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INSTITUTO UNIVERSITARIO DE INGENIERÍA DE ALIMENTOS

PARA EL DESARROLLO

Propiedades de films de almidón de maíz. Influencia de la incorporación de lípidos, biopolímeros y compuestos bioactivos

TESIS DOCTORAL

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CONSIDERAN: que la memoria titulada PROPIEDADES DE FILMS DE ALMIDÓN DE MAÍZ. INFLUENCIA DE LA INCORPORACIÓN DE LÍPIDOS, BIOPOLÍMEROS Y COMPUESTOS BIOACTIVOS que, para aspirar al grado de Doctor, presenta D**. Alberto Jiménez Marco**, realizada bajo su dirección en el Instituto Universitario de Ingeniería de Alimentos para el Desarrollo de la Universitat Politècnica de València, reúne las condiciones adecuadas para su presentación como tesis doctoral, por lo que **AUTORIZAN** al interesado su presentación en ese doctorado.

Valencia, 26 de febrero de 2013

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Resumen

Se han desarrollado y caracterizado films biodegradables a base de almidón de maíz y glicerol como plastificante, evaluando al mismo tiempo el efecto de la adición de componentes lipídicos (ácido palmítico, esteárico y oleico), otros polímeros (hidroxipropilmetilcelulosa y caseinato de sodio) y compuestos bioactivos (α-tocoferol, aceite esencial de naranja y D-limoneno) sobre las propiedades de los films (propiedades barrera al vapor de agua y al oxígeno, ópticas, mecánicas, micro y nanoestructurales). Asimismo se evaluó la influencia del tiempo de almacenamiento en las propiedades de los films.

La adición de ácidos grasos no mejoró notablemente la permeabilidad al vapor de agua excepto en el caso de los films con ácidos grasos saturados y solo en films no almacenados. Los resultados de difracción de rayos X mostraron que la cristalinidad aumentó con el tiempo de almacenamiento, incrementándose la rigidez, y disminuyendo el brillo de los films. Del mismo modo, la cristalinidad afectó a la capacidad de sorción de agua de los films en función de la humedad relativa y la temperatura. La temperatura de transición vítrea de los films de almidón se vio afectada por la adición de ácidos grasos saturados pero no por la adición de ácido oleico. La presencia de dichos componentes promovió la formación de estructuras cristalinas tipo V, indicando la formación de complejos entre los lípidos y las cadenas de amilosa e inhibiendo la formación de otros tipos de formas cristalinas.

Se analizó también el efecto de la incorporación de otros biopolímeros en la posible mejora de la funcionalidad de los films de almidón. En las mezclas con hidroxipropilmetilcelulosa (HPMC), se inhibió la retrogradación del almidón en los films *composite*, pero se observó un efecto negativo en las propiedades barrera de los mismos, que fueron más permeables, principalmente al oxígeno. La adición de

HPMC produjo separación de fases en los films (observada por microscopía electrónica de barrido). Por el contrario, la incorporación de caseinato de sodio (NaCas) permitió formar films homogéneos y menos permeables al oxígeno. Los films presentaron una resistencia mecánica algo menor que los films de almidón puro pero una mayor flexibilidad sin incrementar los valores de permeabilidad al vapor de agua. La reorganización de las cadenas de los polímeros con el tiempo de almacenamiento provocó la disminución de la resistencia mecánica, la deformabilidad y el brillo de los films *composite*. Atendiendo a los efectos observados, se eligió como formulación más adecuada el film *composite* formado por almidón y NaCas con un ratio de polímeros del 50:50.

El film *composite* de almidón y NaCas (50:50) se estudió como matriz para la incorporación de compuestos bioactivos como son el α-tocoferol y el aceite esencial de naranja o su principal componente, el D-limoneno. El efecto de la adición de α-tocoferol se comparó con la influencia de la adición de ácido oleico y también con la adición de ambos compuestos. La adición de lípidos provocó una separación de fases entre el almidón y el NaCas debido a la diferente interacción entre cada polímero y los lípidos. Asimismo la adición de ácido oleico incrementó significativamente la permeabilidad al oxígeno, al contrario que el α-tocoferol, que además impartió a los films una elevada capacidad antioxidante.

La incorporación de aceite esencial de naranja y D-limoneno se realizó utilizando nanoliposomas de lecitina de soja y lecitina de colza que encapsularon los compuestos activos. La incorporación de nanoliposomas en los films se realizó directamente en las dispersiones acuosas sin posterior homogeneización para evitar su ruptura. La adición de los compuestos bioactivos en forma de nanoliposomas no confirió capacidad antimicrobiana a los films, salvo en el caso de los nanoliposomas de lecitina de soja con aceite esencial, debido probablemente a la dificultad de los compuestos encapsulados para difundir en el film por la gran estabilidad de los liposomas y a la baja actividad antilisteria del D-limoneno y el aceite esencial de naranja.

Abstract

Biodegradable starch-glycerol based films were obtained. The influence of lipid compounds (palmitic, stearic and oleic acid), other polymers (hydroxypropylmethylcellulose and sodium caseinate) and bioactive compounds (α-tocoferol, D-limonene and orange essential oil) on film properties (oxygen and water vapour barrier, optical, mechanical, nano- and microstructural). Furthermore the effect of storage time on films' properties was also considered.

Fatty acids addition did not improve the water vapour ability of films except for non-stored saturated fatty acids containing films. X-ray diffraction results showed that cristallinity of films increased with storage time, thus increasing the stiffness and decreasing the gloss of films. Furthermore, crystallinity affected the water sorption capacity of films as function of relative humidity and temperature. Glass transition temperature of starch films varied with saturated fatty acids addition. However, oleic acid did not affect this parameter. The presence of fatty acids promoted the formation of V-type structures, thus indicatin the formation of amylose-lipid complexes that inhibited the developmet of other crystalline structures.

The effect of the incorporation of other biopolymers to improve the functionality of starch films was also studied. Hydroxypropylmethylcellulose (HPMC) addition inhibited starch retrogradation. However, obtained films were more permeable, specially in case of oxygen. HPMC addition produced phase separation as it was observed by scanning electron microscopy. On the contrary, sodium caseinate incorporation (NaCas) allowed to obtain homogeneous films and less permeable to oxygen. Obtained films showed less mechanical resistance in comparison with pure starch films but a greater flexibility without increasing the water vapour permeability. Rearrangement of polymers chains during storage reduced the mechanical resistance, the extensibility and the gloss of composite

films. Regarding the obtained results, the film including a starch:protein ratio of 50:50 was choosen as the film with the most adequate properties.

Composite film (starch:Nacas ratio = 50:50) was studied as a matrix for the incorporation o active compounds (α-tocopherol, D-limonene and orange essential oil). The effect of α-tocopherol addition was compared with the incorporation of oleic acid and their mixture. Lipids addition promoted phase separation between starch and NaCas due to the different interactions between each polymer and the lipids. Furthermore, oleic acid addition increased significantly the oxygen permeability whereas α-tocopherol greatly improved the antioxidant capacity of films without affecting the oxygen permeability.

D-limonene and orange essential oil incorporation was carried out by forming rapeseed and soy nanoliposomes, which acted as carriers of bioactive components. Nanoliposomes incorporation was performed directly in starch-NaCas dispersions without any homogenization, to avoid nanoliposomes damages. Bioactive compounds addition did not confer antimicrobial capacity to the films (except for soy-orange oil nanoliposomes containing film) probably due to the high stability of nanoliposomes and the low antibacterial activity of D-limonene and orange essential oil.

Resum

S'han desenvolupat i caracteritzat films biodegradables a base d’almidó de dacsa i glicerol com plastificant, avaluant al mateix temps l'efecte de l'addició de components lipídics (àcid palmític, estearic i oleic), altres polímers (hidroxi-propil-metilcelulosa (HPMC) i caseïnat sòdic (NaCas)) i compostos bioactius (α-tocoferol, oli essencial de taronja i D-limoneno) sobre les propietats dels films (propietats barrera al vapor d'aigua i a l'oxigen, òptiques, mecàniques, micro i nanoestructurals). Així mateix es va avaluar la influència del temps d'emmagatzematge en les propietats dels films.

L'addició d'àcids grassos no va millorar notablement la permeabilitat al vapor d'aigua excepte en el cas dels films amb àcids grassos saturats i solament en films no emmagatzemats. Els resultats de difracció de rajos X van mostrar que la cristalinitat va augmentar amb el temps d'emmagatzematge, incrementant-se la rigidesa, i disminuint la brillantor dels films. De la mateixa manera, la cristalinitat va afectar a la capacitat de sorció d'aigua dels films, en funció de la humitat relativa i la temperatura. La temperatura de transició vítria dels films d’almidó es va veure afectada per l'addició d'àcids grassos saturats però no per l'addició d'àcid oleic. La presència d'aquests components va promoure la formació d'estructures cristal·lines tipus V, indicant la formació de complexos entre els lípids i les cadenes d’amilosa i inhibint la formació d'altres tipus de formes cristal·lines.

Es va analitzar també l'efecte de la incorporació d'altres biopolímers en la possible millora de la funcionalitat dels films d’almidó. En les barreges amb HPMC, es va inhibir la retrogradació de l’almidó en els films *composite*, però es va observar un efecte negatiu en les propietats barrera dels mateixos, que van ser més permeables, principalment a l'oxigen. L'addició de HPMC va produir separació de fases en els films (observada per SEM). Per contra, la incorporació de NaCas va permetre formar films homogenis i menys permeables a l'oxigen. Els films van

presentar una resistència mecànica menor que els films d’almidó pur però una major flexibilitat sense incrementar els valors de permeabilitat al vapor d'aigua. La reorganització de les cadenes dels polímers amb el temps d'emmagatzematge va provocar la disminució de la resistència mecànica, la deformabilitat i la brillantor dels films *composite*. Atenent als efectes observats, es va triar com a formulació més adequada el film *composite* format per almidó i NaCas amb un ràtio de polímers del 50:50.

El film *composite* d’almidó i NaCas (50:50) es va estudiar com a matriu per a la incorporació de compostos bioactius com són el α-tocoferol i l'oli essencial de taronja o el seu principal component, el D-limoneno. L'efecte de l'addició de α-tocoferol es va comparar amb la influència de l'addició d'àcid oleic i també amb l'addició dels dos compostos. L'addició de lípids va provocar una separació de fases entre l’almidó i el NaCas a causa de la diferent interacció entre cada polímer i els lípids. Així mateix l'addició d'àcid oleic va incrementar significativament la permeabilitat a l'oxigen, al contrari que el α-tocoferol, que a més va impartir als films una elevada capacitat antioxidant.

La incorporació d'oli essencial de taronja i D-limoneno es va realitzar utilitzant nanoliposomes de lecitina de soia i lecitina de colza que van encapsular els compostos actius. La incorporació de nanoliposomes en els films es va realitzar directament en les dispersions aquoses sense posterior homogeneïtzació, per a evitar la seua ruptura. L'addició dels compostos bioactius en forma de nanoliposomes no va conferir capacitat antimicrobiana als films, excepte en el cas dels nanoliposomes de lecitina de soia amb oli essencial, probablement per la dificultat dels compostos encapsulats per a difondre en el film per la gran estabilitat dels liposomes i a la baixa activitat antilisteria del D-limoneno i l'oli essencial de taronja.

ÍNDICE PÁGINA

Justificación e interés del trabajo

Durante los últimos años se ha mostrado un interés creciente en el desarrollo de films comestibles y/o biodegradables que puedan sustituir, al menos parcialmente, a los polímeros sintéticos derivados del petróleo. Esto se debe a la cada vez mayor disminución de las reservas de crudo y al incremento significativo de los problemas medioambientales derivados del uso de plásticos sintéticos, con la consiguiente concienciación social sobre la protección del medio ambiente.

Los films y recubrimientos comestibles y biodegradables presentan dos ventajas competitivas frente a los polímeros sintéticos. Por un lado, se trata de compuestos de origen natural provenientes de fuentes renovables, cuya biodegradabilidad garantiza su inocuidad medioambiental. Por otra parte, y en relación también a su carácter inocuo y natural, son susceptibles de combinarse con compuestos bioactivos, también naturales, para incremententar su funcionalidad en la protección de alimentos. Entre los compuestos bioactivos destacan los compuestos antioxidantes como tocoferol, ácido ascórbico o aceites esenciales y los compuestos con poder antimicrobiano, muchos de ellos de origen vegetal como los constituyentes de los aceites esenciales de plantas (terpenos en su mayoría).

En la formulación de films comestibles y biodegradables se han utilizado una gran variedad de polímeros que pueden dividirse en dos grandes grupos: polisacáridos y proteínas, aunque también los lípidos han sido ampliamente utilizados, principalmente en combinación con los anteriores para formar films *composite*. Entre los polisacáridos podrían destacarse el almidón y sus derivados, la celulosa y sus derivados, el quitosano, los alginatos, los carragenatos, etc. En cuanto a las proteínas es común encontrar trabajos relacionados con las proteínas de la leche (caseínas, caseinatos y proteínas de suero), proteínas vegetales (soja principalmente) y gelatinas, ya sean de origen bovino, porcino o de pescado.

La mayor parte de los polímeros enumerados pueden formar films con propiedades más o menos adecuadas, limitanto la permeabilidad al vapor de agua, a gases permeantes y evitando procesos de transferencia de masa no deseados durante el almacenamiento de los productos y proporcionando a los mismos una protección mecánica y un aspecto adecuados. Entre los polímeros mencionados hay uno que tiene especial interés debido a que, correctamente formulado, puede procesarse utilizando sus propiedades termoplásticas mediante equipos industriales usualmente empleados con los polímeros sintéticos, facilitandose así el escalado de los procesos desde el laboratorio a las plantas de procesado. Este polímero es el almidón, que puede pasar de su estado nativo, a formar un film en cuestión de minutos si se añaden los aditivos necesarios (plastificantes como el glicerol) con equipos de extrusión o termomoldeado. El almidón es además un producto ampliamente extendido en la naturaleza y asequible en el mercado a un precio muy competitivo. No obstante, los films de almidón presentan el inconveniente de su gran higroscopicidad, que constituye un inconveniente en su interacción con el ambiente, limitando sus propiedades barrera, altamente dependientes de su contenido en agua, así como su resistencia mecánica. A su vez la ganancia en agua de los films incrementa fuertemente la movilidad molecular, haciendo que ocurran en gran extensión procesos de retrogradación que modifican las propiedades de los films durante el almacenamiento de los mismos. La incorporación de compuestos que puedan disminuir su capacidad de adsorción de agua y los fenómenos de retrogradación, sin menguar su resistencia mecánica o su extensibilidad es un tema de interés a la hora de definir formulaciones adecuadas de films de almidón que permitan optimizar sus propiedades funcionales como material de envase o recubrimiento. A su vez, es importante conocer como se modifican las propiedades de estos films cuando se incorporan diferentes compuestos bioactivos en su utilización como vehículos de antioxidantes o antimicrobianos para mejorar la conservación de los productos.

El presente trabajo se centra en el desarrollo de films de almidón de maíz y glicerol y en el análisis de la aptitud de distintos componentes (lípidos, polisacáridos y proteínas) para mejorar las propiedades de los films limitando la retrogradación del almidón e incrementando en la medida de lo posible su hidrofobicidad. También se ha estudiado el efecto de antioxidantes y antimicrobianos en las propiedades de films formulados a base de almidón. A pesar de que los estudios se han realizado a partir de dispersiones acuosas sometidas a una gelatinización previa, los resultados obtenidos establecen un punto de partida de gran interés para el desarrollo de materiales basados en almidón y obtenidos siguiendo tanto un procesado en húmedo (a partir de dispersiones acuosas) como en seco (utilizando equipos convencionales de procesado de termoplásticos).

1. INTRODUCCIÓN

En la introducción de la tesis se incluye una revisión sobre los diferentes estudios publicados relativos a las propiedades del almidón como polímero para formar films comestibles-biodegradables.

En una primera parte se analizan precisamente las características básicas del almidón como compuesto capaz de formar films, así como la necesidad de desestructurar los gránulos de almidón cuando se trabaja con almidón nativo. La gelatinización del almidón ocupa una buena parte de este primer apartado debido a que se trata de un proceso complejo que tiene lugar en un intervalo de temperatura determinado en agua, dependiendo de las condiciones del medio y del tipo de almidón y que comprende una serie de fenómenos sucesivos: hidratación e hinchamiento de los gránulos, lixiviación de la amilosa, fusión de los cristales de amilopectina.

Los dos principales métodos de obtención de films de almidón se analizan en un segundo apartado. Por un lado, se analiza el proceso en húmedo, en el que es necesaria una cantidad de agua superior al 90 % (p/p) para gelatinizar el almidón y por otra parte el proceso en seco, en el que es necesario utilizar una cantidad adecuada de plastificantes (además de un porcentaje pequeño de agua) y además disponer de una adecuada fuerza de cizalla que permita la destrucción de los gránulos de almidón. Para ambos métodos se revisan distintas condiciones utilizadas por los investigadores, concluyéndose que dependiendo de los componentes incluidos en la formulación, el tipo de almidón (nativo, modificado, etc.), la fuente (maíz, trigo, yuca, etc.) y otros factores, las condiciones de procesado tienen que ser optimizadas de forma particular para cada caso.

A continuación se analizan distintas propiedades de los films de almidón así como la influencia que la cristalinidad del polímero tiene sobre éstas. Con el objetivo de ilustrar esta información se incluyen distintas tablas en las que se recogen valores de propiedades mecánicas, permeabilidad al vapor de agua y a gases y temperaturas de transición vítrea de los films. Asimismo, se recoge lo publicado por diferentes autores en relación a la modificación de las propiedades de los films de almidón por la adición de lípidos y otros polímeros, así como diferentes materiales de refuerzo (*fillers*) como arcillas o celulosa microcristalina. Este efecto de refuerzo consiste básicamente en la adición de micro y nanopartículas que pueden mejorar la resistencia mecánica y disminuir la permeabilidad de los films, principalmente al vapor de agua.

La revisión finaliza ofreciendo una panorámica sobre la adición de compuestos bioactivos (antimicrobianos y antioxidantes) en matrices de almidón.

EDIBLE AND BIODEGRADABLE STARCH FILMS: A REVIEW

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ABSTRACT

Mainly due to enviromental aims, petroleum-based plastics are being replaced by natural polymers. In the last decades, starch has been evaluated in its film forming ability for applications in the food packaging area. Characteristics of the starch film matrices, the film formation methods and physicochemical properties of the starch films are reviewed in this paper. The influences of different components added in casting methods and thermoplastic processes have been also analysed. Comparison of mechanical properties of newly prepared starch films and stored films reveals that the re-crystallization phenomenon made the films more rigid and less stretchable. These effects can be inhibited by adding other polymers to the starch matrix. Other approaches to improve the starch films' properties are the reinforcement by adding organic or inorganic fillers to the starch matrix as well as the addition of functional compounds. In this way starch films have improved mechanical and barrier properties and can act as a bioactive packaging. Physicochemical properties of the starch films showed a great variability depending on the compounds added to the matrix and the processing method. Nevertheless, dry methods are more recommendable for film manufacturing because of the greater feasibility of the industrial process. In this sense, a better understanding of the nano and microstructural changes occurring in the matrices and their impact on the film properties is required.

Keywords: biopolymer, crystallinity, film formation, casting.

1. INTRODUCTION

Edible starch films and coatings are used for food protection to increase the shelf-life of the foodstuffs. Nowadays, a great number of research studies focus on solving the problems produced by plastic waste in order to obtain environmentally friendly material. To this end, several works study the possibility of substituting the petro-based plastics with biodegradable, low cost materials with similar properties (Psomiadou *et al*., 1996; Mali *et al*., 2002; Mali *et al*., 2006; Famá *et al*., 2007; Jiménez *et al*., 2012). These biodegradable polymeric films offer an alternative packaging option which does not contribute to environmental pollution and is obtained from renewable sources (Lu *et al*., 2005; Tharanathan, 2003; Yu & Chen, 2009).

This review analyses the film forming ability of starch and the physicochemical properties of these types of films, focusing on the way to obtain biodegradable starch films and the relationship between the structure and physico-chemical properties. Other aspects related to the obtention of starch based films or packages, such as the effect of the addition of other components (lipid, hydrocolloids, fillers or active compounds) and applications of starch based materials have been also reviewed.

2. STARCH: A BIODEGRADABLE POLYMER MATRIX

Starch is one of the most abundant natural polysaccharide raw materials. It is a renewable resource, inexpensive and widely available (Lourdin *et al*., 1995). This polymer constitutes more than 60% of cereal kernels and is relatively easy to separate from the other chemical components (Arvanitoyannis & Kassaveti, 2009). Native and modified starches play an important role in the food industry because they can be used to change the physical properties of food products, such as sauces, soups or meat products (Thomas & Atwell, 1997), mainly modifying the texture, viscosity, adhesion, moisture retention, gel formation and film formation (Thomas & Atwell, 1997).

Starch granules can vary in shape, size, structure and chemical composition, depending on the origin of the starch (Smith, 2001). Chemically, native starch is composed of two main macromolecular components: amylose and amylopectin. Amylose is a nearly linear polymer of α-1,4 anhydroglucose units that has excellent film-forming ability, rendering strong, isotropic, odorless, tasteless, and colorless film (Campos *et al*., 2011). Meanwhile, amylopectin is a highly branched polymer of short α-1,4 chains linked by α-1,6 glucosidic branching points occurring every 25-30 glucose units (Durrani & Donald, 1995; Liu, 2005). Physically, native starches take the form of granules where both amylose and amylopectin are structured by hydrogen-bonding, containing crystalline and non-crystalline regions in alternating layers (Jenkins *et al*., 1993). In fact, most native starches are semi-crystalline, having a crystallinity of about 20-45% (Whistler *et al*., 1984). Amylose and the branching points of amylopectin form the amorphous regions while the short-branched chains in the amylopectin are the main crystalline components in granular starch. So, the higher content of amylopectin in native starch means greater crystallinity (Cheetham & Tao, 1998). The ratio of amylose/amylopectin depends on the source and age of the starch. Starch generally contains 20 to 25 % amylose and 75 to 80 % amylopectin (Brown & Poon, 2005). For instance, wheat, corn and potato starch contain 20-30% amylose, while its content in waxy starches is lower than 5% and in high-amylose starches is as high as 50-80 % (Liu, 2005).

Starch granules are not soluble in cold water due to the fact that strong hydrogen bonds hold the starch chains together. However, when starch is heated in water, the crystalline structure is disrupted and water molecules interact with the hydroxyl groups of amylose and amylopectin, producing the partial solubilisation of starch (Hoover, 2001). Heating starch suspensions in an excess of water or of another solvent able to form hydrogen bonding (e.g. liquid ammonia, formamide, formic acid, chloroacetic acid and dimethyl sulphoxide) and at high temperatures (between 65-100 ºC approximately depending on the type of starch) provokes an irreversible gelatinization (destructuration) process. This process is highly affected by the kind of solvent and the starch/solvent ratio. This is needed to obtain a homogeneous solution with which to form films. This process introduces irreversible changes in starch granules, such as the loss of crystallinity, water absorption and the swelling of the granules (Zhong *et al*., 2009, Carvalho, 2008). Gelatinization involves two steps: the hydration and diffusion of the solvent into the starch granules and the melting of starch crystals (Donovan, 1979, Liu *et al*., 1991; Jenkins & Donald, 1998). The gelatinization process and its relevance in the production of starch-based films are thoroughly reviewed in the next section.

3. FILM FORMATION: STARCH AS A MATRIX OF EDIBLE FILMS

Edible or biodegradable starch films can be obtained from the native starch or its components, amylose and amylopectin, by two main techniques: solution casting and subsequent drying (wet method) and thermoplastic processing (dry method) (Paes *et al*., 2008). Modified (López *et al*., 2008) and soluble or pre-gelatinized starch have also been used (Pagella *et al*., 2002) to obtain starch films.

As mentioned previously, starch films can be formed from a film forming dispersion, or an emulsion, which contains a high percentage of water. Using this method, many authors have formulated films from different sources, such as whey protein (Osés *et al*., 2009a), caseinates (Fabra *et al*., 2009), gelatin (Limpisophon *et al*., 2010), hydroxypropylmethylcellulose (Jiménez *et al*., 2010) or chitosan (Vásconez *et al*., 2009). Otherwise, starch films may be obtained by using a dry process (thermoplastic or thermal processing) in which the water content is lower when compared to the wet process. A dry process can be used with those raw materials which present thermoplastic properties; this means that they become soft (melted or rubbery) at a temperature lower than decomposition temperature and so, they can be molded into a determined shape when submitted to a thermal/mechanical process. Although starch does not present this characteristic in its native state, it is capable of becoming a thermoplastic material if it is treated correctly. According to Carvalho (2008), thermoplastic starch (TPS) is generally produced by processing a starch-plasticizer(s) mixture in an extruder at temperatures of between 140°C and 160°C at high pressure and high shear. Additionally, batch mixers can also be used, operating in conditions similar to those of the extrusion process. The result of the process, in which starch granules are disrupted and mixed with one or a mixture of plasticizers is the TPS. The presence of plasticizers (not only water) is necessary in order to obtain a rubbery material, without brittleness, when equilibrated at the ambient relative humidity (Forsell *et al.*, 1997).

In order to obtain starch based films, an essential requirement which must be considered is that, if native starch is used, the granules have to be disrupted previously through a gelatization process in an excess of water media (> 90 % w/w, Carvalho, 2008), where they undergo an irreversible order–disorder transition, or destructuration, as has been commented on above. Starch gelatinization is a process in which granules swell, depending on the available water, provoking the breakage of the amylopectin matrix and releasing the amylose. In other words, it can be considered as a first step, in which the solvent diffuses through the starch granules and a second, in which the melting of the starch crystallites takes place (Carvalho, 2008). Although the gelatinization process seems to be simple, it is a very complex process. According to Ratnayake & Jackson (2007), the gelatinization process initiates at low temperatures and continues until the granules are completely disrupted. These authors studied seven types of starch by scanning electron microscopy and observed differences in the granule structure when treated at different temperatures. Furthermore, they summarized the gelatinization as a three stage process during which different structural events take place:

1. The absorption of water by starch granules promotes an increase in starch polymer mobility in the amorphous regions.
2. Starch polymers in the amorphous regions rearrange often forming new intermolecular interactions.
3. With increasing hydrothermal effects, the polymers become more mobile and lose their intermolecular interactions and overall granular structure.

Wet process

As mentioned above, the wet process consists of forming a film by means of dispersion or as an emulsion. This is the most commonly used method for obtaining edible films, regardless of the matrix used. The polymer dispersion is generally poured on a levelled surface like a petri dish (Bertuzzi *et al*., 2007a) or a Teflon® plate (Jiménez *et al*., 2012) in order to obtain films by casting.

Many researchers have studied starch films obtained by casting (Bertuzzi *et al*., 2007ab; Bourtoom & Chinnan, 2008; Dias *et al*., 2010; Talja *et al*., 2007; 2008). The complete process (from native, modified or pre-gelatinized starch to final film) could be divided into several steps: gelatinization and dispersion, homogenization of the mixture (in the case of emulsions or mixes), casting and drying.

The initial stage is always the gelatinization of the starch, or dispersion if the pre-gelatinized kind is used. The main method by which granules are disrupted is gelatinization in an excess of water. Researchers optimize the process according to the origin of the starch, since the granule structure depends on the starch source (Srichuwong *et al*., 2005). Starch gelatinization takes place at different temperatures, depending on the type of starch (Ratnayake & Jackson, 2007; Ronda & Roos, 2008) and the plasticizer content (Tan *et al*., 2004). This is why different authors consider different conditions. Likewise, there is a wide variation of gelatinization times. As an example, some authors reported times of 1 min at 70 ºC (Bergo *et al*., 2008) and 2 hours at 90 ºC (Pagella *et al*., 2002).

Pagella *et al*. (2002) used various types of maize starch in order to obtain films. A heating step was necessary in the case of native starches, whereas pregelatinized starch only needed 3 hours stirring in cool water. According to Hodge & Osman (1976), pregelatinized starches are those which have been precooked and drum dried to yield products that readily disperse in cool water to form moderately stable suspensions.

