolysis of the PLA films took place. The potential of this novel active nanoadditive for food contact applications is demonstrated provided that dosage, matrix and foods are adequately selected and screened to ascertain compliancy with the stringent specific migration restrictions for silver migration defined by the EFSA.

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4. ANTIMICROBIAL BIOCOMPOSITES OF MELT-COMPOUNDED POLYLACTIDE FILMS CONTAINING SILVER-BASED ENGINEERED CLAYS

Abstract

This paper reports on two polylactide (PLA) composites obtained by melt compounding containing two silver-based biocide montmorillonites. The active clays are differentiated by the biocide agent oxidation state, i.e. in one sample silver is in ionic form and in the other as native nanoparticles. In both cases, composites with enhanced thermal stability and good dispersion and distribution of the antimicrobial compounds were obtained. The active engineered organoclays and the corresponding biocomposites showed strong antimicrobial activity against *S. aureus*. The biocidal effect was larger for the ionic silver sample, as expected. The results support these composite material's potential as antimicrobial additives in PLA active packaging applications obtained via melt compounding.

Keywords: Silver, polylactides, antimicrobials, composites.

Introduction

Consumers are becoming increasingly concerned about the potential toxicity of microorganisms to which they are daily exposed. The apprehensiveness to contamination by consuming infected food goods, or during routine medical procedures or surgery, or just by contact to exposed surfaces and accessories in public places is increasing as food poisoning alerts and infectious diseases emerge. In addition to this, and more specifically concerning food products, consumers demand minimally processed and low additive food goods with high fresh-like quality, while at the same time modern distribution chains require extended shelf-life packaged products. Antimicrobial agents which include organic acids, bacteriocins, spice extracts, chelating agents and metals, and others, have been tested in the last years for developing biocide materials to respond to these requests.[1-7] Since ancient times silver is well known for its antimicrobial effects, although it was not until the end of the 19th century that the first scientific papers describing the medical uses of silver were published. Among metallic ions like silver, copper, iron and zinc, the silver ion has the strongest antimicrobial activity.[1] The bactericidal mechanism of silver is not yet well understood but silver ions are thought to be first adsorbed on the microbial cell surface and then incorporated within them by active transport, which inhibits metabolic enzymes and their functions.[1] Cationic silver can also bind with the negatively charged components in proteins and nucleic acid, thereby causing structural changes in bacterial cell walls, membranes and nucleic acid that affect viability.[8] On the other hand, silver nanoparticles (< 100 nm) exhibit similar effects on bacteria as silver ions.[9] The antimicrobial activity of silver nanoparticles may be related to several mechanisms including, oxidative stress induction due to reactive oxygen species generation (ROS) which may cause the cell membrane structure degradation and ion release from the nanoparticle surface.[10] It has been demonstrated that metallic silver can release silver ions in aqueous environments and inhibit microbial activity.[8] Silver's antimicrobial activity is attributed to the silver cation Ag^+. Silver as a metal is inert and can be oxidized to the metallic silver to the active species Ag^+ through a water-induced oxidation process.[11-13] Hence silver-based antimicrobial polymers, i.e., have to release

Ag^+ ions to a pathogenic environment in order to be effective.[11, 14] In addition to its strong antimicrobial activity, silver is safe to human beings at relatively high dosages and relatively inert as native metal. The amount of silver that can be safely consumed by humans (i.e., the reference dose, RFD) is 5.0 µmg/kg/day. Two liters of drinking water/day would already provide around 200 mg of silver, and the metal is found in variable quantities in other foods as well. Following ingestion, silver is poorly absorbed from the intestine. Studies in mice, rats, monkeys, and dogs indicate that only 10 percent of the ingested dose is actually absorbed; and the absorption rate in man of 18 percent after a single administration. Regardless of the administration route, silver excretion from the body is mainly gastrointestinal and urinary. The metal half-life varies with the administration route. Approximately 24 hours after oral administration, up to 1.7 days after inhalation.[15] The acute silver toxicity is dependent on its bioavailability. It was found that marine living things (i.e. oysters, gastrops and arthropods) can incorporate silver, but the quantity in which this occurs depends on the biological availability of silver and age, size, sex, general health and metabolism of the organism Also, water temperature, salinity, dissolved oxygen, turbidity, and presence of other compounds may influence bioaccumulation.[16, 17] In general, silver accumulation by terrestrial plants from soils is low, even if the soil is amended with silver-containing sewage sludge or the plants are grown on tailings from silver mines, where silver accumulates mainly in the root systems.[17] Additionally, this antimicrobial effect does not cause rejection by consumers, in principle. All of these features have made silver one of the most studied and applied antimicrobial agents in recent years.

Cationic and metallic silver are currently used as antimicrobial agents in many matrixes, by direct addition or well supported in vehicles in order to produce active materials in different areas, such as polymers (oil based and biopolymers), coatings and paints, fabrics, paper and ceramics. Some silver-containing fillers such as zirconium phosphate, zeolites and bentonitic clays, have been used in successful silver carriers for preparing antimicrobial materials. The synthesis and intercalation of metallic silver nanoparticles in kaolinite and montmorillonite, and silver modification of montmorillonites, have facilitated studying both metallic silver and ionic silver in modified clays.[18-21] Silver based engineered clays have the advantage of having been designed to be incorporated in polymeric matrixes.[7, 22] The uniform silver distribution across the matrixes, and thus, antimicrobial capacity, is achieved by dispersion and distribution of the silver containing clay tactoids throughout the polymer matrix. In addition to its activity against pathogenic microorganisms, engineered clays show other synergetic advantages regarding another commercial systems based on silver, i.e. their inherent ability to enhance polymer physical characteristics such as gas, water and aroma barrier and mechanical properties, among others.[7, 23-25.]

Currently an important antimicrobial material use is in the active packaging of foods, where there is an increasing demand for environmentally friendly, low cost and oil independent packaging materials. As it is well known, oil-based resins are being slowly replaced by renewable bioplastics, facilitated by continuous oil price fluctuations due to the various geopolitical factors of the producing countries. Polylactides are an important biodegradable polymers derived from renewable resources. It has relatively low cost and interesting physical properties, which can be tailored by adding fillers such as nanoclays and others.[26]

A previous paper detailed the characterization and performance of PLA containing silver based clays was designed to be dispersed in solvent cast biopolymers for coating applications, not in thermally processed polymers for packaging.[7, 22] The use in thermally processed materials yielded coloured composites. The current paper deals with the morphology; thermal properties and antibacterial performance of both cationic and metallic melt compoundable silver containing organoclays added to PLA. Melt mixing is the most commonly used way to process packaging polymers and hence the applied interest of this work. Thus, antimicrobial PLA composites based on silver containing clays are expected to meet the current needs of the food packaging market regarding product quality and safety extension with potentially lower environment impact due to its renewable character.

Experimental

Materials

Nanobiomatters S.L. (Paterna, Spain) supplied their experimental proprietary antimicrobial silver-based organomodified layered silicate additives, trademarked Bactiblock®.

Bactiblock® R1.51 is a cation exchanged ionic silver organomodified montmorillonite. Bactiblock® T1.51 is an organomodified montmorillonite which contains silver nanoparticles in the platelets. Both clays are organomodified to help dispersion/distribution into PLA. These additives are white powders with a 3 micron D50 particle size. We will refer to them as R1.51 and T1.51, respectively. They also provided corresponding unmodified clay (natural montmorillonite) for comparative purposes. No additional information regarding the additive components was supplied.

Tryptone soy broth (TSB), tryptone soy agar (TSA) and Mueller Hinton broth (MHB), for antimicrobial analysis, were purchased from Conda Laboratories (Madrid, Spain). The TSB was said to contain in grams per liter of distilled water at final pH equal to 7.3 +/- 0.2 at 25 °C:

- Pancreatic digest of casein: 17.0
- sodium chloride: 5.0
- papaic digest of soy bean: 3.0
- dipotassium phosphate: 2.5
- glucose monohydrate: 2.5

A semicrystalline polylactide, film extrusion grade produced by NatureWorks^{TM} (USA) with D-isomer content ca. 2%, was used. The number-average molecular weight (M_n) was circa 130,000 g mol^{-1}, and the weight-average molecular weight (M_w) was circa 150,000 g mol^{-1}.

Preparation of films

PLA composites with 2.5 weight percent filler content, as well as the blank without filler, were prepared by internal melt mixing using a Brabender Plastograph® EC (Duisburg, Germany). Before mixing, both PLA and the clays were dried overnight in a vacuum oven at 60 °C. Mixing conditions were 180 °C, 60 rpm for 3 min. Composite films were prepared by compression moulding using a Carver 4122 hydraulic press

(Indiana, USA) at 180 °C and 4000 psi. Films were quenched, and film thickness was about 100 microns.

Chemical analysis

Chemical analysis of unmodified montmorillonite and the corresponding silver based grades were performed to determine silver content by using energy dispersive X-ray fluorescence, using an EDXRF Bruker Tiger S8 (Bruker Española, Madrid, Spain). Pressed clay powder pellets were prepared using boric acid as a support. Each sample was vacuum dried overnight at 105 °C, weighed together with the support and ground in planetary mill (Retsch RS200, Haan, Germany) at 700 rpm for 20 s. Then the specimens were pressed with 80 Ton pressure for 20 s.

Thermogravimetric analysis (TGA)

The PLA composite thermal stability was assessed using a TGA Q500 (TA Instruments Spain, Barcelona, Spain) with analysis software Universal 2000 V4.4A. Composite samples were analyzed from 30-600 °C, at a rate of 10 °C /min, under a nitrogen flow.

Morphology evaluation

The structure and dispersion of R1.51 and T1.51 clay in the PLA matrix were evaluated by wide angle X-ray scattering (WAXS) as well as by transmission electron microscopy (TEM). WAXS diffractograms were acquired using a Bruker Advance D8 diffractometer (Bruker Española, Madrid, Spain), equipped with Cu Kα filament (0.1546 nm, 45 KV, 45 mA), time step of 8s at a scanning rate of 0.5 °/s. Transmission electron micrographs (TEM) were made in a JEOL1010 transmission electron microscope (Jeol Europe, Croissy-sur-Seine, France) equipped with a digital Bioscan image acquisition system on microtomed layers. The accelerating voltage was 90 KV.

Antimicrobial tests

A pathogenic microorganism *S. aureus* (CECT 86T, ATCC 12600) was used to determine the antimicrobial activity of the silver-based clays. The strain was stored in Tryptone Soy Broth (TSB) with 20% glycerol at -80 °C until needed. For experimental use, the stock cultures were maintained by regular subculture on agar Tryptone Soy Agar (TSA) slants at 4 °C and transferred monthly. A loopful of bacteria was removed to 10 ml of TSB and incubated at 37 °C overnight. A 100 μl aliquote from the overnight culture was again transferred to TSB and grown at 37 °C to the mid-exponential growth phase. This culture served as the inoculum in the susceptibility study, starting with approximately 10^5 CFU ml^{-1} in the tests tubes. These CFU counts were accurately and reproducibly obtained by inoculation of 0.1 ml of the culture having an absorbance value of 0.2, as determined by optical density at 600 nm by ultraviolet visible (UV) spectroscopy.

To perform the antimicrobial tests of R1.51 and T1.51, 0.01 or 0.1 g of clay was added to the 10 ml Mueller Hinton Broth (MHB). After 5 hours, the clay sample was inoculated with 0.1 mL of *S. aureus* culture. Control experiments without clay and samples with unmodified clay were performed under the same conditions. Control and samples were incubated at 37°C for 24 hours. Afterwards, 0.1 ml of each was sowed in agar-triptone-soybean (TSA) plates and incubated for 24 h at 37 °C. Finally, viable cells were counted.

Susceptibility tests of PLA and active composites were performed following the standard JIS Z 2801 (ISO 22196). The tested microorganism was *S. aureus* (CECT 86T, ATCC 12600). Each piece 5x5 cm^2 was inoculated with circa 1 x 10^5 CFU and incubated at 37 °C, 100%RH, for 24 hours. Viable cells were determined by the agar plate count method. Four specimens of each sample were tested.

The antimicrobial activity value of the samples tested by the Standard JIS Z 2801 was determined through the following equation:

$$R = \log (B/C) \qquad (1)$$

Where B = average of the number of viable cells of bacteria on the untreated test piece after 24 h; C= average of the number of viable cells of bacteria on the antimicrobial test piece after 24 h. If $R \geq 2.0$, it is considered that the sample presents biocide properties.

Results and discussion

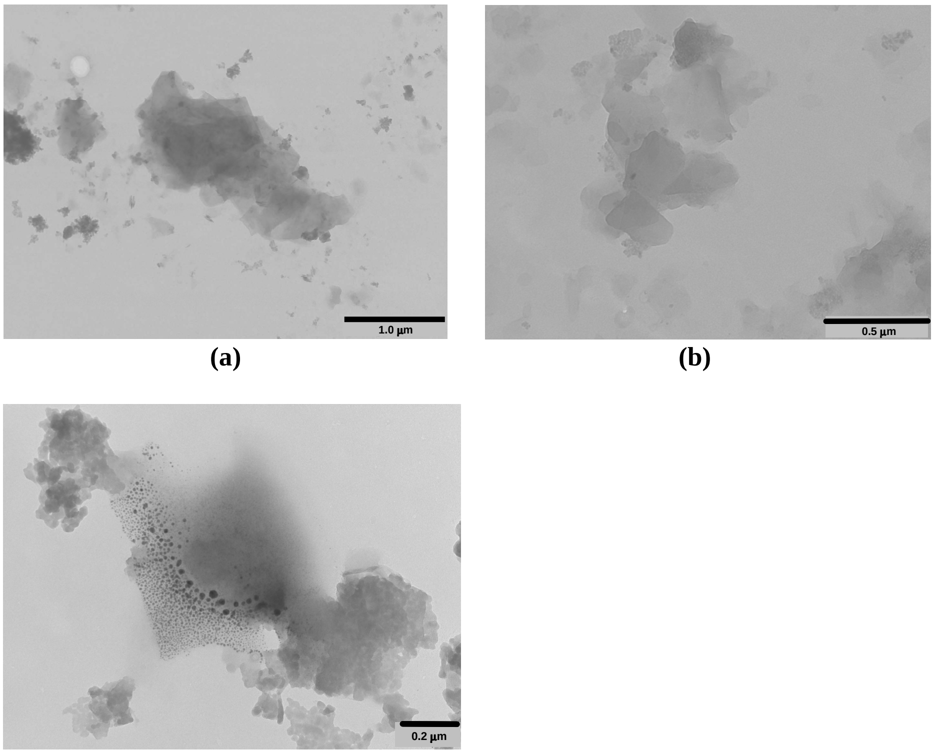
Characterization of the silver-based engineered clays

Semi-quantitative chemical analysis of the silver clays showed silver contents of 2.8 to 3.1% in the composition, with highest level of silver in the grade T1.51. According to the results in Table 1, no silver is present as expected in the unmodified clay.

Table 1. Semi-quantitative silver content of clays

Sample	% Ag
Unmodified clay	Not detected
R1.51	2.8 ± 0.3
T1.51	3.1 ± 0.3

Figure 2 shows typical TEM images for the silver-based clays and the comparative unmodified clay. The unmodified clay (Figure 1a) and R1.51 (Figure 1b) do not differ significantly, they show the same appearance of regular mineral sheets. However, micrograph of T1.51 (Figure 1c), which contains metallic silver, shows silver nanoparticles as spherical dark nanodots up to 20 nm diameter. It can be inferred that the largest metallic silver nanodots are located on the surfaces and edges of the clay mineral sheets, because particles may have less limitations for grow.[27] In addition, nanoparticles could have a preference to link to some specific sites of the clay sheets as evidenced by the uneven distribution across the clay platelets. This observation agrees with those previously reported for silver based montmorillonite and kaolinite.[7, 18]



(a) (b)

(c)

Figure 1. Typical TEM image of the (a) unmodified clay; (b) R1.51 and; (c) T1.51.

Figure 1 shows the XRD patterns for R1.51, T1.51 and the unmodified clay. The basal reflection of unmodified clay (montmorillonite) is located at 5.7° (2θ), and cristobalite as an impurity is evidenced by the reflection at 21.8 and 36.0° (2θ). The intercalation of organomodifier and silver (cation or metallic nanoparticles as well) increased the intensity of the (0 0 1) reflection, and greatly enhanced the structural organization of the clay; the basal peak shifted to 2.3° (2θ), which corresponds to the opening in the basal distance from 15.4 to 38.5 Å. Based on the diffractograms one could not distinguish the changes produced by organomodification from those occurred by silver modification. Typically, the surface modification of clays increases the basal spacing according to the chain length of the organomodifier.[25] On the other hand, montmorillonites modified with silver have been reported to maintain the distinctive diffraction peak of the unmodified clay, but shape and intensity is markedly different by the structural changes occurred during the cationic replacement.[20] Thus from the above, it can be deducted that the peak at 2θ= 2.3° corresponds to the organomodified clay characteristic diffraction peak.

Considering that the structural changes in organomodified montmorillonite take place during the modification with Ag ions, it is possible to estimate the location of the silver in the galleries or on the surface of the platelets, as already observed.[20] However, as seen in the TEM micrographs (Figure 1), silver nanoparticles can grow without limits up to 20 nm (200 Å) on the surface and edges of the platelets, so given the abovementioned space between adjacent layers in the modified clay, the larger particles are clearly not confined between the galleries. The silver based clays R1.51 and T1.51 have distinctive multiple diffraction peaks at higher angles, which are likely ascribed to the second and third order features of the basal peak, suggesting high regular platelet stacking in the modified clay. The reflections of metallic silver particles in T1.51 could not be detected between 38-42° (2θ).

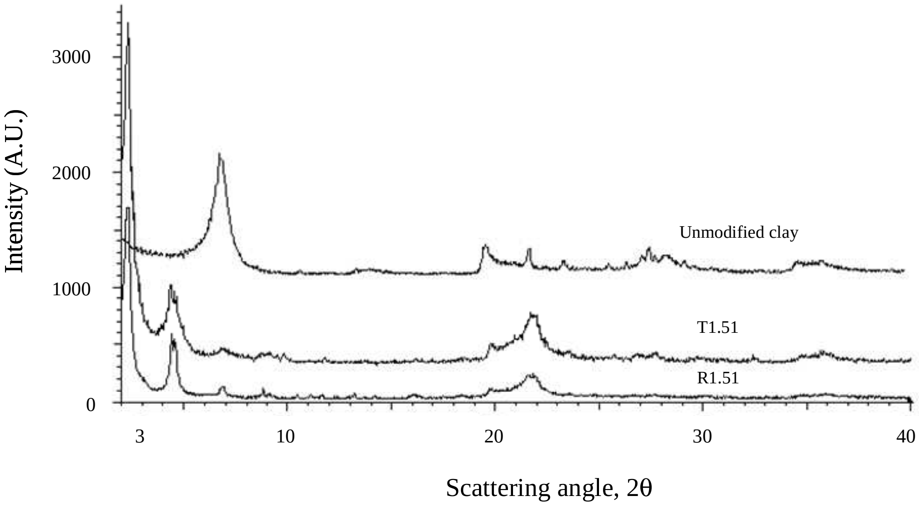


Figure 2. XRD patterns of silver based clays and comparison with corresponding unmodified clay

Antimicrobial tests of silver clays. The Gram-positive *S. aureus* was selected for testing antibacterial activity of the silver based clays because it is a common pathogen in food. *S. aureus* causes foodborne illness by growing in temperature abused food and producing a heat stable toxin. Consumption of this toxin (not the bacteria) can cause the food intoxication.[28] Unlimited bacterial growth as control was evaluated in nutrient broth without clay. As seen in Table 2, the bacterial growth was not inhibited by the unmodified clay. The silver based clays, R1.51 and T1.51 exhibited a different antibacterial performance. While silver's antibacterial mechanism is still not well understood, it is believed to be different for silver ions than for silver nanoparticles. Silver ions can react with cell proteins as well as penetrate inside the bacterial cell and the DNA molecule loses its replication ability leading to cell death.[10, 29] Silver nanoparticles are expected to be more efficient due to their larger surface area which provides better contact with the microorganisms; nanoparticles get attached to the cell membrane and penetrate inside the bacteria, attacking preferably the respiratory chain, but silver ion release from nanoparticles enhances the antibacterial activity.[10, 29]

The results in Table 2 indicate that the clay with silver nanoparticles, T1.51, has a bacteriostatic effect when 0.1g of clay was used, whereas no effect was observed at the lowest sample weight (0.01g). This can be explained by insufficient incubation time

(24 h) to allow sufficient Ag^+ ion release from silver nanoparticles. On the other hand, silver cations in R1.51 are able to inhibit completely the bacterial growth. The antibacterial activity of R1.51 can be explained by the Ag^+ ions released from the clay, as already reported.[19]

In the case of the clay R1.51, silver is completely available in its biologically active ionic form. Thus, although this clay is more effective and it can be suitable for potential applications where high Ag^+ concentrations are required immediately, it is also expected to undergo slow migration over time.[30] Although T1.51 has low bactericidal effect after 24 hours, its increased activity at longer exposures should not be discarded, because it is fully documented that the controlled release of active Ag^+ cations from silver nanoparticles occurs in the medium to long term under humid conditions.[11-14, 30]

Table 2. Growth of *S. aureus* in the presence of unmodified clay and silver based clays

	Sample weight: 0.1 g	Sample weight: 0.01g
Sample	**CFU/ml**	**CFU/ml**
Control*	1.88E+08	1.40E+08
Unmodified clay	3.00E+08	1.14E+08
R1.51	0.00E+00	0.00E+00
T1.51	3.80E+05	3.00E+08

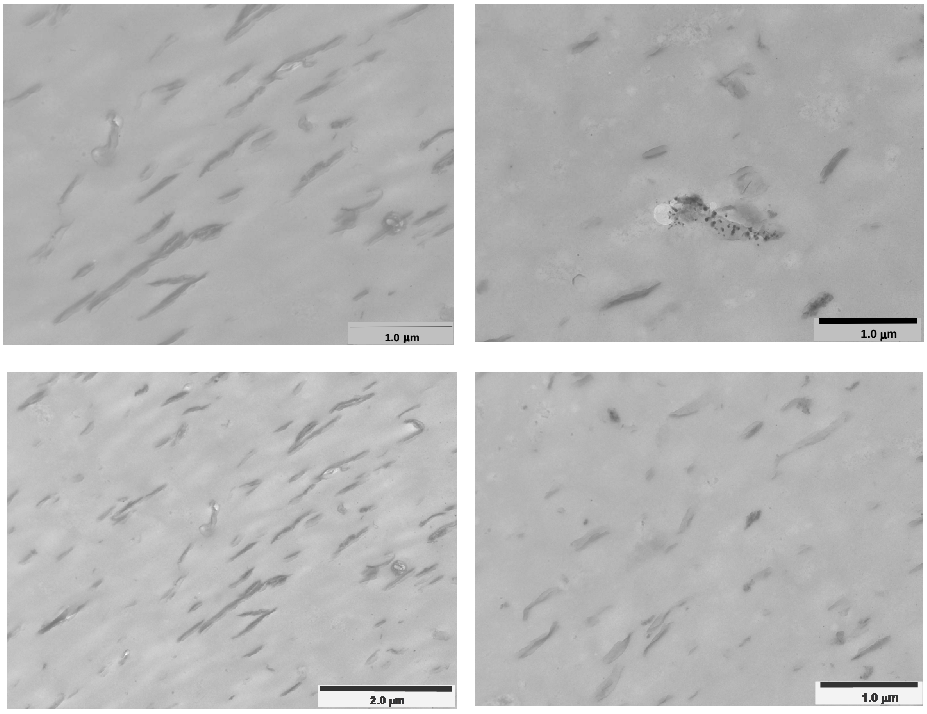
* Without clay

Characterization of the PLA nanobiocomposite films

Morphology evaluation. Silver based clay intercalation into the PLA matrix was assessed using both XRD and TEM. Depending on the layered silicate sheet exfoliation the nanocomposite's structure can be classified into different categories. In a polymer / clay blend or microcomposite the polymer chains are unable to penetrate the layered silicate structure. In intercalated nanocomposites the layered silicates form stacks (tactoids) and the polymer chains can penetrate. A fully exfoliated nanocomposite is produced when mineral clay layers are found as single sheets throughout the material and the basal plane peak is no longer observed by WAXS.[31]

Figure 3 shows the TEM images of composites samples made from R1.51 and T1.51. In all cases, tactoids with different sizes randomly distributed in the PLA matrix are observed. Exfoliation of the clay layers has, therefore, not occurred and the structure can be best described as a reduced tactoid size mixture, intercalation and some exfoliation morphologies. Complete exfoliation is rarely achieved during melt blending even when an appropriate surface modification and high shear forces combine during twin screw extrusion.[32] However, in the systems studied here, it can be said that a reasonable clay dispersion and distribution into the PLA matrix was achieved. Figure 3b shows the spherical dark particles corresponding to metallic silver on the platelets for the T1.51 clay composite.