An important aspect to consider is that, in most cases, heating is used to mix the starch with other materials prior to gelatinization. In this sense, it is mainly plasticizers (Han *et al*., 2006; Rodríguez *et al*., 2006; Bergo *et al*., 2008; Abdorreza *et al*., 2011) lipids (Han *et al*., 2006) or clays (Wilhelm *et al*., 2003) which are added. These materials may affect the gelatinization process, which has to be taken into account when the dispersions are prepared. Tan *et al*. (2004) reported that the gelatinization temperature varied as the glycerol content increased. However, when triglycerides (Chiotelli & Le Meste, 2003) or fatty acids (Nakazawa & Wang, 2004; Zhou *et al*., 2007) were added to various types of starch in different conditions, the gelatinization temperature was not affected.

Nevertheless, additives may be prepared independently and mixed with starch after gelatinization. Petersson & Stading (2005) made films with native potato starch and commercial monoglyceride by preparing both raw materials separately and subsequently mixing them. Firstly, they gelatinized the starch in a Brabender viscograph while the monoglyceride was emulsified with water by means of a rotor-stator homogenizer. Finally, processed starch was mixed with the monoglyceride emulsion in a Brabender viscograph, in order to obtain a homogeneous mixture.

Although most researchers usually disrupt the starch granules by means of a heating step, it is also possible to achieve a similar result using an alkaline medium. This method of granule disruption is called cold gelatinization. In this way, García *et al*. (2000a) obtained corn starch films in which the gelatinization process was carried out using an alkaline solution of 10 g/L NaOH. They also used 7M H3PO4 to neutralize the suspensions. The use of an alkali produces starch hydrolysis, a fact that can be taken into account in order to use a combined method with which to disrupt granules. Araujo-Farro *et al*. (2010) obtained films from quinoa (*Chenopodium quinoa*) starch. They processed the starch from a quinoa starch powder and treated it with NaOH in order to promote the disruption of starch granules during the gelatinization process, which was carried out at 97 ºC for 30 min. Before, Bertuzzi *et al*. (2007b) carried out a gelatinization step preceded by different periods of alkaline treatment and studied its effect on the final properties of high-amylose corn starch films. They concluded that the use of combined starch disruption allows the use of low gelatinization temperature instead of the traditional high temperature process. Although cold gelatinization seems to be a friendly treatment for the raw materials, there are two aspects which must be considered: a) an additional step related with solution neutralization is necessary and b) the resulting films may show undesirable properties in comparison with those films obtained by thermal gelatinization, such as those derived from the possible hydrolysis and reduction of the polymer molecular weight: low mechanical resistance and poor barrier properties. Romero-Bastida *et al*. (2005) carried out an interesting study in which they compared different properties between films obtained by both cold and thermal gelatinization. They used non-conventional starch sources, such as banana, mango and Okenia, to compare the structural, mechanical and water vapour barrier properties of films. SEM observations showed a homogeneous matrix when hot gelatinization was carried out, whereas cold gelatinization led to a cracked structure. This fact was linked with the poorer water barrier and mechanical resistance shown by cold gelatinization obtained films.

Once the starch has been gelatinized and other compounds have been added to the mixture, the following step is homogenization. This step can be avoided depending on the components in the film forming dispersion. Many researchers have obtained films from different starch sources in combination with plasticizers. In these cases, a homogenization step was not necessary. As an example, potato, banana (Hernández *et al*., 2008), high-amylose corn (Bertuzzi *et al*., 2007ab) and sago starch (Abdorreza *et al*., 2011) have been used to obtain films without a homogenization step. In these cases, the solutions were cooled after gelatinization and cast directly.

In formulations in which lipids or other non-miscible components are added, the homogenization step is compulsory in order to obtain a stable emulsion and an adequate integration of all components. In general, this step is carried out by means of a rotor-stator homogenizer (Jiménez *et al*., 2012) or a Brabender viscograph (Petersson & Stading, 2005).

In films containing lipids, the rotor-stator homogenizer is the main piece of equipment used to obtain emulsions. García *et al*. (2000a) and Han *et al*. (2006) used this system (Ultra Turrax and Fischer Scientific high-speed homogenizer, respectively) to achieve stable emulsions of sunflower oil-corn starch and beeswax-pea starch, respectively. Recently, Jiménez *et al*. (2012) also used an Ultra Turrax for homogenizing fatty acid-glycerol-corn starch mixtures.

In cases in which different hydrocolloids are added, such as chitosan, decolorized hsian-tsao leaf gum or egg albumen protein, a mixing step is also required to obtain film forming dispersions. Working with tapioca starch-chitosan blends, Vásconez *et al*. (2009) aimed to obtain films with antimicrobial properties by preparing both polysaccharides separately and mixing them with a rotor-stator homogenizer. Lai *et al*. (2011) also used this type of equipment to obtain film forming dispersions based on starch, hsian-tsao leaf gum and cinnamon oil or grape seed extract. Meanwhile, Wongsasulak *et al*. (2006) obtained emulsions with egg albumen suspension (16 % protein) and sunflower oil by means of a two-stage high-pressure homogenizer, in which cassava starch was dispersed. As a consequence of the homogenization step, bubbles are frequently observed to become incorporated in the film forming dispersions. If this occurs, vacuum devices are used (Famá *et al*, 2006; Rodríquez *et al*., 2006; Talja *et al*., 2008) to remove these bubbles, which can provoke microholes in the final films.

As previously mentioned, after gelatinization or homogenization, film forming dispersions must be poured or cast on dishes and allowed to dry in controlled conditions. Most researchers have obtained films after casting on levelled Petri dishes. In this way, starch films can be peeled off from polystyrene (Han *et al*., 2006; Paes *et al*., 2008) or polyethylene (García *et al*., 2000a; Osés *et al*., 2009b) Petri dishes. Polytetrafluoroethylene (Teflon®) has also been used by different authors (Talja *et al*., 2007; Reddy & Yang, 2010; Jiménez *et al*., 2012) due to this polymer being highly inert. Other surfaces, such as stainless steel (Hu *et al*., 2009) or glass (Flores *et al*., 2007), have likewise been considered in order to achieve a good appearance in starch films. Film forming dispersions can be poured immediately after the homogenization step (Jiménez *et al*., 2012) or cooled to a fixed temperature (Bertuzzi *et al*., 2007a). Regardless of the pouring temperature, cast films have to be dried in controlled conditions in order to eliminate the excess of water and make them easier to handle. The film drying conditions vary greatly in the reviewed literature. As an example, Rodríguez *et al*. (2006) used a climate chamber in which temperature and relative humidity were 60 ºC and 60 % respectively, but lower temperatures can also be used. Bergo *et al*. (2008) dried cassava starch films at 30 ºC for 18-24 hours, whereas Jiménez *et al*. (2012) obtained corn starch films by drying at room temperature and at 45 % relative humidity for 60 hours.

According to Paes *et al*. (2008), there is no agreement on a standard method for the preparation of starch films to obtain one with the required functional and physicochemical characteristics. Therefore, it can be concluded that the preparation of starch films is a process which depends on several factors, such as the type of starch and plasticizer, and must be optimized in order to obtain films with adequate properties. Table 1 summarizes the different casting methods with which to obtain starch films and highlights the differences between the methods used by different authors.

Dry process

One of the major constraints when replacing synthetic polymers with natural ones is that, in most cases, edible films can not be produced using industrial processes. Some of these polymers are manufactured by casting, which involves drying times that are too long to permit large scale manufacturing. As a solution to this problem, the production of starch composites by thermoplastic treatment should be considered. As mentioned above, although native starch is not a thermoplastic material, it can be processed like conventional polymers if it is treated properly. Carvalho (2008) described TPS as an amorphous or semi-crystalline material composed of gelatinized or destructurized starch containing one or a mixture of plasticizers. TPS can be repeatedly softened and hardened so that it can be moulded/shaped by the action of heat and shear forces, thus allowing its processing to be conducted with the techniques commonly used in the plastics industry. Liu *et al* (2009) reviewed the traditional and new technologies used in starch treatment. According to these authors, starch can be processed thermally by sheet/film extrusion, foaming extrusion, injection molding, compression molding and reactive extrusion (a special type of extrusion in which chemical reaction and typical extrusion take place).

Table 1. Different methods used to obtain starch edible and biodegradable films by casting

Type of starch	Other components	Plasticizer	Gelatinization conditions	Drying conditions of films	Reference
Sago starch		Glycerol Sorbitol Polyethylene glycol	85 ºC, 30 min	40 ºC 20 hours	Abdorreza *et al.*, 2011
Quinoa starch		Glycerol	97 ºC, 30 min	34 - 50 ºC, 55 % RH for different times	Araujo-Farro *et al.*, 2010
High amylose corn starch		Glycerol	78 - 80 ºC, 10 min in alkaline medium	35 ºC 50 % RH	Bertuzzi *et al.*, 2007ab
Cassava starch		Glycerol	70 ºC, 1 min	30 ºC 18 - 24 hours	Bergo et al., 2008
Cassava starch	Potassium sorbate	Glycerol	73.5 ºC (5% higher than the temperature of the gelatinization plateau)	25 ºC after a 52 ºC / 2 hour step	Famá *et al.*, 2006
Tapioca starch	Potassium sorbate	Glycerol	Over 70 ºC	Different methods with temperatures of between 25 and 50 ºC	Flores *et al.*, 2007
Corn starch High amylose corn starch	Sunflower oil	Glycerol Sorbitol	Alkaline medium (10 g/L NaOH)	60 ºC 8 hours	García *et al.*, 2000a
Pea starch	Beeswax	Glycerol	Boilling temperature, 15 min	Room temperature of at least 40 hours	Han *et al.*, 2006
Sago starch Banana starch Potato starch Maize starch		Glycerol	98 ºC, 30 min	4 ºC during 10 days and after at 40 ºC during 48 hours in silica gel	Hernández *et al.*, 2008
Normal and oxidized potato starch		Glycerol	85 ºC, 3 hours	45 ºC for 8 hours	Hu *et al.*, 2009
Native and modified corn starches		Glycerol	80 - 90 ºC, 5 - 30 min	50 ºC in a ventilated oven until constant weight	López *et al.*, 2008
Potato starch	Sunflower oil	Glycerol	70 ºC, 30 min	60 ºC 60 % RH for 24 hours	Osés *et al.*, 2009b

(Cont.)

Type of starch	Other components	Plasticizer	Gelatinization conditions	Drying conditions of films	Reference
Standard maize starch Waxy maize starch High amylose maize starch Modified maize starch Pre-gelatinized maize starch	Wax of operculum	Glycerol Sorbitol	90 ºC, 2 hours 90 ºC, 15 min + autoclaving	23 ºC 60 % RH	Pagella *et al*., 2002
Native potato starch	Grindsted Acetem 70-001		95 ºC, 30 min	23 ºC 50 % RH 35 - 50 ºC in an oven	Petersson & Stading, 2005
Corn starch	Citric acid as cross-linking agent	Glycerol	On a hotplate at 90 ºC, 20 min	Air dry for about 48 hours before a cross-link step at 165 ºC	Reddy & Yang, 2010
Potato starch	Tween 20 Soy lecithin	Glycerol	70 ºC, 30 min	60 ºC 60 % RH 24 hours	Rodríguez *et al*., 2006
Mango, banana and okenia starch		Glycerol	Cold gelatinization (10 g/L NaOH) Thermal gelatinization, 95 ºC, 10 min	65 ºC 5 hours	Romero-Bastida *et al*., 2005
Native potato starch		Glycerol Sorbitol Xylitol	90 ºC, 5 min	35 ºC for at least 4 hours	Talja *et al*., 2007; 2008
Tapioca starch	Chitosan	Glycerol	On a hotplate set at 125 ºC, 30 min	25 ºC 48 hours	Vásconez *et al*., 2009
Native starch from Cará roots	Mineral clay	Glycerol	Boilling temperature, 30 min	40 - 50 ºC	Wilhelm *et al*., 2003

Some of these techniques are generally used with starch in combination with both natural and petroleum-derived polymers (Fishman *et al*., 2000; Kim & Lee, 2002; Frost *et al*., 2011).

Without physical forces, the gelatinization process mainly depends on water content and temperature conditions. However, in extrusion or thermal processes, gelatinization is typically achieved at low moisture content due to the high-shear and high-pressure conditions used which tear down the starch granules, allowing faster water transfer into the starch molecules (Burros *et al*., 1987). So, the loss of crystallinity is caused by the mechanical disruption of molecular bonds due to the intense shear conditions applied in the extruder.

The thermal processing of starch biopolymers also involves several chemical and physical changes (water diffusion, granule expansion, gelatinization, decomposition, melting and crystallization) in the starch granules in which gelatinization plays an important role in the conversion of starch to a thermoplastic (Liu *et al*., 2009). Although the complete gelatinization of starch in an extrusion process seems to be a simple mechanism in which shear forces and high pressures are involved, the whole process is much more complex.

During the last few decades, researchers have obtained starch-based films by means of thermal processes. This way of processing starch usually includes two main steps. Firstly, the starch is mixed with plasticizers and extruded in order to disrupt the starch granules, followed by a final step in which the obtained paste (or pellets) is thermo-moulded to form films. Once the starch is in an amorphous state, it can be injection-moulded (Averous & Boquillon, 2004), extruded with a film-blowing die (Thunwall *et al*., 2008) or thermopressed (Chung *et al*., 2010; Müller *et al*., 2011). The main variable that researchers consider when thermally processing starch is the temperature. They can vary this parameter throughout the screw in the extruder, during thermopressing or in the extruder die. In this way, Li *et al*. (2011) varied the temperature between 60 and 180 ºC along the extruder screw. Pushpadass *et al.* (2008) studied the influence of the temperature (110 or 120 ºC) in the sheeting die on the different properties of the final product and found significant, temperature-dependent differences. Studies focusing on the structural changes which occur in the polymer when submitted to temperature or mechanical action, by applying microscopic and spectroscopic techniques, would be necessary to better understand the dry process in this kind of matrices.

4. PHYSICOCHEMICAL PROPERTIES OF STARCH BASED FILMS

Starch is one of the polysaccharides used to obtain biodegradable films due to its ability to form a continuous matrix, its low permeability to oxygen (Liu, 2005; Dole *et al.*, 2004) and, compared to other non-starch films, its lower cost. However, like other hydrocolloids, when compared to plastic polymers, starch films exhibit several drawbacks, such as their hydrophilic character and poor mechanical properties. Films based on starch are transparent (Mali *et al.*, 2004, Jiménez *et al.*, 2012), odourless, tasteless and colourless.

As concerns the structure of the starch biopolymers, they are often semicrystalline, containing both amorphous and crystalline zones which are characterized by the glass transition temperature and the degree of crystallinity, respectively. Thus, physical and chemical properties, such as tensile and gas barrier properties will be influenced by both amorphous and crystalline zones (Liu, 2005) and their cohesive energy density. As commented on above, native starch with a high amylopectin content is more crystalline, which is contrary to the fact that starch films containing more amylose have a higher degree of crystallinity (García *et al.*, 2000b). This can be explained by the phenomenon of retrogradation that occurs in starch after gelatinization, from an initially amorphous state to a crystalline state (Liu & Thompson, 1998). During aging, starch molecules reassociate in more ordered structures by forming simple juncture points and entanglements, helices, and crystal structures (Vázquez & Álvarez, 2009). When the starch is dissolved in hot water, the crystalline structure of amylose and amylopectin are lost and they are hydrated. However, during film-formation, both macromolecules are rearranged and linear fractions of amylose and amylopectin form re-associations by hydrogen bonds. Therefore, the crystallinity of starch based films depends on several factors, such as drying and storage conditions (temperature and relative humidity), as well as the content of plasticizer (Rindlav *et al*., 1997). The presence among the film polymer molecules of these attractive forces can lead to the undesirable property of brittleness and, to overcome this limitation, plasticizers (usually polyols) are added to the film forming dispersions to decrease intermolecular forces (Arvanitoyannis *et al*., 1997; Arvanitoyannis & Biliaderis, 1998; Sothornvit & Krochta, 2001; Talja *et al*., 2008). Plasticization occurs in the amorphous region which has higher molecular mobility, and the ability of plasticizers to interrupt hydrogen bonding along the polymer chains depends both on the plasticizer type and the amount. The main advantage of using plasticizers is that the film becomes more flexible, tensile strength (TS) is decreased and elongation at break (E %) increases, as can be seen in the data shown in Table 2 (Sothornvit & Krochta, 2001). However, plasticizers increase film permeability to moisture, oxygen and aroma compounds (some data are shown in Tables 3 and 4). An effective plasticizer needs to be compatible with the polymer matrix. In this sense, glycerol is one of the most commonly used plasticizers in film formation. Depending on the content and type of the plasticizer, the botanical origin of the starch and the storage conditions, biopolymers can be obtained with different mechanical properties. Generally, starch-glycerol films have lower TS and EM (elastic modulus) values and higher E values than starch-sorbitol films, as can be seen in Table 2. So, films prepared with glycerol are more flexible and stretchable than those prepared with sorbitol, thus indicating the greater plasticizing effect of glycerol. Similar effects have been reported by other authors for different protein or polysaccharide matrices, such as soy protein (Audic & Chaufer, 2005) or sodium caseinate (Fabra *et al*., 2008). This can be explained by the more marked hydrophilic nature of glycerol compared to sorbitol, which leads it to absorb more water molecules which act as an additional plasticizer. Glycerol molecules are small and easily gain access between the polysaccharide chains through hydrogen bonds, reducing intermolecular polysaccharide interactions, increasing intermolecular spacing and lowering the tensile strength of starch films. Consequently, as the moisture content and plasticizer concentration of starch films increase, so does the water vapour transfer rate (Table 3). Dias *et al*., (2010) reported an increase in water vapour permeability when the plasticizer content increased in starch films, with either glycerol or sorbitol as plasticizer. Starch films plasticized with sorbitol were more effective moisture barriers than those plasticized with glycerol in the same test conditions (temperature and relative humidity gradient). This can be related with the relatively higher hydration ratio of glycerol, since sorbitol is less hygroscopic. Similarly, as shown in Table 4, starch-sorbitol films were less permeable to gas than starch-glycerol films (García *et al*., 2000a).

The glass transition temperature (Tg) is the parameter related to the softening point from which moment onwards the polymer can exhibit a thermoplastic nature. Likewise, in semi-crystalline polymers, the melting temperature (Tm) and degree of crystallinity limit the softening point. When both Tg and Tm are lower than the polymer decomposition temperature by a sufficient amount, thermoplastic treatment can give rise to thermoformed material which is useful as films or packages. Likewise, Tg, the ratio of both crystalline and amorphous zones and the degree of recrystallization are intimately related with the film physicochemical properties, mainly the tensile and transport properties. The addition of plasticizers, such as water or glycerol, tends to decrease the film Tg (García *et al*., 2000a), as can be seen by the data shown in Table 5.

Table 2. Mechanical properties of starch-based films (vs synthetic films)

STARCH GELATINIZED IN EXCESS OF WATER					
Film composition	**Films conditioned before and/or during tests**	**Elastic modulus EM (MPa)**	**Tensile strength TS (MPa)**	**Elongation E (%)**	**Reference**
Yam starch + 20 % glycerol	20 ºC; 64 % RH, Fresh films	546 (80)	30 (5)	13 (5)	Mali *et al*., 2006
	20 ºC; 64 % RH, Stored films	605 (161)	29 (5)	9 (3)	
Cassava starch + 20 % glycerol	20 ºC; 64 % RH, Fresh films	409 (30)	10 (3)	33 (1)	
	20 ºC; 64 % RH, Stored films	482 (86)	11 (3)	21 (2)	
Corn starch + 20 % glycerol	20 ºC; 64 % RH, Fresh films	550 (50)	17 (6)	9 (5)	
	20 ºC; 64 % RH, Stored films	551 (150)	13 (2)	11 (5)	
Rice starch + 20 % glycerol	25 ºC; 58 % RH	532.8 (115.6)	10.9 (1.2)	2.8 (0.7)	Dias *et al*., 2010
Rice starch + 30 % glycerol		21.3 (5.0)	1.6 (0.2)	59.8 (9.9)	
Rice starch + 20 % sorbitol		1052.6 (146.0)	22.3 (1.9)	2.8 (0.7)	
Rice starch + 30 % sorbitol		456.3 (81.0)	11.2 (1.0)	3.9 (1.9)	
Corn starch:glycerol (1:0.25)	25 ºC; 53 % RH, Fresh films	800 (70)	9.2 (0.6)	8 (4)	Jiménez *et al*., 2012
	25 ºC; 53 % RH, Stored films	1470 (160)	20 (2)	2.2 (0.2)	
Corn starch:glycerol: palmitic acid (1:0.25:0.15)	25 ºC; 53 % RH, Fresh films	640 (110)	6.8 (0.3)	4 (3)	
	25 ºC; 53 % RH, Stored films	828 (13)	11 (3)	1.6 (0.3)	
Corn starch:glycerol: stearic acid (1:0.25:0.15)	25 ºC; 53 % RH, Fresh films	870 (170)	8.3 (1.3)	2.2 (0.7)	
	25 ºC; 53 % RH, Stored films	1300 (200)	12 (2)	1.0 (0.2)	
Corn starch:glycerol: oleic acid (1:0.25:0.15)	25 ºC; 53 % RH, Fresh films	170 (30)	3.3 (0.3)	13 (7)	
	25 ºC; 53 % RH, Stored films	1100 (300)	6.1 (0.7)	0.8 (0.2)	

(Cont.)

STARCH GELATINIZED BY EXTRUSION OR THERMAL TREATMENT					
Composition	**Films conditioned before and/or during tests**	**EM (MPa)**	**TS (MPa)**	**E (%)**	**Reference**
Soluble starch:methyl cellulose:glycerol: water (0.325:0.325:0.3:0.05)[1]			33.5 (3.1)	34.2 (3.5)	Arvanitoyannis & Biliaderis, 1999
Corn starch:mycrocrystalline cellulose:glycerol:water		53.4 (3.2)	25.0 (1.7)	8.5 (0.5)	Psomiadou *et al*., 1996
Corn starch + 25 % glycerol + stearic acid (0.25 %) Extruded at 110 ºC	25 ºC; 50 % RH	64.1	3.1	39.9	Pushpadass *et al*., 2008
Corn starch + 25 % glycerol + stearic acid (0.25 %) Extruded at 120 ºC		57.6	3.1	45.8	
Corn starch + 30 % glycerol	25 ºC; 43 % RH	840 (61)	11.82 (0.57)	4.62 (1.06)	Chung *et al*., 2010
Corn starch + montmorillonite (nanocomposite:gly = 1:0.3)	25 ºC; 43 % RH	1390 (166)	15.49 (0.67)	4.34 (1.15)	
Corn starch + chitosan/montmorillonite (nanocomposite:gly = 1:0.3)	25 ºC; 43 % RH	805 (79)	12.46 (1.45)	5.35 (1.17)	
Cassava starch:gly 1:0.3 + bentonite nanoparticles		789 (42)	16.47 (1.17)	2.60 (0.49)	Müller *et al*., 2011
SYNTHETIC FILMS					
Low-density polyethylene (LDPE)	25 ºC; 50 % RH		9-17	500	Briston (1986)
High-density polyethylene (HDPE)			17-35	300	
Polypropylene (PP)			42	300	
Oriented polypropylene (OPP)			165	50-75	

[1] Water content in film before analysis was 10 %

Table 3. Water vapour permeability of starch films (vs synthetic films)

STARCH GELATINIZED IN EXCESS OF WATER			
Composition	**Test conditions**	**WVP ($g\cdot m^{-1}\cdot s^{-1}\cdot Pa^{-1}$) $\cdot 10^{11}$**	**Reference**
[1]HCMS + 20 % glycerol	25 ºC; 50-100 % RH	120 (10)	Kim *et al.*, 2002
[1]HCMS + 20 % sorbitol		180 (30)	
[1]HCMS + 20 % xylitol		140 (20)	
[1]HCMS + 20 % mannitol		220 (30)	
Yam starch + 20 % glycerol	25 ºC; 0-75 %, RH Fresh[2] films	44.6 (10.5)	Mali *et al.*, 2006
	25 ºC; 0-75 %, Stored[3] films	40.0 (5.5)	
Cassava starch + 20 % glycerol	25 ºC; 0-75 %, Fresh films	40.2 (12.6)	
	25 ºC; 0-75 %, Stored films	40.2 (10.1)	
Corn starch + 20 % glycerol	25 ºC; 0-75 %, Fresh films	53.7 (2.1)	
	25 ºC; 0-75 %, Stored films	50.0 (7.5)	
[4]Composite:sorbitol 1:0.1 + 15 % lauric acid (d.b.)	25 ºC; 0-75 % RH	3.01 (0.02)	Fakhouri *et al.*, 2009
[4]Composite:sorbitol 1:0.1 + 15 % miristic acid (d.b.)		3.55 (0.35)	
[4]Composite:sorbitol 1:0.1 + 15 % palmitic acid (d.b.)		3.48 (0.07)	
Rice starch + 20 % glycerol	25 ºC; 2-75 % RH	4.64 (0.69)	Dias *et al.*, 2010
Rice starch + 30 % glycerol		8.67 (0.22)	
Rice starch + 20 % sorbitol		2.67 (0.11)	
Rice starch + 30 % sorbitol		3.03 (0.36)	
Corn starch:glycerol 1:0.25	25 ºC; 53 %, RH Fresh films	9.14 (0.23)	Jiménez *et al.*, 2012
	25 ºC; 53 %, RH Stored films	8.91 (1.16)	
Corn starch:gly:palmitic acid 1:0.25:0.15	25 ºC; 53 %, RH Fresh films	6.83 (0.69)	
	25 ºC; 53 %, RH Stored films	8.68 (1.16)	
Corn starch:gly:stearic acid 1:0.25:0.15	25 ºC; 53 %, RH Fresh films	6.71 (0.35)	
	25 ºC; 53 %, RH Stored films	7.99 (0.46)	
Corn starch:gly:oleic acid 1:0.25:0.15	25 ºC; 53 %, RH Fresh films	8.33 (1.04)	
	25 ºC; 53 %, RH Stored films	8.72 (0.17)	

(Cont.)

STARCH GELATINIZED BY EXTRUSION OR THERMAL TREATMENT			
Soluble starch:sodium caseinate:glycerol:water 0.325:0.325:0.3:0.05	25 ºC; 22.6-84.3 % RH	15.1 (1.3)	Arvanitoyannis & Biliaderis, 1998
Soluble starch:sodium caseinate:glycerol:water 0.3:0.3:0.3:0.1		19.5 (1.3)	
Corn starch + 25 % glycerol + stearic acid (0.25 %) Extruded at 110 ºC	25 ºC; 50-100 % RH	12.62	Pushpadass *et al*., 2008
Corn starch + 25 % glycerol + stearic acid (0.25 %) Extruded at 120 ºC		14	
Cassava starch:glycerol 1:0.3 + bentonite nanoparticles	25 ºC; 2-75 % RH	8.33 (0.56)	Müller *et al*., 2011
SYNTHETIC FILMS			
Polyvinyl chloride (PVC)	28 ºC; 0-100 % RH	0.7176	Shellhammer & Krochta, 1997
Polyethylene terephthalate (PET)	25 ºC; 0-100 % RH	0.1968	
LDPE	28 ºC; 0-100 % RH	0.0358	

[1] HCMS: Highly carboxymethylated starch

[2] Fresh films: Newly prepared films, non stored

[3] Stored films: Films analyzed after a storage period after preparation

[4] Composite: Lipophilic starch + gelatin

Table 4. Gas barrier properties of starch and synthetic films

Composition	Test conditions	O_2 permeability · 10^{12} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	CO_2 permeability · 10^{12} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	N_2 permeability · 10^{12} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	Reference
Soluble starch:sodium caseinate:glycerol:water 0.325:0.325:0.3:0.05	22 ºC	350 (20)	1400 (100)	11 (1)	Arvanitoyannis & Biliaderis, 1998
Soluble starch:sodium caseinate:glycerol:water 0.3:0.3:0.3:0.1		3000 (40)	430 (40)	32000 (2000)	
Soluble starch:methyl cellulose:glycerol:water 0.3:0.3:0.3:0.1	22 ºC	30000 (7000)	320000 (20000)	43 (5)	Arvanitoyannis & Biliaderis, 1999
Corn starch	20 ºC; 63.8 % RH	1592 (299)	29210 (1389)		García *et al*., 2000a
Corn starch + 34.4 % glycerol		461 (51)	5690 (970)		
Corn starch + 34.4 % sorbitol		248 (32)	4190 (810)		
Amilomaize starch		2645 (248)	28050 (370)		
Amilomaize starch + 34.4 % glycerol		321 (19)	3850 (1280)		
Amilomaize starch + 34.4 % sorbitol		228 (26)	2960 (460)		
Corn starch:cellulose:lignin 40:50:10	25 ºC; 0 % RH	1.427 (0.074)	1.51 (0.11)		Wu *et al*., 2009a
Corn starch:cellulose:lignin 25:73:2		1.479 (0.115)	2.44 (0.13)		
Quinoa starch +21.2 % glycerol	25 ºC; 58 % RH	0.0502 (0.0119)			Araujo-Farro *et al*., 2010
Silver nanoparticle-loaded chitosan-starch blend + 20 % glycerol	23 ºC; 0 % RH	0.00642 (0.00001)			Yoksan & Chirachanchai, 2010
SYNTHETIC FILMS					
LDPE	23 ºC; 50 % RH	21.644			Salame, 1986
HDPE		4.942			
Polyester		0.181			Hanlon, 1992
Ethylene Vinyl Alcohol (EVOH; 70 % VOH)	23 ºC; 95 % RH	0.139			Salame, 1986

Table 5. Glass transition and melting temperatures observed in biodegradable films based on starch

Composition	Films conditioned before test	Glass transition temperature Tg (ºC)	Melting temperature (Onset) To (ºC)	Melting temperature (Peak) Tp (ºC)	Reference
Corn starch + 34.4 % glycerol (15 days' storage)	20 ºC; 63.8 % RH	45.21			García *et al.*, 2000a
Corn starch + 34.4 % glycerol (30 days' storage)		47.75			
Corn starch + 34.4 % sorbitol (15 days' storage)		43.42			
Corn starch + 34.4 % sorbitol (30 days' storage)		45.79			
Cassava starch	25 ºC; 58 % RH	131.9			Bergo *et al.*, 2008
Cassava starch + 15 % glycerol		97.9			
Cassava starch + 30 % glycerol		62.2 -55.7			
Cassava starch + 45 % glycerol		62.2 -55.8			
[1]Nanocomposite	23 ºC; 50 % RH	52.36 (1.90)			Tang *et al.*, 2008
[1]Nanocomposite + 10 % glycerol		53.42 (2.25)			
[1]Nanocomposite + 20 % glycerol		48.97 (2.12)			
Sago starch	23 ± 2 ºC; 50 ± 5 % RH		123.65 (1.87)	157.20 (2.76)	Abdorreza *et al.*, 2011
Sago starch + 30 % glycerol			149.00 (3.52)	168.89 (2.29)	
Sago starch + 40 % glycerol			151.45 (3.93)	174.53 (3.52)	
Sago starch + 50 % glycerol			140.56 (0.85)	164.21 (1.19)	
Sago starch + 30 % sorbitol			125.60 (3.14)	157.86 (2.45)	
Sago starch + 40 % sorbitol			118.40 (1.80)	151.01 (3.27)	
Sago starch + 50 % sorbitol			121.53 (1.42)	154.46 (1.24)	
Corn starch:gly 1:0.25	25 ºC; 53 % RH	37.35 (1.85)			Jiménez *et al.*, unpublished data
Corn starch:gly:stearic acid 1:0.25:0.15		31.75 (1.65)			
Corn starch:gly 1:0.25	25 ºC; 75 % RH	5.17 (5.58) -48.75 (6.57)			
Corn starch:gly:stearic acid 1:0.25:0.15		0.66 (5.22) -39.05 (0.65)			

[1] Nanocomposite: Corn starch + montmorillonite nanoparticles

This phenomenon has also been recently observed in extruded waxy maize and rice starches in presence of glycerol and water (Enrione *et al*., 2010). If the storage temperature is higher than the Tg, starch tends to recrystallize (Liu, 2005). As is known, water molecules have a plasticizing effect on polysaccharide films. The insertion of plasticizer molecules into the polymer matrix facilitates the movement of chains thus increasing the film flexibility. However, too much plasticizer weakens the cohesion of the polysaccharide chains and the number of plasticizer-plasticizer bonds increases, which can give rise to phase separation by forming glycerol enriched zones and polysaccharide-plasticizer zones. The starch-plasticizer ratio at which this phase separation occurs depends on the type of starch and the relative humidity conditions. Bergo *et al*. (2008) reported two glass transition temperatures in cassava starch films, attributed to the starch enriched phase and to the glycerol phase respectively, taking into account the fact that the glass transition temperature of glycerol ranges between -50 and -80ºC, depending on the water content (Claudy *et al*., 1997).

Considering that recrystallinization phenomena in starch films occur, starch films are greatly affected by ageing (defined by the storage time), inducing important physical and chemical changes in the films' properties (Delville *et al*., 2003; Famá *et al*., 2007; Jiménez *et al*., 2012) that could affect their functionality as a consequence of the reassociation into crystalline segments and, thus, in amorphous and crystalline zones. Generally, starch based films become stronger and stiffer but less flexible after several weeks of storage (Jiménez *et al*., 2012). However, Mali *et al*. (2006) did not report significant differences in TS and EM values of cassava, corn and yam starch based films containing 20% of glycerol, though these films were less deformable as storage time increased. They concluded that the crystallinity of starch films increased the longer they were stored and glycerol-free films were more seriously affected; the plasticizer seemed to limit crystal growth and recrystallization due to the interaction with the polymeric chains. However, neither Mali *et al*. (2006) nor Jiménez *et al*. (2012) reported significant differences in water vapour permeability values as a consequence of the storage time.

Liu (2005) affirmed that stored films are expected to exhibit lower carbon dioxide permeability values due to their increased crystallinity. In this way, García *et al*. (2000b) reported lower values of CO_2 permeability for corn starch and amylomaize stored films (20 days at 20ºC/63.8 % RH) in comparison with newly prepared ones. The O_2 and CO_2 permeability values of newly prepared films are compared in Table 4. Carbon dioxide permeates faster than oxygen, probably due to its greater solubility in the water molecules of the film. Gontard *et al*. (1996) and Alves *et al*. (2010) observed similar behaviour for wheat gluten films and composite films based on kappa-carrageenan/pectin blends and mica flakes, respectively.

Effect of lipid addition and hydrocolloid mixture

The incorporation of lipophilic materials can effectively modify starch film properties, improving the barrier properties of starch films to water vapor due to an overall increase of the film’s hydrophobicity (Jiménez *et al*., 2012; García *et al*., 2000a). The optimum lipid concentration depends on the use of the film and it is a balance between the hydrophilic-hydrophobic ratio and the crystalline-amorphous ratio (García *et al*., 2000a). Adding saturated fatty acids to corn starch films did not notably improve the water vapour transfer of non-aged films and saturated fatty acids only provoked a slight reduction in water vapour permeability as compared to oleic acid (Jiménez *et al*., 2012; Fakhouri *et al*., 2009). However, García *et al*., 2000a observed that by increasing the sunflower oil concentration above a critical ratio, the water vapour permeability of starch films also increased due to the migration of the oil and the decrease of the crystalline-amorphous ratio. They observed that films containing oil showed lower fusion enthalpy values, which were associated with a lower crystalline-amorphous ratio. Jiménez *et al*., (2012) observed that corn starch-fatty acid films also developed crystallinity throughout the storage time, which implied an increase in the film's stiffness and brittleness and a loss of stretchability, gloss and transparency.

Since the physicochemical properties of starch based films are limited by their degree of crystallinization, recent studies have analysed the effect of other carbohydrates, such as agar, chitosan and cellulose, on matrices mixed with starch in order to improve the physico-chemical properties of the films. Table 6 summarizes some of these studies. Films made with agar and starch were elaborated by Wu *et al*. (2009b) and Phan The *et al*. (2009) and tested for their potential use as food packaging. They used Fourier-transform infrared spectroscopy (FTIR) to demonstrate that agar and starch were compatible and the addition of agar improved the microstructure of starch films as well as the tensile and water vapor properties at high moisture contents, probably due to the fact that the addition of agar could restrain the mobility of starch chains, thus preventing the starch retrogradation. Similarly, Ghanbarzadeh *et al*. (2010) affirmed that the carboxyl-methyl-cellulose-starch-glycerol composite films show better physico-mechanical properties than starch films. However, Müller *et al*. (2009a) demonstrated that films with cellulose fibers were more crystalline and had higher tensile strength and rigidity, although the addition of these fibers increased the stability of starch based films submitted to relative humidity variations, solving the problem encountered with these types of films.

Likewise, several studies analyzed the effect of chitosan on starch based films (Fernández-Cervera *et al*., 2004; Mathew & Abraham, 2008; Bourtoom & Chinnan, 2008; Shen *et al*., 2010; Zhong *et al*., 2011) and underlined the known antimicrobial property of this carbohydrate. In these pieces, although the mechanical resistance of starch films was improved by chitosan addition, oxygen permeability, water vapor permeability and water solubility all decreased.

Table 6. Effect of other polysaccharides on some characteristics of edible and biodegradable films based on starch

Type of starch	Hydrocolloid added	Film characteristics and improvement	Reference
kudzu starch	Chitosan	The two film-forming components were compatible. Films showed antimicrobial activities against *Escherichia coli* and *Staphylococcus aureus*. Acids (acetic acid, lactic acid and malic acid) used to dissolve chitosan affected the properties of the films, making them stronger or more flexible with different solubility depending on the acid used to dissolve the chitosan.	Zhong *et al.*, 2011
Rice starch		Mechanical resistance of the starch films was improved by incorporating chitosan into the starch film. Addition of chitosan increased the crystalline peak structure of the starch film, but phase separation between both components was observed when chitosan was added in high concentrations. Water vapor permeability of blended films was significantly lower than for chitosan based films.	Bourtoom & Chinnan, 2008
Tapioca starch		Properties of starch-based edible films were influenced by chitosan and glycerol concentrations. Optimum concentrations of chitosan and glycerol in tapioca starch-based films were 0.55% and 0.5%, respectively.	Chillo *et al.*, 2008
Cassava starch		Coating starch film with chitosan, by using an automatic film coater, produced a chitosan-coated film with improved performance, including water vapour barrier capability, surface and mechanical properties.	Bangyekan *et al.*, 2006
Corn starch		Tensile strength and flexibility of the starch films were improved greatly after incorporating 20% chitosan. Irradiated starch/chitosan blend film showed good antibacterial activity against *E. coli*.	Zhai *et al.*, 2004
Potato starch	Chitosan / Potassium sorbate	Chitosan addition improved the tensile properties of films but decreased oxygen permeability, water vapor permeability and water solubility. However, the addition of potassium sorbate produced the opposite effect probably due to the fact that it disturbed the crystallinity of the starch which increased the permeable properties of the films.	Shen *et al.*, 2010
	Agar	Agar and potato starch were compatible. Addition of agar improved microstructure, mechanical properties and WVP in a high moisture environment.	Wu *et al.*, 2009b
Cassava starch	Cellulose fibers	The addition of cellulose fibers to starch-based films is an effective way to prepare stronger and more stable films and they increase the stability of starch-based films to relative humidity changes.	Müller *et al.*, 2009a
	Agar	Films prepared with mixtures of agar and cassava starch increased the surface wettability of agar films but not that of cassava starch films. Adding agar to cassava starch induces an improvement in elongation and tensile strength of cassava starch based films. They concluded that agar is able to provide a very good cohesive matrix.	Phan The *et al.*, 2009
Corn starch	Carboxy-methyl-cellulose (CMC)	CMC-starch biocomposite films show better physico-mechanical properties than starch films and can be replaced by starch films. For instance, CMC improved the moisture resistance of the resulting starch based composites. Although the blend film, composed of 15% W/W CMC /starch, had the lowest water vapor permeability value, their moisture absorption and solubility in water properties exhibited similar trends.	Ghanbarzadeh *et al.*, 2010
Pea starch	Konjac glucomannan	Strong hydrogen bonding formed between macromolecules of starch and konjac glucomannan, resulting in a good miscibility. This additive increased the moisture uptake of the blend films as well as their tensile strength. The melting temperature of starch crystallinities decreased as the glucomannan content increased.	Chen *et al.*, 2008

The reinforcement of starch based films

As has been commented on previously, starch films are mostly water-soluble and exhibit poor mechanical properties (Carvalho *et al*., 2001). One way to mitigate these drawbacks is the reinforcement of starch films by using organic and inorganic fillers to form biocomposites, as has been reported in different works over the last few years. The resistance to water (or water vapour barrier) can be improved by adding microparticles (Carvalho *et al*., 2001) or nanoparticles (Tang *et al*. 2009; Yu *et al*., 2009). Mechanical resistance of starch based films has also been improved by using different reinforcement agents such as clays (Carvalho *et al*., 2001; Avella *et al*., 2005) or cellulosic fibers (Curvelo *et al*., 2001; Müller *et al*., 2009b).

Carvalho *et al*. (2001) used kaolin (a hydrate aluminosilicate) as reinforcement agent in a thermoplastic starch matrix. They found that when kaolin was added, at 50 per cent (dry basis), to thermoplastic starch, the elastic modulus and tensile strength showed an increase with respect to the clay-free matrix of around 130 and 50 %, respectively. Furthermore, the addition of 20 phr of kaolin allowed the moisture uptake to be reduced significantly in comparison with the pure starch matrix.

The addition of nanoparticles is also an option to modify positively the properties of starch films. A nanoparticle is an ultrafine particle in the nanometer size order (Hosokawa *et al*., 2008) which is able to form nanobiocomposite films when it is combined with natural polymers (Falguera *et al*., 2011). According to Rhim and Ng (2007), the nanometer-size dispersion of polymer-clay nanocomposites exhibited a large-scale improvement in the mechanical and physical properties in comparison with pure polymer or conventional composites. Tang *et al*. (2009) obtained a starch/poly(vinyl alcohol) biodegradable film reinforced with silicon dioxide nanoparticles. The addition of the reinforcement agent improved the mechanical resistance of the polymer without affecting the final biodegradability of the film. The addition of nanoparticles is also able to reduce the water vapour permeability of starch based films, as has been mentioned above. In this way, Yu *et al*. (2009) used ZnO nanoparticles in combination with pea starch and found that water vapour permeability decreased markedly with the addition of nanoparticles. They related this phenomenon to the introduction of a tortuous path for water molecules to pass through due to the presence of the nanoparticles in the matrix (Kristo & Biliaderis, 2007).

Besides the improvement in water and mechanical resistance of reinforced films, some studies on the influence of reinforcement agents in the glass transition of the polymer were carried out. Carvalho *et al*. (2001) found that Tg diminished as the kaolin microparticle content increased, and related this to the heterogeneity of the matrix. On the contrary, Curvelo *et al*. (2001), working with regular corn starch, reported an increase in the glass transition temperature as a consequence of the addition of cellulosic fibers, in agreement with water sorption experiments. The physicochemical properties of starch-cellulosic composites and starch-cellulosic fiber blends have been reviewed by Arvanitoyannis & Kassaveti (2009) and Vázquez & Álvarez (2009), respectively.

Incorporation of functional compounds and applications of starch based films

Edible and biodegradable films and coatings can be formulated with antimicrobial and antioxidant ingredients to improve the overall protection they provide. As regards the antimicrobial effects, chitosan has shown great potential to be used in packaging for food preservation against a wide variety of microorganisms due to the fact that it shows antimicrobial activity (Dutta *et al*., 2009). Several studies evaluated the effectiveness of chitosan in starch films (Vásconez *et al*., 2009; Zhai *et al*., 2004; Zhong *et al*., 2011). For instance, Salleh *et al*. (2009) reported that adding chitosan to plasticized starch films containing 8% lauric acid led to material with good antimicrobial and oxygen barrier properties, showing an oxygen transmission rate reduction of 91% with respect to pure chitosan. Vásconez *et al*. (2009) observed a decrease in water vapour permeability values of starch/chitosan films, which was attributed to the fact that chitosan is more hydrophobic than starch (Bangyekan *et al*., 2006). In addition, hydrogen bond interactions between tapioca starch and chitosan reduce the availability of hydrophilic groups, diminishing their interactions with water molecules. They also observed that when chitosan coating was applied to salmon fillet pieces, the antimicrobial effectiveness was greater than when chitosan-tapioca starch blends were applied.

In more recent work, Pyla *et al*. (2010) obtained a starch based film with both antioxidant and antimicrobial effects, by using tannic acid (a water-soluble polyphenol containing sugar esters and phenol carboxylic acids). They studied the effect of adding tannic acid to the corn starch matrix and found antimicrobial activity against *Escherichia coli* O157:H7 and *Listeria monocytogenes* and an antioxidant effect on soybean oil.

One of the main problems of precooked meat products is that they are susceptible to lipid oxidation, which results in a rancid flavor during refrigerated storage (St. Angelo *et al*., 1987; Love, 1988). Edible coatings containing antioxidant have been studied as an alternative to control the quality of these products. For instance, coatings of starch-alginate, starch-alginate-tocopherol and starch-alginate-rosemary have been reported to reduce off-flavours in precooked, refrigerated pork chops and beef patties (Hargens-Madsen, *et al*., 1995; Ma-Edmonds *et al*., 1995, Handley *et al*., 1996). Films containing tocopherol were more effective than the tocopherol-free controls. Wu *et al*. (2001) reported that starch-alginate-stearic acid composite films were effective at controlling lipid oxidation, off-flavours and moisture loss in precooked ground beef patties. Nevertheless, these films were not as effective as polyester vacuum bags at retarding moisture loss and lipid oxidation.

Ediflex®, an extruded hydroxypropylated high-amylose starch film, was developed and used as a wrap for frozen meats and poultry in the late 1960s (Anonymous 1967; Kroger & Igoe, 1971; Morgan, 1971; Sacharow, 1972). It was flexible, a good oxygen barrier, oil-resistant and heat-sealable, and was effective at protecting meat products during frozen storage. The coating also dissolved during thawing and cooking (Kroger & Igoe 1971; Morgan 1971; Sacharow 1972).

Besides avoiding lipid oxidation, packaging has to be able to limit moisture transfer. For example, edible starch based films can retard microbial growth by lowering the water activity within the package, thereby reducing drip loss of meat products and binding water that otherwise would be available for microbial growth (Wong *et al*., 1994).

Nowadays, several starch based films are commercialized. For instance, Novamont, an Italian company, commercializes MaterBi®, a starch based material available in granular form that can be processed as thermoplastic material to form films or bags. Other available products are Bio-P-TM®, commercialized by Bioenvelope (Japan) and BIOPAR® (Biop Biopolymer Technologies AG, Germany) which is a material based on potato starch and fully biodegradable.

5. CONCLUSION

Starch is becoming an environmentally-friendly alternative to petroleum-based polymers due to its low cost, biodegradability and its capability to form films by thermoplastic processing. Starch films have been obtained by casting or by thermal processing when thermoplastic starch was used. A great number of components have been added to the matrix, and process variables changed, in order to improve film properties. When the conditions are optimized, the obtained films are transparent, odourless, tasteless and colourless, with good mechanical, barrier and optical properties. Nevertheless, retrogradation phenomenon and the highly hydrophilic nature of starch films limit their usefulness. The incorporation of some additives, such as lipids, other hydrocolloids or reinforcement agents, can solve these problems to quite an extent, giving rise to more stable materials with improved properties. However, most of the studies have been carried out by using casting methods with very limited industrial application. More studies using thermal processing are required in order to optimize formulation of starch films that can be produced on a commercial scale. In this sense, the analysis of nano and microstructural changes occurring in the starch matrices as a function of the composition and process conditions, and their relation to the film properties, is necessary to optimize film formulation and process conditions.

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II. OBJETIVOS

1. OBJETIVOS

1.1. OBJETIVO GENERAL

El objetivo general de la presente tesis doctoral consiste en desarrollar y caracterizar films biodegradables a base de almidón de maíz y glicerol, evaluando la influencia de la adición de componentes lipídicos, otros biopolímeros y compuestos bioactivos (antioxidantes y/o antimicrobianos) sobre las propiedades físicas y estructurales relevantes en su funcionalidad como materiales de envase o recubrimiento.

1.2. OBJETIVOS ESPECÍFICOS

- Analizar la influencia de la adición de ácidos grasos saturados (palmítico y esteárico) e insaturados (ácido oleico) sobre las propiedades físicas (mecánicas, ópticas y de barrera al vapor de agua) y microestructurales de films de almidón acondicionados a distintos tiempos de almacenamiento, evaluando la cristalización de los componentes mediante difracción de rayos X.
- Analizar la capacidad de adsorción de agua de films de almidón y ácidos grasos en función de la temperatura y la humedad relativa y su relación con los fenómenos de cristalización (analizados por difracción de rayos X), transición vítrea de la matriz polimérica y fusión de los lípidos y el comportamiento mecánico de los films.
- Evaluar la influencia de la adición de hidroxipropilmetilcelulosa, las condiciones de homogeneización de las dispersiones formadoras de films y el tiempo de almacenamiento, sobre las propiedades microestructurales, mecánicas, ópticas y de barrera (vapor de agua y oxígeno) en films a base de almidón de maíz y glicerol.

- Analizar el efecto de la adición de caseinato de sodio en las propiedades microestructurales, mecánicas, ópticas y de barrera (vapor de agua y oxígeno) de films de almidón en distintas condiciones de almacenamiento.
- Evaluar el efecto de la adición de ácido oleico y/o α-tocoferol en las propiedades de films de almidón y caseinato sódico a través del análisis de las propiedades microestructurales, mecánicas, ópticas y de barrera así como su capacidad antioxidante a diferentes tiempos de almacenamiento.
- Desarrollar films a base de almidón y caseinato de sodio con propiedades antimicrobianas, incorporando nanoliposomas portadores de compuestos activos (aceite esencial de naranja y D-limoneno) y estudiando la influencia de la adición de nanoliposomas (con y sin compuestos activos) sobre las propiedades de las dispersiones formadoras de films y de los propios films.

III. RESULTADOS Y DISCUSIÓN

El almidón presenta la ventaja, frente a otros biopolímeros, de que puede procesarse industrialmente con las técnicas convencionales de los materiales termoplásticos, gracias a que presenta termoplasticidad, cuando se combina con una cantidad adecuada de plastificante. Sin embargo, como ocurre con otros polisacáridos, presenta dos inconvenientes de gran relevancia. Por un lado se trata de un polímero muy hidrofílico, y por tanto, muy sensible en sus propiedades a la acción de la humedad. Por otro lado, presenta fenómenos de retrogradación, que comprometen seriamente el comportamiento mecánico de los films formados y su evolución con el tiempo, dependiendo de la condiciones ambientales.

En una primera aproximación para minimizar estos problemas se adicionaron ácidos grasos (saturados e insaturados) a las formulaciones de los films de almidón-glicerol y se estudió su influencia sobre su comportamiento. Este trabajo constituye el Capítulo 1 del presente apartado. En éste se recojen una serie de análisis realizados en los films acondicionados en su humedad durante una semana (tiempo inicial) y durante cinco semanas (tiempo final) donde se evaluan distintas propiedades como son las propiedades mecánicas, de barrera y ópticas, en relación a la microestructura y a los fenómenos de cristalización observados mediante los espectros de difracción de rayos X. Por otra parte, se analizó la capacidad de adsorción de agua de los films, las transiciones de fase, la cristalización y el comportamiento mecánico de dichos films en condiciones variables de humedad relativa y temperatura.

En base a los resultados obtenidos que se recogen en el primer capítulo, se optó por utilizar la mezcla con otros polímeros con el fin de limitar la retrogradación del almidón con el tiempo. En este sentido, en el Capítulo 2 se utilizaron dos polímeros de distinta naturaleza. En primer lugar se utilizó hidroxipropilmetilcelulosa (de carácter hidrofílico como el almidón) y en segundo lugar caseinato de sodio (de carácter anfifílico). En el primer caso, y debido a la falta de miscibilidad entre ambos polímeros, se utilizaron dos tipos de homogeneización (rotor-estator y microfluidificación) de las dispersiones formadoras de films, con el objetivo de mejorar su integración. En ambos trabajos se analizaron las diferentes propiedades de los films a dos tiempos distintos (1 semana y 5 semanas de almacenamiento) y se realizaron análisis de difracción de rayos X para verificar la idoneidad de los polímeros ensayados como inhibidores de la retrogradación. En relación a esto, se utilizaron distintas proporciones de polímero para determinar las formulaciones óptimas.

Elegida una formulación óptima a partir de los resultados del capítulo anterior, en el Capítulo 3 se recogen los resultados de un trabajo en el que se evalúa la influencia de la adición de α-tocoferol (antioxidante) y ácido oleico en las propiedades de films a base de almidón y caseinato sódico. En este trabajo se analizaron las propiedades microestructurales, mecánicas, ópticas y de barrera así como la capacidad antioxidante de los films no almacenados y almacenados durante cinco semanas.

En el Capítulo 4, tras haber estudiado anteriormente la posibilidad de incorporar un antioxidante, se evalúa la aptitud de los films *composite* de almidón y caseinato de sodio como portadores de compuestos antimicrobianos de origen natural (aceite esencial de naranja y su componente mayoritario limoneno). En este sentido, y para evitar la pérdida de los compuestos volátiles durante el secado de los films, se realizó una encapsulación de los mismos en nanoliposomas de lecitina de colza y lecitina de soja. Los resultados correspondientes a la caracterización de los nanoliposomas, las dispersiones formadoras de films y los films secos aislados se recogen en este capítulo.

Capítulo I

Influencia de la adición de ácidos grasos saturados e insaturados en las propiedades físicas de films a base de almidón de maíz y glicerol

EFFECT OF RE-CRYSTALLIZATION ON TENSILE, OPTICAL AND WATER VAPOUR BARRIER PROPERTIES OF CORN STARCH FILMS CONTAINING FATTY ACIDS

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ABSTRACT

Starch films are very hygroscopic and undergo crystallization during processing and storage. In this work, fatty acids (SA, PA and OA) were incorporated into starch films containing glycerol as plasticizer, in order to reduce the hygroscopic character of the films and to improve water vapour permeability. Microstructural, mechanical, barrier and optical properties were studied in both non-stored films and those stored for 5 weeks. Fatty acid addition affected the main properties of films since mechanical resistance was decreased, water vapour permeability was reduced and gloss and transparency were reduced. The degree of starch and FA crystallinity increased with storage time and gave rise to changes in film properties: films became stiffer, less effective as water vapour barriers and less transparent and glossy. Saturated fatty acids were more efficient at reducing WVP as compared to oleic acid, but these differences decreased after storage due to the greater increase in crystallization of the former as it has been observed by X-ray diffraction.

Keywords: Starch, re-crystallization, SEM, physical properties, films storage.

1. INTRODUCTION

Starch is more and more commonly used to obtain polymer matrices for packaging due to its wide availability in many natural products and low cost (Mali & Grossmann, 2003; Rodríguez, Osés, Ziani, & Mate, 2006). Furthermore, it is known to be completely biodegradable in soil and water which is of great advantage from the environmental point of view (Kampeerapappun, Aht-ong, Pentrakoon, & Srikulkit, 2007). In starch biopolymers, physical and chemical changes, associated with retro-gradation, which occur during ageing have a great impact on the material properties (Delville, Joly, Dole, & Bliard, 2003) affecting the material functionality. Retro-gradation implies that starch molecules are reassociated into crystalline zones. Several works point out that initial crystallinity of starch films depends on the drying temperature and relative humidity (Liu, 2005) as well as the chain lengths, concentration of starch, pH and composition of the system. The development of the degree of crystallinity of starch films during storage (ageing) is greatly affected by the storage conditions, such as the temperature, relative humidity and time (Osés, Fernández-Pan, Mendoza, & Maté, 2009). For instance, if the storage temperature is higher than the glass transition temperature (Tg), starch matrix tends to crystallize. Thus, even though recently prepared starch films are almost amorphous (García, Martino, & Zaritzky, 2000a; Myllärinen, Buleon, Lahtinen, & Forssell, 2002; Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998), in time they develop crystallinity (García *et al*., 2000a). As a consequence, starch films usually become stronger, stiffer and less flexible (Forssell, Mikkilä, Moates, & Parker, 1997).