Figure 4 illustrates the differences between neat PLA and the two different composites prepared from the R1.51 and T1.51 clays. The neat PLA shows the typical amorphous halo with a reflection at 29.5°. On the other hand, the composites exhibit in addition to the same amorphous halo, and the basal peaks of clays, but they were not shifted. Particularly relevant is the first order of the basal peak at low angles suggesting that the clays retained the tactoid-like structure once incorporated into the polymer. These results suggest that the morphology obtained corresponds to a microcomposite. For the T1.51 clay composite the 2.3° peak corresponds to a 38.5 Å *d*-spacing. For the R1.51 clay composite the 2.2° peak corresponds to a 39.4 Å *d*-spacing. These spacing values are similar to those reported for organoclay-PLA composites, between 31.5-35 Å.[26, 33]



(a) **(b)**

Figure 3. TEM images of PLA composites with 2.5 weight percent filler content: (a) R1.51; (b) T1.51

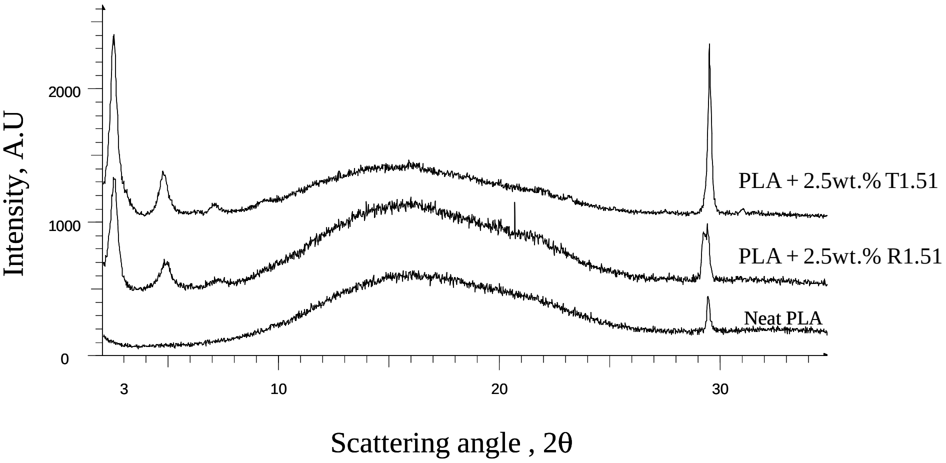


Figure 4. XRD patterns of neat PLA and PLA composites with 2.5 weight percent R1.51 and T1.51

Thermogravimetric analysis. Silver based composite thermal stability was compared with neat PLA by thermogravimetric analysis (Figure 5, Table 3). The composites demonstrated enhanced thermal properties over the pure polymer. As seen in Table 3, the composite's degradation onset shifted to higher temperatures, specifically 17.9 °C and 16.5 °C for composites made from R1.51 and T1.51, respectively. Thus, it has been theorized that thermal stabilization in these clay systems is from the increased diffusion pathway of combustion products caused by the clay layers.[33] In this case, the silver oxidation state does not affect the polymer degradation behavior, as both systems exhibited similar thermal behavior.

Table 3. DTG data of PLA-silver based clays composites

Sample	Onset (°C)	Peak (°C)	% Weight change
Neat PLA	329.4	367.9	99.4
PLA + 2.5 wt.-% R1.51	347.3	375.1	98.1
PLA + 2.5 wt.-% T1.51	345.9	368.9	97.3

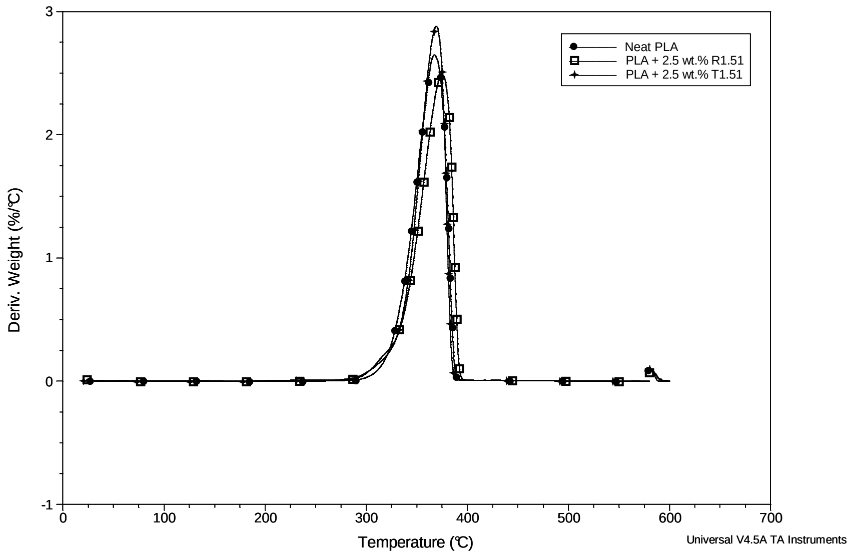


Figure 5. DTGA of neat PLA and PLA composites with 2.5 weight percent R1.51 and T1.51

Antimicrobial activity of the nanobiocomposites. Table 4 shows that PLA composites containing R1.51 and T1.51 exhibited a R value > 2; which means that the biocidal properties previously demonstrated by both reduced and non reduced silver clays are retained in the PLA matrix. The antimicrobial performance of silver-based antimicrobial technologies is governed by silver ion release from the matrix, which at the same time depends on the water diffusion through the polymer matrix, as was already observed in polypropylene-silver composites and in PLA-silver based composites prepared by solvent casting.[6, 7, 34] For metallic silver contained in T1.51, albeit some of the original silver in the clay could be already in its ionic form, water sorption is needed to produce additional active silver cations by oxidation of the nanoparticles surface. Hence, the best results in the short-term antimicrobial activity are expected for the samples in which silver is in its ionic form as observed in Table 4.

It was already demonstrated that antimicrobial polymer materials based on silver nanoparticles exhibit long-term performance (i.e. several weeks) because of the controlled Ag^+ release mechanism, which is activated under humid conditions, thus cation exchange from the environment with silver from the insoluble inorganic carrier located at the surface of polymer matrix occurs and, bacterial concentration on silver-containing surface decreases.[11, 30] The controlled release of Ag^+ can be a clear advantage in any long-term antimicrobial applications (i.e. contact surfaces, medical devices, and food-manufacturing equipment).[30]

Table 4 . Comparative antimicrobial activity against *S. aureus* of PLA-silver based composites and neat PLA

Sample	R
Neat PLA	0.00 ± 0.25
PLA + 2.5 wt.-% R1.51	5.10 ± 0.00
PLA + 2.5 wt.-% T1.51	2.90 ± 0.25

Conclusions

Two experimental organomodified silver containing montmorillonites in which the silver is different –ionic vs. nanoparticles- were evaluated in melt compounded PLA film composites. Antimicrobial tests in the clays showed strong biocidal effect against the growth of *S. aureus*. The resulting composites containing 2.5 weight percent filler exhibited good optical properties, filler dispersion/distribution and excellent antimicrobial performance. Differences between composites containing metallic or cationic silver were observed in the antimicrobial tests, suggesting that the clay containing nanoparticles has less active ionic species available immediately after compounding, as expected.

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5. OXYGEN SCAVENGING POLYOLEFIN NANOCOMPOSITES CONTAINING AN IRON-BASED KAOLINITE OF INTEREST IN ACTIVE FOOD PACKAGING APPLICATIONS

Abstract

A synthetic iron containing kaolinite was evaluated as an oxygen scavenger additive for food packaging plastics having an active performance of ca. 43 ml of oxygen/g of additive. The corresponding active composites were obtained by double-screw extrusion of HDPE and LLDPE resins and 10 wt.-% of the iron-based kaolinite. The resulting composites exhibited significant oxygen scavenger activity (up to 4.3 ml of oxygen/g composite), being the kinetics of absorption more pronounced in the case of the LLDPE composites due to its higher water and oxygen permeability compared to HDPE. Oxygen permeability tests carried out after consumption of the active role of the additive suggest that the iron containing clay plays in fact a dual oxygen fighting role, i.e. active performance (trapping and reacting with molecular oxygen) as well as a passive barrier performance (allegedly imposing a more tortuous path to the diffusion of the spoiling gas). Migration tests into two food simulants were finally carried out which indicated that iron (accounting for the active ingredient) and aluminum (accounting for the clay migration) are hardly detectable in the migration media. This study suggests that there is significant potential for the use of this novel oxygen scavenger additive to constitute active packaging of value in the shelf-life extension of oxygen sensitive food products.

Keywords: Oxygen scavengers, nanoclays, polyolefins, active food packaging

1. Introduction

Oxygen is one of the major factors in food spoilage. Among the many adverse effects provoked by oxygen penetration on food and beverages are oxidative rancidity of unsaturated fats, loss of vitamin C, browning of fresh meat, oxidation of aromatic flavor oils and pigments and fostering the growth of aerobic spoilage microorganisms. These undesirable effects on the product quality indicate that the elimination or exclusion of oxygen is one of the main targets for preserving foods and beverages. Up until now, antioxidants, absorbers, interceptors and scavengers have been used to remove oxygen or to prevent this from entering into the packaging environment. Specifically, oxygen scavengers are materials which, once incorporated into the packaging structure, bind chemically to this and are capable of eliminating large volumes of oxygen that either permeate through the packaging or from the head space (1-3). Oxygen scavenging commercial technologies are based on enzymatic and chemical systems. The latter ones are, for example, based on catechol, ferrous salts, active zero-valent iron, ascorbic acid and titania among others (2- 4).

Many of the commercially available oxygen scavengers contain iron and are marketed in a sachet format (5), to prevent imparting color, odor and taste to the product. Considering that the use of sachets have the potential risk of being misused by the consumer or being ingested, as well as to contaminate the product by leakage from the sachet, the use of packaging materials that contain incorporated the active agent within the packaging monolayer or multilayer structure seems more suitable, acceptable by the consumer and safer. The incorporation of scavenging agents into packaging materials such as polymers present some advantages such as the potential use in retortable packaging, elimination of food product distortion that may occur when a sachet contacts the food, and potential cost savings due to increased production efficiency and convenience. In this sense, the active packaging research is currently focused in the incorporation of oxygen scavenger compounds into packaging materials.

The iron-zero valent nanoparticles have not only being considered as oxygen scavenging agents, but they have also been applied for remediation and removal of toxic metals and organic contaminants of waste waters (6-13) or for inactivation of bacteria and fungus (14). However, the incorporation of iron-based proprietary montmorillonite in polymeric matrixes has not been reported until recently, when polymers and especially biopolyester-based nanocomposites were described as an efficient biodegradable oxygen scavenger material for potential use in food packaging (15). The use of active nanoclays to produce active materials has advantages such as raw material availability and low cost, and relative simple processability and dispersion. Also, the incorporation of active nanoclays into polymers could additionally improve the passive barrier due to the alteration of the diffusion path imposed by the clay platelets in the polymer matrix (16-17).

The use of polyolefins for containers and bottles for the food packaging market has increased significantly in recent decades due to their low cost, chemical inertness, good mechanical properties and light weight. However, this increase in usage has also created many challenges associated with waste management and the corresponding environmental impact. Even though polyolefins are not biodegradable, increasing the shelf-life of products packed in polyolefins could result in both reductions in the consumption of oil-based resources (reduction in oil-based polymer consumption) and also in the polymer mass per packaged used, i.e. the so-called downgauging. In this context, this work presents the development of novel composites containing an iron-based kaolinite incorporated into polyolefins to produce oxygen fighting nanocomposites to increase the shelf-life of oxygen sensitive products. Besides the scavenging activity, other properties of the resulting nanocomposites were determined, such as barrier properties and migration of the active agent into food simulants, to evaluate their potential in polyolefin based active food packaging applications.

2. Materials and methods

A proprietary natural kaolinite and its corresponding experimental iron containing homonymous grade were kindly supplied in powder form by Nanobiomatters S.L. (Valencia, Spain). The black colored iron-based kaolinite exhibited a particle size of 27 microns in the so-called d90. No further details about the additive composition or modification process were disclosed by the manufacturer.

High density polyethylene, HDPE Rigidex® HD5226EA (Ineos Polyolefins, Hampshire, UK), and linear low density polyethylene, LLDPE, Dowlex™ NG5056G (Dow, USA) were used as polymeric matrixes for preparing the nanocomposites.

2.1 Active nanocomposites preparation

The active polyolefin composites were prepared by extrusion. Since the presence of potential moisture can result in porosity in the processed material, this has to be removed by pre-drying the additive before processing (*18*). Before the extrusion process, the iron kaolinite was dried at 60ºC under reduced pressure during 4 hours.

Polymer pellets were melt-blended with the iron-based kaolinite (10 wt.-% filler content) in a ZE25X42DUTX co-rotating twin-screw extruder (Berstorff, Hannover, Germany). Composite strands were chopped into small granules using a pelletizer. Finally, the pellets were stored in high barrier plastic film bags (PET/aluminum foil/LDPE) under vacuum prior to their use.

2.2 Optical microscopy

Films of 1cm^2 samples were observed in a digital microscopy system (Nikon Eclipse 90i) fitted with a 12 V, 100 W halogen lamp and equipped with a digital camera (Nikon DS-5Mc) attached to the microscope. Images processing and analysis were performed using the Nis Elements BR 2.30 software (Nikon Instruments Inc., New York, USA).

2.3 XRD patterns

The dispersion of the iron-based kaolinite in the polymeric matrixes (intercalation) was evaluated by wide angle X-ray scattering scans. The diffractograms were acquired using a Bruker Advance D8 diffractometer (Wisconsin, USA), with Cu Kα filament (0.1546 nm, operating at 45 KV and 45 mA), time step of 8s at scanning rate of 0.5 º/s. The basal spacing d was calculated applying the Bragg´s law (eq. 1):

$$\lambda = 2d \sin \theta \qquad \text{(eq. 1)}$$

where λ is the wavelength of the radiation and θ is the measured diffraction angle.

2.4 Thermogravimetric analysis (TGA)

Thermal stability of HDPE and LDPE nanocomposites were assessed using a TGA Q500 equipment (TA Instruments, USA) with analysis software Universal 2000 V4.4A. Samples were analyzed from 30 to 600ºC under nitrogen flow.

2.5 Oxygen scavenging capacity

Oxygen scavenging capacity of iron kaolinite and its nanocomposites was determined by measuring the oxygen content in the headspace as a function of time. The clay (0.05g) and film pieces of the composites (1.5 g) were placed inside gas-tight septum-equipped vials of 40 ml capacity. Each vial also contained another one full of water to achieve 100% RH. Blank vials with unmodified kaolinite and with pieces of film of the neat polymer matrixes were also prepared as controls. The vials were subsequently closed and placed in a room at 24ºC. No modified atmosphere was used for this test, just atmospheric air. The headspace percentage of oxygen (%O_2) was determined using a PBI Dansensor CheckPoint (PBI Dansensor AS., Ringsted, Denmark) at different time intervals.

2.6 Oxygen transmission rate (OTR)

The oxygen transmission rate (OTR) was measured using an Oxtran 100 equipment (Mocon, MN, USA) at 24ºC and 80% relative humidity. The samples were previously purged with nitrogen before being exposed to the oxygen flow. The oxygen permeability was calculated from the oxygen transmission rate at equilibrium conditions by eq. 2:

$$PO_2 = (OTR * L) / \Delta p \qquad \text{(eq. 2)}$$

where *OTR* is the oxygen transmission rate, *L* is the sample thickness, and *Δp* is the partial pressure gradient across the sample.

2.7 Migration tests

Migration tests from neat processed HDPE and HDPE-iron-based kaolinite films were performed following the European Standard UNE-EN 13130-1 (*19*) in a certified laboratory (Red Control S.L., Valencia, Spain). The samples (film pieces of 6 dm^2 of area and 200 microns thick) were immersed in water and isooctane as food simulants for aqueous and fatty foods, respectively, at the specific conditions established by the standard (see Table 1). Each sample was evaluated by triplicate. After completing the exposure time, film samples were removed from the liquids, and the simulants were stored for further determination of iron and aluminum by inductively coupled plasma mass spectroscopy (ICP-MS Agilent 7500CX).

Table 1. Applied simulants and conditions in the migration tests

Simulant	Simulant volume (ml)	Temperature (ºC)	Time (days)
Water	100	40	10
Isooctane	100	20	2

3. Results and discussion

3.1 Characterization of the active kaolinite

A typical TEM micrograph (see Figure 1) of the kaolinite additive in which rounded morphologies of iron have been attained is seen in Figure 1. From this figure, kaolinite platelets can be easily discerned in which iron metallic particles are located inside the material and also on the edges. An average iron particle diameter of ca. 115 nm is observed with particle diameters varying from 78 to 220 nm. These observations agree with results reported earlier for the formation of iron nanoparticles (14, 20) as well as for the generation of kaolinite-supported zero-valent iron nanoparticles (*6,10*). As a result, the iron particles present within the clay are in average at the verge of the definition of a nanomaterial, which upper limit in size is typically set at 100 nanometers. This is the reason why the terminology nanoadditive or nanocomposite, the latter a nanoadditive inserted in the plastic matrix, is used throughout the paper. Additionally, the iron particles are used to modify the clay. Modified clays also-called nanoclays are known to undergo delamination into the nanoscale during processing with plastics.

Figure 2 shows the changes in the basal plane of kaolinite after modification with iron as determined by WAXS. The unmodified kaolinite diffractogram shows the basal peak relection at 12.4º (2θ) and the reflection of residual illite at 8.9º (2θ). After modification with iron, the reflections of kaolinite and illite disappeared most likely as a consequence of the structural disorder produced by the intercalation of the active metallic compound in accordance with previous work (10).

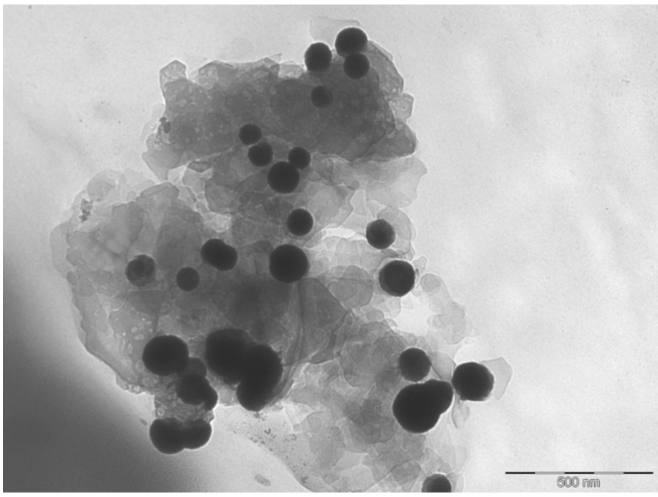

Figure 1. TEM image of iron kaolinite

The data referring to the oxygen depletion generated by the iron containing kaolinite at 100%RH is presented in Figure 3. The natural kaolinite did not exhibit scavenging activity (results not shown), which confirms that the structural iron (0.72% in the nanoclay as indicated by the manufacturer), in the form of iron compounds within the clay, is not active, as expected, for oxygen scavenging purposes. The absorption of the active kaolinite was tested at 100%RH.