Similarly to many polysaccharide based films, pure starch films are too brittle and need the incorporation of plasticizers to make them easier to handle and achieve the optimum mechanical properties. Plasticizers reduce the cohesive forces in hydrocolloid films, through the limitation of intermolecular forces responsible for the chain-to-chain interactions, which allows this drawback to be overcome. So, plasticizers improve flexibility, elongation and toughness of films and they must be compatible with the film forming polymer (Sothornvit & Krochta, 2005). In this sense, different polyols such as glycerol (Rodríguez *et al*., 2006; Famá, Goyanes & Gerschenson, 2007; Talja, Helén, Roos & Jouppila, 2007), xylitol (Talja *et al*., 2007) and sorbytol (Pagella, Spigno & De Faveri, 2002; Talja *et al*., 2007) have been used in edible film formation. Nevertheless, given that polyols plasticize the polymer matrix, they can favour crystallization because of the increase in the polymer chain mobility. However, previous studies into yam starch films, did not report a significant effect of glycerol on the amylose re-crystallization throughout film storage (Mali, Grossmann, García, Martino & Zaritzky, 2002). In thermoplastic starch (TPS), obtained by the extrusion of potato starch, an increase in crystallinity was obtained when the amount of glycerol was reduced in the blend, which is attributed to the greater viscosity of the blend, thus implying greater orientation requirements of the amylose for crystallization (Van Soest, Hulleman, de Wit & Vliegenthart, 1996a).

In some biopolymer matrices, such as polylactic acid (PLA), polyol plasticizers also induce hydrolysis parallel to crystallization (Courgneau, Domenek, Guinault, Avérous & Ducruet, 2011). This may induce the formation of a less dense polymer network which favours the mobility of small molecules, thus promoting the film permeability. Hydrolysis of the starch polymer chains is promoted during the processing of TPS, mainly due to the effect of shear stress forces. This is inhibited by the presence of great amounts of glycerol because of the lower requirements of shear forces in these less viscous systems (Carvalho, 2008).

Fatty acids have been incorporated into the biopolymer films in order to reduce their water vapour permeability (Zahedi, Ghanbarzadeh, Sedaghat, 2010; Jiménez, Fabra, Talens & Chiralt, 2010, Fabra, Jiménez, Atarés, Talens & Chiralt, 2009a; Fernández, Díaz de Apodaca, Cebrián, Villarán, & Maté, 2007), which is relatively high in polysaccharide based films, such as starch, due to their highly hydrophilic nature (Kester & Fennema 1986; Lacroix & Le Tien 2005). Several works reported the formation of amylose-fatty acid complexes (Biliaderis & Seneviratne, 1990ab; Kugiyama & Donovan, 1981; Sievert & Holms, 1993; Singh, Singh & Saxena, 2002) that may affect the starch film properties and crystallization behaviour during the film formation and storage. Van Soest *et al*. (1996a) reported that by means of X-Ray diffraction no long range order crystalline forms are observed, although by solid state NMR spectroscopy, the presence of a single helical conformation of amylose and the occurrence of a fatty acid methylene resonance are evident (Morisson, Law & Snape, 1993a; Morrison, Tester, Snape, Law & Gidley, 1993b). This suggests a rather imperfect lattice organisation of the amylose complexes in the system. Extrusion cooking of manioc starch in the presence of fatty acids, caused the formation of two long range order amylose crystal structures (Mercier, Charbonniere, Grebaut & Gueriviere, 1980). The single helical structure, stable at low moisture content, transforms into another crystalline form by increasing the water content of the sample (Mercier, 1989). So, differences between the two morphologies can be found in arrangement of the single helices in the crystal lattice, depending on the starch processing conditions. On the other hand, fatty acids (palmitic and steric acids) have been seen to reduce glass transition temperature of the gelatinized corn starch films containing glycerol at low water contents (below 10 %) (Jiménez *et al*., unpublished results) which will also affect the diffusion dependent film properties.

In this work, the influence of fatty acids, saturated (palmitic and stearic acids) and unsaturated (oleic acid), on the microstructural and physical (tensile, optical and water vapour permeability) properties of starch films was analysed, in both newly prepared films and those stored for 5 weeks. Crystallization of film components during storage was analysed through X-ray diffraction.

2. MATERIALS AND METHODS

2.1. Materials

Corn starch was purchased from Roquette (Roquette Laisa España, Benifaió, Spain) Fatty acids (minimum purity 96%) were obtained from Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain). The fatty acids used were palmitic (PA, C16:0), stearic (SA, C18:0) and oleic (OA, C18:1). Glycerol was also provided by Panreac Química, S.A. as plasticizer.

2.2. Preparation and characterization of films

Four different formulations based on starch, glycerol and fatty acids were prepared. Corn starch was dispersed in water in order to obtain 2 % (w/w) polysaccharide suspensions. These dispersions were maintained, under stirring, at 95 ºC for 30 minutes to induce starch gelatinization. Afterwards, plasticizer was added in a starch:glycerol ratio of 1:0.25 and dispersions were homogenized (13,500 rpm for 1 min and at 20,500 rpm for 5 min) at 95 ºC, under vacuum, using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany). In the case of emulsions containing fatty acids (1:0.15 starch:fatty acid ratio), these were incorporated prior to the homogenization step. The homogenization at high temperature favoured the lipid dispersion in the system.

Newly homogenized film forming dispersions, containing 1.5 g of total solids, were spread evenly over a Teflon casting plate (15 cm diameter) resting on a level surface. Films were formed by drying for approximately 60 h at 45% RH and 20 °C. These conditions were established after previous experiments to ensure that homogeneous, flawless films were obtained. At a higher relative humidity, films did not dry adequately. Dry films could be peeled intact from the casting surface. Film thickness was measured with a Palmer digital micrometer to the nearest 0.0025 mm at 6 random positions.

2.2.1. Film equilibration and storage

To characterize the films, samples were equilibrated in dessicators at 25 ºC and 53 % RH, by using magnesium nitrate-6-hydrate saturated solutions (Panreac Quimica, SA, Castellar del Vallés, Barcelona) for one week where the first analyses were carried out. One part of the samples was stored under the same conditions for five weeks, when the second series of analyses was performed.

2.2.2. Moisture content

Film samples were dried at 60 ºC for 24 h in a natural convection oven and another 24 h in a vaccum oven in order to determine their moisture content. The reported results represent the average of, at least, four samples.

2.2.3. Tensile Properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, according to ASTM standard method D882 (ASTM, 2001) EM, TS, and E were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 10 cm long). Equilibrated samples were mounted in the film-extension grips of the testing machine and stretched at 50 mm min^{-1} until breaking. The relative humidity of the environment was held constant at 53 (±2) % during the tests, which were performed at 25 (±1) °C. At least six replicates were obtained from each sample.

2.2.4. X-ray diffraction

X-ray diffraction patterns were recorded using a Rigaku Ultima IV multipurpose X-ray diffraction system (Rigaku Corporation, Tokyo, Japan). All samples were analyzed at 25ºC and 53% RH, between $2\theta = 5º$ and $2\theta = 50º$ using

Kα Cu radiation (λ = 1.542 Å), 40 kV and 40 mA with a step size of 0.02 º. For this analysis, samples were cut into 2 cm squares, prior to storage, in order to avoid breakage during handling.

2.2.5. Water Vapour Permeability (WVP)

WVP of films was determined by using the ASTM E96-95 gravimetric method, taking into account the modification proposed by Mc Hugh, Avena-Bustillos & Krochta (1993). Films were selected for WVP tests based on the lack of physical defects such as cracks, bubbles, or pinholes. Distilled water was placed in Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle /s Argenteau, Belgium) to expose the film to 100 % RH on one side. Once the films were secured, each cup was placed in a relative humidity equilibrated cabinet at 25 ºC, with a fan placed on the top of the cup in order to reduce resistance to water vapour transport, thus avoiding the stagnant layer effect in this exposed side of the film. RH of the cabinets (53 %) was held constant using oversaturated solutions of magnesium nitrate-6-hydrate (Panreac Quimica, SA, Castellar del Vallés, Barcelona). The free film surface during film formation was exposed to the lowest relative humidity to simulate the actual application of the films in high water activity products when stored at intermediate relative humidity. The cups were weighed periodically (0.0001 g) and water vapour transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film area.

From WVTR data, the vapour pressure on the film's inner surface (p2) was obtained with eq 1, proposed by McHugh *et al*. (1993) to correct the effect of concentration gradients established in the stagnant air gap inside the cup.

$$WVTR = \frac{P \cdot D \cdot Ln\left[{P - p_2}\big/{P - p_1}\right]}{R \cdot T \cdot \Delta z} \qquad \text{Equation 1}$$

where P, total pressure (atm); D, diffusivity of water through air at 25 °C (m^2/s); R, gas law constant (82.057×10^{-3} m^3 atm $kmol^{-1}$ K^{-1}); T, absolute temperature (K); Δz, mean stagnant air gap height (m), considering the initial and final z value; p_1, water vapour pressure on the solution surface (atm); and p_2, corrected water vapour pressure on the film's inner surface (atm). Water vapour permeance was calculated using eq 2 as a function of p_2 and p_3 (pressure on the film's outer surface in the cabinet).

$$permeance = \frac{WVTR}{p_2 - p_3} \qquad \text{Equation 2}$$

Permeability was obtained by multiplying the permeance by the average film thickness.

2.2.6. Scanning Electron Microscopy (SEM)

Microstructural analysis of the films was carried out by SEM using a scanning electron microscope (JEOL JSM-5410, Japan). Film samples were maintained in a desiccator with P_2O_5 for two weeks to ensure that no water was present in the sample. Then, films were frozen in liquid N_2 and cryofractured to observe the cross-section of the samples. Films were fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

2.2.7. Atomic Force Microscopy (AFM)

The surface morphology of dried film samples (equilibrated with P_2O_5) was analyzed by using AFM with a Nanoscope III.a, Scanning Probe Microscope (Digital Instruments, Inc. Santa Barbara, California) with a 125 × 125 μm scan size and a 6 μm vertical range. The resulting data were transformed into a 3D image. Measurements were taken from several areas of the film surface (50 × 50 μm) using the tapping mode. According to method ASME B46.1 (ASME, 1995), the following statistical parameters related with sample roughness were calculated: average roughness (R_a: average of the absolute value of the height deviations from a mean surface), root-mean-square roughness (R_q: root-mean-square average of height deviations taken from the mean data plane). A minimum of three replicates were considered to obtain these parameters.

2.2.8. Optical Properties

The film transparency was determined by applying the Kubelka-Munk theory (Hutchings, 1999) for multiple scattering to the reflection spectra. The surface reflectance spectra of the films were determined from 400 to 700 nm with a spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) on both a white and a black background. As the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the scattering (S) coefficients. Internal transmittance (T_i) of the films was quantified using eq 3. In this equation R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by eqs 4 and 5, where R is the reflectance of the sample layer backed by a known reflectance R_g. Measurements were taken in triplicate for each sample on the free film surface during its drying.

$$T_i = \sqrt{(a - R_0)^2 - b^2} \qquad \text{Equation 3}$$

$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \qquad \text{Equation 4}$$

$$b = (a^2 - 1)^{1/2} \qquad \text{Equation 5}$$

The gloss was measured on the free film surface during its drying, at 60º incidence angles, according to the ASTM standard D523 method, (ASTM, 1999), using a flat surface gloss meter (Multi Gloss 268, Minolta, Germany). Measurements were taken in triplicate for each sample and three films of each formulation were considered. All results were expressed as gloss units, relative to a highly polished surface of black glass standard with a value near to 100.

2.3. Statistical Analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used for carrying out statistical analyses of data through analysis of variance (ANOVA). Fisher′s least significant difference (LSD) was used at the 95% confidence level.

3. RESULTS

3.1. Microstructural properties

Film components (polysaccharide, lipids and plasticizer) can be arranged in different ways in the dried film, depending on the nature of the hydrocolloid and the interactions between film components developed in the film forming dispersions and during film drying. Interfacial tension between the two liquid phases (starch-glycerol solution /fatty acid) will determine the dispersion feasibility of the components. In this sense, the surfactant action of fatty acids is remarkable as is, furthermore, the high temperature during the emulsification step which decreases interfacial tension, favouring the formation of small droplets in the dispersed phase. The smaller the droplets, the greater the emulsion stability (Dickinson, 1988). Water evaporation during the film drying induces structural changes in dispersion, such as an increase in concentration and viscosity which, in turn, affects its stability. Flocculation and coalescence phenomena of the dispersed phase are promoted by the concentration increase and, when lipid aggregates reach a critical value, difference in density between lipid and continuous phases provokes creaming or the migration of lipid aggregates to the film surface. Ostwald ripening could also contribute to the increase in the size of lipid aggregates, but the water solubility of fatty acids is too low for the quantitative progression of this destabilization mechanism (Dickinson, 1988). The increase of the viscosity of the continuous phase, due to water loss, mitigates all these phenomena, but they can occur to quite an extent. In the case of gelling hydrocolloids (such as starch or other polysaccharides) the gelling process can be promoted during this period. All of this determines the final arrangement of components in the film and its internal and surface structure which affect transport, mechanical and optical properties. Different studies revealed significant correlations between microstructural and functional properties of films (Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005, Fabra, Talens, Chiralt, 2009b; Souza, Cerqueira, Casariego, Lima, Teixeira, & Vicente, 2009; Jiménez *et al*., 2010). In this work, the microstructure of the cross sections of the films was qualitatively analyzed by using SEM and the surface structure was analyzed through AFM.

SEM micrographs of the cross-section of starch based films (with and without lipid) are given in Figure 1. While control films showed a continuous and smooth aspect, the addition of saturated fatty acids (palmitic and stearic acids), as well as the oleic acid, gave rise to a heterogeneous film matrix due to presence of the lipid dispersed phase in the starch-glycerol matrix.

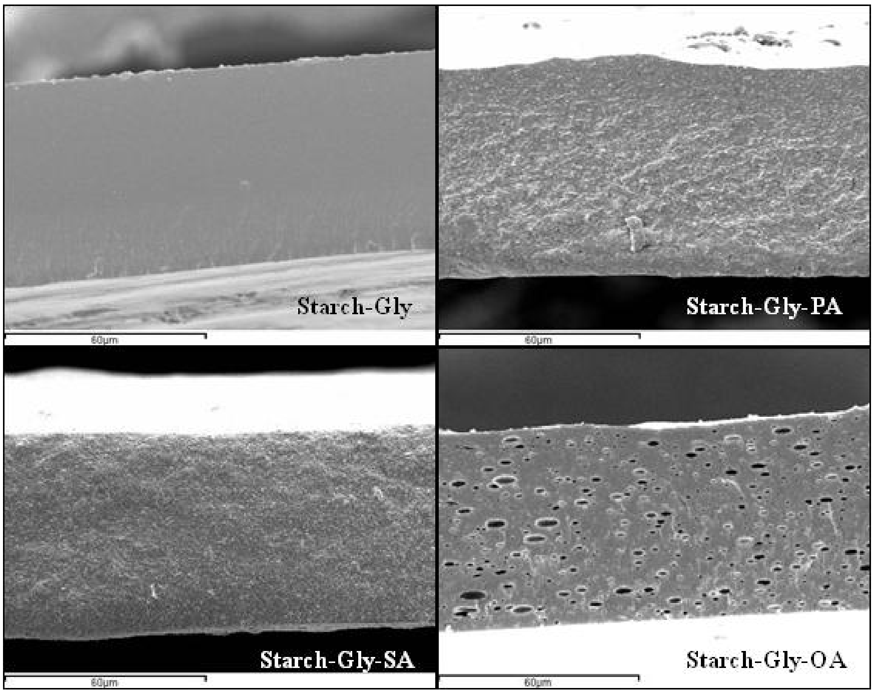

Figure 1. SEM micrographs of the cross-sections of the studied films.

The degree of heterogeneity depended on the nature of the lipid. For a same energy input, the droplet size of the initial emulsion only depends on the interfacial tension of liquid phases (McClements, 2005), this being very similar for the different fatty acids. Nevertheless, saturated fatty acids (SFA) seem to be more finely distributed in the polymer matrix giving rise to a more homogenous network where particles can hardly be appreciated in the continuous matrix, while OA can be more clearly observed in droplet form, dispersed in the polymer network. In this sense, it is remarkable that only voids of the oleic acid droplets can be seen in the micrographs, which suggests that the liquid lipid at the observation temperature evaporated under the high vacuum conditions in the SEM observations. These results differ from that previously observed (Fabra, Jiménez, Atarés, Talens & Chiralt, 2009a; Jiménez *et al.*, 2010) for other polymer matrices (sodium caseinate and hydroxyl-propyl-methylcellulose) where the saturated fatty acids (lauric, palmitic and stearic acids) were clearly separated in a dispersed phase forming a multilayered structure where layers are constituted by lipid crystals. The formation of this type of structure was attributed to the lipid molecular self association in the initial dispersion (lamellar phase) which progresses during drying, leading to the final crystallization of fatty acids in the polymer matrix (Larsson & Dejmek, 1990). The formation of these multilayered structures greatly contributed to the reduction of film capacity for mass transfer since they represented a perpendicular resistance to mass flow. The fact that SFA did not form this kind of structures in the starch matrix suggests that other lipid-starch interactions occur in the film forming dispersion, such as the formation of the amylose-fatty acid complexes, described by other authors (Singh *et al*., 2002), thus limiting the lipid self association phenomena and its final laminar crystallization in the dried film.

For oleic acid, the different microstructural arrangement, observed in the polymer matrix, where lipid droplets appear distributed throughout the cross-section micrograph, could be due to the fact that its non linear chain (because of the double bond) makes the formation of complexes with the helical conformation of amylose difficult. In previous studies where oleic acid was incorporated to sodium caseinate and HPMC matrices, no visible droplets of OA were observed by SEM which indicates that OA was better integrated than in the starch matrix. In these studies, no layers of OA were obtained probably due to the fact that no bi-layer self association of lipid molecules occurred, although other molecular interactions were developed (Fabra, Talens & Chiralt, 2010a).

The surface structure of the films analyzed through AFM is shown in Figure 2 where the 3D plots obtained for the height of the film with respect to a reference plane are plotted.

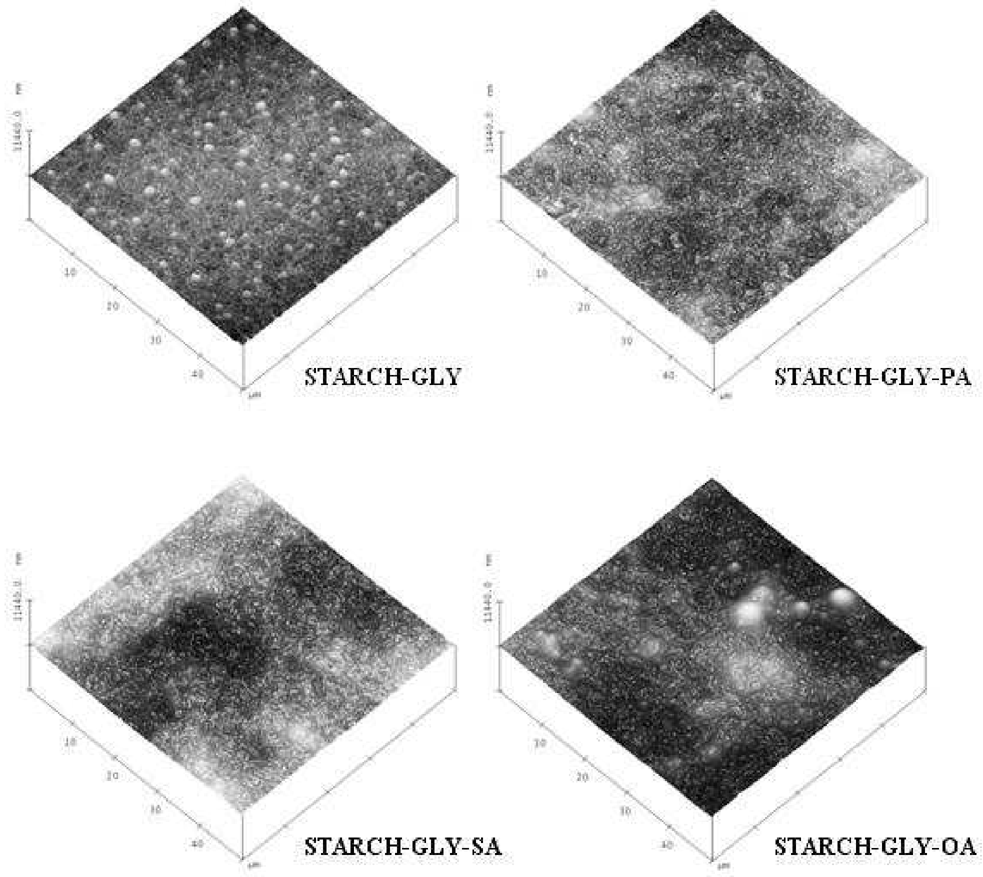

Figure 2. 3D-plots obtained from AFM of films with and without fatty acids.

Table 1 shows the values of the roughness parameters (Ra and Rq) which quantitatively described the 3D images. A similar pattern was observed for Ra and Rq parameters. Films containing oleic acid showed the highest Ra and Rq, as compared to the control film (without lipid) and films containing saturated fatty acids. This can be attributed to the low degree of integration of the oleic acid in the starch matrix, as can be observed in Figure 1.

Table 1. Mean values (and standard deviation) of roughness parameters obtained from atomic force microscopy. (Three images were analyzed in each case).

Film	Ra (nm)	Rq (nm)
Starch-Gly	87 (20)^{a}	120 (30)^{a}
Starch-Gly-PA	43 (4)^{a}	60 (7)^{a}
Starch-Gly-SA	63 (15)^{a}	78 (17)^{a}
Starch-Gly-OA	200 (90)^{b}	230 (140)^{b}

$^{a\text{-}b.}$ Different superscripts within a column indicate significant differences among formulations ($p < 0.05$).

This lesser degree of integration probably also occurs in the film forming dispersion, with the formation of large lipid aggregates (no complexes with amylose). The formation of great lipid droplets favours their migration to the film surface during film drying, giving rise to surface roughness. Palmitic and stearic acids did not significantly ($p<0.05$) modify Ra and Rq values of control films, although the mean values tend to decrease when SFA are incorporated. The great roughness of the fat free film, revealed by the presence of white spots on the surface, could be due to the re-crystallization of amylose during film drying, after the homogenization step. Film forming dispersion was cast at 95ºC and, during cooling, the helical conformation of amylose could be formed on the film surface where fast evaporation and cooling occur. The helical amylose could form larger crystalline aggregates at the film surface than inside the film, where slower temperature and water content reduction occurs. The internal zone of the film did not show these crystalline forms probably because the helical forms did not aggregate to such an extent that they could be observed at the magnification used in SEM. These crystalline formations can also occur in saturated fatty acid containing films, where the linear chains of the lipid were entrapped in the helical conformation of amylose (Singh *et al.*, 2002).

Directly related with the film microstructure are the optical properties of the films, gloss and transparency, both respectively affected by the surface and internal heterogeneity of the structure. Table 2 shows the values of the internal transmittance (Ti) of studied films obtained at 500 nm. According to the Kubelka-Munk theory, high values of Ti are associated with greater film homogeneity and transparency. As previously reported (Fabra *et al*., 2009ab; Jiménez *et al*., 2010), lipid incorporation to the hydrocolloid films provokes a decrease in the transparency due to the differences in the refractive index of dispersed and continuous phase. The highest Ti values were found for starch based films without lipids (control films), whereas in films prepared with saturated fatty acids or oleic acid Ti values decreased. So, control films exhibited the greatest transparency. No significant differences ($p>0.05$) were obtained between films containing palmitic and stearic acids, whereas for OA films, a slightly lower transparency was detected in line with its larger dispersed particles. No significant changes in film transparency were observed due to film ageing for both fat-free films and those containing palmitic acid, although a slight decrease was observed for the two other cases. This decrease in transparency could be associated to the formation of crystalline zones with a different refractive index from that of the amorphous phase.

Gloss of the films at 60º showed great variability, which means that particles (such as crystals) on the film surface are heterogeneously distributed, giving rise to a different degree of roughness in the different surface zones. In non-stored films, the fat-free film and that containing SA were glossier than those containing PA and OA.

Table 2. Tensile properties, moisture content, water vapour permeability and optical properties of starch based films, with and without fatty acids, non-stored (initial) and stored for 5 weeks (final). Mean values (standard deviation).

	EM (MPa)		**TS (MPa)**		**E (%)**		**Moisture (g water·g film^{-1})**	
Film	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Starch-Gly	800 (70)^{a1}	1470 (160)^{a2}	9.2 (0.6)^{a1}	20 (2)^{a2}	8 (4)^{a1}	2.2 (0.2)^{a2}	0.076 (0.004)^{a1}	0.092 (0.003)^{a2}
Starch-Gly-PA	640 (110)^{b1}	828 (13)^{b2}	6.8 (0.3)^{b1}	11 (3)^{b2}	4 (3)^{ab1}	1.6 (0.3)^{b1}	0.114 (0.007)^{b1}	0.090 (0.003)^{a2}
Starch-Gly-SA	870 (170)^{a1}	1300 (200)^{ac2}	8.3 (1.3)^{ab1}	12 (2)^{b2}	2.2 (0.7)^{b1}	1.0 (0.2)^{c2}	0.108 (0.002)^{bc1}	0.093 (0.004)^{a2}
Starch-Gly-OA	170 (30)^{c1}	1100 (300)^{bc2}	3.3 (0.3)^{c1}	6.1 (0.7)^{c2}	13 (7)^{c1}	0.8 (0.2)^{c2}	0.097 (0.01)^{c1}	0.109 (0.002)^{b1}

	WVP (g· mm· kPa^{-1}· h^{-1}·m^{-2})		**T$_i$ (500 m)**		**Gloss 60º**	
Film	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
Starch-Gly	7.9 (0.2)^{a1}	7.7 (1.0)^{a1}	86.1 (0.3)^{a1}	85.9 (0.1)^{a1}	71 (5)^{a1}	67 (9)^{a1}
Starch-Gly-PA	5.9 (0.6)^{b1}	7.5 (1.0)^{a2}	85.3 (0.2)^{b1}	84.9 (0.1)^{b1}	56 (10)^{b1}	47 (12)^{b1}
Starch-Gly-SA	5.8 (0.3)^{b1}	6.9 (0.4)^{a2}	85.22 (0.12)^{b1}	84.6 (0.3)^{b2}	73 (8)^{a1}	49 (9)^{b2}
Starch-Gly-OA	7.2 (0.9)^{c1}	7.53 (0.15)^{a1}	84.8 (0.1)^{c1}	83.6 (0.1)^{c2}	58 (5)^{b1}	47 (10)^{b2}

a-c: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences among formulations ($p < 0.05$).

Gloss of the films analysed after 5 weeks of storage showed the same tendency as initial films, but, in every case, gloss was reduced after film storage, this reduction being more significant for SA and OA. The growth of crystalline zones in the film surface induces more surface roughness, which leads to light dispersion and gloss loss.

So, optical parameters related with the film microstructure, revealed the growth of fatty acid or complex crystals during storage which provokes a slight loss of transparency and gloss. This takes place to a slightly greater extent for fatty acid containing films, where the crystallization of both fatty acid and fatty acid-amylose complexes can occur.

3.2. Tensile properties

Table 2 shows tensile properties (EM: elastic modulus, TS: tensile strength and E(%): elongation at break) of studied films at 25 ºC and 53% RH. The effect of lipid addition, type (saturated fatty acid or oleic acid) and storage time was evaluated. Lipid addition in the non-stored films provoked a decrease in the EM and tensile strength and elongation at break, except in the case of oleic acid which favours the film stretchability (deformation at break was duplicated as compared to the starch film). This was expected since lipids introduce discontinuities in the polymer matrix which contribute to a reduction in the polymer cohesion forces and, thus, the film's resistance to break. Size and distribution of solid particles in the polymer matrix also affect its mechanical properties. If fatty acid is better dispersed as nanoparticles instead of micro/macro particles, they also could act as a lubricant for the polymer chain slipping during the film stretching, thus inducing an increase in stretchability and no change, or a very small one, in TS and elastic modulus (Bonilla, Atarés, Vargas & Chiralt, 2011; Vargas, Perdones, Chiralt, Cháfer & González-Martínez, 2011). So, both phenomena could occur synergistically or antagonistically.

SA made the film shorter (it breaks at the lowest deformation) and slightly more rigid (greater EM) and resistant to break than PA. Films with PA showed twice as much deformation at break. Films with OA behaved very differently, showing a much lower rigidity and break resistance and two times the stretchability. Similarly to that observed in other polymer matrices, oleic acid seems to act as a plasticizer in the starch matrix leading to an increase in the film's stretchability and flexibility (Fabra *et al*. 2010a; Monedero, Fabra, Talens & Chiralt, 2009; Rhim, Wu, Weller & Schnepf, 1999). When SFA were added to sodium caseinate EM increased due to the formation of solid lipid laminar structures (Fabra *et al*., 2009a) which did not appear in the starch systems. A decrease in the elongation at break was also observed in this case.

Ageing the film for 5 weeks induced changes in the starch film's mechanical behaviour. Whereas elongation at break decreased in every case, EM and TS significantly increased. The zone of plastic deformation practically disappeared in the stretching curves after the storage time (Figure 3). Similar changes were also observed by other authors analysing starch films obtained by casting (Mali, Grossmann, García, Martino & Zaritzky, 2006) or extrusion (Van Soest, Hulleman, de Wit & Vliegenthart, 1996b). TS values of all films were practically duplicated during storage, whereas EM and elongation at break changed to a different extent depending on the composition. The smallest change in EM occurred for films containing SFA, whereas in pure starch films it was duplicated and in films containing OA, it increased tenfold. Deformation at break was approximately 3 times lower, except in films containing OA where it was over 10 times lower. This indicates that the structural changes which occurred in the films during storage depended on the lipid composition. These changes can be related with the crystallization phenomena that took place in both starch matrix and lipids. In fact, newly prepared films will show a smaller crystalline fraction due to the fact that, during the preparation of film forming emulsions, starch was gelatinized thus losing the crystallinity of native starch granules.

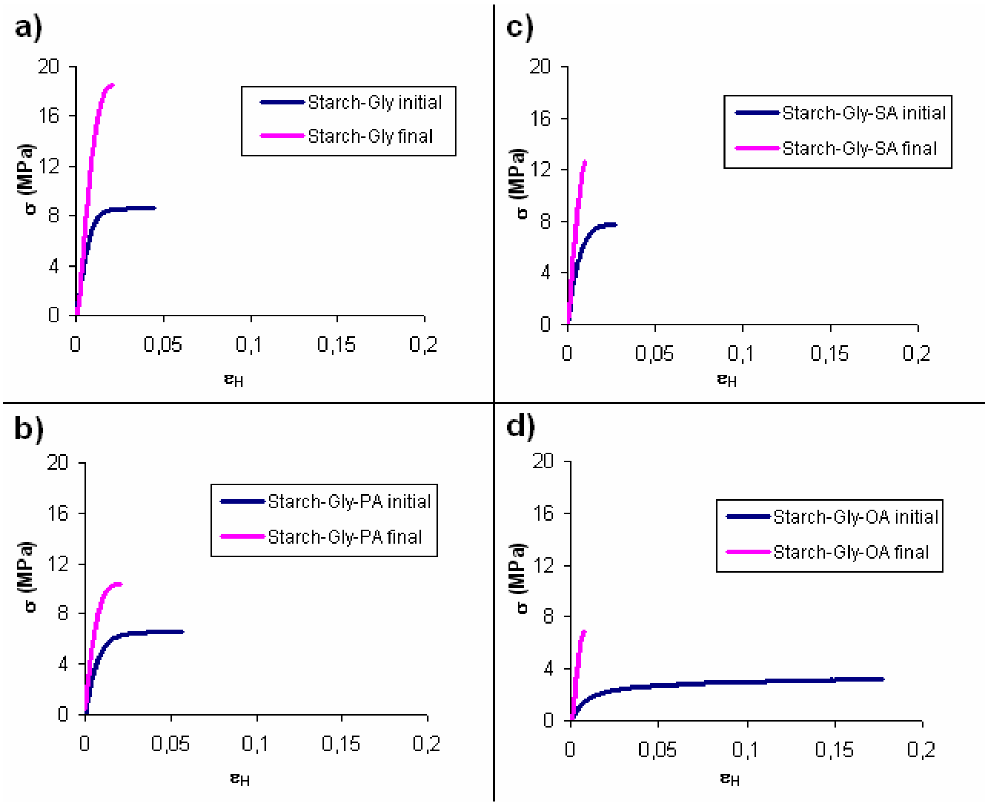

Figure 3. Stress-strain curves of non-stored and stored films for 5 weeks.

Nevertheless, during the drying period amylose and lipid re-crystallization can occur. During the first cooling step after casting of the film-forming dispersion, helical conformation of amylose and fatty acid complexes could be formed that give rise to crystalline zones in dried film which may increase in size during storage if there is enough molecular mobility in the system (Roos, 1995). In this sense, it is remarkable that in films equilibrated at 53% relative humidity, the film glass transition temperature was 37, 22, 32 and 49 ºC for fat free films and those containing PA, SA and OA, respectively (unpublished results), higher than the storage temperature (except for film containing PA film) and so a glassy state is expected for the amorphous starch matrix with limited molecular mobility.

To corroborate the hypothesis about the re-crystallization process in the dried-stored films, X-ray diffraction patterns of the starch films with and without lipids are characterized. Figure 4 shows the X-ray diffraction spectra of the non-stored and stored films where the sharp peaks corresponding with crystalline diffraction can be observed, as stated by Mali *et al*. (2006).

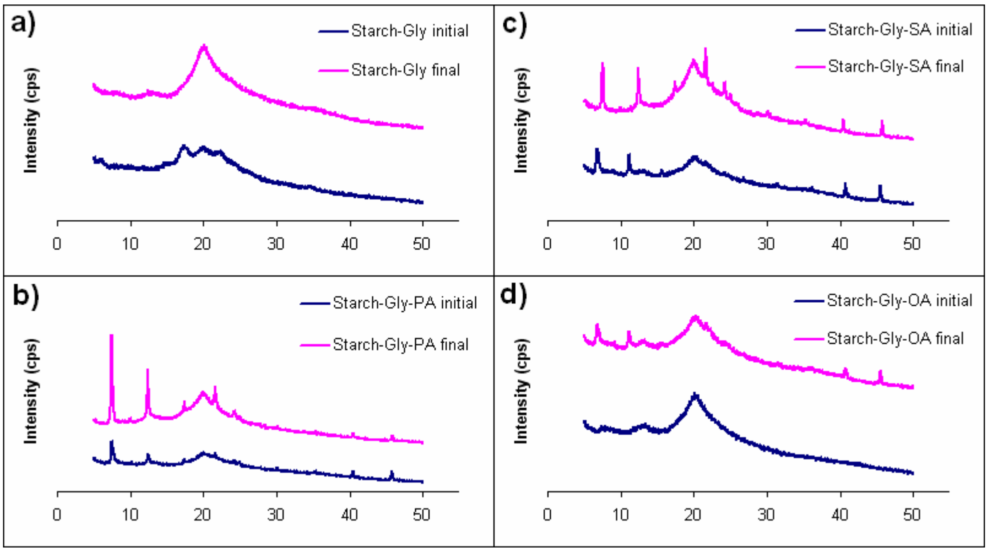

Figure 4. X-ray diffraction patterns of non-stored and stored films for 5 weeks.

All the samples showed differences in crystallinity between the initial and final time according to the different area of the peaks. In all spectra, a main peak located at 20º can be observed which is attributed to the starch crystalline region. A similar peak was previously observed in tapioca starch-sorbate films (Famá, Rojas, Goyanes & Gerschenson, 2005) and in tapioca starch-decolorized hsian-tsao leaf gum films (Chen, Kuo & Lai, 2009). Other sharp peaks observed at low and high angles are attributed to the fatty acid crystals.

For films containing SFA, Figure 4b and Figure 4c show the X-ray diffraction patterns where the lipid peaks can be observed in both non-stored and stored films but in the latter their magnitude increased. This indicates that, as a consequence of molecular mobility, the lipid crystallization progressed during storage. It is remarkable that, at the initial time, the oleic acid film showed only the main starch peak (Figure 4d) but, after storage, four peaks appeared which were characteristic of fatty acid crystal. This indicates that, despite oleic acid being liquid at the storage temperature, its partial crystallization was induced in the starch matrix. This can be attributed to the formation of an ordered molecular arrangement for AO at the starch lipid interface, which could develop to form crystals. Oleic acid molecules will have limited mobility when adsorbed in the interface, thus promoting the molecule organization into a crystalline structure.

The crystalline zones in the starch films, although in less quantity than the amorphous ones, tend to strengthen the films due to the fact that they behave like hard particles.

3.3. Water Vapour Permeability (WVP)

Table 2 shows WVP of starch based films (with and without lipid) analyzed at 25 ºC and 53-100 % RH gradient. WVP values of films containing SFA (palmitic and stearic acid) were significantly ($p<0.05$) lower than those obtained for control and films containing oleic acid. The increase in the water barrier efficiency of biopolymer films when lipids were incorporated has been reported in previous works for different types of lipids and hydrocolloid matrices, such as chitosan (Sánchez-González, González-Martínez, Chiralt & Cháfer, 2010), HPMC (Hagenmaier & Shaw, 1990), sodium and calcium caseinates (Fabra, Talens & Chiralt, 2010b), fish water soluble proteins (Tanaka, Ishizaki, Suzuki, & Takai, 2001), iota-carrageenaan (Hambleton, Debeaufort, Beney, Karbowiak & Voilley, 2008) or corn starch (García, Martino & Zaritzky, 2000b). Nevertheless, some authors claim that water vapour permeability was reduced as the chain length of fatty acids increased (Mc Hugh & Krochta, 1994, Tanaka *et al*., 2001) but no significant differences ($p<0.05$) between palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) on WVP were observed, underlining the fact that there are other effects related with the structural arrangement of the lipid in the films which play an important role. As commented on above, both palmitic and stearic acid were homogeneously distributed in the film matrix and they are solid at the control temperature, which contributes to a reduction in the water transfer rate. On the contrary, despite the hydrophobic nature of the oleic acid, it did not contribute to an increase in the water barrier properties of film as much as SFA. Similar results were observed by Fabra, Talens & Chirat (2010c) and Jiménez *et al*. (2010) working with sodium caseinate and HPMC matrices, although SFA were more efficient at controlling water vapour barrier properties in these cases than in starch films due to the formation of multilayered structures (Fabra *et al*., 2010c, Jiménez *et al*., 2010).

Storage time (5 weeks) did not modify significantly WVP values of control films and films containing oleic acid ($p>0.05$). In this sense, a similar pattern was observed by Mali *et al*. (2006) for corn starch films without lipids, stored for 90 days, where no changes in WVP values were detected due to storage. However, an increase in the WVP values was observed for films containing SFA stored for 5 weeks. This could be attributed to the increase in the previously described degree of crystallinity in the matrix as is also deduced from the moisture content reduction observed for these two films, since re-crystallisation induces water release by the network. Crystal growth in the amorphous matrix could promote ruptures in the amorphous zones leading to the formation of channels where water molecules can diffuse more easily. In fact, as shown in Figure 3, the greatest increase in the peak area during storage occurred for SFA, thus indicating that a greater crystallization occurred in these cases. Hydrolysis of the polymer chains in the amorphous phase, as has been reported for other polymers (Courgneau *et al*. 2011) could also occur, thus inducing the formation of a less compact network where small molecule permeation can occur more easily.

4. CONCLUSIONS

Fatty acid incorporation in starch-glycerol films did not notably improve water vapour properties of the films and only saturated fatty acids promoted a slight reduction of WVP in non-aged films. The degree of crystallinity of the matrix increased during storage time in all cases, which implied an increase in the film stiffness and brittleness and a loss of stretchability, gloss and transparency, which was also induced by lipid incorporation into non-aged films. Other organic non polar compounds must be tested to improve the starch film's functional and ageing properties.

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PHASE TRANSITIONS IN STARCH BASED FILMS CONTAINING FATTY ACIDS. EFFECT ON WATER SORPTION AND MECHANICAL BEHAVIOUR

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ABSTRACT

The water sorption capacity (WSC) of starch films containing, or not, glycerol (1:0.25 starch:glycerol ratio) and fatty (palmitic, stearic and oleic) acids (1:0.15 starch:fatty acid ratio) was analysed at 10, 25 and 40ºC in the entire range of a_w. Starch re-crystallization during the equilibration time was also analysed by X-ray diffraction. Likewise, the glass transition and lipid melting properties as a function of the film water activity were determined. Mechanical behaviour was also analysed at 25ºC for film samples equilibrated at 0, 53, 68 and 75% RH. Crystallization was promoted at low temperature and high relative humidity which modifies the expected tendencies in WSC as a function of the temperature. In relationship with this, V-type structures were formed during time, mainly in fatty acids containing films. Glass transition of the starch matrix at low moisture contents was affected by the presence of saturated fatty acids. Microstructural observations seem to corroborate the complex formation between these and the polymer chains. The mechanical behaviour of the films, as a function of the moisture content, is coherent with the results of the phase transition analysis and microstructural observations. The plasticization effects and the structural discontinuities in the polymer matrices determine the value of the elastic modulus and the behaviour at break.

Keywords: Starch, fatty acids, isotherms, glass transition, tensile properties.

1. INTRODUCTION

Starch alone cannot form films with satisfactory mechanical properties (high elongation at break and tensile strength), unless it is plasticized, blended with other materials, chemically modified or modified with a combination of these treatments (Liu, 2005). Generally, polyalcohols are the most common types of plasticizers for biodegradable films, including propylene glycol, glycerol, sorbitol and other polyols. There are many publications where various types of polyols were studied for their effects on the resulting films (Arvanitoyannis, Psomiadou & Nakayama, 1996; Arvanitoyannis, Psomiadou, Nakayama, Aiba & Yamamoto, 1997; Psomiadou, Arvanitoyannis & Yamamoto, 1996; Parra, Tadini, Ponce & Lugão, 2004; Famá, Rojas, Goyanes & Gerschenson, 2005; Rodríguez, Osés, Ziani & Maté, 2006). In most studies, starch based films are plasticized most effectively with glycerol, which has specific advantages. Moreover, the strength of the films has also been demonstrated to be dependent upon their moisture content, which is in turn influenced by the ambient relative humidity (Chang, Cheah & Seow, 2000; Bertuzzi, Armada & Gottifredi, 2003). So, knowing the water sorption equilibrium conditions of starch based films is useful to understand the performance of films under varying RH conditions. Different factors such as phase transition, like crystal formation, or phase separation can also affect the water sorption behaviour of polymers such as gelatinized starch.

Several works have focused on overcoming the inherent hydrophilicity of starch films, by surface esterification (Zhou, Ren, Tong, Xie & Liu, 2009; Ren, Jiang, Tong, Bai, Dong & Zhou, 2010), surface photocrosslinking (Zhou, Zhang, Ma & Tong, 2008) or blending starch with other hydrophobic materials, such as polycaprolactone and cellulose acetate (Koenig & Huang, 1995; Averous, Moro, Dole, Fringant, 2000; Fang & Fowler, 2003). Other non-polar compounds, such as lipids, can also be combined with starch either by incorporating lipids in the hydrocolloid film-forming solution (emulsion technique) or by depositing lipid layers onto the surface of the pre-formed hydrocolloid film to obtain bilayers. Multi-component films have been extensively reviewed by Wu, Weller, Hamouz, Cuppett & Schnepf (2002). Jiménez, Fabra, Talens & Chiralt (2012) have recently studied the effect of fatty acid addition (by the emulsion technique) on physico-chemical properties and their changes during ageing, of starch based films. They observed that re-crystallinization process in starch matrix during storage was not inhibited by the lipid addition which could modify water-starch relationships and phase transitions due to the crystal formation in the amorphous phase.

The plasticizing effect of water and polyols as well as the interactions and compatibility of biopolymers in blend films have been studied by several authors (Psomiadou *et al*., 1996; Arvanitoyannis & Biliaderis, 1999; Kristo & Biliaderis, 2006) by using differential scanning calorimetry (DSC) and thermo-mechanical analysis. The glass transition temperature (Tg) is strongly dependent on both the film composition and moisture content, and can define the stability of a film. The structure–property relationship of hydrated biopolymers and their broader implications on processing and product quality can be better understood within the theoretical concept of glass transition, commonly used in polymer science (Fennema, 1996; Karel, Buera, & Roos, 1993; Levine & Slade, 1988, 1992; Slade & Levine, 1991). As generally observed, glass transition temperature of an amorphous material decreases as water content increases. For a given storage temperature, an amorphous matrix with water content below a critical value is glassy state and remains stable against molecular changes because of the highly reduced molecular mobility. When the water content exceeds the critical value, the same matrix becomes rubbery and molecular reorganization and diffusion dependent processes take place at a determined rate. Thus, in the rubbery state, polymer chains are mobile enough to form crystalline zones if the polymer chains can form ordered associations for a determined molecular conformation. It is important to know the water content-glass transition relationships for a determined film-forming polymer since they determine the rate of diffusion dependent processes, such as crystallization, while having a great impact on mechanical and barrier properties of the formed film. Formation of crystalline regions in the film greatly affects mechanical properties of the films, since crystals behave as hard particles which contribute to the film strengthening, decreasing their extensibility. Forsell, Hulleman, Myllärinen, Moates & Parker (1999) attributed the increase in tensile modulus throughout the storage time to the crystallization of starch components. Van Soest & Knooren (1997) pointed out that even when crystallinity is low, entangled in the starch network and chain–chain association can be formed during storage promoting changes in mechanical properties of the starch films.

The aim of this work is to analyse the water sorption capacity of starch films containing glycerol and fatty acids, as affected by starch re-crystallization (analysed by X-ray diffraction) at different temperature and relative humidity conditions, as well as the glass transition and lipid melting properties as a function of the film water activity. Mechanical behaviour was also analysed taking into account the film moisture content, phase transition behaviour and microstructural observations.

2. MATERIALS AND METHODS

2.1. Materials

Corn starch was purchased from Roquette (Roquette Laisa España, Benifaió, Spain) Fatty acids (minimum purity 96%) were obtained from Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain). The fatty acids used were palmitic (PA, C16:0), stearic (SA, C18:0) and oleic (OA, C18:1). Glycerol, used as plasticizer, and P_2O_5, $Mg(NO_3)_2$, $CuCl_2$ and NaCl salts were also provided by Panreac Química, SA, Castellar del Vallés, Barcelona.

2.2. Preparation and characterization of films

Four different formulations based on starch, glycerol and fatty acids were prepared. Corn starch was dispersed in water in order to obtain 2 % (w/w) polysaccharide dispersions. These were maintained, under gentle stirring, at 95 ºC for 30 minutes to induce starch gelatinization. Afterwards, plasticizer was added in a starch:glycerol ratio of 1:0.25 and dispersions were homogenized (13,500 rpm for 1 min and at 20,500 rpm for 5 min) at 95 ºC, under vacuum, using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany). In the case of emulsions containing fatty acids (1:0.15 starch:fatty acid ratio), these were incorporated prior to the homogenization step. The homogenization at high temperature favoured the lipid dispersion in the system.

Newly homogenized film forming dispersions, containing 1.5 g of total solids, were spread evenly over a Teflon casting plate (15 cm diameter) resting on a level surface. Films were formed by drying for approximately 60 h at 45% RH and 20 °C. These conditions were established after previous experiments to ensure that homogeneous, flawless films were obtained. At a higher relative humidity, films did not dry adequately. Dry films could be peeled intact from the casting surface.

2.2.1. Film conditioning and storage

Before tensile and DSC analysis, samples were equilibrated for two weeks at four different relative humidities (0, 53, 68 and 75 %) at 25 ºC by using, respectively, P_2O_5 and oversaturated solutions of $Mg(NO_3)_2$, $CuCl_2$ and NaCl. Film thickness was measured with a Palmer digital micrometer to the nearest 0.0025 mm at 6 random positions, previous to tensile analysis.

2.2.2. Water sorption isotherms

Triplicate film samples (1.5–2.0 g), accurately weighed, were placed in desiccators (placed individually in three different climate chambers) at 10, 25 and 40 ºC and equilibrated to different water activity using oversaturated solutions of NaOH, LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, $CuCl_2$, NaCl and KCl (different a_w values are shown in Table 1). Samples were weighed periodically (0.00001 g precision) for 4 weeks, when the equilibrium was reached. Finally, the equilibrium moisture content was determined by drying in a vacuum oven at 60 ºC and 50 Torr for 2 days. Experimental sorption isotherms were fitted to Guggenheim-Anderson-deBöer (GAB) model (Eq. (1)) in the entire a_w range. Solver tool, included in Microsoft Excel 2003, was used to carry out the non-linear fitting of experimental data.

$$w_e = \frac{w_o \cdot C \cdot K \cdot a_w}{(1 - K \cdot a_w) \cdot (1 + (C-1) \cdot K \cdot a_w)} \tag{1}$$

Table 1. Water activity values of the oversaturated salt solutions used at the different temperatures (Greenspan, 1977).

10 ºC		25 ºC		40 ºC	
Salt	**a_w**	**Salt**	**a_w**	**Salt**	**a_w**
LiCl	0.113	LiCl	0.113	NaOH	0.063
CH_3COOK	0.234	CH_3COOK	0.225	LiCl	0.112
$MgCl_2$	0.335	$MgCl_2$	0.328	$MgCl_2$	0.316
K_2CO_3	0.431	K_2CO_3	0.432	$Mg(NO_3)_2$	0.482
$Mg(NO_3)_2$	0.574	$Mg(NO_3)_2$	0.529	NaBr	0.532
NaCl	0.757	$CuCl_2$	0.675	NaCl	0.747
KCl	0.868	NaCl	0.753	KCl	0.832
		KCl	0.843		

In Eq. (1) W_e is the equilibrium moisture content on dry basis, aw is water activity, W_0 is the monolayer moisture content, C and k are equation parameters, both being temperature dependent and related to the water sorption energy in the film.

2.2.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry analyses (DSC) were carried out using a DSC 220 CU-SSC5200 (Seiko Instruments). Small amounts (15-20 mg approx.) of samples were placed into aluminium pans (Seiko Instruments, P/N SSC000C008) and hermetically sealed. All pans were then heated between -80 and 180 ºC in order to analyze phase transitions. The exact temperature range for each sample depended on the sample a_w. In general, samples were cooled from room temperature to a temperature at least 80 ºC below its Tg (Tg-80 ºC) and then heated to a temperature 20 ºC above lipid fusion temperature. Both cooling and heating scans were performed at 10 ºC/min. An empty aluminium pan was used as reference. The Tg was determined as the midpoint temperature of the glass transition.

In samples containing lipids, the lipid melting endotherm was also characterized in the heating scan (onset, peak and end temperature of the endotherm and fusion enthalpy). To this end, samples were first cooled and, during the heating scan, a constant temperature was maintained for 30 min at a slightly lower value than the initial temperature of the endotherm (determined in the first run) to promote the free lipid crystallization. Each sample was analyzed in triplicate.

2.2.4. Tensile properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, according to ASTM standard method D882 (ASTM, 2001) EM, TS, and E were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 10 cm long). Six replicates were analyzed per film formulation. Equilibrated specimens were mounted in the film-extension grips of the testing machine and stretched at 50 $mm \cdot min^{-1}$ until breaking. The temperature was maintained constant at 25 (±1) °C during the tests.

2.2.5. X-ray diffraction

X-ray diffraction patterns were recorded using a Rigaku Ultima IV multipurpose X-ray diffraction system (Rigaku Corporation, Tokyo, Japan). All the samples were analyzed between $2\theta = 5^{o}$ and $2\theta = 50^{o}$ using Kα Cu radiation (λ = 1.542 Å), 40 kV and 40 mA with a step size of 0.02 º. For this analysis, samples were cut into 2 cm squares, prior to storage, in order to avoid breakage during handling. The temperature and the relative humidity of the room in which the diffractometer was located were controlled in order to avoid changes in the samples during analyses.

2.2.6. Scanning Electron Microscopy (SEM)

Microstructural analysis of the films was carried out using a Scanning Electron Microscope (JEOL JSM-5410, Japan). Film samples were maintained in a desiccator with P_2O_5 for two weeks. Then films were frozen in liquid N_2 and gently and randomly broken to investigate the cross-section of the samples. Films were fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

2.3. Statistical Analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used for carrying out statistical analyses of data through analysis of variance (ANOVA). Fisher′s least significant difference (LSD) was used at the 95% confidence level.

3. RESULTS

3.1. Water sorption capacity of the films

Figure 1 shows the water sorption isotherms obtained for starch and starch films containing glycerol, at 10, 25 and 40ºC. The curves show the typical sigmoid shape characteristic of polar polymers. As expected, the presence of glycerol significantly modified water sorption behaviour of the starch matrix; in the low and intermediate a_w range, it provoked a slight decrease in the water sorption capacity of the matrix, while this was enhanced at high a_w values. This can be explained by the highly competitive water retention in low molecular weight glycerol molecules, which can retain a great amount of water, without notable changes in the a_w values, when the water solvent effects predominate at higher a_w values. Similar effects of glycerol on the sorption behaviour of other biopolymers have been previously reported by other authors (Kristo & Biliaderis, 2006; Fabra, Talens & Chiralt, 2010).

In samples containing glycerol, the effect of temperature was not coherent with the endothermic nature of the water sorption process which implies an increase in the water sorption capacity as the temperature decreases. The coherence can only be observed for a_w values lower than 0.30, whereas at higher a_w values water sorption capacity at 10ºC is lower than at 25 and 40ºC. This suggests that structural changes, such as crystallization, can occur to a different extent depending on the temperature, affecting water sorption behaviour.

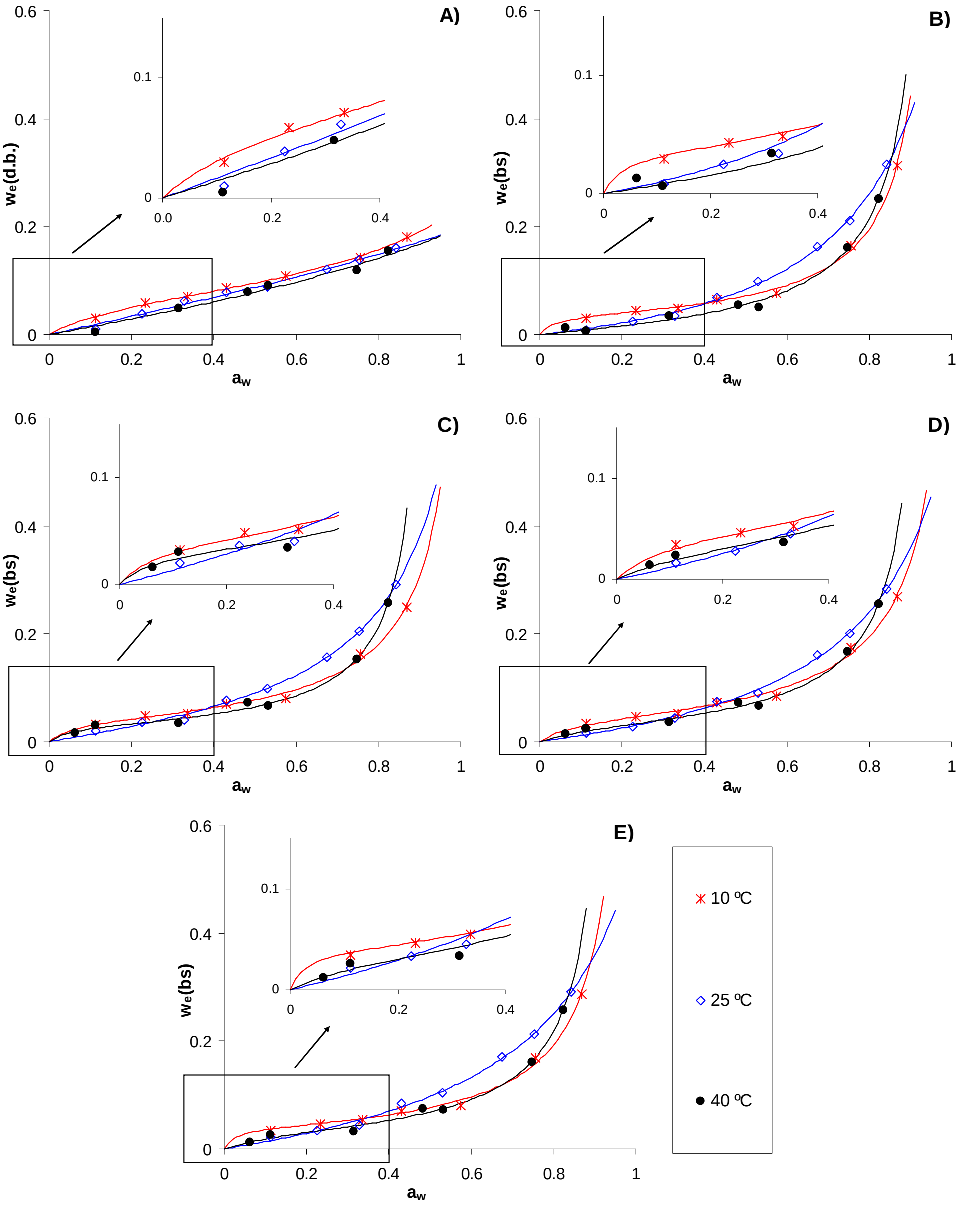

Figure 1. Sorption isotherms of starch based films, at 10, 25 and 40 ºC. A: Starch, B: Starch-Gly, C: Starch-Gly-PA, D: Starch-Gly-SA, E: Starch-Gly-OA. Experimental points and GAB fitted model (lines).