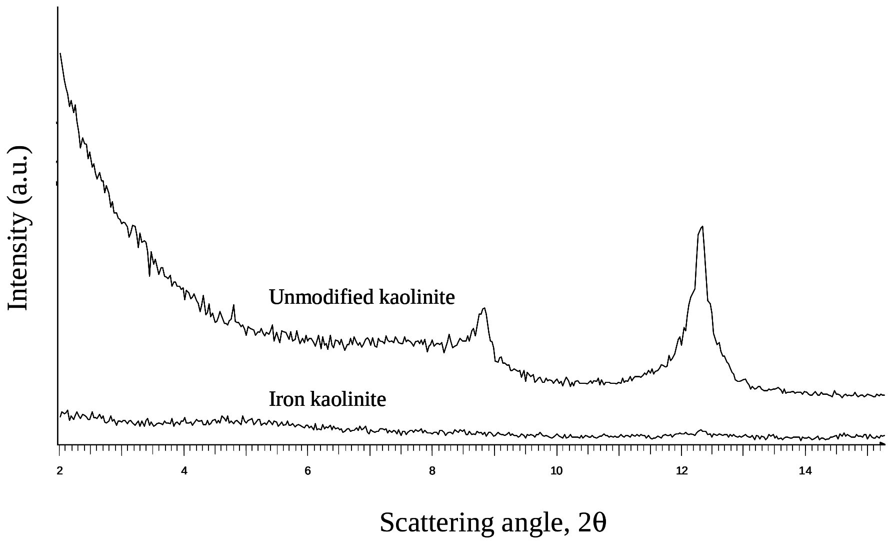

Figure 2. Comparative zoom in the basal plane region of diffractograms of iron kaolinite and unmodified one

The reason for testing at high humidity is that at lower moisture levels, the activity of iron is known to reduce its kinetics of absorption, since iron needs moisture to scavenge oxygen according to eq. 3. The active kaolinite showed strong efficiency at short times, i.e. the first day of exposure the active kaolinite was able to uptake 30 ml of oxygen per gram of additive and up to 43 ml after 60 days. The mechanism of action for iron is well-known (5) and is extremely efficient since it is expected that 1 g of iron should inactivate 300 ml of oxygen in the presence of moisture.

$$4\ Fe + 3O_2 + 6H_2O \rightarrow 4\ Fe(OH)_3 \rightarrow 2Fe_2O_3 \cdot 3H_2O \qquad \text{(eq. 3)}$$

The lower efficiency of the additive compared to pure iron is related to the relatively low targeted loading of ion (cited but not disclosed by the manufacturer) into the clay formulation. The low iron loading was set in the formulation in order to achieve better dispersion, passive barrier and good melt flowability and strength to make thin films, but the manufacturer claims the additive can be tailor made to aim specific oxygen scavenging requirements. Additionally, low efficiency regarding theoretical absorption values are also ascribed to the formation of iron oxide as a coating on iron particles. Thus, it has been found that iron oxide shells can have an almost invariable thickness independent of the particle size (*20*). Therefore, the mass contained inside the larger particles can be inaccessible once the shell is oxidized. However feasible the latter argument might be, it is also known from basic corrosion theory that iron oxide does not adhere well to the underneath iron material, i.e. it does not undergo self-passivation, and hence oxidation may in principle continue below the shell. However, this process may be prevented by the fact that the iron particles are confined inside a plastic matrix, which could impede oxide shell delamination. As a result, having iron particles in the nanorange, as observed for this additive, is thought to be beneficial to enhance efficiency due to enhanced surface to volume ratios, particularly in composites where iron particles are confined.

Milz and Perry (*5*) studied some commercial iron-based oxygen scavengers in the form of sachets, and they concluded that in fact the limiting factor in the scavenging process is the rate of oxygen diffusion to the surface of the scavenger. In this process, oxygen molecules may diffuse through the membrane and, then, through the solid material and the scavenging reaction takes place when oxygen meets metallic iron centers. This process is expected to be more efficient as iron particle sizes become smaller as mentioned above, but also at higher relative humidity conditions, because the presence of moisture is an important governing kinetic factor in the process. As a result, it is expected that smaller particles and/or nanoparticles uniformly distributed along the surfaces and edges of the nanoclay dispersed surfaces must show high efficiency. A comparative on this will be shown in further studies.

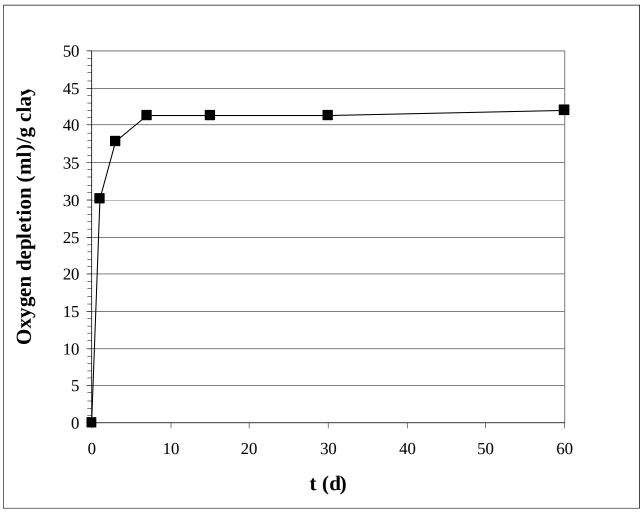

Figure 3. Oxygen scavenging behavior of iron containing kaolinite at 100 %RH (conditions: 0.05 g of clay; 24ºC, in air)

3.2 Morphology and thermal properties of the active nanocomposites

Optical microscopy images of the active polyolefin composites (Figure 4) showed a good distribution of the additive in the matrix but not a good dispersion (exfoliation) of the nanoclay. The nanoclay was seen to exhibit a heterogeneous size distribution most likely due to some agglomeration. Lack of nanoclay dispersion may be related to insufficient sheer during processing and/or affinity with the matrix. Insufficient sheer forces can be due to ineffective double-screw extrusion choice of processing parameters and/or due to insufficient clay swelling after modification. Agglomeration can also occur when a relatively high loading of the nanoclay is added to the matrix, as it is the case here. Figure 4a corresponds to the HDPE-iron kaolinite nanocomposite, where due to the high filler content, aggregates are formed after melt-mixing, which can achieve up to an average of 40 microns in diameter. The iron kaolinite is black colored, which together with the significant load used causes the dark color of the matrix. The situation is similar in the LLDPE-iron kaolinite composite (Figure 4b), where some of the clay agglomerates in the matrix to an average size of about 44 microns. Of course smaller size clay particles and some exfoliation and intercalation of the nanoclay should have also been achieved from previous observations on how nanoclays perform during double-screw extrusion processes (21-24). It should also be borne in mind that optical microscopy observations from a top-view are prone to reveal the non-nano size of the platelets, i.e. length and width, in this technology due to the parallel laying of the platelets in the flow direction. Unfortunately, in this case transmission electron microscopy (TEM), which is usually used to study the morphology of clay containing nanocomposites, was not of value due to the high clay loading and high metal content, which made difficult the electrons beam transmission through the specimens and provided meaningless observations.

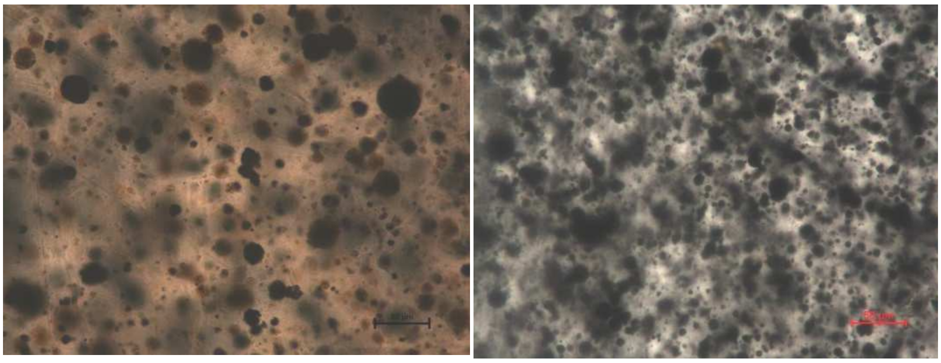
(a) **(b)**

Figure 4. Optical microscopy images of HDPE-iron kaolinite composite (a); LLDPE-iron kaolinite composite (b)

Figures 5 and 6 show the WAXS diffractograms of the active HDPE and LLDPE composites, respectively. As the iron containing kaolinite basal peak was not observed in the diffractogram of the active additive (see Figure 2), there are no patterns related to clay in the diffractograms of the composites either. The absence of kaolinite reflections in the composite diffractograms does not, therefore, imply exfoliation into the polymeric matrix. Optical microscopy images (Figure 4) showed that iron kaolinite does aggregate or remained aggregated to some extent inside the polyolefin matrixes. Thus, complete exfoliation by miscibility of the iron containing clay in the polymeric matrixes is not expected. As mentioned above, exfoliation cannot occurs unless there is a good thermodynamic affinity between the nanoclay and the matrix and/or the stresses imposed during melt-mixing are intense enough to break up the aggregates and shear the stack of platelets into individual ones. Additionally, a high filler loading necessary here to increase the active performance, may exceed the solubility limit of the additive in the matrix, resulting in agglomerates. If tactoids or agglomerates are internally disordered or not well aligned to one another, the peak intensity will still be low and may not appear to be completely absent (*16*) in the diffractogram as may be the situation here.

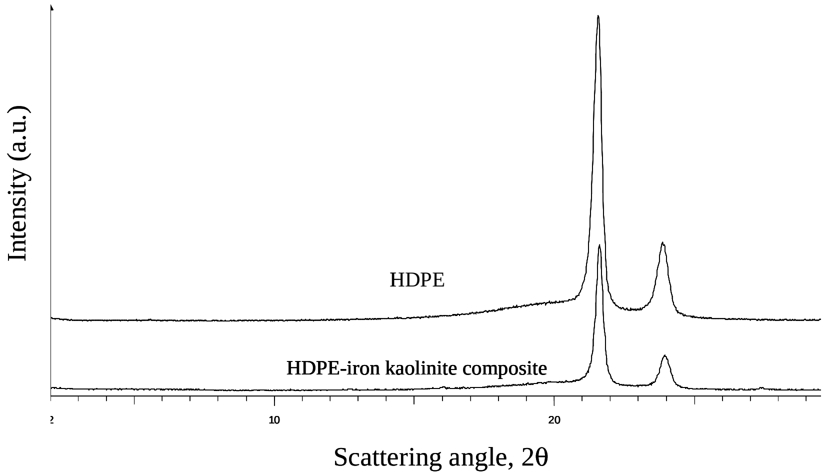

Figure 5. Diffractograms of the HDPE-iron kaolinite composite and of the neat HDPE resin

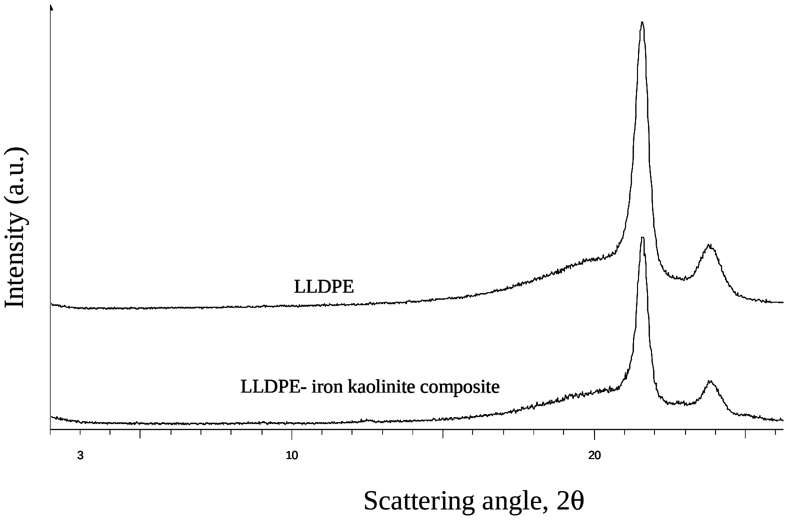

Figure 6. Diffractogram of the LLDPE-iron kaolinite composite and of the neat LLDPE resin

The thermal stability of the composites was assessed by TGA. The TGA runs were done in duplicate to study whether the presence of the iron additive can affect the thermal stability of the polyolefin matrix (see Figures 7 and 8). In all cases, the thermal stability was characterized by the peak onset and midpoint, so-called $T_{0.5}$, of the degradation process (see Table 2). The total weight change in the specimens and the mentioned temperatures are also gathered in Table 2. From this Table, it can be seen that similar mass drops occurred above 450ºC for both composites, suggesting that the inorganic content is higher than 9% in the additive. From the results, the high filler content used (almost 10 wt.-%) in the polyolefin composites did not provoke or accelerate degradation of the matrixes; in fact the thermal stability seemed to increase few degrees in the onset temperature as well as in the degradation midpoint. Some authors (*24-25*) have claimed that structural iron in montmorillonite resulted in increased thermal stability due to the iron capacity to trap free radicals.

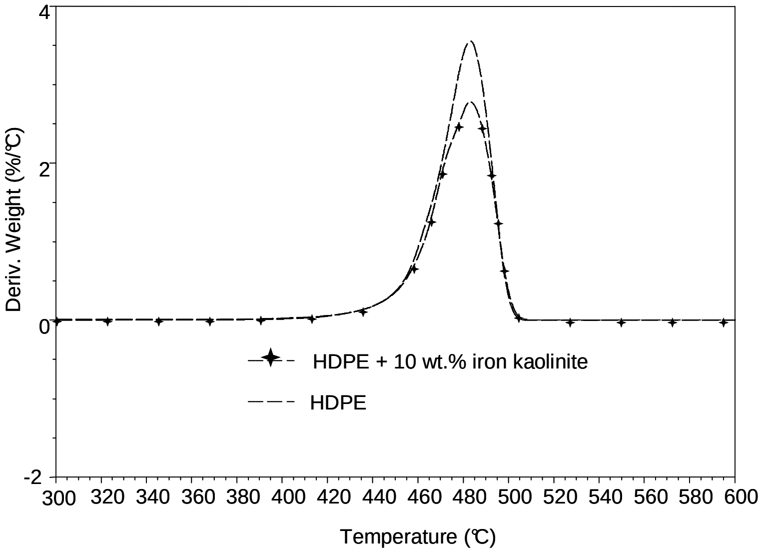

Figure 7. DTG curves of HDPE-iron kaolinite composite and neat HDPE

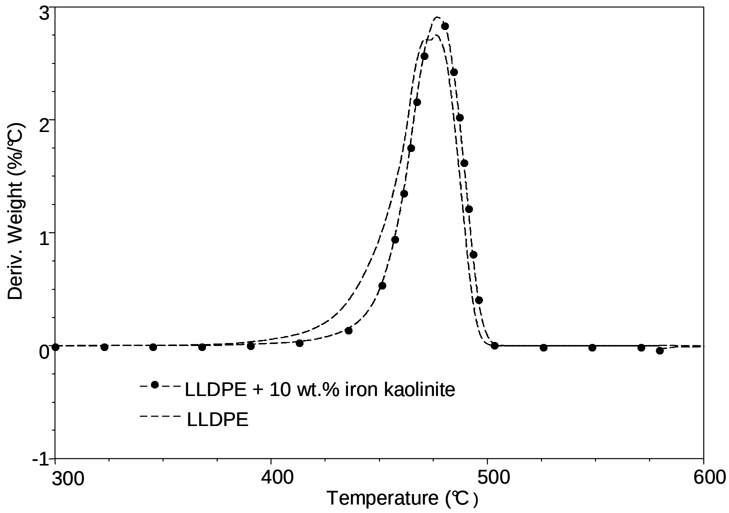

Figure 8. DTG curves of LLDPE-iron kaolinite composite and neat LLDPE

Table 2. TGA data for the neat polymers and their iron containing kaolinite nanocomposites

Sample	Total weight change (%)	Onset temperature (°C)	$T_{0.5}$ (°C)
HDPE	100	458.2	479.4
HDPE+10 wt. % iron kaolinite	90.5	462.9	480.9
LLDPE	100	448.7	469.2
LLDPE +10 wt.% iron kaolinite	90.6	452.1	475.6

3.3 Passive barrier and active properties of the polyolefin composites against oxygen

The barrier properties of polymers can be significantly altered by inclusion of inorganic platelets of sufficient aspect ratio to alter the diffusion path of permeant molecules, their solubility and other structural parameters (*16-17*). In the case of polyolefins containing active iron kaolinite, one must assume that two effects take place simultaneously. The first one corresponds to the, generally assumed, more tortuous gas permeation path that the kaolinite platelets imposed onto the polymeric matrix. This is the so-called “passive barrier” effect.

The second one corresponds, of course, to the discussed iron metallic centers contained on the surface and edges of the kaolinite platelets, trapping molecular oxygen and hence causing the reduction of gas pressure permeating through the composite. In order to evaluate the passive effect on oxygen, the oxygen transmission rate through inactive HDPE+10% wt.-% iron kaolinite films, as well as through neat HDPE films were assessed. Inactivation of the iron-based composite was achieved by continuous exposure to room ambient conditions until no further oxygen scavenging activity was detected.

The oxygen transmission rate through inactive films was measured at high relative humidity conditions in order to determine the passive barrier effect of the iron kaolinite. Table 3 shows that the barrier effect just by incorporation of the nanoclay can reduce the oxygen permeability by 28 %.

Table 3. Oxygen permeability of iron-based HDPE composites (24ºC, 80% RH)

Film sample	P(m^3 m/m^2sPa)	Improvement (%)
HDPE	6.46 × E^{-18}	-
HDPE+10 wt. % iron kaolinite (active)	4.65 × E^{-18}	28.0
HDPE+10 wt. % iron kaolinite (inactive)	5.32 × E^{-18}	17.7

Regarding the active role, the comparative oxygen scavenging activity of LLDPE and HDPE – iron containing kaolinite composites (for the same filler content at 100% RH) during 60 days is presented in Figure 9. The LLDPE-iron kaolinite composite is up to twice more active that its counterpart based on HDPE at the same conditions. After 60 days at 100% RH, the LLDPE composite absorbed ca. 4.3 ml of oxygen/g composite while the HDPE composite absorbed ca. 2.4 ml/g composite. The ability of oxygen molecules to encounter iron metallic centers and moisture inside the composite will determine the final absorption characteristics of the composites. Thus, the differences in absorption kinetics are mainly ascribed to the different inherent water and oxygen permeability properties of the two types of resins (see Table 4). Since water is needed to trigger the oxygen absorption reaction, the more permeable LLDPE clearly exhibits the faster activity after 60 days of exposure.

Table 4. Comparative reported barrier properties for an LLDPE and a HDPE (26)

Polymer	Water vapor transmission rate [g m m^{-2} s^{-1}]	Oxygen permeability [m^3 m m^{-2} s^{-1} Pa^{-1}]
LLDPE	2.3-4.6×10^{-9}	8.9-17.9×10^{-18}
HDPE	1.5-2.2×10^{-9}	4.5-9.0×10^{-18}

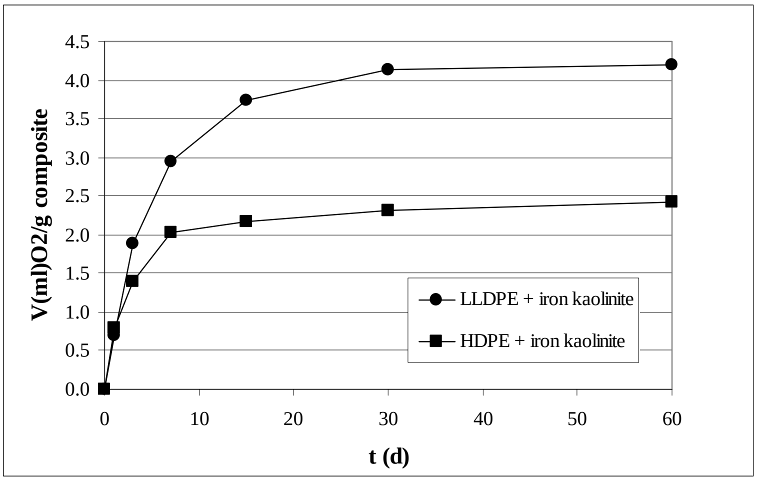

Figure 9. Oxygen scavenging behavior of polyolefins + 10 wt.-% iron containing kaolinite, at 100% RH, 24 ºC.

These results confirm that the active iron containing kaolinite does have a dual effect in oxygen inactivation from within the composites, that is, it promotes a simultaneous passive and active barrier and inactivation mechanisms against the deleterious oxygen permeant.

3.4 Migration data from the active HDPE composite

One of the main issues concerning the food safety of packaging materials is the non-intended migration of packaging constituents. In active oxygen scavengers, migration is not required to exert the active role and therefore should be avoided.

The typical valid route to assess this mass transport process is to evaluate the specific and global migration of the substances into food simulants to avoid dealing with the complexity of analysis in real foods. In Europe the "Commission Regulation (EU) Nº 10/2011" (*27*) for food contact materials has approved the use of both iron and clays as substances allowed to be in direct contact with food, with a specific migration limit of 48 mg/kg of packaged food for iron and with no specific migration limit for some layered clays. In any case, the global migration of permitted substances from plastics to be into contact with foodstuffs should be less than 60 mg/kg of packaged food (*28*). Nonetheless, this legislation has recently being amended and now deals with the specific case of listed substances that have been engineered as nanomaterials. Thus, in the case of the so-called nanomaterials intended for food contact, whether listed or not listed, the amendment states that they have to be re-assessed by the EU authorities regarding its potential toxicity, and hence no specific or global migration limits are defined at the moment.

With this framework in mind as an example or as a reference for migration, the release to water and isooctane of iron and aluminum, as representatives of active iron and nanoclay, respectively, from the active HDPE composites was investigated. The obtained migration data is gathered in Table 5.

Table 5. Characteristics of iron-based composite samples and migration data from water and isooctane as food stimulants as determined by ICP-MS

Sample	Food Simulant	Average migration (mg/kg simulant)*	
		Fe	Al
HDPE-Blank	Water	$<(1.10\pm0.00)\times10^{-2}$	$<(3.62\pm0.00)\times10^{-3}$
HDPE+10 wt.% iron kaolinite	Water	$(1.58\pm0.02)\times10^{-2}$	$(33.1\pm21.8)\times10^{-3}$
HDPE-Blank	Isooctane	$<(1.10\pm0.00)\times10^{-2}$	$<(3.62\pm0.00)\times10^{-3}$
HDPE+10 wt.% iron kaolinite	Isooctane	$<(1.10\pm0.00)\times10^{-2}$	$<(3.62\pm0.00)\times10^{-3}$

*The symbol < means that the obtained data is below the value the testing laboratory is certified for.

From this, very small/negligible migration values are found for both constituents, which for the case of isooctane are similar to those measured in the blank polymer, suggesting that migration is hardly detectable for these components. In any case, the migration of the additive components is much less than that currently permitted by the EU legislation for the no-nanoform. Migration was perhaps more detectable in water than in isooctane, since water is a more likely solvent for both substances. On the overall, migration of the nanoadditive into foods should be anticipated close to negligible, even in direct contact.

4. Conclusions

The oxygen scavenging capacity of an experimental nanoiron-containing kaolinite grade, designed for thin film applications, as well as its polyolefinic nanocomposites was evaluated for the first time. The clay was seen to contain metallic iron in the form of nanoparticles with an average particle diameter of about 115 nm intercalated and deposited on the surface and edges of the clay platelets. It was shown, that the iron kaolinite exhibits a rapid oxygen uptake at 100% RH, of up to 43 ml per gram of additive. Active polyolefin composites with 10.-wt% filler content were manufactured by extrusion into HDPE and LLDPE. The iron kaolinite contained in the active composites was seen to play a dual oxygen fighting role: As a passive barrier due to the tortuous path that imposes to gas diffusion; and secondly, the iron contained in the kaolinite traps and reacts with the molecular oxygen, causing the active reduction of gas pressure through the composite. Humidity is a key factor in the kinetics for oxygen depletion, the active composites are able to uptake between 2.4 and 4.3 ml of oxygen per gram of composite, in what it thought as a dependency with the permeability to water and oxygen of the polymeric matrix. Iron and aluminum migration from active composites to food simulants (water and isooctane) show very small or negligible migration levels, especially in isooctane.

Acknowledgments

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6. POLYLACTIDE (PLA) FILMS CONTAINING AN OXYGEN SCAVENGING IRON-BASED MONTMORILLONITE

Abstract

The morphology and oxygen scavenging activity of a zero-valent iron containing montmorillonite and its composite with PLA have been evaluated. The iron particles carried by the clay were seen to be of up to 150 nm and were responsible for the high absorption behavior exhibited by the developed additive. The active clay was seen to absorb up to 62 mL of oxygen/g clay, at 100% RH and 24ºC. The zero-valent iron montmorillonite was incorporated by melt mixing into polylactide to obtain a composite with significant oxygen scavenging properties (of up to 4.2 mL of oxygen/g composite). The thermal stability of the iron composite was reduced by the catalytic effect of iron on polylactide degradation processes, however, other thermal parameters were not affected and the water vapor permeability was slightly improved.

Keywords: Oxygen scavenger, polylactide, composites, active food packaging

Introduction

In active food packaging there are positive synergistic interactions between the product, the package and the environment. These interactions are beneficial to food products because they translate into shelf-life extension. Active packaging techniques for preserving or improving the quality and safety of packaged foods can be divided into three classes: absorbing systems, releasing systems and, other specialty systems based on temperature UV light or microwave control. Developments in active packaging include prevention or delay of oxidation processes and microbial growth, carbon dioxide absorbers/emitters, ethylene removers, among others. The oxygen scavengers constitute one of the most widely spread active packaging technologies, as they contribute to keep the optimal concentration of oxygen inside packaging in order to preserve the quality (appearance, odor, taste and texture), nutritional values and prolong the shelf-life of oxygen sensitive products.

Oxygen permeating through packaging films (even with high barrier packaging materials) or trapped inside foods cannot be efficiently removed by the use of MAP or vacuum packaging. The oxygen scavengers can absorb the residual oxygen present in the headspace of packaging below 0.01%, thus oxidation of products is minimized, hence reducing proliferation of molds, aerobic bacteria and insects.[1] The development of oxygen scavenging systems has followed two lines, depending upon whether the oxydizable substance was designed to be a part of the package (incorporation of scavenging agent into the packaging film) or to be inserted into it with the food. Packaging inserts are commonly based on the oxidation of iron-based compositions. The evaluation of some commercial iron-based oxygen scavengers in the form of sachets confirmed that the oxygen molecules diffuse through the membrane and the solid material, thus the scavenging reaction takes place when oxygen meets metallic iron centers. The scavenging reactions are of first order and the dominant factor is the rate of oxygen diffusion to the surface of the scavenger.[2,3] It was found also that the increase of oxygen depletion rate results in higher surface area of corrosion products and decrease of O_2 diffusivity though active particles, as consequence of loss of porosity.[4] Sachets can also contain sulfur compounds, oxydase enzymes, catechols, glycols, ascorbic acid and photosensitive dyes.[5-9] Nevertheless, the current research trends make also use of other technologies such as organometallic complexes,inclusion in cyclodextrin complexes, and also biological systems.[10-13] The incorporation approach includes self-adhesive labels, adhesive devices or free sachets included within foods. Even though the performance of oxygen-absorbing sachets is satisfactory for a wide range of food storage conditions, important limitations restrict their use. The esthetics of inserts (that causes low consumer acceptance), the food product distortion, the risks of possible ingestion or rupture, as well as their unsuitability for use with beverages, led researchers to develop package-based solutions. The most promising way to overcome these issues is the introduction of the active oxygen scavenger directly into the polymeric matrix even though the design and the production of a functional and efficient active package can be difficult to carry out because of the complexity of the systems.[1, 5-6, 13-14] The incorporation of active systems into the packaging materials allow some extra advantages such as the potential use with retort packaging, and potential cost savings due to increased production efficiency and convenience. Active agents can be melt extruded with other polymers to form a film or coating and used to produce the barrier/active package all as one structure. Modified oxygen-scavenging packaging materials includes monolayer and multilayer materials, as well as reactive closure liners for bottles and jars, and the scavenging active agents consist of inorganic, organic or polymeric materials. The active agent proper selection depends on the matrix selection, on the food characteristics and on the shelf-life required.14 Most of them include iron compounds, such as free flowing pellets containing iron powder blended with low density polyethylene as carrier resin, with the convenience of co-extrusion possibilities with EVOH, but there are other commercial systems based on ascorbic acid, sulfites, catechol, some nylons and ligands, metallic salts, photosensitive dyes and enzymes.[1, 7, 15-16]

More sophisticated technologies include a combination of a natural free radical scavenger (α-tocopherol) and a transition metal, the catalytic reaction of oxygen and hydrogen onto a catalyst surface, or aerobic microorganisms such as *Kocuria varians* and *Pichia subpelliculosa* as active components of hydroxyethyl cellulose and polyvinyl alcohol environmentally friendly oxygen scavenger films.[17-20] Some patents claim the use of UV light as an activator triggering the oxygen scavenging reaction, and there are commercial products based on this principle.[21-22] However, these UV activation steps can reduce packaging line speeds and hence, profits. In addition, there is a significant cost increase for oxygen scavenging films production due to the high costs of photoinitiators, as well as operation and maintenance of the UV machinery.[17] On this basis, the development of ease application, low cost and efficient oxygen scavenging systems is valuable to the food packaging industry.

Iron-based scavengers consist on the oxidation of iron into $Fe(OH)_3$, and they require water for their action at ambient or chilled temperatures.[3] Recently, the iron scavenging reactions have been described as electrochemical process in which the water adsorbed on the surface of iron forms electrolyte solutions that accelerate the electrochemical reaction:[13]

$$Fe \rightarrow Fe^{+2} + 2e^- \qquad \text{(anodic reaction, eq. 1)}$$
$$H_2O + \tfrac{1}{2} O_2 + 2e^- \rightarrow 2\,OH^- \qquad \text{(catodic reaction, eq. 2)}$$
$$Fe^{+2} + 2\,OH^- \rightarrow Fe(OH)_2 \qquad \text{(eq. 3)}$$
$$2\,Fe(OH)_2 + \tfrac{1}{2} O_2 + H_2O \rightarrow Fe_2O_3 \cdot 3H_2O \qquad \text{(eq. 4)}$$

The abundance and environmental-friendly character of clays are advantages to use them as supporting materials of iron-zero valent nanoparticles for applications of remediation of waste waters, for the production of flame retardant materials, and also for heterogeneous catalysis.[24-36] However, the use of iron nanoparticles as active agents and their support on clays as vehicle for the preparation of active composites was not described until recently.[36-37] It has been demonstrated the oxygen absorption capacity of iron-based kaolinite as well as the activity of its corresponding polyolefins composites for potential use in food packaging. The use of modified clays to produce active materials has advantages such as raw material availability and low cost, and relative simple processability and dispersion. Also, the incorporation of active clays into polymers could additionally improve passive barrier due to the alteration of the diffusion path imposed by the clay platelets or tactoids. [37-40]

Polylactide, PLA, is one of the polymers most widely used for the preparation of biocomposites. The combination of PLA with natural inorganic solids such as clays provides reinforced biocompatible and biodegradable materials with enhanced properties (barrier, storage modulus, flexural modulus and heat distortion). Melt intercalation as well in situ polymerization are procedures employed to produce these green biocomposites that show improved thermomecanical and gas-barrier properties. The biodegradability of the reinforced PLA bioplastics depends on the nature of the layered silicates and the organic modifiers.[41] The development of biocomposites based on PLA is particularly useful to reduce waste and diminish environmental pollution by replacing non-degradable materials without compromising quality and properties that consumers demand. In this sense, incorporation of active clays with oxygen scavenging capabilities to PLA will generate active biocomposites for potential application in packaging of products sensitive to oxidation.

In this work, we report on the characterization of a zero-valent iron montmorillonite with strong oxygen scavenging capacity, and its subsequent incorporation into PLA to produce bio-based active packaging materials with oxygen absorption activity and reinforced properties compare to neat PLA.

Materials and methods

A natural montmorillonite and its corresponding zero-valent iron grade marketed as O_2Block®OS, were supplied in powder form by Nanobiomatters S.L. (Valencia, Spain). The dark black colored iron montmorillonite (Fe-MMT) exhibited a d(0,9) particle size of 25 μm, as disclosed by the manufacturer.

Polylactide 7000D, film extrusion grade, D-isomer content ca. 2%, was purchased from NatureWorks^{TM} (Minnesota, USA). The average molecular weight (Mw) is 150,000 g/mol.

Active composites preparation

Composites of PLA with 10 wt.-% natural clay and Fe-MMT, and its blank without filler, were prepared by melt mixing using a Plastograph® EC (Brabender, Duisburg, Germany). Before mixing, both PLA and clays were dried overnight in a vacuum oven at 40°C. Mixing conditions were 180ºC, 60 rpm during 3 min. Films of blank PLA and of the resulting composites were prepared by compression moulding using a Carver 4122 hydraulic press (Carver, Indiana, USA) at the same melt mixing temperature and 4000 psi of pressure, then were cooled down by quenching. Typical composite film thickness was 150 microns average.

Optical microscopy

Films samples about 3 cm x 3 cm were observed in a digital microscopy Leica DM2500M (Leica Microsystems GmbH, Wetzlar, Germany) fitted with a 12 V, 100 W halogen lamp and equipped with a Leica DFC295 digital camera attached to the microscope. Images processing and analysis were performed using the Leica Application Suite software version 3.7.0.

WAXS patterns

The structure of Fe-MMT was evaluated by wide angle X-ray scattering scans (WAXS). The diffractograms were acquired using a Bruker Advance D8 diffractometer (Wisconsin, USA), with Cu Kα filament (0.1546 nm, operating at 45 KV and 45 mA), time step of 8s at scanning rate of 0.5 °/s. The basal spacing *d* was calculated applying the Bragg´s law (eq. 5):

$$\lambda = 2d \sin \theta \qquad \text{(eq. 5)}$$

where λ is the wavelength of the radiation and θ is the measured diffraction angle.

TEM imaging

The morphology of composites was evaluated by transmission electron microscopy (TEM) using a JEOL1010 microscope (Jeol Ltd., Tokyo, Japan) equipped with a digital Bioscan image acquisition system. For clay observations, diluted clays suspensions in ethanol were prepared: two drops of suspension were poured onto a carbon coated grid to evaporation. The observations on composites were performed on ultra thin sections of microtomed composites slices. The accelerating voltage was 90 KV.

Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) of clays and the PLA composites were performed using a TGA Q500 equipment (TA Instruments, New Castle, USA) with analysis software Universal 2000 V4.4A. Samples were run from 30-1000 ºC, at heating rate of 10 ºC/min, under nitrogen flow.

Oxygen scavenging capacity

Oxygen scavenging capacity of materials was determined by measuring the oxygen content in the headspace as a function of time. Samples of 0.05 g clay were placed inside gas-tight septum-equipped vials of 40 mL capacity. The vials also contained another one of 1 mL full of water to achieve 100% RH. Each sample was measured by triplicate, and blank vials with unmodified montmorillonite were also prepared as controls. The vials were subsequently closed and placed in a room at 24 ºC. No modified atmosphere was used for this test, just atmospheric air. The headspace percentage of oxygen (%O_2) was determined using a PBI Dansensor CheckPoint (PBI Dansensor AS, Ringsted, Denmark) at different time intervals. The same procedure was followed to evaluate the activity of composites but weighing 0.5g of film.

DSC analysis

The thermal properties of composites and changes respecting neat PLA were analyzed by differential scanning calorimetry (DSC). The tests were performed on samples of approximately 5 mg in a Perkin Elmer DSC7 (Perkin Elmer Inc., Massachusetts, USA). The thermal program consisted of a first heating scan from 30ºC up to 190ºC, at a scanning speed of 10ºC/min under N_2 atmosphere. The samples were then cooled back to 30^{o}C in order to eliminate the thermal history and reheated to 190ºC at a heating rate of 10ºC/min. The thermograms were subtracted to similar runs of an empty pan.

The crystallinity (%X_c) of the PLA composites was estimated using the equation 6:

$$\%X_c = \frac{\Delta H_f}{\Delta H_f^0} x100 \qquad \text{(eq. 6)}$$

where ΔH_f is the enthalpy of fusion of the studied specimen, and ΔH_f° of 91 J/g is the estimated enthalpy of fusion of a 100% crystalline PLA-L at the equilibrium melting temperature.[42]

Water vapour permeability (WVP)

The WVP of the films was determined according to the ASTM method E96, using aluminium cells with internal and external diameters of 3.5 and 6 cm, respectively. Each sample film was sealed to a permeation cell containing liquid water, and then the permeation cells were placed under controlled environmental conditions (40 ºC and 80% relative humidity) and weighed regularly until steady-state was reached. The water vapour transmission rate was easily determined from the slope of the cell weight loss vs. time plot following equation 7.

$$WVP = (G * L) / (A * \Delta p) \qquad \text{(eq. 7)}$$

where G is the slope of weigh loss vs. time straight line, L is the film thickness, and Δp is the vapour partial pressure differential across the film. All tests were conducted in duplicate, and aluminium films were used as blanks to evaluate water vapour loss through the sealing.

Results and discussion

Characterization of Fe-MMT

One of the characteristics of the Fe-MMT is its deep opaque black color due to the content of zero-valent iron, which is consistent with previous works on iron metallic supported bentonite and kaolinite.[29, 37] The TEM micrograph of Fe-MMT (shown in Figure 1) revealed the typical spherical iron zero-valent particles as already reported, with particle size up to 150 nm, in some cases with chain-like morphology due to the inherent magnetism of particles located on the surface and also on the edges the montmorillonite platelets.[23-24, 43-44] The formation of iron dispersed nanoparticles on the surface and edges, as well as the chain-like morphology are characteristics of zero-valent iron clays.[27, 29, 37]

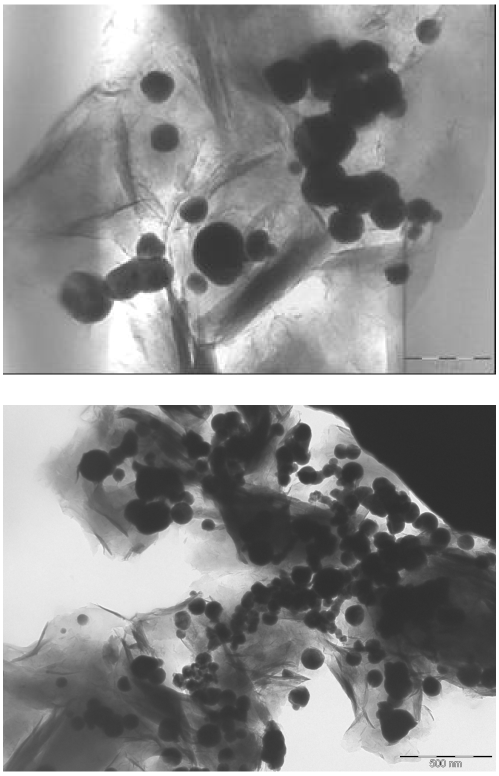


Figure 1. TEM images of Fe-MMT

The XRD diffraction patterns and scattered angles of clays are shown in Figure 2. The *d*-spacing of 0.78 nm (5.75º, 2θ) of the natural clay corresponds to the 001 reflection of MMT at normal conditions. In the case of Fe-MMT, a flat pattern (no basal peak) is evidence of some disorder of the lattices as a consequence of modification with iron. The broadening of the basal peak or its disappearance as a consequence of the iron modification was already observed in previous works.[45] The interpretation of large *d*-spacings is unclear, but they can be the result of the structural disorder produced by the penetration of iron nanoparticles into the galleries of montmorillonite.

PLA-Fe-MMT composite evaluation

Morphology and thermal properties. A typical microscopy image of the opaque and black colored PLA-Fe-MMT composite with 10 wt.-% of filler content is presented in Figure 3a. The clay particle size varied between 4 and 38 microns. The TEM micrograph of the composite (Figure 3b) exhibits tactoid-like structures where the clay is distributed but not dispersed.[7] In general, the microcomposite structure indicates that insufficient shear and the presence of iron particles may impair the complete clay platelets exfoliation during the melt compounding. A completely delaminated morphology is considered important because it maximizes the polymer–clay interactions, it may also bring legislation barriers related to the existence of nanoparticles. [46]

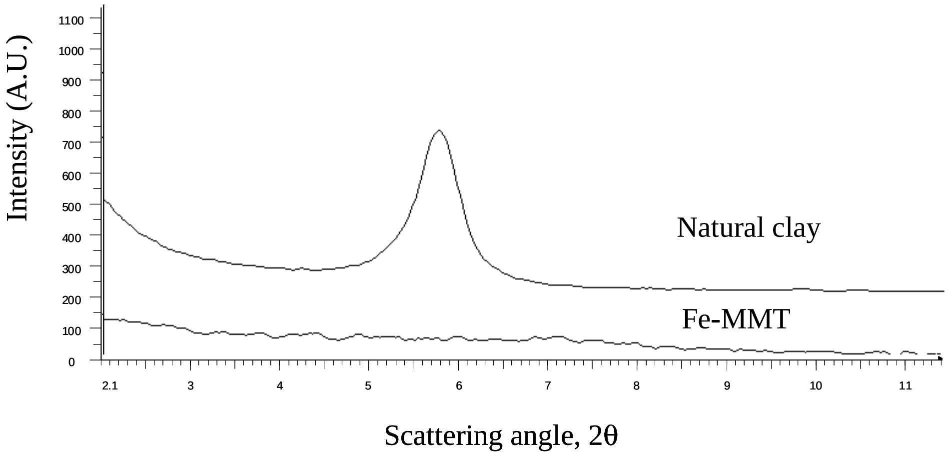
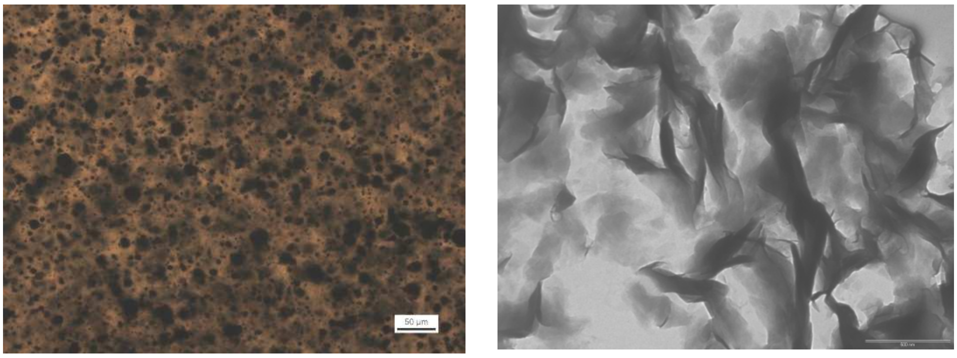


Figure 2. Powder comparative XRD patterns of natural clay and Fe-MMT



(a) **(b)**

Figure 3. Images of PLA + 10 wt.-% iron clay: (a) optical microscopy (scale marker is 50 microns); (b) TEM (scale marker is 500 nm)

The stability to thermal degradation of PLA composite containing 10 wt.-% of zero-valent iron clay was analyzed by thermogravimetric analysis (TGA) and compared with processed neat PLA and PLA composite with natural clay (Figure 4). The values for the onset of degradation are summarized in Table 1. In all cases, with a heating rate of 10 ºC/min, both unfilled PLA and its composites volatilize completely in a single step, beginning at about 331ºC for neat PLA. It can be noticed that the addition of clay can cause a reduction in the thermal stability around 4ºC due to hydrolytic and adiabatic degradation. The most important effect in the composite thermal stability was due to the presence of iron, causing the rapid degradation of PLA (more than 60°C lower than neat and processed PLA). It has been reported that iron can catalyze chain transfer, trans-esterification, anhydride formation and depolymerization reactions in PLA; and that these effects influence more drastically than moisture or hydrolysed monomers.[47-48] The very relevant degradative effect of iron on PLA could be explained by the high capacity of transition metals to coordinate ester groups and accelerate the transesterification and depolymerization reactions above 240°C. As a result, the presence of iron in the clay may promote further the reduction in the thermal stability of PLA composites.