Amorphous materials have greater water sorption capacity than crystalline forms due to the steric restrictions, given in the ordered crystalline arrangements, which limit the location of water molecules. The obtained water sorption results suggest that, above a_w 0.5, crystal formation occurs to a greater extent at 10ºC, thus reducing the water sorption capacity of the starch matrices, as compared with data obtained at 25 and 40 ºC. On the other hand, differences in sorption capacity at 25 and 40 ºC were enhanced in the 0.5 and 0.75 a_w range. This could indicate that the sorption enthalpy in this moisture range was higher than that corresponding to the low moisture levels. This is unlike the thermodynamic basis of sorption process, which establishes the maximum for the sorption enthalpy at the monolayer moisture content (a_w about 0.2). This behaviour seems to indicate that chain rearrangement occurs at high moisture content in the presence of glycerol, due to the increase in molecular mobility. In this process, hydration layers of the polymer, where interactions with glycerol are expected, are modified as is the water affinity of the matrix. These effects could be more sensitive to the temperature (greater sorption enthalpy). Previous studies reported that amylose and glycerol are only partially miscible which give rise to phase separation with the subsequent formation of amylose rich and glycerol rich phases (Moates, Noel, Parker & Ring, 2001). This phenomenon could also contribute to the increase of the temperature effect on water sorption capacity from determined moisture content.

The presence of fatty acids in the starch matrix (0.15:1 ratio with respect to starch) did not notably modify sorption behaviour despite their non-polar character. If moisture content was estimated per g of non-fatty solids, the obtained values, compared with those corresponding to the lipid-free starch-glycerol matrix, were slightly higher (1-2 g water/g non-fatty solids). This reflects that the presence of fatty acids enhanced the water sorption capacity of the starch matrix. This was slightly more marked for oleic acid (2-3 g water/g non-fatty solids). Nevertheless, no changes in the sorption pattern were induced by the presence of fatty acids in the films.

Table 2 shows the values of the GAB parameters obtained from the fitting of the experimental values. In agreement with that previously commented on, the glycerol incorporation to the starch matrix supposed notable changes in the GAB parameters due to the change in the sorption pattern provoked by the presence of this polar low molecular weight compound. Differences between lipid-free matrices and those containing lipids are not relevant taking into account the mathematical correlation between the three parameters.

Table 2. Parameters of the GAB model.

Sample	**10 ºC**			**25 ºC**			**40 ºC**		
	k	**C**	**W_o**	**k**	**C**	**W_o**	**k**	**C**	**W_o**
Starch	0.694	7.82	0.077	0.353	1.84	0.255	0.460	1.64	0.183
Starch-Gly	1.020	26.72	0.036	0.843	0.71	0.142	1.028	1.56	0.044
Starch-Gly-PA	0.956	14.98	0.043	0.890	1.71	0.087	1.066	16.57	0.032
Starch-Gly-SA	0.959	11.62	0.046	0.837	1.12	0.114	1.041	6.22	0.038
Starch-Gly-OA	0.995	37.17	0.039	0.807	1.21	0.129	1.043	6.80	0.037

Figure 2 (A to D) shows the X-ray diffraction patterns of starch-glycerol films and starch-glycerol-fatty acid films equilibrated at different relative humidity and temperature for 5 weeks, in order to differentiate the crystallization pattern of the films depending on the equilibration conditions. This pattern was affected by the equilibration conditions as deduced from the water sorption data, commented on above. In the case of starch-gly films (Figure 2A), the number of peaks in the

spectra increased when the moisture content in the film rose, thus indicating that different crystalline structures were formed.

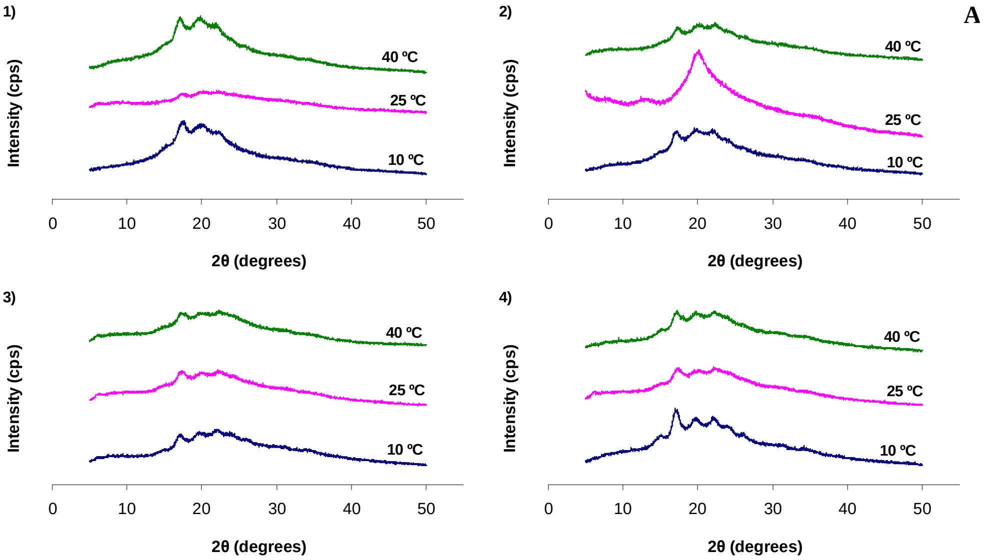

Figure 2. X-ray diffraction patterns of starch films (without and with fatty acids) equilibrated a different relative humidity. A: Starch-Gly, B: Starch-Gly-PA, C: Starch-Gly-SA, D: Starch-Gly-OA (1: a_w=0; 2: a_w=0.53; 3: a_w=0.68; 4: a_w=0.75).

This effect is especially notable from a_w=0.68 onwards and it was enhanced at 10ºC, when the intensity of the peaks also increased. Table 3 shows the angles (2θ) and the intensity (counts per second) corresponding with the main peaks registered in each sample. In general, native starches present A and B-type crystallinity, whereas C-type is a form intermediate between A and B (Carvalho, 2008). Another forms that can be observed from X-ray diffraction patterns are the V-conformation helices, which are a result of amylose being complexed with substances such as aliphatic fatty acids, surfactants,emulsifiers, n-alcohols, glycerol or dimethyl sulfoxide (Famá *et al*., 2005).

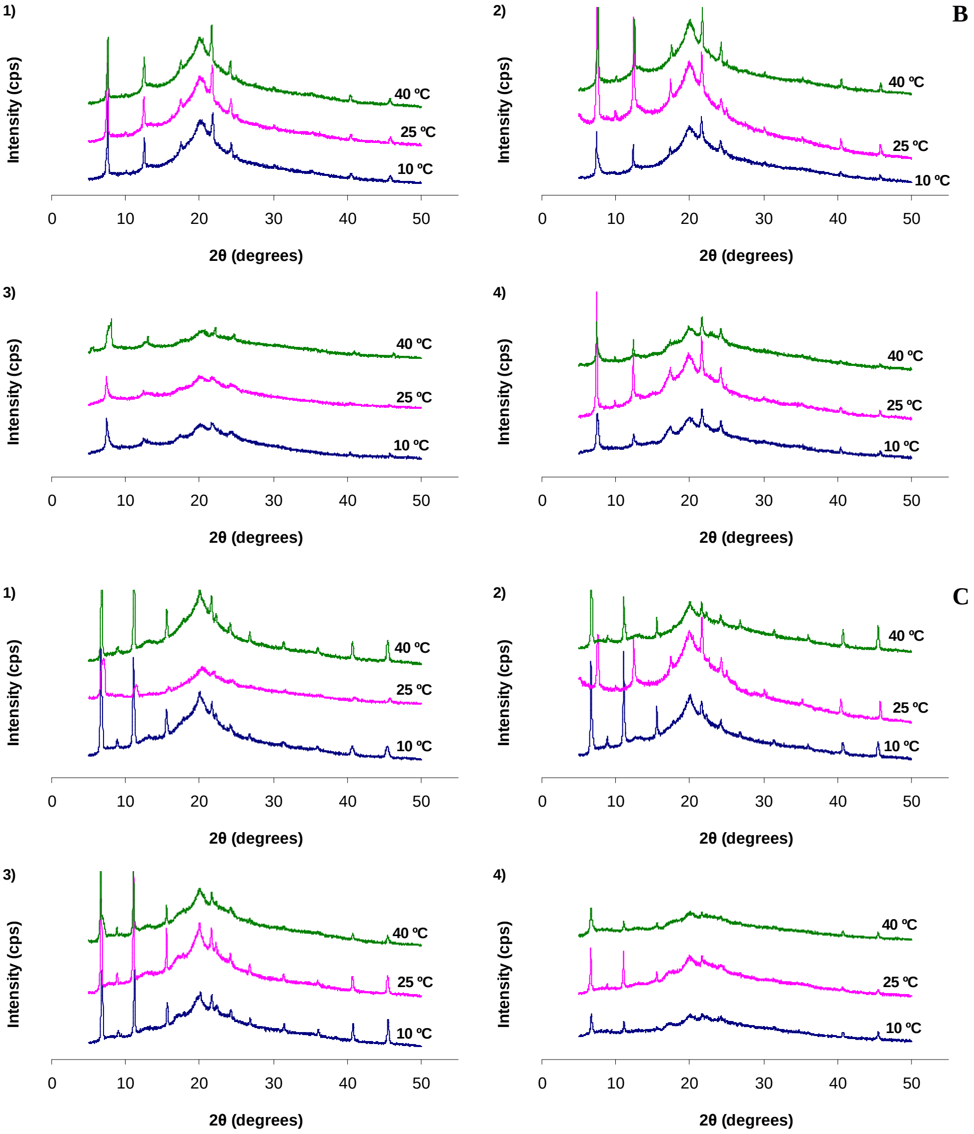

Figure 2. Cont.

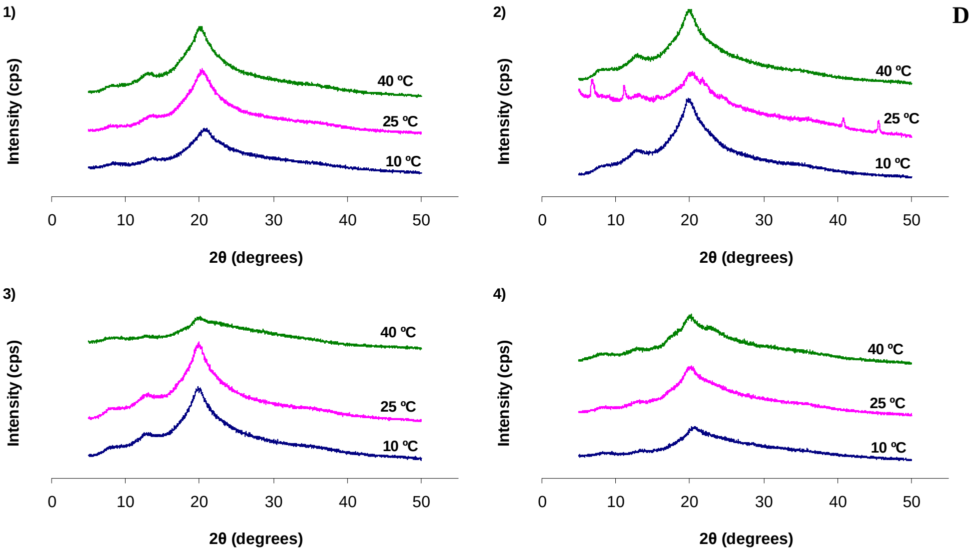

Figure 2. Cont.

In samples without fatty acids the obtained patterns show small differences between them with a crystallization pattern near to B-type. Although starch based films normally present B-type crystallinity, A-type crystalline structure can be formed by heating and/or moisture increase, which promotes the mobility of macromolecular chains and thus allows the rearrangement of the crystalline structure (Liu, 2005). The V-conformation helices can also be formed where endogenous lipids are complexed within the amylose helices (Gelders, Vanderstukken, Goesaert & Delcour, 2004). Particularly, in sample equilibrated at 53% relative humidity and 25 ºC, only one main peak was observed at 2θ 20º which is characteristic of the V-type crystalline structure. This seems to indicate that, in these conditions, amylose helices form the main crystalline structures, probably entrapping the endogenous lipids or other organic non polar compounds of the starch (Gelders *et* al., 2004).

Table 3. Angles and intensity (cps·10^{-3}) of crystalline peaks (2θ) associated to starch crystal forms in the studied films conditionted at different temperature and relative humidity.

		Starch-Gly				Starch-Gly-PA				Starch-Gly-SA				Starch-Gly-OA			
	2θ (º)	**0**	**0.53**	**0.68**	**0.75**	**0**	**0.53**	**0.68**	**0.75**	**0**	**0.53**	**0.68**	**0.75**	**0**	**0.53**	**0.68**	**0.75**
10 ºC	16				24												
	17	42	35	25	45												
	20	40	37	27	38	51	45	29	34	55	52	44	22	36	64	58	27
	22	35	36	29	39												
	24			27	32												
	26			22	26												
25 ºC	16				18												
	17	16		28	29												
	20	18	69	27	28	56	79	26	53	30	74	59	33	52	37	64	40
	22	18		28	30												
	24			24													
	26																
40 ºC	16			17	18												
	17	44	26	27	32												
	20	45	30	27	32	57	70	23	36	60	41	46	24	56	61	26	40
	22	39	29	28	32												
	24																
	26																

When saturated fatty acids were added (Figure 2B and 2C), the typical X diffraction peaks of the fatty acids were observed, as deduced from the X-ray spectra database of the used difractometer. These peaks overlap in the spectra with the starch diffraction patterns. In this sense, it is remarkable that only one peak at 2θ 20º, characteristic of the V-type structure was observed for starch, thus indicating the predominating formation of amylose-lipid complexes, and inhibiting the formation of other crystalline forms.

When oleic acid was added to starch-glycerol matrices, no peaks of fatty acid was observed since it was liquid at the temperatures of the analysis but the predominating formation of V-type crystals was also observed. Only for samples equilibrated at 25 ºC and 53% RH weak peaks characteristic of oleic acid crystals (2θ: 7.1, 11.2, 22.0 and 45.5) unexpectedly appeared in the spectrum which could be related to the formation of an ordered molecular arrangement of oleic acid at the starch-lipid interface where the limited molecular mobility promotes the molecule organization into a crystalline structure (Jiménez *et al*., 2012).

From the peak intensities (Table 3) it can be concluded that, except for the films containing oleic acid, a greater intensity was observed for the typical peak of V-type crystal in samples equilibrated at 25ºC and 53% relative humidity. No clear effect of temperature was observed in the peak intensity, although, in general, this decreased when moisture content increased in the films equilibrated at the highest relative humidity.

3.2. Glass transition of the starch matrix and lipid melting

Table 4 shows the values of glass transition temperature (Tg) of the starch films as a function of a_w, together with the corresponding equilibrium moisture content. Additionally, DSC curves of starch-gly films and lipids melting endotherms were shown in Figures 3 and 4, respectively.

Table 4. Values of the glass transition temperature (mid point) and moisture content (x_w: g water/g film) of starch based films equilibrated at different relative humidity.

Sample	$a_w = 0$		$a_w = 0.53$		$a_w = 0.68$		$a_w = 0.75$		
	x_w	Tg (ºC)	x_w	Tg (ºC)	x_w	Tg (ºC)	x_w	Tg_1 (ºC)	Tg_2 (ºC)
Starch	0*	50 (4)^{a1}	0.081 (0.001)^{a1}	48.3 (3)^{a1}	0.11 (0.03)^{a2}	10.3 (1.4)^{a2}	0.121 (0.000)^{a3}	1 (3)^{ab3}	
Starch-Gly	0*	45 (4)^{a1}	0.090 (0.001)^{b1}	37.4 (1.9)^{b1}	0.139 (0.001)^{b2}	11.2 (1.2)^{a2}	0.174 (0.003)^{b3}	5 (6)^{b2}	-49 (7)^{a}
Starch-Gly-PA	0*	22.1 (0.9)^{b1}	0.090 (0.002)^{b1}	22.5 (1.3)^{c1}	0.135 (0.006)^{b2}	15.6 (1.1)^{b2}	0.170 (0.006)^{b3}	-5.3 (1.2)^{b3}	-41 (3)^{a}
Starch-Gly-SA	0*	30 (1)^{b1}	0.083 (0.005)^{a1}	31.8 (1.7)^{b1}	0.138 (0.009)^{b2}	10.9 (0.4)^{a2}	0.166 (0.008)^{b3}	0.7 (6)^{ab3}	-39.1 (0.7)^{a}
Starch-Gly-OA	0*	53 (5)^{a1}	0.094 (0.000)^{b1}	49 (3)^{a1}	0.146 (0.001)^{b2}		0.175 (0.004)^{b3}		

a-c: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).

1-3: Different superscripts within the same line indicate significant differences among samples equilibrated at different relative humidity ($p < 0.05$).

*: This a theoretical value. Previous studies (Moates *et al*. 2001) reveald that amylose-glycerol films equilibrated in P_2O_5 contained about 2% moisture content.

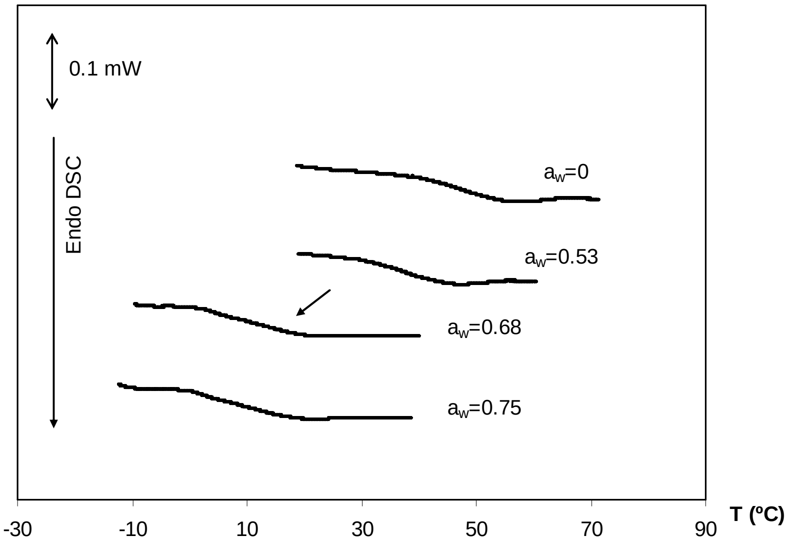

Figure 3. DSC curves showing glass transition at different a_w values for starch-gly films.

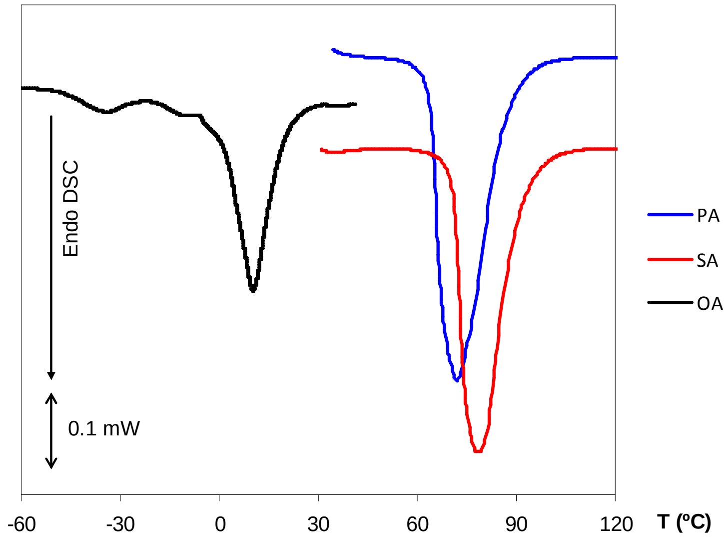

Figure 4. Lipid melting endotherms por pure palmitic acid (A), stearic acid (B) and oleic acid (C).

The incorporation of glycerol significantly decreased Tg values of the starch matrix, according to its low molecular weight and plasticizing nature. Nevertheless, at a_w 0.75, a phase separation seems to occur since two glass transitions were observed, one at higher temperature attributed to the starch-rich phase and one in the range of -40/-50ºC, attributed to the glycerol-rich phase. Therefore, no glycerol plasticizing effect would be expected in the starch matrix in this situation, where mainly water molecules would contribute to this effect. The great water affinity of glycerol molecules is responsible for water-glycerol interactions predominating over glycerol-starch interactions and the small hydrated molecules separate from the polymer network. Examination of the calorimetric behaviour of glycerol plasticised amylose films (25±50% w/w glycerol) showed the presence of a weak glass transition in the region of -50ºC, regardless the glycerol ratio in the film, which was attributed to a separated glycerol rich phase. This temperature was slightly lower than that obtained for glycerol (-76ºC) under the same conditions (Moates *et al.*, 2001). This phenomenon was also observed in films of methylcellulose plasticized with polyethylene glycol 400 by Debeaufort & Voilley (1997) and in iota-carrageeenan films plastized with glycerol (Hambleton, Perpiñan-Saiz, Fabra, Voilley & Debeaufort, 2012).

Fatty acids affected the Tg values of the starch to a different extent, depending on the nature of the lipid. OA did not have a significant effect at low a_w values where Tg could be observed, since at higher a_w values it overlapped with the lipid fusion endotherm. Nevertheless, SA and PA provoked a significant decrease in Tg for the low a_w range. This seems to indicate that saturated fatty acids, with linear chain, interact with the polymer contributing to its plasticization. This effect was greater when the acid chain length increased and was inhibited when the chains showed a double bond (OA), probably due to the loss of the chain linear arrangement. This suggests that interactions could occur through complex formations, where helical conformation of the polymer chains (amylose) entrapped the linear chain of fatty acid, as described by other authors for some lipid molecules (Biliaderis & Seneviratne, 1990a, 1990b; Singh, Singh & Saxena, 2002; Zhang, Huang, Luo, Fu, 2012). In this sense, changes in the amylopectin/amylose ratio could occur in the amorphous region due to the non-availability of one of these (mainly amylose) involved in the complex. This change in the polymer ratio could imply a reduction of the amorphous region Tg value, since Tg value of pure amylose is greater than that of amylopectin (Guo, Liang & Du, 2011). Bizot, Le Bail, Leroux, Davy, Roger & Buleon (1997) reported that pure amylose exhibited somewhat higher Tg than branched amylopectin. The α-(1-6) linkages theoretically offer three rotational degrees of freedom whereas α-(1-4) linkages offer two degrees. Thus, amylopectin with a higher degree of branching has greater chain flexibility than the relatively linear amylose. The longer molecular weight and chain length, the higher the Tg; the more branched and flexible, the lower the Tg. This interprets the reason why the Tg of amylopectin is lower than the Tg of amylose.

The melting behaviour of pure lipids and the lipid fraction in the films was analysed to observe possible differences associated to the lipid-starch complex formation. Table 5 shows the initial, peak and final temperature of the melting endotherm, as well as the melting enthalpy, expressed per mg of lipid. Neither the embedding of lipid in the starch matrix nor water activity of the films caused notable differences in the temperature range of the melting endotherm for a determined fatty acid. Nevertheless, significant differences in the melting enthalpy were observed for a given fatty acid, depending on the water activity of the film. Whereas for high a_w values (a_w >0.68) the enthalpy values were similar to those obtained for the pure lipids, at lower a_w values the melting enthalpy values were lower than those of pure lipids for all fatty acids, in agreement with a lower crystallization degree in these cases. This could be explained by the lipid-starch complex formation when the films contain a low water content.

Table 5. Values of melting enthalpy and melting temperature range of pure fatty acids and those present in the films equilibrated at different relative humidity.

Sample	To (ºC)	Tp (ºC)	Te (ºC)	ΔH (J/g of lipid)
Pure fatty acids				
PA	54 (2)^{a}	71.8 (0.4)^{a}	101.3 (0.7)^{a}	183 (2)^{a}
SA	61.0 (0.8)^{1}	78.0 (0.5)^{1}	106.0 (1.6)^{1}	188.6 (0.2)^{1}
OA	-21.5 (0.9)^{w}	10.0 (0.3)^{w}	36.2 (0.4)^{wx}	84 (5)^{x}
Starch based films				
a_w = 0				
Starch-Gly-PA	46 (3)^{b}	67.7 (0.7)^{bc}	100 (9)^{a}	141 (12)^{b}
Starch-Gly-SA	58 (3)^{12}	74.7 (0.6)^{23}	105 (3)^{1}	175 (3)^{2}
Starch-Gly-OA	-7.16 (0.3)^{x}	7.3 (0.9)^{x}	26 (3)^{y}	39 (5)^{y}
a_w = 0.53				
Starch-Gly-PA	46.6 (0.2)^{b}	67.1 (0.4)^{c}	97 (2)^{a}	176 (4)^{a}
Starch-Gly-SA	54.9 (0.5)^{2}	73.6 (0.1)^{4}	104.3 (0.7)^{1}	181 (2)^{1}
Starch-Gly-OA	-12.2 (1.5)^{y}	6.8 (0.9)^{x}	30 (3)^{xy}	34 (10)^{y}
a_w = 0.68				
Starch-Gly-PA	50.0 (0.8)^{ab}	67.2 (0.2)^{c}	99.8 (1.2)^{a}	194.3 (0.2)^{a}
Starch-Gly-SA	57.6 (0.1)^{12}	74.0 (0.4)^{34}	105.2 (2)^{12}	190 (10)^{1}
Starch-Gly-OA	-16.3 (1.2)^{z}	7.6 (0.5)^{x}	42.2 (0.2)^{wz}	64 (3)^{z}
a_w = 0.75				
Starch-Gly-PA	48.2 (0.1)^{b}	68.8 (0.1)^{b}	100 (3)^{a}	174 (16)^{a}
Starch-Gly-SA	57.1 (1.3)^{2}	75.5 (0.1)^{2}	111 (3)^{2}	195 (7)^{1}
Starch-Gly-OA	-16.7 (1.8)^{z}	10.6 (0.4)^{w}	47 (4)^{z}	96 (7)^{x}

a-c; 1-4; w-z: Different superscripts in formulations containing the same fatty acid (in columns) indicate significant differences among formulations ($p < 0.05$).

With more water availability and the subsequent greater molecular mobility, complexes could be destroyed by the predominance of starch chain hydration and so lipids remain free to crystallize. In fact, no significant differences in the Tg values of the starch matrices containing different fatty acids were observed from a_w 0.68 onwards. It is remarkable that, despite the fact that oleic acid did not contribute to modify the Tg value of the starch matrix, its crystallization was also inhibited at low moisture content in the film. This could indicate that another type of complexes could be formed without modification of the amylose/amylopectin ratio in the amorphous region.

The formation of complexes between starch polymers and saturated fatty acids can be also deduced from the microstructural observations. Figure 5 shows the SEM micrographs of the film cross section (samples equilibrated with P_2O_5).