Table 1. Data from DTG curves for PLA composites

Composite (10 wt.-% filler)	Peak Onset (°C)	Weight change (%)
Processed PLA	331.4	-
PLA+ 10 wt.-% natural clay	327.5	8.2
PLA+ 10 wt.-% Fe-MMT	269.5	10.2

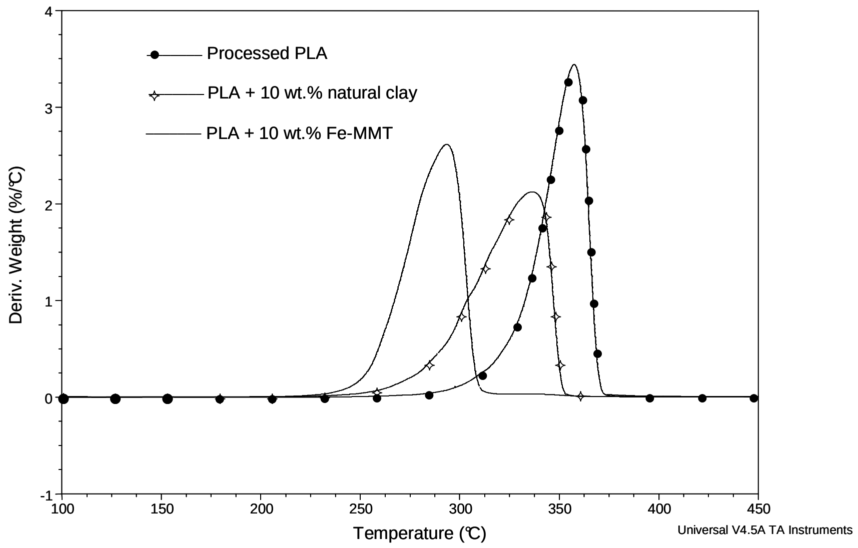


Figure 4. Comparative differential thermogravimetric curves of processed neat PLA and PLA clay composites

The effect of distributing the zero-valent iron clay and natural clay on the crystallinity and thermal parameters of PLA was investigated by DSC. Glass transition temperature (*Tg*), cold-crystallization temperature (*Tc*), melting temperature (*Tm)* and crystallinity data are summarized in Table 2. The *Tg* values were not seen to change with regard to pure PLA. The cold crystallization peak of both composites appeared at a similar temperature as in neat PLA. Cold crystallization has been reported to be more significantly influenced by a high dispersion and distribution of the filler.[49] The crystallinity values were lower than those for neat PLA, which means that Fe-MMT unlike the natural clay, does not act as a nucleating agent. Crystallization depends on the size of the inclusions, the filler dispersion and interactions between the constituents. In this case, it seems that there were some restrictions to polymer chains crystallization by adding Fe-MMT into PLA. These results indicate that the effects in physical properties are, therefore, not likely the result of crystallinity development.

Table 2. DSC transition phases and crystallinity of melt mixing PLA and its composites

Sample	**T_g (ºC)**	**T_c (ºC)**	**T_f (ºC)**	**% X_c**
Neat PLA	57.13	116.10	149.43	6.29
PLA+10 wt.-% natural clay	57.12	117.85	148.27	5.40
PLA+10 wt.-% Fe-MMT	56.61	114.10	147.10	0.54

Water vapor permeability (WVP). Biopolymers have in general several shortcomings in terms of barrier, thermal and mechanical performance in comparison to oil-based plastics currently used. The used of clay-based additives to overcome, particularly, barrier properties, is one of the most active areas of research.[50] Clay layers can constitute a barrier to gases and moisture once they are introduced into the polymer matrix. This has been shown to significantly improve barrier properties by forcing the permeant molecules to follow a tortuous path, minimizing one of the main limitations of biopolymeric films.[8, 40] The barrier properties of non-interacting gases in composites depends on the aspect ratio of the silicate particles and the extend of the distribution of these particles within the matrix.[51] The WVP of PLA composites containing iron clays (or other metals) have not been reported before, however food contact complying PLA-organomodified MMT composites were seen to exhibit a reduction of 27% in water permeability.[52] In this study, the incorporation of 10 wt.-% of the active clay into PLA was seen to lead to a reduction in the water vapour permeability between 11.35 and 12.43% (Table 3).

Active properties (oxygen scavenging capacity). The oxygen absorption activity of Fe-MMT and its PLA composite was measured by the depletion of the headspace oxygen inside vials at 100% RH (Figure 5). The natural clay did not show activity over time as expected (results not shown). As previously observed for an iron based kaolinite, the Fe-MMT showed strong absorption kinetics the first 24 hours in a high humidity environment.[36-37] The O_2 absorption volume increased very quickly to 58 mL O_2/g clay, near the saturation value, after 48 h of exposure. Complete saturation was seen after 30 days, when oxygen absorption stabilized at 62.8 mL O_2/g clay. The presence of water is required for triggering the uptake reaction, which is extremely efficient since it is expected that 1 g of iron inactivates 300 mL of oxygen in the presence of moisture.[3, 23]

The scavenging effect can be explained by the diffusion of the oxygen molecules through the clay, thus the reaction occurs when oxygen meets metallic iron centers.[3, 23] The higher kinetics exhibited during the first hours of exposure of Fe-MMT can be due to irreversible O_2 absorption by oxygen diffusion through the clay. As reaction proceeds, porosity is reduced because corrosion products (Fe_2O_3) are formed thus diffusivity of gas is consequently reduced, limiting the scavenger kinetics reaction.[4]

Figure 5 also shows the variation of the oxygen content in the headspace of vials containing PLA-Fe-MMT composites with 10 wt.-% of the active filler, as a function of time. Both neat PLA and the unmodified clay composite did not show oxygen absorption activity as expected (results not shown). The Fe-MMT composite also showed high absorption kinetics in the first 24 hours of exposure, then it was able to absorb 3.7 mL of oxygen from the head space within the first 15 days, and the stabilization occurred after 30 days, at a saturation value of ca. 4.2 mL oxygen/g composite. The absorption process is in this case controlled, among other factors, by the diffusion of moisture and oxygen through the PLA matrix, which can be limited by the intrinsic water vapour and oxygen transmission rates and the degree of crystallinity of the material. It was already established that the higher the permeability of the matrix to the diffusion of gases and moisture, the higher the absorption kinetics of the systems.[37]

Table 3. Water permeability of neat PLA and PLA-Fe-MMT composites films

Sample	**Permeability [(g*m)/(m2 *s *Pa)]**	**% Permeability reduction**
Neat PLA	1.500E-11	----
PLA+ 10 wt.-% natural clay	1.330E-11	11.35
PLA+ 10 wt.-% Fe-MMT	1.684E-11	12.43

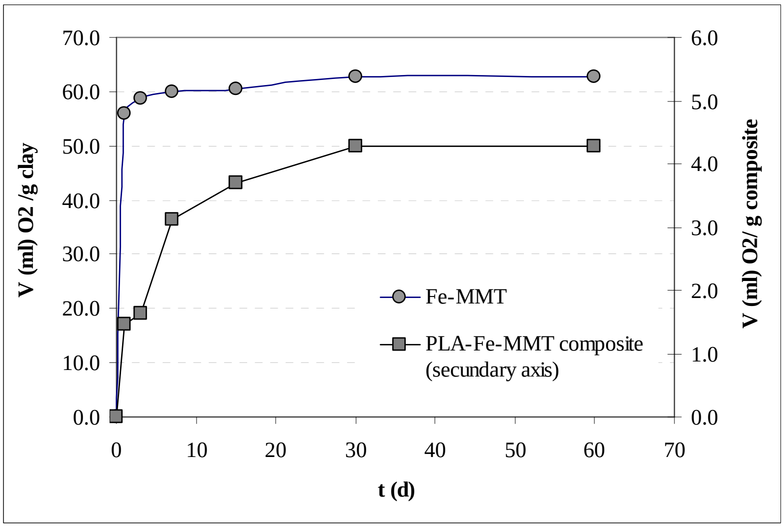


Figure 5. Oxygen scavenging behavior of Fe-MMT and its PLA composite containing 10 wt.-% of the active filler (Conditions: 100% RH; 24 ºC)

Conclusions

The morphology, thermal and barrier properties and the oxygen scavenging activity of an iron zero-valent montmorillonite (Fe-MMT) and of its PLA composite were evaluated. The Fe-MMT exhibited strong oxygen scavenging kinetics behavior, at 100% RH and 24 ºC, being able to absorb up to 62.8 mL of oxygen/g clay. The high absorption kinetics exhibited by the clay was due to the presence of zero-valent iron particles with an average particle size of 150 microns, which were seen to be primarily located inside the layers and onto the surface of the clay as observed by TEM. By adding 10 wt.-% of Fe-MMT into PLA by melt mixing, it was possible to prepare an active composite with a good clay distribution across the matrix and with strong absorptive behavior (up to 4.2 mL oxygen/g composite). The thermal stability of the PLA-Fe-MMT composite was seen to decrease by ca. 60ºC in comparison with the neat PLA, since iron has been reported to catalyze some degradation processes. Finally, the water vapor barrier was seen to increase to some extent in the composite due to the blocking presence of the laminar filler.

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7. ANTIOXIDANT POLYETHYLENE FILMS BASED ON A RESVERATROL CONTAINING CLAY OF INTEREST IN FOOD PACKAGING APPLICATIONS

Abstract

The phenolic antioxidant resveratrol, which is naturally produced by plants under stress conditions, was seen to exhibit high thermal stability and strong antioxidant capacity in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The antioxidant behavior was compared to the benchmark food contact permitted butylated hydroxytoluene (BHT). Active linear low density polyethylene (LLDPE) composites were manufactured by immobilization of resveratrol in the polymeric matrix and also by pre-incorporation of the resveratrol into a food contact permitted montmorillonite clay prior to melt mixing with the polymer. Migration tests from active films in water and isooctane showed extremely low migration levels in water (below 0.01 mg/kg) and was not detectable in isooctane. The resulting composites not only showed strong antioxidant activity, but also antimicrobial activity. The Thiobarbituric Acid Reactive Substances method (TBARS) was also applied to assess the comparative oxidative behavior in air of fresh meat in direct contact with the LLDPE film containing the active clay. The results suggested that this technology could potentially extend the shelf-life of red meat over a few days by a mechanism of free radicals trapping and the subsequent arrest in food oxidation processes.

Keywords: Free radical scavengers, resveratrol, antimicrobial, food packaging, active packaging.

1. Introduction

The oxygen and its reactive oxygen species, ROS, are responsible for most of the degradation reactions of packaging materials and food products, either directly or indirectly. Direct oxidation reactions on foodstuffs cause losses of organoleptic and nutritional properties (degradation of oils, fats, pigments and vitamins), while indirect action of O_2 includes food spoilage by aerobic microorganisms. In the case of meat and processed meat products, lipids and myoglobin oxidation in muscle foods occurs in a concurrent manner and each process appears to enhance the other (Chaijan, 2008). Oxidative deterioration myoglobin causes surface discoloration which gives an unattractive brown color, and the fat degradation causes rancidity (Nerín, 2006). Aldehydes are the major contributors to the off-flavors of meat; proteins are damaged by the action of free radicals producing the loss of their functions. Free radicals generated during the lipid oxidation, plus transition metals (if they are also present), promote the accumulation of oxidized proteins.

Ultra low oxygen environment (<0.05 wt. %) in which meat is stored is critical for the extension of shelf life and subsequent adequate blooming of the meat when exposed to oxygen (Brandon *et al.*, 2009). Packaging options such as skin vacuum packaging and low O_2 modified atmosphere packaging (MAP) with anoxic gases can significantly extend the shelf life of raw fresh meat (Zhou, Xu and Liu, 2010); however, restrict the access of oxygen inside the package is not effective if residual oxygen levels are high enough to promote food spoilage. Oxygen scavenger systems are claimed to reduce the headspace oxygen to < 0.01 wt.-% within 1-4 days at room temperature, and most of them are marketed as sachets containing ferrous oxide (Brody, Strupinsky and Kline, 2001). However, while the scavenger sachets are effective in removing oxygen, the rate of removal would appear not to be fast enough to create the anoxic conditions to prevent metmyoglobyn formation in meat (Brandon *et al.*, 2009). On the other hand, the use of sachets can create safety issues by accidental ingestion or food contamination by leakage from the sachet. In order to preserve the meat tissue, antioxidants (carotenoids and polyphenols) can be incorporated in muscle through dietary delivery (Descalzo and Sancho, 2008). In addition, when antioxidants are used, the kinetics of oxidation can be reduced even in the presence of oxygen, such as during modified atmosphere packaging of red meats. In fact, direct addition of natural antioxidants as preservatives to meat, such as carotenoids, tocopherols, extracts (i.e., rosemary, sage, oregano, thyme, ginger, and mustard) and vitamin E has been proven efficient in delaying oxidation (Shibamoto *et al*, 1995: Mitsumoto, 2000; Grün, *et al.*, 2006). Limitations in the use of additives or the low acceptance of them as well as the potential changes in flavor and odor that they produce reduce the possibilities of using preservatives, especially natural ones.

The incorporation of scavenging agents into the packaging material (i.e. polymer films) offers several advantages such as elimination of the potential safety risks of sachets, the use with retort packaging, and potential costs saving due to increased production efficiency and convenient (Brody et al., 2001). Nowadays, the food industry has shown increasing interest in the development of polymeric films with antioxidant properties (Arrua et al. 2010). In this sense, antioxidants, free radical scavengers and oxygen scavengers can be incorporated into the packaging materials to produce active materials, reduction of the oxygen and ROS effects on the products. Common synthetic antioxidants have been incorporated into packaging materials for many years. Their function is to interrupt the free radical chain reactions by reacting with ROS by hydrogen donation, hence preventing the ROS attack to the unsaturated molecules present in foods. Antioxidant materials are particularly interesting in those systems where an specific quantity of oxygen is beneficial for making some packaged products appear fresher and more desirable to the consumer, i.e., red meat fresh appearance. The antioxidants which are incorporated into plastic packaging materials could exert the dual role of protecting the polymer as well as the packaged food from oxidation (Lee, 2004). The antioxidants can be incorporated into mono or multi-layer structures (Brody et al., 2001), or coated on the packaging layer, remain stable there for the required time and finally be released from the packaging surface in a controlled manner (Lee, 2004). Flexible and thermoformable plastic packaging materials containing t-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), propyl gallate (PG), butylated hydroxyanisole (BHA), among others, have been developed and commercially applied. However, potential toxicity and carcinogenic effects of BHA, TBHQ, PG, BHT have been reported (Kahl and Kappus, 1993; Miyauchi et al., 2002) and their use in food packaging had to be restricted, as was the case with others synthetics antioxidants long used. Because of the growing concern about the use of food chemicals due to the possibility of migration of additives and of their degradation products into foods that may affect the sensory properties or even lead to toxicity, the replacement of synthetic and toxic free radical scavengers by natural compounds is thought to provide added benefits and positive consumer acceptance because they are perceived as safer, healthier and much less toxic than synthetic compounds (Lee, 2004; Pezo et al., 2007; Arrua et al., 2010; López-de-Di Castillo et al., 2010; Gülcin, 2010).

Antioxidant compounds can occur naturally in plants or can be synthetized by them as a response to oxidative stress produced by adverse environmental conditions (Inzé and Van Montagu, 1995; Michalak, 2006). Natural antioxidants coming from plants include phenolic acids, stilbenes, flavones, carotenoids, catechins, hydrolysable tannins, among others, and they form an array of antioxidants that may act by different ways, and even can have a synergistic effect between them (Shahidi, 1997; Dapkevicius *et al*., 1998). Antioxidants which contain in their structure at least one phenolic moiety with a specific arrange of hydroxyl groups and double bond conjunction that allows electron delocalization act as radical scavengers (Inzé and Van Montagu, 1995; Michalak, 2006). Resveratrol, trans-3,4,5-trihydroxystilbene, is a naturally occurring polyphenol phytoalexin in the fruits and leaves of edible plants, peanuts, mulberries, grapes and many others, but the *Polygonum cuspidatum* or Giant Knotweed root is the richest in resveratrol content (Cravotto *et al.,* 2012). Resveratrol is considered as one of the most active and bioactive antioxidant and it is characterized by its known and proven beneficial effects on the human body such as anti-cancer, antiviral, cardioprotective, neuroprotective, anti-aging, anti-inflammatory and life-prolonging effects (Gülcin, 2010; Li *et al.,* 2012; Cantos-Villar *et al.*, 2012). Several studies have shown that polyphenolic compounds, such as resveratrol, can directly scavenge molecular species of active oxygen (superoxide $\cdot O_2^-$, hydrogen peroxide H_2O_2, hydroxyl radical $^{\cdot}OH$, or singlet oxygen $^1O_2$) by donating one of their delocalized electrons or hydrogen atoms due to both their acidic nature and ability to transfer electrons while remaining relatively stable (Inzé and Van Montagu, 1995; Arora *et al.*, 1998). It was suggested that resveratrol can be used for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality, and prolonging the shelf-life of pharmaceuticals due to its proved antioxidant activity (Gülcin, 2010). In the same way, resveratrol could also reduce the adverse effects of free radicals on food during its storage and until its consumption.

The growing interest in using natural antioxidants as food additives and food packaging materials has led not only to improvement of isolation and characterization techniques of these compounds from spices, extracts and essential oils, but also their incorporation to packaging materials. Natural antioxidants from rosemary, oregano and barley husks have been extracted and immobilized by coating into polypropylene and polyethylene to manufacture active films (Nerín *et al.,* 2008; Camo *et al.,* 2008, Pereira de Abreu *et al*., 2011); cellulose acetate solutions containing L-tyrosine and L-ascorbic acid have been cast onto polypropylene substrate for producing control release systems (Gemili *et al.,* 2010); caffeic acid can be grafted to polypropylene to obtain high antioxidant films (Arrua *et al.,* 2010); EVOH composites containing quercetin, catechin and green tea extract have been manufactured by direct addition of the antioxidants during extrusion (López-de-Di Castillo *et al.*, 2010; López-de-Di Castillo 2011). In this sense, antioxidant packaging prototypes made from natural compounds are being tested to preserve quality and properties of raw meat: polypropylene films containing immobilized rosemary and oregano extract has been tested for reducing the oxidation process in fresh meat (Nerín *et al*., 2006; Camo *et al.,* 2008; Bolumar *et al.,* 2011);

phenolic compounds from barley husks have also been used to prepare an active material by coating onto a LDPE film (Pereira de Abreu *et al.*, 2011). On the other hand, the combined effect of antioxidants with other active functions such as oxygen scavenger or antimicrobials have also been tested and reported as advantageous properties of active food packaging materials. An active system made from EVOH containing iron (II) as oxygen scavenger and α-tocoferol as antioxidant showed capacity for reducing oxygen concentration in the headspace of packaging systems (Byun *et al.*, 2011); and nisin and α-tocoferol were coated onto paper using a binder medium of vinyl acetate-ethylene copolymer to prepare a dual antimicrobial and antioxidant packaging for the food industry (Lee *et al*., 2004).

The abundance and environmentally friendly character of clay minerals have advantages for their use as active agents supporting materials that allow better dispersion of these inside the polymeric matrix, thus yielding more efficient materials (Busolo and Lagaron, 2010; Busolo and Lagaron, 2012). In this sense, the use of resveratrol as an active ingredient in the development of antioxidant engineered clays for manufacturing active packaging materials is a commercial proprietary technology (Lagaron *et al.,* 2011). These systems, where clays play a role as vehicles of active compounds, present the advantage of clay availability and low cost, synergistic enhancements in the final properties of the composite matrix (i.e. thermal, mechanical and barrier properties) and good processability. These advantages, plus the active antioxidant activity, allow composite materials with interesting both passive and active properties of interest in active food packaging applications.

The current study reports on the development and characterization of resveratrol as an antioxidant component directly incorporated into melt compounded active films of interest in food packaging applications, both by direct addition and supported on clays. The study reports on the characterization of the thermal, antioxidant and antimicrobial properties of the composite materials as well as on the antioxidative properties in contact with fresh red meat.

2. Materials and Methods

The natural antioxidant compound, resveratrol 98%, extracted from the *Polygonum Cuspidatum* root, was purchased from Solchem (Barcelona, Spain). A natural montmorillonite (MMT) and its corresponding experimental grade containing resveratrol (O_2Block® series), from now on RS-MMT were supplied by Nanobiomatters S.L. (Paterna, Valencia, Spain). The RS-MMT exhibited an average particle size of 6 microns. The commercial antioxidant butylated hydroxytoluene (BHT), provided by Guinama (Valencia, Spain), was also tested for comparative purposes.

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), thiobarbituric acid and isooctane (density 0.69 g/mL), all reagent grades, were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Trichloroacetic acid, methanol and formic acid HPLC grade were purchased from Panreac (Barcelona, Spain). MiliQ water was used for migration tests. Linear low density polyethylene (LLDPE) Dowlex NG5056G from DOW (USA) was used to manufacture the corresponding composites.

2.1 *Composites preparation*

Two grades of active composites of LLDPE with resveratrol were manufactured. The composite C1 (1 wt.-% resveratrol) was obtained by direct addition of resveratrol to the polymer. The composite C2 was prepared by incorporation into the polymer of a suitable amount of RS-MMT in order to achieve 1 wt.-% of the final content of resveratrol. The two antioxidant composites were manufactured in a co-rotating twin screw extruder (Berstorff, Hannover, Germany) at 180ºC (flat profile). Composite streams were cooled down in a quenching bath, and most of the water carried along the filaments was wiped by deflecting rods placed immediately after the bath. Residual moisture was removed by evaporation with air blowers. Composite strands were chopped into small granules using a pelletizer. Finally, pellets were maintained in a high barrier plastic film bag (PET/aluminum foil/LDPE) under vacuum. Neat LLDPE was also processed under the same conditions and used as a blank.

Films from processed LLDPE and the active composites were manufactured in a monolayer cast film line machine E25P (Collin, Munich, Germany) at 180ºC. Typical film thickness was 60 microns.

2.2 *Optical properties*

The color of the composite films was determined with a Konica Minolta CM-600d spectrophotometer with Color Data software CM-S100W. To obtain the parameters *L**, *a**, *b**, the films samples were placed on a standard white surface. Two samples of each film were measured in triplicate.

The films were also observed in a digital microscopy Leica DM2500M (Leica Microsystems GmbH, Wetzlar, Germany) fitted with a 12 V, 100 W halogen lamp and equipped with a Leica DFC295 digital camera attached to the microscope. Image processing and analysis were performed using the Leica Application Suite software version 3.7.0.

2.3 *Morphology evaluation*

The structure of the RS-MMT and its intercalation degree in the LLDPE was evaluated by X-ray diffraction, XRD. The diffractograms were acquired using a Bruker Advance D8 diffractometer (Wisconsin, USA), with Cu Kα filament (0.1546 nm, operating at 45 KV and 45 mA), time step of 8s at a scanning rate of 0.5 º/s. The basal spacing *d* was calculated applying the Bragg´s law (eq. 1):

$$\lambda = 2d \sin \theta \qquad \text{(eq. 1)}$$

where λ is the wavelength of the radiation and θ is the measured diffraction angle.

2.4 *Thermogravimetric analysis (TGA)*

Thermogravimetric analysis was employed for evaluation of the thermal stability of the samples, determination of resveratrol content in the modified montmorillonite and, for confirming the inorganic filler content in the composite C2. For these purposes, a TGA Q500 equipment (TA Instruments, USA) with analysis software Universal 2000 V4.4A was used. Analysis of pure antioxidants, RS-MMT and blank and antioxidant LLDPE films were carried out under nitrogen flow, from 30-800 ºC, at a heating rate of 10 ºC/min.

2.5 FT-IR analysis

Fourier Transform Infrared spectroscopy (FT-IR) was applied to evaluate the concentration of resveratrol in the composite C1. The FT-IR spectra of pure resveratrol was collected at 24ºC and 40%RH by coupling the ATR accessory Golden Gate of Specac Ltd. (Orpington, UK) to a FTIR Tensor 37 equipment (Bruker, Rheinstetten, Germany). Based on the absorptive features, the band at 1010 cm^{-1} was chosen for further concentration evaluation in the composite C1.

The Beer-Lambert law was applied,

$$A = \varepsilon \; l \; c \qquad \text{(eq. 2)}$$

where A is the absorption of the sample at 1010 cm^{-1} and l and c are the thickness of film and the concentration of resveratrol in the composite, respectively. The extinction coefficient ε of resveratrol was determined by the slope of the straight line A/l as a function of c, plotted from the absorption data vs. standard concentrations.

The calibration line was plotted from absorbance data of blank LLDPE and corresponding composites containing 0.1; 0.5 and 1 wt.-% of resveratrol (M1, M2 and M3, respectively). These were prepared by melt mixing, using a Brabender Plastograph® EC (Duisburg, Germany). Mixing conditions were 170ºC, 100 rpm during 3 min. Films of these composites and from C1 pellets were obtained by compression moulding using a Carver 4122 hydraulic press (Indiana, USA) at the same melt mixing temperature and cooled down by quenching. Film thickness in each case was measured by using a thickness gage Mitutoyo Absolute (Mitutoyo, Japan). Typical film thickness was 150 microns.

The FTIR spectra of blank LLDPE, M1, M2, M3 and C1 samples were collected in a FTIR Tensor 37 equipment (Bruker, Rheinstetten, Germany). The Beer-Lambert law was applied, using the resveratrol absorption band at 1010 cm^{-1}.

2.6 Free radical scavenger capacity

The DPPH inhibition assay (Yen and Duh, 1994) with some modifications was used for evaluating the free radical scavenging activity of the antioxidant compounds (BHT and resveratrol), the clay RS-MMT and, of the films. Samples were weighed by triplicate in cap vials, then an aliquot of DPPH solution (0.05g/L in methanol) was added to each one. Vials without samples were also prepared as controls. Table 1 shows the weight of sample used in each case to allow a maximum resveratrol content of 1,000 ppm, depending on the nominal content of the antioxidant in the sample.

All the samples were prepared and immediately stored at room temperature during 24 h in darkness. After this the absorbance of the solution was measured at 517 nm in a UV-VIS Helios Omega spectrophotometer (Thermo Scientific, USA). Samples containing the clay were previously centrifuged, while for the samples containing the films, these were removed from the solutions. Results were expressed as % inhibition to DPPH, as follows:

$$\% \, Inh \, DPPH = (1 - (A_{sample}/A_{control})) \times 100 \qquad \text{(eq. 3)}$$

Table 1. Sample weight for DPPH assays

Sample	Sample weight (mg)	V (mL) DPPH solution	Maximum concentration of antioxidant (ppm)
Resveratrol; BHT	0.1; 1.0; 10	1.0	100; 1,000; 10,000
RS-MMT	11	3.0	1,000
C1	100	1.0	1,000
C2	39	1.0	1,000

2.7 Antimicrobial tests

Susceptibility tests of LLDPE, C1 and C2 films were performed following the standard method JIS Z2801 (ISO 22196). The tested microorganism was *S. aureus* (CECT 86T, ATCC 12600). Each piece 5x5 cm^2 was inoculated with ca. 1×10^5 CFU and incubated at 37ºC, 100% RH, during 24 hours. Viable cells were determined by the agar plate count method. Four specimens of each type of sample were tested.

The antimicrobial activity value of the samples tested by the Standard method JIS Z 2801 was determined through the following equation:

$$R = \log (B/C) \qquad \text{(eq. 4)}$$

Where B is the average of the number of viable cells of bacteria on the untreated test specimen after 24 h, and C is the average of the number of viable cells of bacteria on the antimicrobial test piece after 24 h. If $R \geq 2.0$, the sample is considered to exhibit biocide properties.

2.8 Migration tests

Migration tests from blank and C2 films were performed following the European Standard Method UNE-EN 13130-1 (AENOR, 2005). Water and isooctane were selected as food simulants for aqueous and fatty foods, respectively, and migration of resveratrol to each simulant was tested in accordance with the standard method (see Table 2). The samples (film pieces of 6 dm^2) were totally immersed in the simulants contained in rounded bottom glass vials of 120 mL capacity, vials were then closed with glass caps and stored in a thermostatic oven at the required temperature during specific time intervals. Each sample was evaluated in triplicate. After completing the exposure time, film samples were removed from the liquids, and the simulants analyzed. The determination of resveratrol in food simulants was performed using an Aquity UPLC® system with a triple quadrupole detector (Waters, Mildford, USA). The column was a Mediterranean Sea 18 (5µm, 10 cm x 0.21 cm; Teknokroma, Valencia, Spain). The mobile phase consisted on a mixture of eluent A (formic acid 0.1%) and eluent B (methanol). The analysis was performed using the following program of eluents: 0-5 min from 0 until 30% eluent B; 5-15 min, from 30 until 95% eluent B; 15-30 min; from 95 until 30% eluent B. The separations were carried out with a flow rate of 0.3 mL/min.

Table 2. Tested simulants and conditions in the migration tests

Simulant	Simulant volume (mL)	Temperature (ºC)	Time (days)
Water	100	40	10
Isooctane	100	20	2

2.9 Evaluation of antioxidant activity of the active films on beef. TBARS method

Antioxidant activity tests were performed by using active film C1 and comparing with blank LLDPE film. Fresh beef meat steaks of about 20 g were packaged individually and aerobically into bags made of LLDPE (control) and C2 films. The bags were inserted in glass jars with airtight closure, and stored at 4 ºC. Each film was used to prepare triplicate samples, and each of them was evaluated in duplicate. Samples were removed after 11, 14 and 17 days to follow up the progress of oxidation.

The thiobarbituric acid index (TBARS) was determined according to a method described elsewhere (Vyncke, 1970). TBARS is expressed as mg malonaldehyde/kg sample. A sample of 20 g of meat was homogenized with 100 mL of trichloroacetic acid 7.5 wt.-% for 1 min. The suspension was then filtered and 5 mL of the filtrate were added to 5 mL of TBA reagent (0.02 M 2-thiobarbituric acid). The mixture was immersed in a boiling water bath for 40 min, then cooled with water and the absorbance was measured at 538 nm.

3. Results and discussion

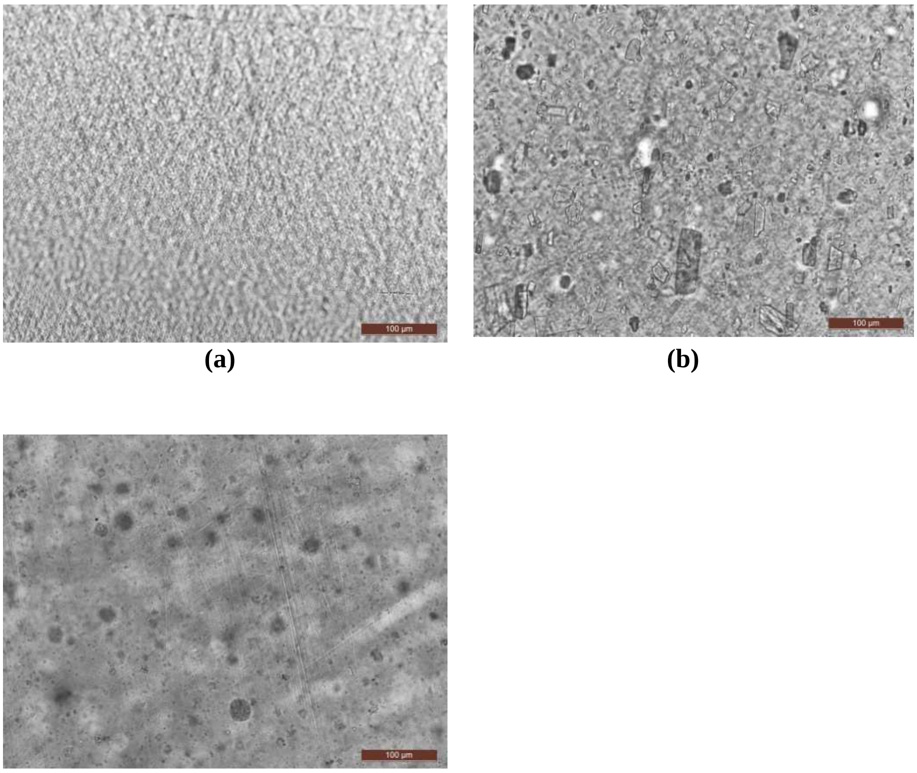
3.1 Optical properties of LLDPE films

The resveratrol was an off-white powder, while the resveratrol containing clay, RS-MMT, exhibited the typical creamy color of the bentonite clays. Once resveratrol was loaded into LLDPE (C1), a transparent film but with a slight brownish appearance was obtained. Both C1 and C2 films were homogeneous as observed by visual inspection, which indicates that the additives were sufficiently well distributed during the extrusion process. The color parameters of the extruded films are presented in Table 3. The luminosity was more affected by the incorporation of RS-MMT than for the case of resveratrol, as can be seen from the decrease of the L^* values. The a^* values for C1 and C2 were almost the same, while the higher rise of the b^* value was obtained in C2, which exhibited a deep brownish tone.

This type of coloring has been associated in the prior literature to caramelization reactions of sugars that remain in antioxidants as impurities, and also by the partial polymerization of phenolic compounds (López de Dicastillo *et al.*, 2011). However, in composites this coloration is typically associated to the usage of clays as fillers.

Table 3. Color parameters of LLDPE composites films

Sample	L*	a*	b*
Standard white surface	94.64	1.54	-6.84
LLDPE blank	94.16 ± 0.06	1.45 ± 0.01	-6.33 ± 0,02
C1	91.37 ± 0.15	1.58 ± 0.02	-1.93 ± 0.32
C2	81.71 ± 0.28	3.02 ± 0.04	8.83 ± 0.51



(a) **(b)**

(c)

Figure 1. Top view optical microscopy images of LLDPE (a), C1 (b) and, C2 (c) films

The optical microscopy pictures taken in the composites are shown in Figure 1. The pictures of the LLDPE films were taken using 200x magnification in order to obtain both a qualitative measure of both the distribution of resveratrol in the bulk polymer (for C1) and of the clay in C2. Figure 1a shows the typical appearance of the blank LLDPE extruded film; in the Figure 1b the resveratrol appears as a thin, translucent, tetragonal or bar-shaped crystals that can be up to 80 microns long. These kind of crystalline structures are typical of resveratrol (Zhang *et al.*, 2012). A possibility is that resveratrol was added at levels above its intrinsic solubility in the molten polymer, thus a saturated solution was produced in the compounding process (Koontz *et al.*, 2010), and partial precipitation of crystals occurred during quenching. The C2 film (Figure 1c) shows uniform distribution of the RS-MMT, even though the clay shows some aggregates of up to 35 microns. It is worth noting that clays during compounding reduce size most typically in the thickness direction.

3.2 Morphology evaluation

The degree of intercalation of resveratrol in RS-MMT, as well as the structure of the C2 composite were assessed using X-ray techniques. Moreover, the blank film of LLDPE and the composite C1, where resveratrol was directly added, were also evaluated for comparative purposes. Figure 2 displays the X-ray diffraction patterns of resveratrol, of the natural clay and of the RS-MMT clay. Resveratrol was highly crystallized and showed intense and sharp reflections between 6 and 20º, being the most intense that at 6.6º (2θ) (Zhang *et al.*, 2012). No impurities of crystalline nature were detected. The basal peak of the natural clay (5.76 º, 2θ) shifted to 6.05º and became less intense after modification with resveratrol. A lack of expansion of the interlayer space may result by the predominant adsorption of resveratrol onto the clay surface (Lagaly *et al.*, 2006); albeit changes in the clay structure which cause narrowing of the basal distance can be also related to the modification process itself or, to the presumable loss of water from interlayer hydration complexes (Brigatti *et al.*, 2006).

The Figure 3 illustrates the differences in the XRD patterns related to composite processing. Even though optical microscopy evidenced crystalline material, no resveratrol reflections were observed in the diffractogram of C1, possibly due to its low concentration (ca. 1 wt.-%). In the case of C2, which contains RS-MMT, the patterns of the LLDPE were also reproduced, but the one corresponding to the clay was shifted towards higher angle (6.4º, 2θ), which suggests lack of polymer intercalation.

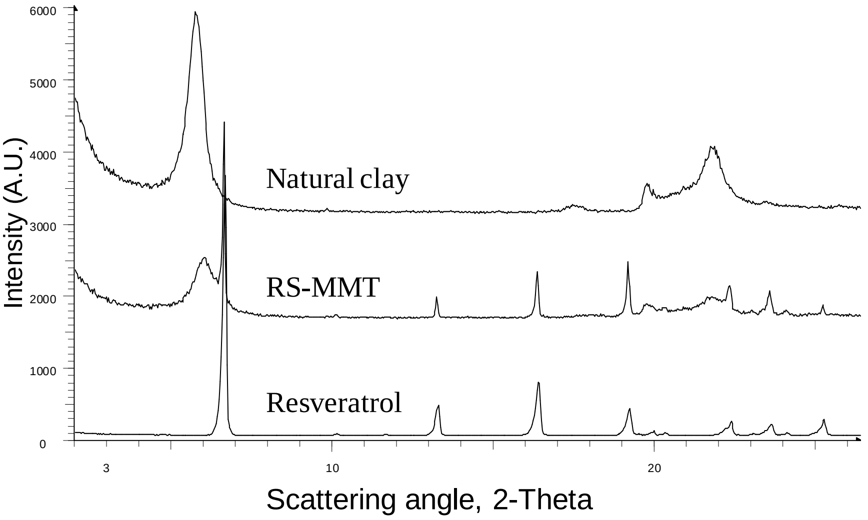


Figure 2. Comparative WAXS patterns of the natural clay, resveratrol and RS-MMT

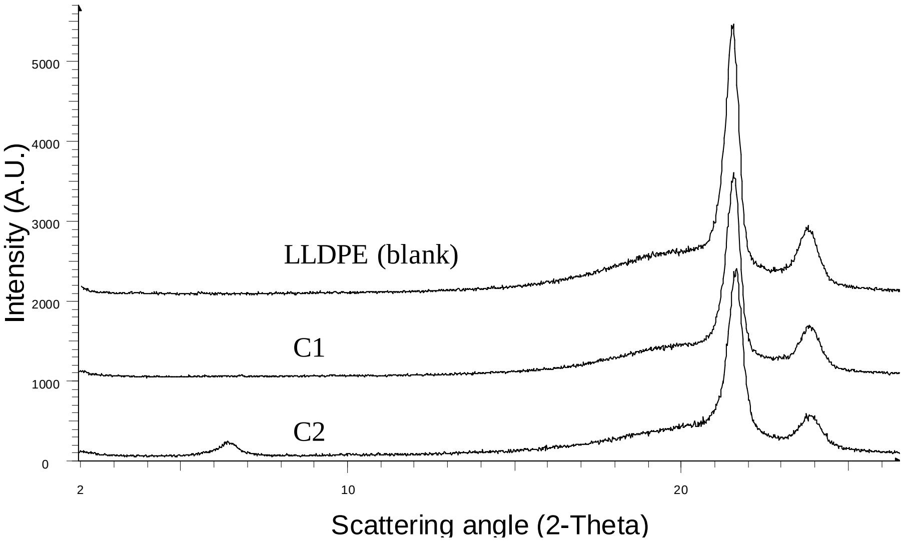


Figure 3. Comparative WAXS patterns of LLDPE and of the composites C1 and C2

3.3 FTIR analysis

The FT-IR spectrum of resveratrol (Figure 4) presented characteristic bands such as a broad feature corresponding to the OH stretching (3190 cm^{-1}) and these of the C-C aromatic double bonds stretching at 1605 cm^{-1}, CH bending at 1385 cm^{-1} and ring stretching at 1010 cm^{-1}. These features agreed with the already data reported (Kumpungdee-Vollrath and Ibold, 2012; Zhang *et al.*, 2012). The ring stretching band at 1010 cm^{-1} was selected to confirm the active concentration in the C1 composite.

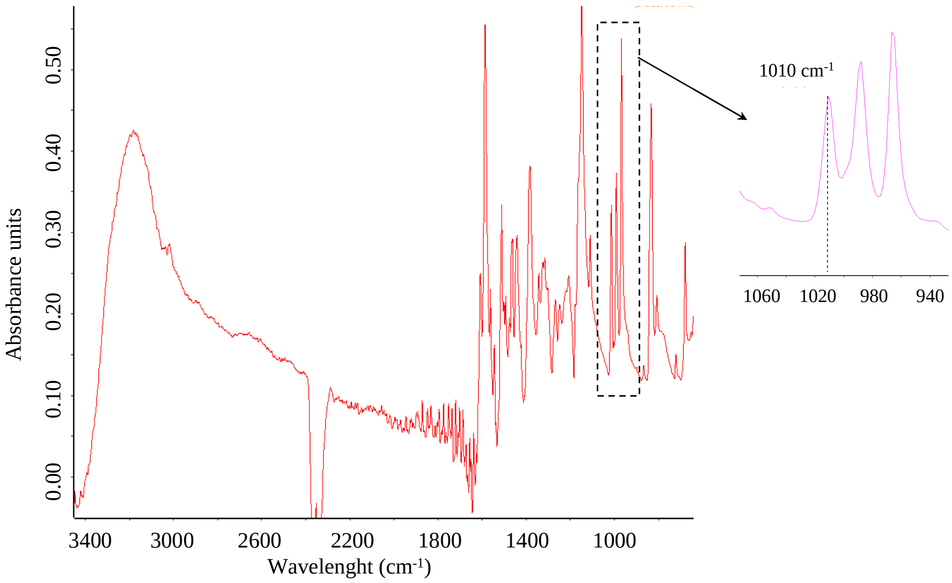


Figure 4. FT-IR spectrum of resveratrol. The inset shows the absorption band at 1010 cm^{-1}, wavelength selected for concentration determination

Table 4 shows the content of resveratrol, thicknesses and absorbance of the films prepared from composites M1, M2, M3 and composite C1. A value of the extinction coefficient, ε=0.7298 [1 /mm %] was obtained from plotting the data in Table 4. By applying the eq. 2, a resveratrol concentration in film C1 of 1.19 wt.-% was inferred, which is in good agreement with the intended loading. This method could not be applied for evaluating the composite C2 because absorptive band of the clay overlapped with those of resveratrol (results not shown).