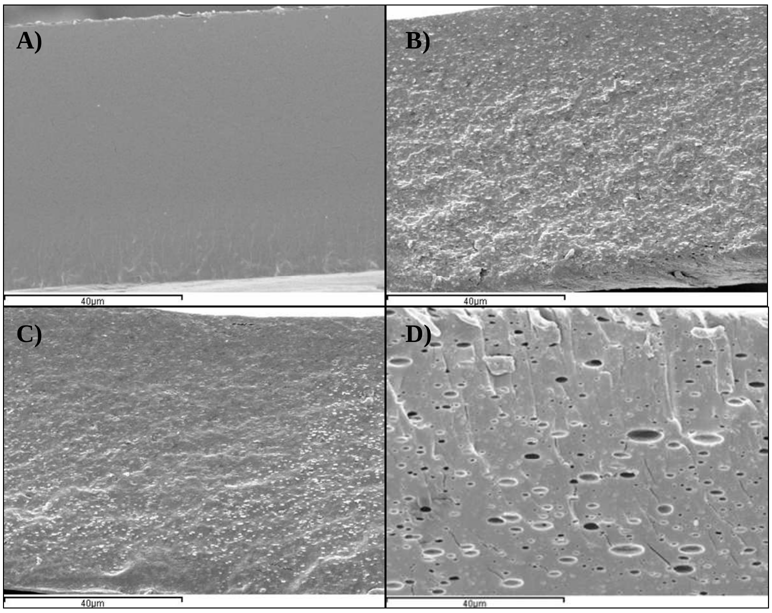

Figure 5. SEM micrographs of the cross-sections of the studied films. A: Starch-Gly, B: Starch-Gly-PA, C: Starch-Gly-SA, D: Starch-Gly-OA.

In the case of samples containing oleic acid, lipid droplets can be seen embedded in the starch matrix, whereas this was not observed for stearic and palmitic acids. This indicates that lipid molecules are well integrated in the polymer matrix and their aggregates (crystals) were very small and can not be observed by SEM at the used magnification level. The gel formation during the film drying step in starch film preparation could contribute to limit the acid crystal growth. However, in other polymer matrices such as sodium caseinate and HPMC, palmitic and stearic acid were separated as molecular lamellar aggregates which had a great impact on the microstructure and physical properties of the films (Fabra, Jiménez, Atarés, Talens & Chiralt, 2009; Jiménez, Fabra, Talens & Chiralt, 2010). The liquid state of oleic acid during the film drying step favours the increase in the droplet size, thus making their observation possible.

The mechanical response of the films is greatly affected by the glassy-amorphous state of the materials and by the presence of crystalline forms. In the studied films, tensile strength curves were obtained for films equilibrated at different relative humidity at 25 ºC. Figure 6 shows the stress-Hencky strain curves for the different film samples containing, or not, different fatty acids and the corresponding values of mechanical parameters are shown in Table 6. At near zero moisture content, elastic modulus of the films reveals that saturated fatty acids induce a slight loss of the matrix strength. This can be attributed to the aforementioned complex formation of the lipids with one of the starch polymers, which reduces the cohesion forces of the amylose network. Nevertheless, the incorporation of oleic acid provoked a much greater reduction of the matrix rigidity due to the discontinuities introduced in the polymer network as could be seen in the SEM micrograph (Figure 5D). Discontinuities imply a loss of the cohesion forces which reduces the elastic modulus of the film. At this near zero water content, the glassy state of the matrices can be deduced since films did not show plastic deformation and they broke when elastic deformability was exceeded.

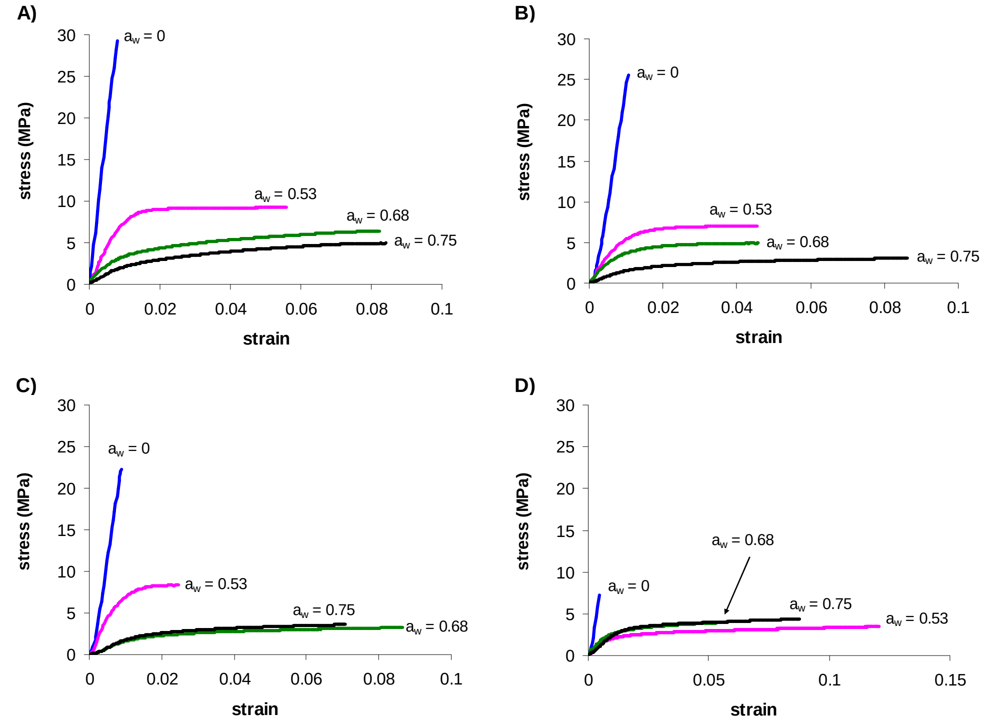

Figure 6. Stress-Hencky strain curves for the starch films equilibrated at different relative humidity. A: Starch-Gly, B: Starch-Gly-PA, C: Starch-Gly-SA, D: Starch-Gly-OA.

Table 6. Tensile properties of starch based films conditioned at different relative humidity. Mean values (standard deviation).

Sample	EM (MPa)				TS (MPa)			
	a_w = 0	**a_w = 0.53**	**a_w = 0.68**	**a_w = 0.75**	**a_w = 0**	**a_w = 0.53**	**a_w = 0.68**	**a_w = 0.75**
Starch-Gly	3500 (400)^{a1}	810 (70)^{a2}	360 (30)^{a3}	180 (20)^{a3}	20 (6)^{a1}	9.1 (0.6)^{a2}	7.3 (1.0)^{a23}	4.7 (0.6)^{a3}
Starch-Gly-PA	3000 (300)^{b1}	640 (110)^{b2}	370 (20)^{a23}	170 (60)^{a3}	27.4 (1.6)^{b1}	6.8 (0.3)^{b2}	4.1 (0.8)^{b3}	3.2 (0.7)^{b3}
Starch-Gly-SA	3000 (200)^{b1}	870 (170)^{a2}	167 (9)^{b3}	220 (40)^{a3}	23 (3)^{ab1}	8.3 (1.3)^{a2}	3.6 (0.5)^{b3}	3.9 (0.5)^{b3}
Starch-Gly-OA	1760 (160)^{c1}	170 (30)^{c2}	233 (10)^{c2}	230 (10)^{a2}	6.1 (1.7)^{c1}	3.3 (0.3)^{c2}	3.72 (0.13)^{b2}	4.1 (0.2)^{ab2}

	E (%)			
	a_w = 0	**a_w = 0.53**	**a_w = 0.68**	**a_w = 0.75**
Starch-Gly	0.54 (0.19)^{ab1}	8 (4)^{a2}	8.4 (1.4)^{a2}	8.5 (1.4)^{a2}
Starch-Gly-PA	0.8 (0.3)^{bc1}	4 (3)^{ab2}	4.2 (1.2)^{b2}	8.3 (1.4)^{ab3}
Starch-Gly-SA	0.85 (0.15)^{c1}	2.2 (0.7)^{b1}	8 (4)^{ab2}	6.5 (0.5)^{b2}
Starch-Gly-OA	0.45 (0.04)^{a1}	13 (7)^{c2}	5.1 (1.4)^{b13}	9.4 (1.4)^{a23}

a-c: Different superscripts within the same column indicate significant differences among formulations at a determined a_w value($p < 0.05$).

1-3: Different superscripts within the same line indicate significant differences for a determined formulation at different a_w values ($p < 0.05$).

At the next a_w value (0.53), the Tg values of the matrices are lower than 25 ºC and a rubbery state was expected for the polymer networks. In fact, plastic deformation was observed for all films at this equilibration relative humidity and differences in the elastic modulus of the lipid-free films and those containing saturated fatty acids were hardly appreciable. The plasticizing effect of water and the possible partial disruption of complexes smooth the differences in the mechanical response of the films containing saturated fatty acids. Nevertheless, in films containing OA, the effect of the lipid discontinuities in the matrix persists at this a_w and it shows a significantly lower elastic modulus. At higher equilibration relative humidity, differences in the elastic modulus of the films were much less appreciable, since the plasticizing effect of water in the starch continuous matrix predominates over the other possible effects, masking them (Table 6).

Tensile strength at break was very similar for pure starch films and those containing saturated fatty acids, although at the highest relative humidity values, pure starch films were slightly more resistant due to the greater cohesion forces in the more homogeneous network. Films containing oleic acid were much less resistant in the entire range of a_w due to the fact that discontinuities in the matrix greatly reduced its cohesion and mechanical resistance. On the contrary, extensibility is promoted by the presence of the OA droplets in the film, especially at low equilibration relative humidity, as compared with the other films (Table 6). The great deformability of the liquid lipid droplets during the film stretching is mainly responsible for this behaviour. At higher relative humidity (75%), the greatest extensibility was shown by pure starch films due to the fact that they are more resistant to the rupture and so, showed a greater margin for deformation.

4. CONCLUSIONS

Starch film water sorption capacity (WSC) was affected by starch crystallization which, in turn depended on the water activity and temperature. Starch crystallization was promoted at low temperature and high relative humidity which modifies the expected tendencies in WSC as a function of the temperature. Glass transition of the starch matrix at low moisture contents decreased when saturated fatty acids are present in the film, but was not affected by oleic acid. When fatty acids are present in the films the only crystalline form which was observed for starch is the V-type structure, thus indicating the predominating formation of amylose-lipid complexes, inhibiting the formation of other crystalline forms. The intensity of the crystalline peak decrease when moisture content increase and different results seems to indicate that complex formation occurs mainly at low moisture content. The mechanical behaviour of the films, as a function of the moisture content, was coherent with the results of the phase transition analysis and microstructural observations. The water plasticization effect and the structural discontinuities determine the value of the elastic modulus and the behaviour at break.

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Capítulo II

Mezclas con otros biopolímeros. Influencia sobre las propiedades físicas y estructurales de los films

INFLUENCE OF HYDROXYPROPYLMETHYLCELLULOSE ADDITION AND HOMOGENIZATION CONDITIONS ON PROPERTIES AND AGEING OF CORN STARCH BASED FILMS

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ABSTRACT

Edible films based on corn starch, hydroxypropyl methylcellulose (HPMC) and their mixtures were prepared by using two different procedures to homogenize the film forming dispersions (rotor-stator and rotor-stator plus microfluidizer). The influence of both HPMC-starch ratio and the homogenization method on the structural, optical, tensile and barrier properties of the films was analysed. The ageing of the films was also studied by characterizing them after 5 weeks' storage. Starch re-crystallization in newly prepared and stored films was analysed by means of X-ray diffraction. HPMC-corn starch films showed phase separation of polymers, which was enhanced when microfluidization was applied to the film forming dispersion. Nevertheless, HPMC addition inhibited starch re-crystallization during storage, giving rise to more flexible films at the end of the period. Water barrier properties of starch films were hardly affected by the addition of HPMC, although oxygen permeability increased due to its poorer oxygen barrier properties.

Keywords: starch, HPMC, storage, microstructure, tensile properties, barrier properties.

1. INTRODUCTION

Nowadays, many researchers are focusing their work on obtaining environmentally friendly materials which are able to protect food products from spoilage but also present an adequate biodegradability. In this way, traditional petroleum-based polymers, such as polyethylene or polypropylene, are being substituted by biopolymers obtained from natural and renewable sources. These polymers are mainly polysaccharides (starch, chitosan, cellulose and its derivatives) and proteins, such as gelatin, caseinates or zein, which are generally processed to obtain edible films or coatings. These structures are thin layers of edible materials applied to food products, which play an important role in their preservation, distribution and marketing (Falguera, Quintero, Jiménez, Muñoz & Ibarz, 2011). One of the most suitable polymers with which to substitute conventional plastics is starch due to the fact that it is able to present thermoplastic behaviour if an adequate amount of plasticizers is used. Starch is a well-known polysaccharide that presents different properties depending on its amylose/amylopectin ratio. Properties, such as glass transition temperature (Liu, Yu, Wang, Li, Chen & Li, 2010) or digestibility (resistant starch; Zhu, Liu, Wilson, Gu & Shi, 2011), vary according to the amylose content. Their properties, as packaging material alone or in combination with other materials, have been widely studied (Phan The, Debeaufort, Luu & Voilley, 2005; Bertuzzi, Armada & Gottifredi, 2007; Flores, Conte, Campos, Gerschenson & Del Nobile, 2007; Chillo, Flores, Mastromatteo, Conte, Gerschenson & Del Nobile, 2008; Tang, Alavi & Herald, 2008; Kuorwel, Cran, Sonneveld, Miltz & Bigger, 2011). Although starch based films and coatings generally present adequate properties, it has been found that storage greatly increases the crystalline fraction in the starch matrix (Mali, Grossmann, García, Martino & Zaritzky 2006; Jiménez, Fabra, Talens & Chiralt, 2012); a fact that may lead to a deterioration of the protective ability of starch-based packaging. Jiménez *et al*. (2012) related the increase in crystallinity with the changes in different properties (gloss, transparency, brittleness) of the films, which can affect the film functionality and consumer acceptance of coated products. One way to avoid the recrystallization of starch is by combining this polymer with others, preferably amorphous. One of the biopolymers with this characteristic is hydroxypropyl-methylcellulose (HPMC). Its ease of use, availability, water solubility, and non-toxicity makes HPMC the most extensively used cellulose derivative (Fahs, Brogly, Bistac & Schmitt, 2010). The amorphous state of HPMC has been reported by Kou, Cai, Xu, Wang, Liu, Yang, & Zhang (2011) through X-ray diffraction analysis. Huang, Chen, Lin & Chen (2011) succeeded in reducing the crystallinity index of bacterial culture cellulose by adding HPMC to the bacterial culture medium, pointing to this polymer's capacity to inhibit crystallization.

HPMC has also been studied as a matrix of edible films in combination with different components, such as fatty acids (Jiménez, Fabra, Talens & Chiralt, 2010) or cellulose nano-particles (Bilbao-Sainz, Bras, Williams, Sénechal & Orts, 2011). Nevertheless, there is no available literature on the mixing of HPMC with starch in order to form edible or biodegradable films or packages.

Starch is able to form films in combination with various polymers, such as agar, arabinoxylan (Phan The, Debeaufort, Voilley & Luu, 2009) or chitosan (Bourtoom & Chinnan, 2008; Vásconez, Flores, Campos, Alvarado & Gerschenson, 2009). Phan The *et al*. (2009), working on films containing mixtures of starch with agar or arabinoxylan, observed that the component integration in the matrix was greatly dependent on the type of polymer. Cassava starch-arabinoxylan film was homogeneous, whereas cassava starch-agar film showed a phase separation and dispersion.

The aim of this work was to evaluate the influence of HPMC addition, the homogenization conditions of the film forming dispersion as well as the effect of ageing on the structural, mechanical, and optical and barrier properties (water vapour and oxygen) of corn starch-glycerol based films.

2. MATERIALS AND METHODS

2.1. Materials

Corn starch was obtained from Roquette (Roquette Laisa España, Benifaió, Spain). Hydroxypropyl-methylcellulose (HPMC) was purchased from Fluka (Sigma–Aldrich Chemie, Steinheim, Germany). Glycerol, used as plasticizer, was provided by Panreac Quimica, S.A. (Castellar Del Vallés, Barcelona, Spain), as well as magnesium nitrate-6-hydrate which was used to equilibrate film samples at 53% RH.

2.2. Preparation and characterization of films

Eight different formulations based on corn starch and/or HPMC were prepared. Firstly, both polysaccharides were dispersed separately: HPMC was dissolved (2 % w/w) in cold water by continuous stirring and maintained under these conditions overnight, whereas dispersions containing 2 % (w/w) starch were mildly stirred with a stirring rod while maintained at 95 ºC for 30 min to promote polysaccharide gelatinization. Afterwards, both hydrocolloid solutions were mixed at room temperature in different ratios to obtain four dispersions with 100:0, 75:25, 50:50 and 0:100 starch:HPMC ratios. Then, the plasticizer was added using a hydrocolloid:plasticizer ratio of 1:0.25. Glycerol was chosen as plasticizer for all formulations due to it being a better plasticizer than other polyols (sorbitol and xylytol) with the same plasticizer content (Talja, Helén, Roos & Jouppila, 2007). The homogenization of film-forming dispersions was carried out under vacuum, to avoid bubble formation, and at 95 ºC using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany) for 1 min at 13,500 rpm and for 5 min at

20,500. A part of each formulation was also homogenized in a second step using a Microfluidizer M-110P (Microfluidics International Corp., Newton, Massachusetts, USA) at 103,390 kPa (microfluidized samples), thus obtaining 8 different film forming solutions (samples A to H).

Controlled amounts of film-forming dispersions (containing 1.5 g of total solids) were spread evenly over a Teflon® casting plate (15 cm diameter) resting on a leveled surface, and films were formed by drying them for approximately 48 h at 45 % RH and 20 ºC. Afterwards, the dried films were peeled intact from the casting surface. Film thickness was measured with a Palmer digital micrometer to the nearest 0.0025 mm at 6 random positions before tests.

2.2.1. Film equilibration and storage

Prior to testing, samples were equilibrated in dessicators at 25 ºC and 53 % RH, by using magnesium nitrate-6-hydrate saturated solutions for one week when the first series of analyses was carried out. One part of the different samples was stored under the same conditions for five weeks, when the second series of analyses was performed.

2.2.2. Tensile Properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, following ASTM standard method D882 (ASTM, 2001) EM, TS, and E were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 10 cm long). Six replicates were analyzed per formulation. Equilibrated specimens were mounted in the film-extension grips of the testing machine and stretched at 50 mm min^{-1} until breaking. The relative humidity of the environment was maintained constant at 53 (±2) % during the tests, which were carried out at 25 (±1) °C.

2.2.3. X-ray diffraction

X-ray diffraction patterns were recorded using a Rigaku Ultima IV multipurpose X-ray diffraction system (Rigaku Corporation, Tokyo, Japan). All samples were analyzed between 5º and 50º (2θ) using Kα Cu radiation (λ = 1.542 Å), 40 kV and 40 mA with a step size of 0.02 º. For this analysis, samples were cut into 2 cm squares, prior to storage, in order to avoid breakage during handling. Analyses were performed at 25ºC and 53% RH. To this end, samples were equilibrated at these conditions which were also maintained in the laboratory where the X-ray difractometer was located.

2.2.4. Water Vapour Permeability (WVP)

A modification of the ASTM E96-95 (1995) gravimetric method (McHugh, Avena-Bustillos, & Krochta, 1993) for measuring WVP of flexible films was employed for all samples using Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle/s Argenteau, Belgium). Films were selected for WVP tests based on lack of physical defects such as cracks, bubbles, or pinholes. Each cup was filled with distilled water to expose the film to 100% RH on one side. Once the films were secured, the cups were placed in a relative humidity equilibrated cabinet fitted with a fan to provide a strong driving force across the film for water vapour diffusion. The RH of the cabinets (53% at 25 ◦C) was held constant using oversaturated solutions of magnesium nitrate-6-hydrate. The free film surface during film formation was always exposed to the lowest relative humidity, whereas the cabinets were maintained at 25 ºC during the tests. The cups were weighed each two hours (0.0001 g) throughout 24 h. Water vapour transmission (WVTR) was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film area.

From WVTR data, the vapour pressure on the film's inner surface (p_2) was obtained using Eq. (1), proposed by Mc Hugh *et al*. (1993), to correct the effect of concentration gradients established in the stagnant air gap inside the cup:

$$WVTR = \frac{P \cdot D \cdot Ln\left[{}^{P - p_2}\!\big/_{P - p_1}\right]}{R \cdot T \cdot \Delta z} \qquad \text{Equation 1}$$

where P, total pressure (atm); D, diffusivity of water through air at 25 ºC (m^2 s^{-1}); R, gas law constant (82.057·10^{-3} m^3·atm·kmol^{-1}·K^{-1}); T, absolute temperature (K); Δz, mean height of stagnant air gap (m), considering the initial and final z value; p_1, water vapour pressure on the solution surface (atm); and p_2, corrected water vapour pressure on the film's inner surface (atm). Water vapour permeance was calculated using Eq. (2) as a function of p_2 and p_3 (pressure on the film's outer surface in the cabinet).

$$permeance = \frac{WVTR}{p_2 - p_3} \qquad \text{Equation 2}$$

Permeability was obtained by multiplying the permeance by the average film thickness.

2.2.5. Oxygen permeability

The oxygen barrier properties of the films were evaluated by measuring oxygen permeability (OP) by means of an Ox-Tran 1/50 system (Mocon, Minneapolis, USA) at 25 ºC (ASTM Standard Method D3985-95, 2002). Measurements were taken at 53% in films previously equilibrated at the same RH.

Films were exposed to pure nitrogen flow on one side and pure oxygen flow on the other side. The OP was calculated by dividing the oxygen transmission rate by the difference in the oxygen partial pressure on the two sides of the film, and multiplying by the average film thickness. At least three replicates per formulation were considered.

2.2.6. Scanning Electron Microscopy (SEM)

A microstructural analysis of the films was carried out using a Scanning Electron Microscope (JEOL JSM-5410, Japan). Film samples were maintained in a desiccator with P_2O_5 for 15 days. Then the films were frozen in liquid N_2 and gently and randomly broken to observe the cross-section and the surface of the film samples. Films were fixed on copper stubs; gold coated, and observed using an accelerating voltage of 10 kV.

2.2.7. Optical Properties

The transparency of the films was determined by applying the Kubelka-Munk theory (Hutchings, 1999) for multiple scattering to the reflection spectra. The surface reflectance spectra of the films were determined from 400 to 700 nm using a spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) on both a white and a black background. As the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the scattering (S) coefficients. The internal transmittance (T_i) of the films was quantified using eq 3. In this equation, R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by eqs 4 and 5, where R is the reflectance of the sample layer backed by a known reflectance R_g. Measurements of each sample were taken in triplicate on the free film surface during drying.

$$T_i = \sqrt{(a - R_0)^2 - b^2}$$ Equation 3

$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right)$$ Equation 4

$$b = (a^2 - 1)^{1/2}$$ Equation 5

The gloss was measured on the free film surface during film formation, at 60° angles from the normal to the surface, following the ASTM standard D-523 method (1999), using a flat surface gloss meter (Multi.Gloss 268, Minolta, Germany). Measurements of each sample were taken in triplicate and three films of each formulation were considered. All results are expressed as gloss units, relative to a highly polished surface of standard black glass with a value close to 100.

2.3. Statistical Analysis

Statistical analyses of data were performed through an analysis of variance (ANOVA) using Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD). Fisher′s least significant difference (LSD) procedure was used at the 95% confidence level.

3. RESULTS

3.1. Microstructural properties

The final structure of the film depends on both the interactions of film components and the drying conditions of the film-forming dispersion and has a great impact on the different film properties (Fabra, Talens & Chiralt, 2009a;

Souza, Cerqueira, Casariego, Lima, Teixeira & Vicente, 2009; Villalobos, Chanona, Hernández, Gutiérrez & Chiralt, 2005). In this sense, Fabra, Jiménez, Atarés, Talens & Chiralt (2009b) showed that the microstructural analysis of the films gives relevant information about the arrangement of the components, which, in turn, allows the values obtained for the water barrier, mechanical or optical properties to be understood.

Figure 1 shows the SEM micrographs obtained from the cross-sections of the obtained films. Generally, pure starch films (Figures 1A and 1E) or pure HPMC films (Figures 1D and 1H) showed a homogeneous structure, whereas a more heterogeneous structure was observed for films prepared with mixtures of both polysaccharides (75:25 or 50:50 starch:HPMC ratio). Slight differences were observed when comparing pure HPMC micrographs (Figures 1D and 1H) with those reported by Jimenez *et al*., (2010), which can be attributed to the presence of glycerol in the present films. Cross-section images reported by Jiménez *et al*. (2010) for pure HPMC films (glycerol-free) were homogeneous and completely smooth, whereas irregularities in the micrographs can be observed when glycerol was added. Even in non-microfluidized samples (Figure 1D), a different phase can be observed on the bottom of the films, indicating that glycerol is not homogenously distributed across the film. When high-pressure was applied, although the components were distributed more homogeneously in the polymer matrix, the matrix obtained was still not completely smooth. This suggests the lack of a complete miscibility of HPMC and glycerol. This is also observed in surface images shown in Figure 2D in which different separate zones in the film surface can be appreciated in agreement with phase separation in HPMC-Gly films. Nevertheless glycerol dispersion was enhanced when the homogenization intensity increased and film surface appeared much more homogeneous (Figure 2H).

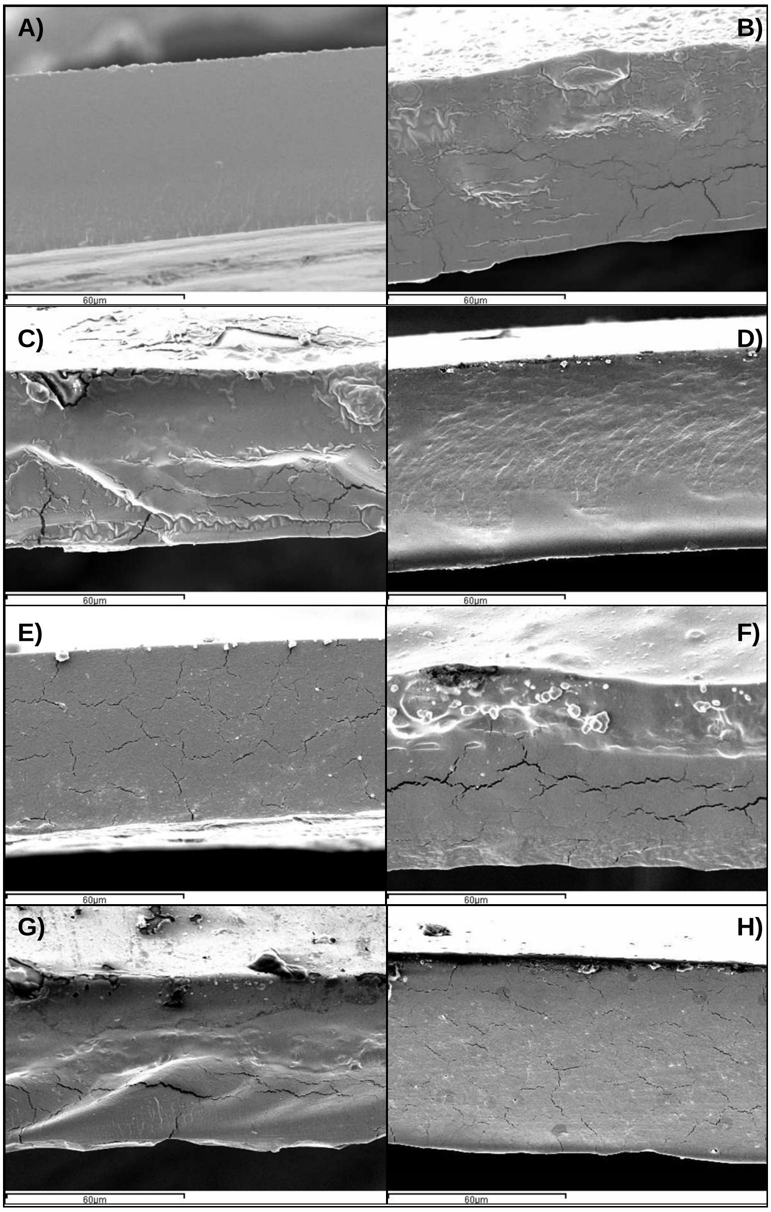

Figure 1. SEM images of the cross-sections of the studied films. A to D correspond to non-microfluidized films, E to H correspond to microfluidized films. A and E: 100:0 starch:HPMC ratio, B and F: 75:25 starch:HPMC ratio, C and G: 50:50 starch:HPMC ratio and D and H: 0:100 starch:HPMC ratio.