Table 4. Characteristics and absorption data (at 1010 cm^{-1}) of resveratrol containing composites

Sample	Concentration of resveratrol, *c* (wt.-%)	Thickness, *l* (mm)	Absorbance, *A*	*A/l* (mm^{-1})
M1	0.1	0.173	0.00311	0.0179
M2	0.5	0.224	0.08172	0.3648
M3	1	0.196	0.14412	0.7353
C1	1.19	0.060	0.05210	0.8683

3.4 Thermal stability of antioxidants and active composites

The purpose of the thermogravimetric analysis was to evaluate the thermal resistance to degradation of the antioxidants under an inert atmosphere. The DTG curves of the pure antioxidants (Figure 5) show that BHT degrades at 166ºC whereas resveratrol degrades at 283.6 ºC. These differences in stability can be associated to the molecular structure of the antioxidants: BHT only has one bencenic ring while resveratrol is stabilized by two conjugated bencenic rings with a double bond (see molecules inserted in Figure 5).

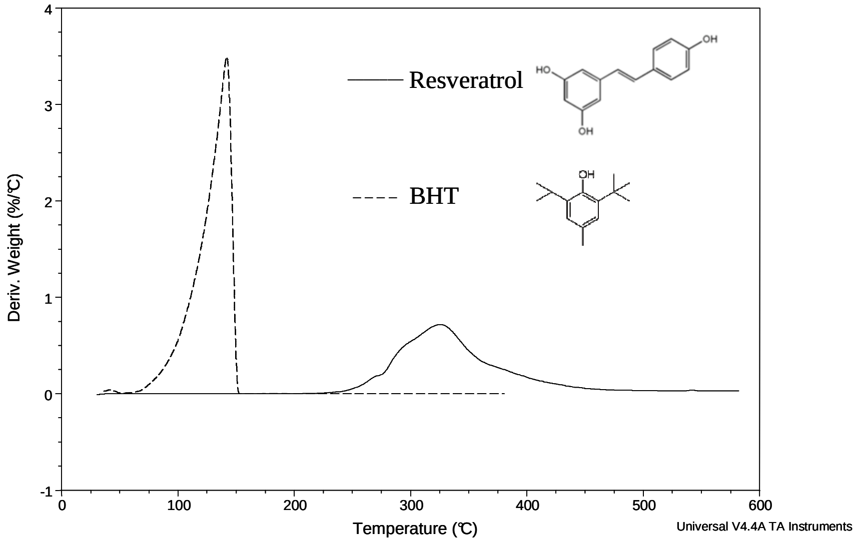


Figure 5. Comparative DTG of resveratrol and BHT (the antioxidants molecular structure are displayed as an inset in the graph)

The resveratrol modified montmorillonite RS-MMT contained ca. 25 wt.-% of resveratrol, as indicated by the weight loss between 150-450 ºC (thermogram not shown), which is the temperature range where organic compounds decomposed. The resveratrol contained in the clay degrades almost at 280 ºC (see Table 5). The thermal stability of antioxidants supported in clays can depend on whether the molecules are completely inserted into the galleries of the mineral or adsorbed on the surface of the clays sheets. The high thermal stability exhibited by resveratrol and its corresponding modified clay constitutes an advantage for composite processing purposes in comparison with more thermally unstable additives which can be easily decomposed.

The thermal stability of both C1 and C2 film samples increased by incorporating resveratrol (see Table 5 and Figure 6), which was expected since resveratrol can exert an antioxidant role in the materials such as other commercially available phenolic antioxidant for polyolefins (Morlat-Therias *et al*., 2005; Ritter *et al*., 2005). The weight loss of the active composite film C2 indicates a content of clay of 3.4 wt.-% (Figure 6), corresponding to a 0.85 wt.-% of resveratrol. The presence of the clay seemed to reduce somewhat the antioxidant effect of resveratrol in comparison with C1 during melt compounding. It was already reported that phenolic antioxidants can be less active in the presence of distributed clay due to adsorption (Morlat-Therias *et al.,* 2005). Nevertheless, the purpose of this technology is to retain the antioxidant capacity of the active compound nor for the processing of the polymer but for shelf-life extension of the food in direct contact with it, and hence the clay exerts a protective role of preserving more intact the antioxidant properties of the compound beyond processing.

Table 5. Degradation onset (in N_2) of RS-MMT clay and active composites

Sample	Onset (ºC)
BHT	117.39
Resveratrol	283.64
RS-MMT	279.79
LLDPE	451.82
C1	463.03
C2	457.82

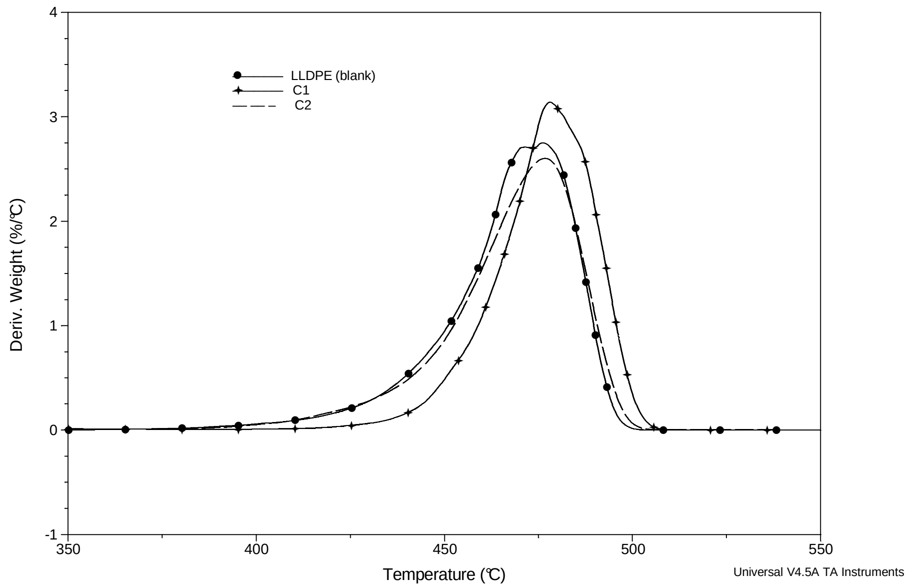


Figure 6. DTG of LLDPE composites C1 and C2, made from resveratrol and RS-MMT, respectively

3.5 Antioxidant capacity of natural substances and composites

The radical-scavenging activity of the materials was assessed by means of DPPH assays in order to compare the activity of resveratrol against BHT. Figure 7 shows that resveratrol and BHT exhibit a comparable antioxidant activity above 85% at 1,000 and 10,000 ppm. These concentrations are very high and differences between the two antioxidants were not significantly different. However, at concentrations of 100 ppm, resveratrol showed to be two-fold more active than BHT. These results agreed with those previously reported, where the activity of low molecular phenolic antioxidants is generally lower than that of multiple biphenyl groups compounds (Okuda, 1997). It is well known that phenolic groups stabilize a radical formed on a phenolic carbon with their resonance structure. Resveratrol has two phenolic rings and hence, the superior performance (see Figure 8).

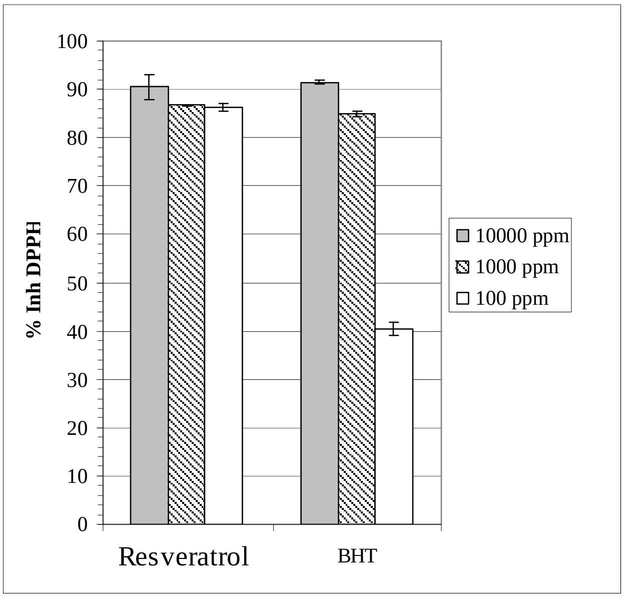


Figure 7. Comparative free radical scavenging activity of resveratrol and BHT, at 100-10000 ppm range concentration

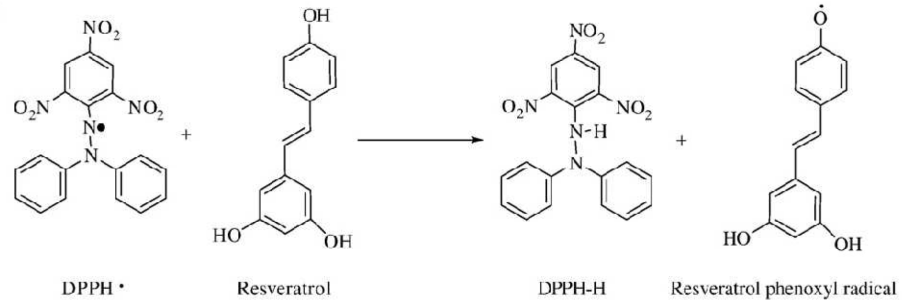


Figure 8. The reaction between DPPH free radical and resveratrol

The clay containing resveratrol shows high inhibition against DPPH (87%), as well as the composites C1 and C2 (Table 6). However, there is a matrix effect by which the antioxidant is less effective inside the composites. The matrix effect can be related to, among others, permeability, polarity differences between solvent and polymer, and moisture levels. Even though, the % inhibition to DPPH of both composites is very high, the C1 sample (film containing neat resveratrol) is slightly less effective than its counterpart C2. This little difference if statistically significant can be possibly explained by the better distribution of the antioxidant across the matrix because the clay acts as a dispersing vehicle. The use of antioxidant containing clays can allow not only to impart active properties to polymers but also to enhance the activity of the agent. In addition to this, passive barrier enhancements to the transport of low molecular weight components due to the presence of impermeable clays can also occur (Paul and Robeson, 2008).

Table 6. Comparative inhibition to DPPH of RS-MMT and of composites made from resveratrol and RS-MMT

Sample	% Inhibition to DPPH
Natural clay	1.2 ± 0.2
RS-MMT	87.3 ± 2.6
Blank film	17.4 ± 3.7
C1	78.8 ± 6.1
C2	81.5 ± 7.0

3.6 Antimicrobial activity of antioxidant substances and composites

Many of the antioxidants substances with high content of polyphenols also exhibit antimicrobial properties that are valuable for producing active packaging (Kubo *et al.*, 1992; Almajano *et al.*, 2008; Camo *et al.*, 2008; Pyla *et al.*, 2010; Siripatrawan and Noipha, 2012; Li *et al.*, 2012a; Ramos *et al.*, 2012). The polyphenols are capable of bactericidal activity by inhibition of DNA and RNA synthesis in the bacterial cells, inhibition of cytoplasmic membrane function of bacteria and interfering with energy metabolisms of bacteria (Siripatrawan and Noipha, 2012). Resveratrol has also shown to exert antimicrobial capacity against *S. Aureus, E. faecalis, P. aeruginosa* (Chan, 2002), and inhibition to filamentous fungi and yeast such as *A. Niger, P. expansum and S. cerevisiae* (Filip *et al*., 2003).

The antimicrobial activity was evaluated in LLDPE blank films and in the active films C1 and C2, to assess the effect of immobilization of resveratrol into a polymeric matrix and when it is supported in a clay as a vehicle for dispersion. The results pointed out that the antioxidant films also exhibit antimicrobial capacity, as shown by the *R* values gathered in Table 7. In addition to confirm the antimicrobial activity already determined for resveratrol (Chan, 2002) and the observations of the high antimicrobial activity observed in samples with high polyphenol concentration and antioxidant activity (Almajano *et al*., 2008), it can be concluded that there is no difference in antimicrobial activity whether the resveratrol is in the composite or supported on the clay.

The incorporation of natural antioxidants such as green tea extract in polymers have allowed to obtain antimicrobial-antioxidant packaging for food preservation (Pyla *et al.*, 2010; Siripatrawan and Noipha, 2012; Li *et al.*, 2012a), however, to the best of our knowledge the use of modified clays with antimicrobial/antioxidant polyphenolic agents for active nanocomposite manufacturing has not been described before. The only references of antioxidant supported clays preparation are the incorporation of glutathione in montmorillonite for drug delivery (Baek *et al*., 2012) and ferulic acid in hydrotalcite for preserving cosmetic formulations (Rossi *et al*., 2005). The described results show that the active agent is supported or modifying the clay and this does not affect its antimicrobial capacity. In fact, the clay acts as an inert vehicle to distribute the active agent across the matrix; thus, the flexibility to incorporate the active agent into the matrix is an additional advantage of these kinds of systems.

Table 7. Growth of *S. aureus* in the presence of blank LLDPE and active LLDPE composites

Film	CFU/mL	R
LLDPE (blank)	1×10^8 1×10^8 1×10^8	-3.0 ± 0.2
C1	10 10 0.01	5.0 ± 0.0
C2	10 10 0.01	5.0 ± 0.0

3.7 Migration tests

Table 8 displays the results obtained when food simulants were analyzed after migration tests. Resveratrol was not detected in the water and isooctane in contact with blank LLDPE, as expected. The LLDPE is most likely to contain some phenolic antioxidant additives as antioxidants and process aids (Morlat-Therias *et al*., 2005; Ritter *et al*., 2005), however it does not contain resveratrol as expected. The migrant concentration was not detected in isooctane when exposed to the C2 films and was only detected to the very low amount of 9.75×10^{-3} mg/kg in contact with water. This value can be actually considered very low or negligible. In spite of this, it should be noted that some foodstuffs, specifically red wines, can contain up to 14 ppm of resveratrol (Fernández-Mar *et al.*, 2012) and that, it has already been proven that this polyphenol has wide beneficial effects on health (Gülcin, 2010; Li *et al.*, 2012b; Cantos-Villar *et al.*, 2012) upon consumption.

Resveratrol is currently not listed in, for instance, the Commission Directive (EU) Nº 10/2011 (European Commission, 2011) for food contact applications. Nevertheless, other similar stilbenes currently listed such as 4,4′-bis(2-benzoxazolyl)stilbene has specific migration limits (SML) of 0.05 mg/Kg. In this case, the active film C2 contains a migration value for the active component that is well below this SML value. Additionally, the currently used compound does well fit the spirit of the new EC legislation regarding active food packaging (European Commission, 450/2009).

Table 8. Concentration of resveratrol in water and isooctane after migration tests

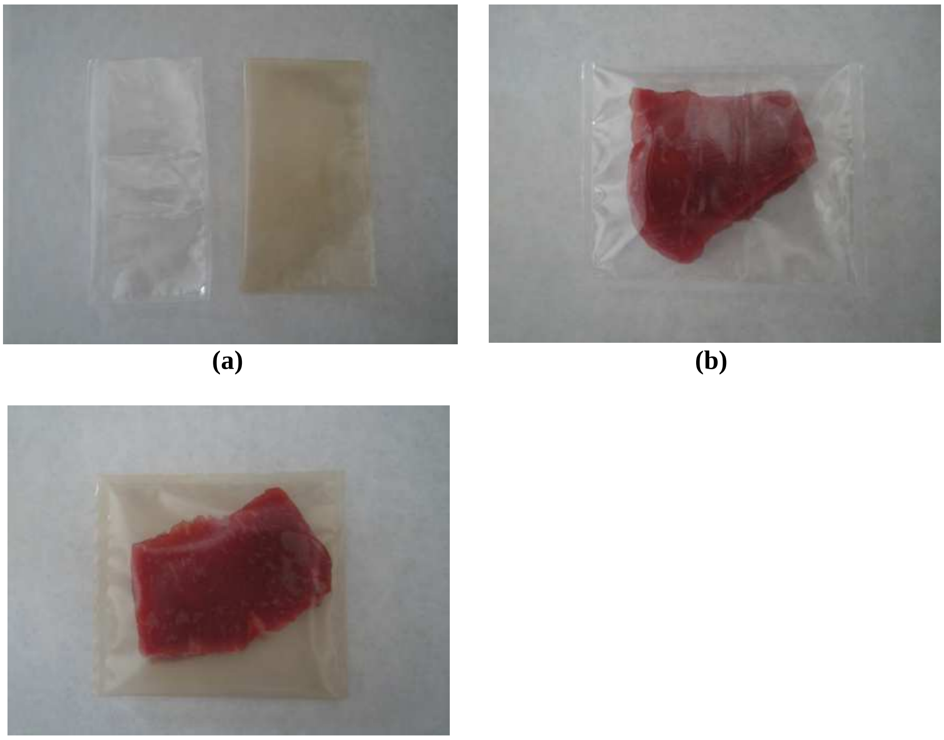
Sample	Food Simulant	Migration (mg/kg simulant)	Average migration (mg/kg simulant)
LLDPE blank	Water	$<1.45 \times 10^{-4}$	$<1.45 \times 10^{-4}$
		$<1.45 \times 10^{-4}$	
		$<1.45 \times 10^{-4}$	
C2		7.60×10^{-3}	9.75×10^{-3}
		10.78×10^{-3}	
		10.86×10^{-3}	
LLDPE blank	Isooctane	$<2.10 \times 10^{-4}$	$<2.10 \times 10^{-4}$
		$<2.10 \times 10^{-4}$	
		$<2.10 \times 10^{-4}$	
C2		$<2.10 \times 10^{-4}$	$<2.10 \times 10^{-4}$
		$<2.10 \times 10^{-4}$	
		$<2.10 \times 10^{-4}$	

3.8 Evaluation of the antioxidant activity of an active film in contact with beef meat

As mentioned above, the film containing RS-MMT (C2) was translucent but with a slight brownish color compared to the blank LLDPE (see Figure 9).

The lipid oxidation in fresh beef meat was studied following the TBARS method, based on the quantification of thiobarbituric acid reactive substances from reaction with malonaldehyde (Shibamoto *et al.,* 1995). The changes in the concentration of malonaldehyde/kg sample in beef meat stored at 4 ºC are displayed in Table 9. The concentration in the samples packed with blank films increased constantly during 17 days up to a maximum value of 4.72 mg/kg sample; this increase is probably due to oxidative degradation. However, the use of the antioxidant film showed strong differences with respect to blank films. The malonaldehyde concentration values were significantly lower in the samples that were packed in active films C2, with a maximum oxidation reduction of 34.7%, which indicates that the antioxidant contained in the film acted as free radical scavenger, thus ending oxidation at the initial stage and avoiding further formation of new radicals by chain oxidation processes. From these results, it could be inferred that shelf-life of fresh meat could potentially be extended by few days using the current antioxidant film as packaging material. Nevertheless, further assays (i.e. under modified atmosphere conditions, specific food quality attributes and sensory analysis to consider consumers acceptance) must be performed to quantify shelf-life extension.

The mechanism of action of antioxidant packaging films is not yet well understood. It has been hypothesized, that either antioxidant molecules can migrate from the active packaging film to the meat or some oxidant molecules from the meat can diffuse onto the active film (Camo *et al.*, 2008). Migration of the antioxidant from the C2 film into water was already determined to be very low, i.e. 0.01 mg/kg, suggesting that it could be a surface contact effect whereby both processes take place simultaneously.



(a) **(b)**

(c)

Figure 9. Comparative images of tested films

Table 9. Changes in concentration of thiobarbituric acid reactive substance, TBARS (average of mg malonaldehyde/kg sample) in samples of beef meat steaks stored at 4ºC at various time intervals.

Time (days)	Blank film	C2 film
0	0.264 ± 0.078	0.264 ±0.078
11	3.20 ± 0.43	2.78 ± 1.33
14	4.27 ± 0.31	2.79 ± 0.44
17	4.72 ± 0.45	3.86 ± 0.36

4. Conclusions

The natural antioxidant resveratrol has exhibited higher resistance to thermal degradation and inhibition to DPPH than the commercial antioxidant BHT. Resveratrol showed strong antioxidant capacity both in free form but also incorporated into a montmorillonite clay. Active composites, with minimal impact in color and transparency, were prepared by direct immobilization of the antioxidant and of the active clay into the polymeric LLDPE matrix. Both composites showed strong antioxidant and also antimicrobial effect; in addition, the active film containing the antioxidant clay seemed to be able to reduce oxidative processes in direct contact with a read meat product. Migration tests conducted in water and isooctane as food simulants evidenced negligible or very low levels (below 0.01 mg/Kg) of the compound, which means that resveratrol more directly acts in direct food contact. The use of a natural antioxidant agent such as resveratrol supported on protective clay additives can maximize shelf-life of food products prone to undergo rapid oxidation, and can potentially constitute very suitable candidates as additives for active food packaging designs and applications.

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8. GENERAL DISCUSSION

This work was designed to prepare and characterize novel active materials, both based on biodegradable and oil-based polymers, coming from clays with antimicrobial, oxygen scavenger and antioxidant properties, for potential applications in food packaging. The active clays which were evaluated in this work are experimental grades made by an external supplier, thus full formulation details were not disclosed. The following clays were supplied:

- Silver organomodified MMT, containing silver as cation (Ag^+), Bactiblock® R1.51
- Silver organomodified MMT, containing silver as metal nanoparticles (Ag^o), Bactiblock® T1.51
- Iron based kaolinite, containing zero-valent iron nanoparticles (Fe^o), O_2Block® series
- Iron based MMT, containing zero-valent iron nanoparticles (Fe^o), O_2Block® series, Fe-MMT
- Resveratrol modified MMT, O_2Block® series, RS-MMT

Polylactide -PLA- was selected for preparing biocomposites from silver based R1.51 and T1.51 and, iron based Fe-MMT. On the other hand, polyethylene (HDPE and LLDPE) were used to obtain composites from iron kaolinite and RS-MMT.

In a fist stage of the experimental work it was attempted the incorporation of metallic silver organomodified clay T1.51 in PLA by applying the solvent casting procedure from chloroform, for observing the compatibility between polymer and clay and to assess the transparency and active properties achieved by different filler contents. The silver nanoparticles were observed to be randomly distributed across the whole morphology of the clay and spotted as spherical dark nanodots up to 20 nm, as detected by TEM. The macrodilution test for antimicrobial evaluation of clay revealed strong antimicrobial effectiveness against Gram-negative *Salmonella spp.* with minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) below 1mg $10mL^{-1}$. Filler loading of 1, 5 and 10 wt.-% exhibited apparent dispersion and transparency. WAXS diffractograms showed no clear intercalation or exfoliation of the clay but TEM micrographs demonstrated high dispersion of the original tactoids across the PLA matrix. Although no exfoliation of clay was achieved, it was determined 99.99% antimicrobial efficiency of composites as well as improved barrier to water up to 40%, depending on the filler content. The silver migration tests from composites to acidic aqueous medium in a range of 6 days, presented high concentration of migrant (4-6 mg/kg of simulant). These results indicates that lower quantities of filler might be recommended for active composites obtained by solvent casting method, or limit the applications of these materials to low humidity environments.

According to the principles of green chemistry, solvents should be made unnecesary and products should be designed to perform their desired function while minimizing the starting raw materials as well as their toxicity, besides other principles. Taking this into account, the solvent casting method for producing PLA-based composites up to 10 wt.% of silver based clay T1.51 with the consequent high silver migration to food simulant seemed to be unadvisable. In order to be more consistent with the green chemistry principles, the next step of experimental program included a no solvent-compositing method (melt mixing) to incorporate the antimicrobial clays to biodegradable matrix and, filler loading were reduced to 2.5 wt.-%. In addition, the silver organomodified MMT containing Ag^+, R1.51, was evaluated together with T1.51 to find out antimicrobial activity differences towards *S. aureus* related to silver state. The R1.51 clay TEM micrographs showed the typical appearance of regular mineral sheets, while micrographs of T1.51 revealed the expected silver nanoparticles as spherical dark nanodots (up to 20 nm diameter). The silver based clays exhibited different antibacterial performance: the T1.51 clay resulted clearly less effective than R1.51 because insufficient release of Ag^+ ions from silver nanoparticles at conditions test. By applying the melt mixing method, suitable macroscale dispersion and distribution of the clays into the PLA matrix was achieved, and transparent composites were obtained. However, as revealed by TEM and WAXS, exfoliation of the clay layers did not occurred and the structures of composites were described as a mixture of tactoids size reduction, intercalation and some exfoliation morphologies. Differences between composites containing metallic or cationic silver were observed in the antimicrobial tests, confirming that the clay containing nanoparticles has less available active species because of the long-term release of active Ag^+ after hydrolysis.

The other active property for potential food packaging application that was considered was oxygen scavenging activity. Based on the knowledge that iron systems are the best-known and most widely used in active packaging, it was proposed the evaluation of zero-valent iron clays and their composites for prepare active materials. PLA was again selected as polymeric matrix following the main idea of obtaining greener materials as possible. The first step consisted on characterize the zero-valent iron MMT, FeMMT. This was deep opaque black clay due to the content of metallic iron nanoparticles, as revealed TEM micrographs. The spherical metallic iron particles were located on the surface and also on the edges the montmorillonite platelets, with a particle size up to 150 nm, and in some cases with chain-like morphology due to the inherent magnetism of particles. The WAXS diffractograms evidenced disorder of the lattices as a consequence of modification with iron. The Fe-MMT exhibited strong oxygen scavenging kinetics behavior, at 100% RH and 24 ºC, being able to absorb up to 62.8 mL of oxygen/g clay. The reason for testing at high humidity is that scavenging reaction needs water to proceed, and this is extremely efficient reaction since it is expected that 1 g of iron inactivate 300 mL of oxygen in the presence of moisture. The incorporation of 10 wt.% of FeMMT in PLA was carried out by melt mixing in order to avoid using chloroform as solvent in casting processes. The resulting dark and opaque composite showed regular clay distribution across the matrix, but tactoid-like structures at microscopic level were detected. The most important effect in the thermal stability of the iron based composite was the rapid degradation of PLA due to the presence of iron, by ca. 60ºC in comparison with the neat PLA. Zero-valent iron seemed to act as catalysts of such degradation processes. However, the composite exhibited oxygen scavenging activity at 24ºC and 100% RH (up to 4.2 mL oxygen/g composite). Finally, the water vapor barrier of composite was slightly increased due to the blocking presence of the laminar filler.

Considering the role of zero-valent iron acts as a catalyst in degradation process of PLA, which could represent a limitation in further up-scaled processes, it was decided to incorporate the iron clays in polymers which are commonly used in the manufacture of food contact materials, such as polyolefins, more chemically resistant. In this new stage, the clay was changed to iron based kaolinite, another experimental grade that was produced by the supplier under the same formulation scheme.

The evaluation started with the determination of the scavenging activity of the zero-valent iron kaolinite, at 100% relative humidity and 24 ºC. The iron kaolinite showed strong efficiency at short times: it was able to uptake 30 mL of oxygen per gram of additive at 24 h, and up to 43 mL after 60 days. The oxygen scavenger activity of the clay was explained by the presence of iron metallic particles located inside the galleries and also on the edges (115 nm average diameter), as TEM micrographs revealed. The corresponding active polyolefin composites with 10wt.-% filler content were manufactured by extrusion into HDPE and LLDPE, resulting in good distribution of the additive in the matrix but not a good dispersion (exfoliation) of the clay, as detected by optical microscopy and WAXS technique. The poor exfoliation of clay was due to insufficient sheer during processing, low affinity with matrix, as well as relatively high loading of filler. The iron kaolinite contained in the composites was seen to play a dual oxygen fighting role: as a passive barrier due to the tortuous path that imposes to gas diffusion; and secondly, the iron contained in the kaolinite traps and reacts with the molecular oxygen, causing the active reduction of gas pressure through the composite. On the other hand, humidity demonstrated to be a key factor in the kinetics for oxygen depletion: the active composites were able to uptake between 2.4 and 4.3 mL of oxygen per gram of composite, in what it thought as a dependency with the permeability to water and oxygen of the polymeric matrix. Because migration should be avoided in oxygen scavenger materials, migration of iron and aluminium from HDPE composites was evaluated in water and isooctane as food simulants. Concentration of Fe and Al as migrants in food simulants were very low or negligible, especially in isooctane, and below the limits currently permitted by the EU legislation.

The last step of this work consisted on the development of antioxidant composites based on LLDPE. They were prepared by the incorporation by extrusion of resveratrol or resveratrol modified MMT (RS-MMT) in LLDPE matrix. A polyolefin matrix was selected taking into account that final active material would be tested at high humidity conditions. It was already found that PLA is easily hydrolysable and not water resistant material thus, may not be the best choice for direct food contact packaging in high humidity applications.

Resveratrol is a naturally occurring polyphenolic compound commonly found in the fruits and leaves of edible plants and is considered as one of the most active and antioxidant which is characterized by its beneficial effects on the human body. Resveratrol is a renewable raw material that can be extracted from plants by using safe solvents or supercritical fluids extraction, as was ascertained after revision of patented procedures. In consequence, it can be said that antioxidant composites had green character.

Resveratrol and RS-MMT (containing ca. 25 wt.-% of resveratrol) were added to LLDPE by extrusion process, in a filler load of 1 wt.-% and 3.4 wt.-%, respectively, as was confirmed by thermogravimetric analysis (TGA). The composite containing resveratrol was transparent, but the one containing RS-MMT showed opacity and light brown color probably caused by caramelization reactions of sugars that remain in antioxidants as impurities, and also by the partial polymerization of phenolic compounds. The films obtained from these composites were homogeneous in a macroscopic scale, even though no significant process induced changes could be deducted from the XRD patterns, and both composites showed strong antimicrobial effect besides the antioxidant activity. The film containing RS-MMT was submitted to migration tests in water and isooctane as food simulants. No migrant was detected in isooctane, and very low concentration was detected in water (below 0.01 mg/Kg). With the reference of a similar stilbene currently listed in European regulations, resveratrol migration levels were below the specific migration limits. The lipid oxidation in fresh beef meat was studied following the method of thiobarbituric acid. The malonaldehyde concentration values were significantly lower in the samples that were packed in active films, with a maximum oxidation reduction of 34.7%. From these results, it could be inferred that shelf-life of fresh meat could be potentially extended by a few days by using the current antioxidant film as packaging material.

The results obtained in the different steps of this work allowed to demonstrate that active composites can be obtained by the incorporation of the corresponding active clays through solvent casting, melt mixing or extrusion methods. The active composites materials may be more sustainable as the matrix selection, compositing method and starting active agents allow it. It was determined that clay acts a vehicle of active agents (silver cation, silver nanoparticles, zero-valent iron nanoparticles or resveratrol), and they contribute to the distribution of the agents through the matrixes. The final activity of material and migration data in food simulants, as well as features such as barrier properties and thermal stability, allow the potential application of these materials in active food packaging.

9. CONCLUSIONS

In this dissertation several active nanoclays and their corresponding composites prepared from polyolefins and PLA were evaluated. The use of clays as vehicles of antimicrobial, oxygen scavenger and antioxidant agents, have special interest because of their potential to produce active materials with enhanced properties for food packaging applications. The following conclusions related to these were obtained from this work:

1) For the case of PLA nanocomposites containing silver organomodified clays

- The experimental grades of organomodified silver montmorillonites, Bactiblock®R1.51 which is a cation exchanged ionic silver clay and Bactiblock® T1.51 which contains nanoparticles of silver intercalated in the platelets, were evaluated. Morphology evaluation of clays allowed distinguishing the metallic from the cationic silver because the first is presented as dark nanodots on the mineral sheets.
- The grades R1.51 y T1.51 showed strong antimicrobial effect against *S.aureus*. The grade R1.51 also showed bactericidal effect against *Salmonella spp*, with minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) below 1 mg/10 ml.
- Solvent casting and melt mixing were the techniques used to produce the composites, from 1 to 10 wt.-% of organomodified silver clays. In general, translucent materials were obtained. However, morphological analysis revealed that exfoliation or intercalation were not achieved in solvent casting compounds, unlike those obtained from melt mixing.
- The water vapour barrier of PLA composites was improved by up to 40%, depending on the filler content and the method applied for clay incorporation into the polymeric matrix.
- Silver migration and antimicrobial activity were shown to be dependent on the availability of Ag^+, which is the active specie. The detected silver concentration was 6-8 ppm after the PLA composites were exposed to a slightly acidic aquous media.
- The behavior of silver based PLA composites showed that they could be successfully applied for food packaging purposes as a biobased alternative for protection of food goods against antimicrobial spoilage.

2) For the case of the composites based on iron clays (oxygen scavenger):

- Two grades or iron based clays were analyzed: kaolinite and montmorillonite. These clays were seen to contain metallic iron in the form of nanoparticles, which were intercalated inside the galleries but also supported on the edges and surfaces of the clay platelets. The iron nanoparticles size can reach between 100-150 microns, depending on the support.
- Both iron based clays showed strong oxygen depletion at high relative humidity, as expected according to the reaction stoichiometry between Fe^o and

water. After 60 days of exposure, iron kaolinite and iron montmorillonite were able to absorb up to 43 mL O2/g clay and 62 mL O2/g clay, respectively.

- Two polyolefin composites containing 10 wt.-% of iron kaolinite were prepared by extrusion with HDPE and LLDPE. The clay contained in the composites exhibited a dual effect against oxygen: as a passive barrier due to the tortuous path imposed by platelets to gas diffusion; and active barrier, by reaction of iron nanoparticles supported on clay with molecular oxygen in the presence of water.
- The iron based polyolefin composites exhibited the same strong absorption kinetics than the iron kaolinite, and they were able to deplete between 2.4 and 4.3 mL O2/g composite. Oxygen scavenger activity of composites showed to be dependent of the water permeability of polymeric matrix, relative humidity and temperature.
- Migration tests of Fe and Al, from HDPE-iron kaolinite composite to water and isooctane as food simulants resulted in very low or negligible concentration levels, especially in isooctane.
- The iron montmorillonite, FeMMT, was successfully incorporated into PLA matrix, with a filler content of 10 wt.%, by applying melt mixing process. The biocomposite was able to absorb up to 4.2 mL O_2/g composite, at 100 % RH.
- While incorporation of iron kaolinite did not caused thermal degradation of polyolefin matrixes, the thermal stability of PLA-iron based composite decreased by 60 ºC because iron seemed to act as catalyst of depolymerization reactions in PLA.
- Although both PLA and polyolefin composites containing iron based clays showed strong oxygen scavenging activity, the active polyolefin composites presented a more suitable balance of thermal stability, barrier properties and activity for constituting packaging of oxygen sensitive products.

3) For the case of the resveratrol containing clay (antioxidants):

- The naturally occurring polyphenol, resveratrol, was shown to be a stronger antioxidant and more thermally stable than the synthetic antioxidant butylhydroxytoluene, BHT, which is currently used as additive in foods, cosmetics and pharmaceuticals. The resveratrol modified montmorillonite, RS-MMT, also exhibited antioxidant activity.
- The incorporation of resveratrol and RS-MMT to LLDPE matrix for achieving materials with nominal content of resveratrol of 1 wt.-% was achieved by extrusion process. The resulting composites were translucent, but the one containing RS-MMT exhibited light brownish color due to the presence of clay. Both composites revealed antimicrobial capacity against *S. aureus* besides antioxidant activity.
- The oxidative patterns of fresh red meet were seen to decrease when the food was packaged with films made from LLDPE/RE-MMT composites. The resveratrol contained in the composites, even though it was supported in clay

as vehicle, was able to exert as antioxidant as antimicrobial agent for retarding the spoilage of meat.

- The resveratrol migration from active composites to water and isooctane as food simulants confirmed insignificant release of active compound. This result pointed out that resveratrol most likely acts by contact.
- The use of active composites containing resveratrol supported on clay can improved the shelf-life of food products such as those packed with modified atmosphere containing oxygen.

ANNEXES

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Novel silver-based nanoclay as an antimicrobial in polylactic acid food packaging coatings

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This paper presents a comprehensive performance study of polylactic acid (PLA) biocomposites, obtained by solvent casting, containing a novel silver-based antimicrobial layered silicate additive for use in active food packaging applications. The silver-based nanoclay showed strong antimicrobial activity against Gram-negative *Salmonella* spp. Despite the fact that no exfoliation of the silver-based nanoclay in PLA was observed, as suggested by transmission electron microscopy (TEM) and wide angle X-ray scattering (WAXS) experiments, the additive dispersed nicely throughout the PLA matrix to a nanoscale, yielding nanobiocomposites. The films were highly transparent with enhanced water barrier and strong biocidal properties. Silver migration from the films to a slightly acidified water medium, considered an aggressive food simulant, was measured by stripping voltammetry. Silver migration accelerated after 6 days of exposure. Nevertheless, the study suggests that migration levels of silver, within the specific migration levels referenced by the European Food Safety Agency (EFSA), exhibit antimicrobial activity, supporting the potential application of this biocidal additive in active food-packaging applications to improve food quality and safety.

Keywords: metals analysis; microbiology; metals

Introduction

Antimicrobial materials and surfaces are of increasing importance in areas such as healthcare, home and personal hygiene, foods, active packaging, automotive and textiles. Antimicrobial technologies are being investigated and developed to control harmful microorganisms. However, these systems need to combine desirable attributes, such as strong antibacterial efficacy, environmental safety, low toxicity, cost effectiveness and ease of fabrication.

Silver is a powerful antimicrobial agent that has been used since ancient times. Recent technical innovations and findings facilitate the availability and incorporation of silver products in a wide range of materials at the manufacturing stage, providing novel antimicrobial formulations. Currently, a specific form of silver does not exist for every application, procedure or matrix. Nanotechnology is a key development in modulating metals, compounds and materials into nanosizes, which often changes their chemical, physical and optical properties, as well of those of the matrices into which they are incorporated. Stable silver nanoparticles can be obtained using soluble starch as both the reducing and stabilising agent (Vigneshwaran et al. 2006), or synthesised via the regular borohydride reduction of Ag^+ ions (Oh et al. 2004; Lok et al. 2007). Silver nanoparticles can also be synthesised in the interlamellar space of a pretreated kaolinite with DMSO by UV radiation or chemical-induced reduction (Dékány et al. 2003; Dékány and Patakfalvi 2004), in layered laponite suspensions via photoreduction (Huang and Yang 2007), or supported on micro and mesoporous structures after ion exchange followed by in situ reduction (Yang et al. 2008; Lv et al. 2009).

Silver(I) nitrate adducts with diverse electronic and steric characteristics can be synthesised with *N* and *P* donor ligands (Pettinari et al. 2007). Thus, Ag/SiO_2 coating solutions have been prepared to serve as antimicrobial refinements of temperature-sensitive materials such as fabrics or wood (Mahltig et al. 2009). Moreover, a suspension of silver nitrate in an ammonium salt medium has been reported as a precursor of stable nanoscale AgBr particles (Zhang et al. 2007).

Research has also been carried out on the development of inorganic materials, such as zeolites, for supporting Ag^+ ions due to their capacity to incorporate and release ionic species. Coleman et al. (2009) prepared Ag^+ and Zn^+ exchanged tobermorites and demonstrated that they have marked bacteriostatic effect with potential as antimicrobial materials for

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Oxygen scavenging polyolefin nanocomposite films containing an iron modified kaolinite of interest in active food packaging applications

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ABSTRACT

A synthetic iron containing kaolinite was evaluated as an oxygen scavenger additive for food packaging plastics having an active performance of ca. 43 ml O_2/g at 100%RH and of ca. 37 ml O_2/g of additive at 50%RH. The corresponding polyolefin films containing 10 wt.% of the active filler also exhibited significant oxygen scavenger activity (up to 4.3 ml of oxygen/g composite, at 24 °C and 100%RH). The effect of oxygen scavenging capacity of HDPE films with temperature and %RH was also assessed. The oxygen permeability tests carried out after inactivation of the additive suggested that the iron containing clay plays in fact a dual oxygen fighting role: active performance (trapping and reacting with molecular oxygen) as well as a passive barrier performance (imposing a more tortuous diffusion path to the permeant). Migration tests into two food simulants indicated that iron (from active ingredient) and aluminum (from clay migration) are hardly detectable in commonly used simulants. This study suggests that there is significant potential for the use of this novel oxygen scavenger additive to constitute active packaging of value in the shelf-life extension of oxygen sensitive food products.
Industrial relevance: Oxygen penetration in food and beverages leads to, for instance, oxidative rancidity of unsaturated fats, loss of vitamin C, browning of fresh meat, oxidation of aromatic flavor oils and pigments and fostering the growth of aerobic spoilage microorganisms. These undesirable effects on the product quality indicate that the elimination or exclusion of oxygen is one of the main targets for preserving foods and beverages in the food packaging industry. The industrial relevance of this study is very significant because it proves that the technology generated and characterized in the paper can uniquely fight oxygen by two means, i.e active and passive performance. This should enable the food packaging industry to package foods in relatively inexpensive, low barrier commodity plastic materials such as polyolefins, that will allow the shelf-life extension of the packaged products with a safe use, since migration of the components is negligible.

1. Introduction

Oxygen is one of the major factors in food spoilage. Among the many adverse effects provoked by oxygen penetration on food and beverages are oxidative rancidity of unsaturated fats, loss of vitamin C, browning of fresh meat, oxidation of aromatic flavor oils and pigments and fostering the growth of aerobic spoilage microorganisms. These undesirable effects on the product quality indicate that the elimination or exclusion of oxygen is one of the main targets for preserving foods and beverages. Active modified atmosphere packaging (active MAP) including N_2, O_2, CO_2 flushing has been used for food preservation over the last 20 years. It is the altered ratio of these gases that makes a difference in the prolongation of shelf life. By reducing the O_2-level and increasing the CO_2-level, ripening of fresh fruit and vegetables can be delayed, respiration and ethylene production rates can be reduced, softening can be retarded and various compositional changes associated with ripening can be slowed down. Besides MAP, antioxidants, absorbers, interceptors and scavengers have been used to remove oxygen or to prevent this from entering into the packaging environment. Specifically, oxygen scavengers are materials which, once incorporated into the packaging structure, bind chemically to this and are capable of eliminating large volumes of oxygen that either permeate through the packaging or from the head space (Brody, Bugusu, Han, Sand, & McHugh, 2008; Brody, Strupinski, & Kline, 2001; De Azeredo, 2009). Oxygen scavenging commercial technologies are based on enzymatic and chemical systems. The latter ones are, for example, based on catechol, ferrous salts, active zero-valent iron, ascorbic acid and titania among others (Brody et al., 2008; De Azeredo, 2009; Restuccia et al., 2010).

Many of the commercially available oxygen scavengers contain iron and are marketed in a sachet format (Miltz & Perry, 2005), to prevent imparting color, odor and taste to the product. Considering that the use of sachets have the potential risk of being misused by the consumer or being ingested, as well as to contaminate the product by leakage from the sachet, the use of packaging materials that contain incorporated the active agent within the packaging monolayer or multilayer structure seems more suitable, acceptable by the consumer and safer. The incorporation of scavenging agents into packaging materials such as

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