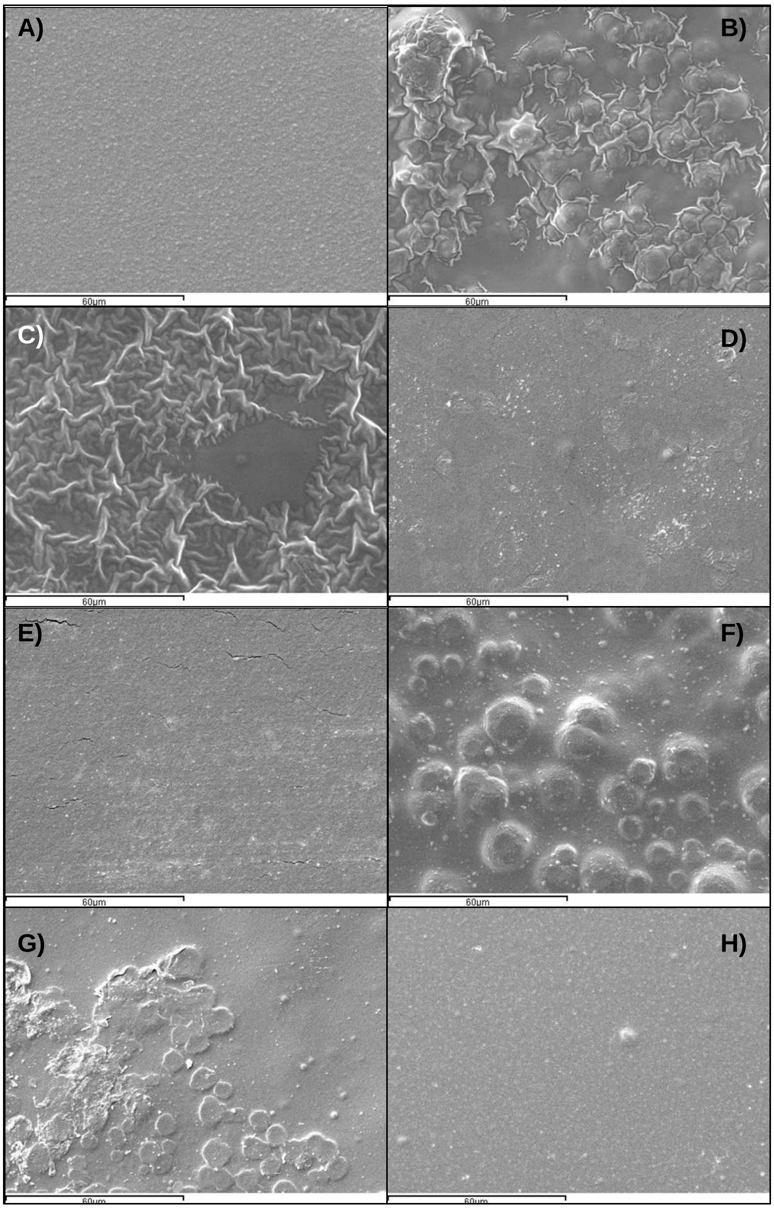

Figure 2. SEM images of the film surface of composite films. A to D correspond to non-microfluidized films, E to H correspond to microfluidized films. A and E: 100:0 starch:HPMC ratio, B and F: 75:25 starch:HPMC ratio, C and G: 50:50 starch:HPMC ratio and D and H: 0:100 starch:HPMC ratio.

Pure starch films showed a smooth surface and a homogeneous microstructure in the cross section images in both microfluidized and non-microfluidized films (Figures 1A, 2A and 1E, 2E), although microfluidification yield a more brittle network as revealed by the micro-cracks provoked by the electron impact. This could be explained by a different arrangement of the amylopectin and amylose fractions in each case due to the high shear applied during microfluidification. Different authors (Rindlav-Westling, Stading & Gatenholm, 2002; Paes, Yakismets & Mitchell, 2008) report that amylose-amylopectin separation can occur during film formation depending on shear and temperature applied and the amylose-amylopectin ratio. A competition between gel formation and phase separation may occur in the amylose-amylopectin-water systems and, if the relative rate of phase separation is lower than the relative rate of gelation, no-phase separation occurs in the system during cooling. Rindlav-Westling *et al*. (2002) reported that when the amylose proportion in the amylose-amylopectin blends was lower than 25%, a phase separation occur, but at higher amylose proportions, the phase separation was apparently prevented by amylose gelation and the formation of a continuous amylose network. The amylose content in native corn starch is around 25% (García, Pinotti, Martino & Zaritzky, 2009). Moreover, Paes *et al*. (2008) reported that amylose-amylopectin phase separation occurred when severe temperature and shear conditions were applied during film formation, while remnants of the granules or ghosts are present for mild temperature or shear conditions. This affects the final film microstructure and its mechanical properties.

Micrographs of films prepared with mixtures of both polysaccharides (starch:HPMC blends) showed a heterogeneous structure, showing phase separation between starch and HPMC due to the lack of polymer compatibility. In any case microfluidization leads to a clearer separation of two phases in the films (top and bottom), despite the high pressure applied to the film forming dispersion.

These two phases will be an HPMC-enriched phase and a starch-enriched phase. The polymer incompatibility leads to films whose heterogeneity cannot be avoided by high pressure homogenization conditions. In fact, composite films homogenized by means of a rotor-stator showed a dispersed phase embedded in a continuous phase; however, when microfluidization was applied, phase separation was more evident probably due to the fact that the promotion of polymer chain interactions at high pressure enhances their incompatibility. From the obtained images, it could be deduced that the starch-enriched phase was located at the bottom of the film, whereas the HPMC-enriched phase was at the top, depending on the relative density of both polymers. In this sense, it is remarkable that liquid phase separation was also visually observed in the film forming dispersions, where the starch phase (less transparent) was also appreciated at the bottom of the system.

The non-homogeneous structure of films obtained by mixing polysaccharides has been previously reported. Mathew & Abraham (2008) found that adding ferulic acid led to starch-chitosan films that were more homogeneous than others which did not have this component, which was explained by the cross-linking activity of ferulic acid. Annable, Fitton, Harris, Philips & Williams (1994) reported that the incorporation of a hydrocolloid, such as galactomannans, into starch dispersion at a sufficiently high concentration leads to the phase separation of mixed gels into two different phases.

The internal network of these kinds of films, formed during drying, usually affects different properties. In this sense, the tortuosity of the internal structure affects the light transmission/dispersion behaviour of the film, and so its transparency-opacity ratio. In previous works, Fabra *et al*. (2009b) and Jiménez *et al*. (2010) found that films became less transparent when fatty acids were added to sodium caseinate and HPMC based films, due to the layered structure of these films; the more layers there are, the less transparent the film.

The film's microstructural irregularities have an impact on the surface level that greatly affects surface roughness, and so film gloss. To evaluate this impact on the composite films, SEM micrographs of the film surface were obtained. Figure 2 shows the surface SEM micrographs of the microfludized and non-microfludized films. Different particles can be observed in all composite films (Figures 2B, 2C, 2F and 2H). These particles are irregular in shape in films obtained with a single rotor-stator homogenization step (Figures 2B and 2C) and more spherical in those obtained following a 2-step homogenization process (Figures 2F and 2G). Microfludized films show a clear reduction in surface irregularities and roughness as compared with non-microfludized films, which coincides with the clearer phase separation observed in the film's cross-section images. Whereas punctual chain aggregates, filamentous in shape, can be observed in non-microfluidized films, in microfluidized films, spherical particles, resulting from the separation of polymer aqueous phases remain on the film surface.

3.2. Optical properties

The different structural arrangement of polysaccharides in the studied films could have an effect on their optical properties, since changes in the refractive index occur through the polymer matrix. Film transparency was evaluated by means of internal transmittance (Ti). Figure 3 plots the spectral distribution curves of Ti for both newly prepared (a) and stored films (b). High values of Ti are associated with more transparent films with a more homogeneous refractive index through their structure, whereas lower Ti values correspond to more opaque films with heterogeneous networks. A similar pattern was observed for every film over the considered wavelength range and there were only small differences in the Ti values. The greatest differences appeared at low wavelength in aged HPMC films, indicating the development of some yellowness.

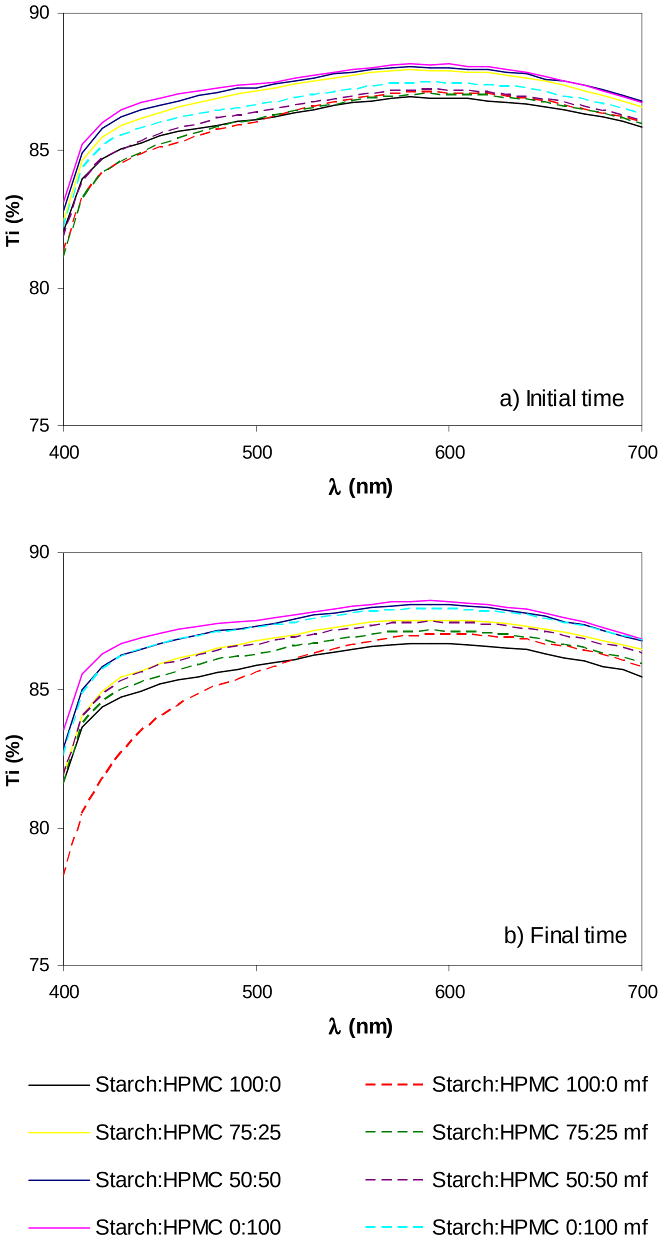

Figure 3. Spectral distribution of internal transmittance (Ti) of microfluidized (mf) and non-microfluidized films containing different starch:HPMC ratios: a) newly prepared and b) conditioned films after 5 storage weeks.

Table 1 shows the Ti values at 450 nm (in the range where the greatest differences among films were observed) where, in newly prepared films, the highest Ti values were found for non-microfluidized pure HPMC and the lowest value for microfluidized pure starch films. In both films, microfluidized and non-microfluidized, the increase in the starch ratio gave rise to a decrease in transparency. In every case microfluidization provoked a decrease in the film transparency, although in pure starch films this was not significant. Nevertheless, differences are small and it can be concluded that HPMC addition and phase separation introduces little discontinuities in the refractive index of the polymer network, thus having only a slight impact on the film transparency. Moreover, the greater transparency of HPMC helps to lend a more transparent character to the starch films, which are more opaque. This is probably due to the fact that they are also composite films made from amylose and amylopectin fractions.

Throughout storage, small changes in film transparency occurred mainly in microfluidized pure starch and pure HPMC films. In this sense, it is remarkable that starch films became more opaque, whereas HPMC films became more transparent. Taking into account the spectral distribution (between 400 and 500 nm) of Ti corresponding to microfluidized pure starch at initial (Figure 3B) and final time (Figure 3B), the Ti decrease is associated with the change in this distribution and was coherent with the yellowness which could be visually observed in samples stored for 5 weeks. This could be attributed to the partial starch hydrolysis during microfluidization, which yielded brown compounds through the reaction of low molecular weight sugars. These sugars can react to some extent in the amorphous film, at low water activity, through the complex caramelization reactions giving rise to brown compounds (Claude & Ubbink, 2006).

Table 1. Optical properties of both non-stored (initial) starch-HPMC based films and stored for 5 weeks (final). Samples A to D: non-microfluidized. Samples E to H: microfluidized. Mean values (standard deviation).

		T_i (450 nm)		Gloss 60º	
Sample	Starch:HPMC ratio	Initial	Final	Initial	Final
A	100:0	85.54 (0.36)^{ab1}	85.21 (0.09)^{a1}	71 (5)^{a1}	67 (9)^{a1}
B	75:25	86.38 (0.22)^{cd1}	85.97 (0.42)^{bc1}	10.32 (0.41)^{b1}	10 (1)^{b1}
C	50:50	86.66 (0.25)^{cd1}	86.69 (0.34)^{cd1}	19.15 (2.71)^{c1}	14.1 (1)^{b2}
D	0:100	86.9 (0.3)^{d1}	87.08 (0.36)^{d1}	71 (5)^{a1}	57 (9)^{c2}
E	100:0	85.12 (0.14)^{a1}	84.03 (0.33)^{e2}	65.5 (4.3)^{d1}	53.8 (8.4)^{c2}
F	75:25	85.23 (0.27)^{a1}	85.5 (0.6)^{ab1}	12.3 (0.5)^{b1}	16.1 (1.6)^{b2}
G	50:50	85.6 (0.5)^{ab1}	85.9 (0.2)^{ab1}	40.34 (4.85)^{e1}	29 (8)^{d2}
H	0:100	86.03 (0.17)^{bc1}	86.69 (0.14)^{d2}	85.24 (1.84)^{f1}	60 (9)^{ac2}

a-f: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences due to storage time ($p < 0.05$).

The increase in transparency of HPMC films during storage suggests that a rearrangement of the polymer chains takes place during this time, since the film is not in a glassy state and molecular mobility permits the changes in the chain entanglements, leading to modifications in macroscopic film properties.

The obtained gloss values of newly prepared and stored films measured at an incidence angle of 60º are shown in Table 1. In all newly prepared films, the gloss values of pure HPMC or pure starch films were the highest, whereas composite films showed very low values coinciding with the roughness of the composite film surface, as observed by SEM (Figure 2), caused by phase separation. The rougher topography is directly related with the loss of gloss (Ward & Nussinovitch, 1996). In general, microfluidization produced an increase in gloss values, except in the case of pure starch films where a decrease was observed and composite films with a 50:50 ratio of polymers, where no significant changes were induced. For composite films, these results are coherent with what can be observed in surface SEM micrographs, where a high level of roughness could be deduced for the film surface. In the case of starch, microfluidization can promote hydrolysis and recrystallization which will affect the film surface topography. In this sense, Augustin, Sanguansri & Htoon (2008) observed that in the case of microfluidization of heated starch, there is an increase in the depolymerisation of the starch chain with increasing microfluidization pressure.

As far as ageing is concerned, the films generally lost gloss probably due to a molecular rearrangement which took place during storage at 25 ºC and which modifies film surface topography. Similar effects were observed by Jiménez *et al.* (2012) working with starch-fatty acid films.

3.3. Tensile properties

The mechanical properties of studied films were obtained at 25 ºC and 53 % RH. According to McHugh & Krochta (1994), elasticity modulus (EM), tensile strength (TS) and elongation at break (E) are useful parameters with which to describe the mechanical behaviour of films, and are closely related with its internal structure. The values of the tensile parameters are shown in Table 2. In newly prepared films, pure HPMC films showed the highest EM and TS values, while pure starch formulations and starch:HPMC blend films were less rigid and mechanically resistant. The presence of glycerol greatly reduced EM and TS values of HPMC films when these values are compared to those reported by Jiménez *et al*. (2010) for HPMC films prepared without glycerol, which must be attributed to the plasticizing effect and the subsequent increase in molecular mobility, providing more stretchable films (Arvanitoyannis, Nakayama & Aiba, 1998; Mali *et al*., 2006; Rodríguez, Osés, Ziani & Maté, 2006). This effect has also been reported in starch based films containing plasticizers (Talja *et al*., 2007). Microfluidization induced a decrease in EM values in almost all of the cases which could be related with the disaggregation/fragmentation of polymer chains due to the high pressure effects, as reported by Augustin, Sanguansri & Htoon (2008), which affect the network compactness and rigidity. With respect to TS values, no significant effect of microfluidization was observed.

The stretchability of films at initial time ranged between 5.7 and 10.4 % with no significant differences between formulations ($p > 0.05$). These values are in agreement with previous works in which films based on corn starch (Mali *et al*., 2006) or HPMC (Jiménez *et al*., 2010) were characterized. Microfluidization did not induce significant modification of the stretchability values.

Table 2 also shows mechanical parameters of stored films. The EM values were similar, or lower, in comparison with those obtained for newly prepared films, except for non-microfluidized pure starch films where a notable increase in this value was observed. This increase in the pure starch film rigidity was attributed to the recrystallization process occurring in the starch matrix, as reported by (Jimenez *et al*., 2012) on the basis of the X-ray data. The obtained X-ray data

Table 2. Tensile properties of both non-stored (initial) starch-HPMC based films and stored for 5 weeks (final). Samples A to D: non-microfluidized. Samples E to H: microfluidized. Mean values (standard deviation).

		EM (MPa)		**TS (MPa)**	
Sample	Starch:HPMC ratio	Initial	Final	Initial	Final
A	100:0	806 (74)^{a1}	1474 (156)^{a2}	9.12 (0.63)^{a1}	20 (2)^{a2}
B	75:25	743 (44)^{ab1}	620 (88)^{b2}	10.90 (0.33)^{ab1}	10.38 (0.35)^{b2}
C	50:50	670 (62)^{c1}	700 (28)^{c1}	13 (1)^{bc1}	13.5 (1.1)^{d1}
D	0:100	1312 (61)^{d1}	1043 (40)^{d2}	24.5 (4.5)^{d1}	23.26 (4.31)^{d1}
E	100:0	747 (33)^{ab1}	400 (56)^{e2}	9.53 (0.43)^{a1}	6.6 (0.4)^{e2}
F	75:25	593 (68)^{e1}	420 (25)^{e2}	8.58 (0.64)^{a1}	6.13 (0.34)^{e2}
G	50:50	683 (49)^{bc1}	761 (59)^{c2}	12.81 (0.25)^{b1}	11.5 (1.2)^{bc1}
H	0:100	1061 (51)^{f1}	1108 (32)^{d1}	15.8 (4.5)^{c1}	22.3 (4.3)^{ad2}

		E (%)	
Sample	Starch:HPMC ratio	Initial	Final
A	100:0	7.6 (4.4)^{abc1}	2.2 (0.2)^{a2}
B	75:25	7 (2)^{ab1}	11.1 (3.3)^{bc2}
C	50:50	9.4 (1.6)^{bc1}	12.5 (1.9)^{dc2}
D	0:100	10.4 (4.)^{c1}	18.52 (2.27)^{e2}
E	100:0	6.5 (2.4)^{ab1}	13.7 (2.3)^{d2}
F	75:25	5.7 (1.4)^{a1}	8.6 (2.6)^{bf2}
G	50:50	9.3 (2.8)^{bc1}	8.08 (1.44)^{f1}
H	0:100	8.43 (4.13)^{abc1}	18.71 (3.15)^{e2}

a-f: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences due to storage time ($p < 0.05$).

commented below confirms this hypothesis. On the contrary, a notable decrease of EM values of microfluidized pure starch film was observed, which could to indicate that no re-crystallization took place in the film throughout storage time. Microfludization promotes starch hydrolysis, thus limiting the rearrangement of amylose and amylopectin fractions in the matrix.

When HPMC was incorporated into starch films the increase in EM values, associated to starch re-crystallization, was not observed, despite the above mentioned phase separation, which suggests that HPMC addition was able to inhibit the starch re-crystallization during storage. In fact, HPMC addition was chosen as a possible mechanism to reduce the recrystallization of starch over time, thus reducing the brittleness of films after storage. As mentioned above, HPMC is characterized by its amorphous structure (Kou *et al*., 2011) and crystallization of the polymer during aging was not expected. Although phase separation was observed, HPMC would be present in both phases (at different concentrations) in a required amount to inhibit the crystal formation in the starch matrix.

For pure starch non microfluidized films the TS value increased, in agreement with the increase in the crystallinity developed over time, as shown in X-ray diffraction patterns (Figure 4), commented below. A small increase was also observed for microfluidized pure HPMC films which suggest a polymer rearrangement during storage, as was also deduced from the analysis of the film transparency. In the rest of the cases no changes or only a slight decrease (in microfluidized starch-rich films) was observed for TS values during storage.

Generally stretchability of pure starch non microfluidized films greatly decreased during storage in line with the formation of crystalline zones which limits displacements of macromolecules. Nevertheless, in the rest of the cases, stored films were more stretchable than newly prepared films, which indicates that HPMC addition or microfluidization inhibit re-crystallization phenomena, leading to less resistant but more flexible films throughout storage. So, mechanical properties of starch based films may be improved with the addition of a determined amount of HPMC or by microfluidization. To corroborate this result, crystallization of polymers in the films was characterized by means of X-ray diffraction.

Figure 4 shows the X-ray diffraction patterns for both newly prepared and stored films. In starch containing films main peaks located around 20º appeared. These are attributed to the starch crystalline domain and this was previously observed in tapioca starch-potassium sorbate films (Famá, Rojas, Goyanes y Gerschenson, 2005) tapioca starch-decolorized-tsao leaf gum films (Chen, Kuo & Lai, 2009) and corn starch-fatty acids films (Jiménez *et al*., 2012). The intensity of the peaks increased with storage time in non-microfluidized pure starch and 50:50 polymer ratio films, but did not change in the rest of the cases, thus indicating that re-crystallization during storage was inhibited. Nevertheless crystallization during film formation also occurred in microfluidized pure starch and 50:50 polymer ratio films. Pure HPMC films showed a totally amorphous pattern in agreement with previous observations obtained by the same technique (Kou *et al*., 2011). These results confirm that an increase of HPMC in starch matrix or microfluidization of the film forming solutions provoke an inhibition of starch re-crystallization during storage. A 50:50 polymer ratio also allows us to inhibit starch crystallization during film formation in both microfluidized and non-microfluidized films. This behaviour coincides with that commented on above, deduced from the mechanical behaviour of the films.

3.3. Barrier properties

The ability of films or packaging to limit the transfer of environmental agents (water vapour, oxygen, CO_2) has been studied (Arvanitoyannis & Biliaderis, 1998; García, Martino & Zaritzky, 2000; Karbowiak, Debeaufort & Voilley, 2007; Mali *et al*., 2006; Navarro-Tarazaga, Massa, & Pérez-Gago, 2011; Souza *et al*., 2009) and reviewed by many researchers (Koelsch, 1994; Miller & Krochta, 1997).

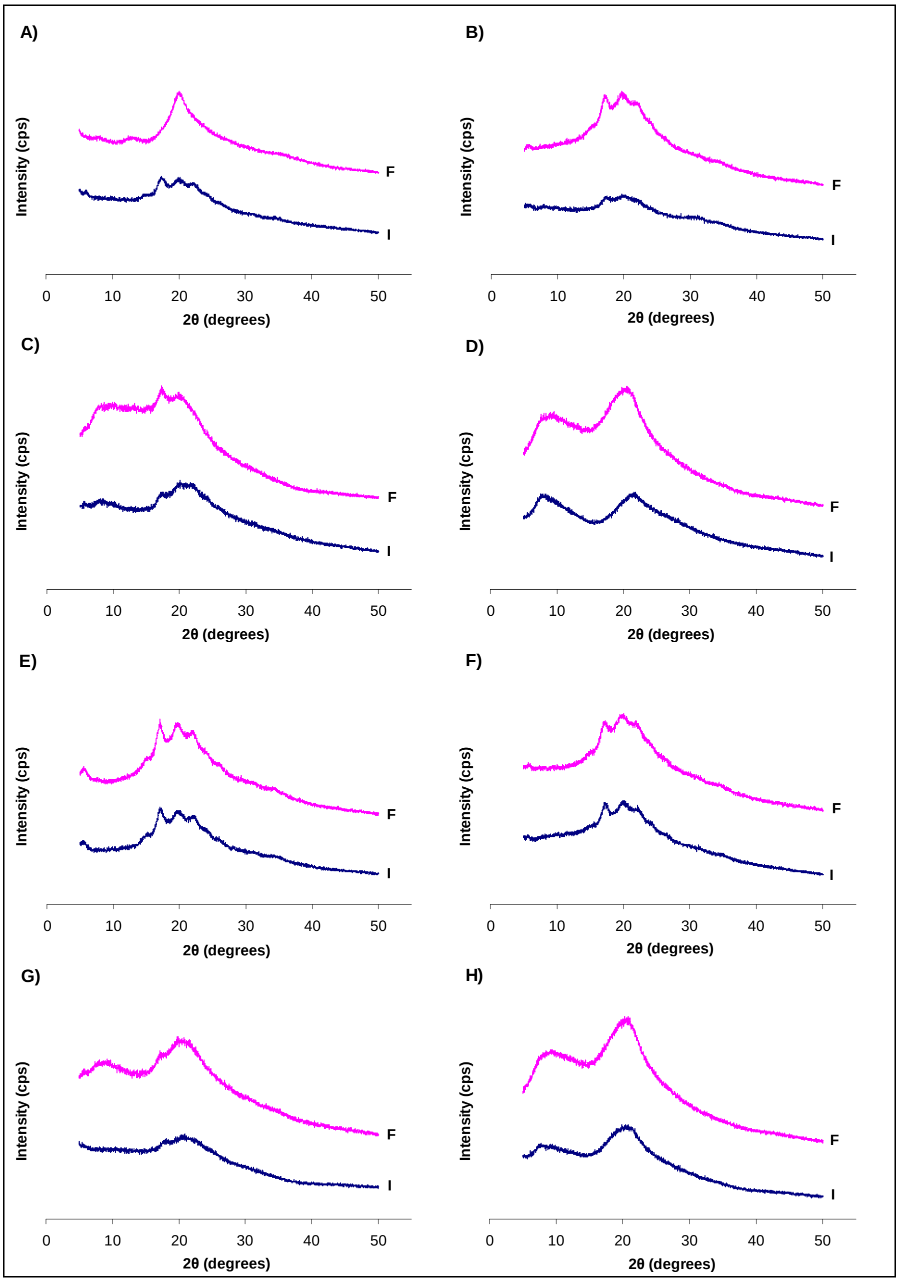

Figure 4. X-ray diffraction patterns of newly prepared (I curve) and stored films (F curve). A to D correspond to non-microfluidized films, E to H

correspond to microfluidized films. A and E: 100:0 starch:HPMC ratio, B and F: 75:25 starch:HPMC ratio, C and G: 50:50 starch:HPMC ratio and D and H: 0:100 starch:HPMC ratio.

Packaging has to be able to avoid moisture and gas exchange between the environment and the product. Both water vapour and oxygen barrier were characterized in the films, both newly-prepared and conditioned and after 5 storage weeks. WVP and OP values are shown in Table 3. WVP values ranged between 5.21 and 10.3 $g·mm·kPa^{-1}·h^{-1}·m^{-2}$ considering both initial and final time. These results agree with those from previous studies in which starch films were obtained. For instance, Pushpadass, Marx, & Hanna (2008) obtained WVP values between 10.9 and 12.9 $g·mm·kPa^{-1}·h^{-1}·m^{-2}$ in starch films containing stearic acid and Jiménez *et al*. (2012) between 6.71 and 9.14 $g·mm·kPa^{-1}·h^{-1}·m^{-2}$ in starch-fatty acid films.

The obtained values for newly prepared non-microfluidized films did not show a clear tendency with the HPMC content since both pure starch and HPMC showed very similar values, in agreement with their very similar hydrophilic character. Microfluidization slightly decreases WVP, which is coherent with the structural effects commented on above (chain disaggregation/fragmentation and formation of a less compact network).

Concerning to the effect of storage time, WVP values generally did not change, or only slightly increased which can be due to the polymer rearrangements commented on above, associated to matrix ageing. For pure starch microfluidized films WVP decreased significantly ($p < 0.05$) over storage time, as reported by Mali *et al*. (2006) for corn, cassava and yam starch. This decrease in WVP values of starch stored films could be explained by a progressive hydrolysis of the starch which led to the formation of a less compact network where small molecule permeation can occur more easily. Hydrolysis of starch is promoted by the effect of

Table 3. Water vapour and oxygen permeability of both non-stored (initial) starch-HPMC based films and stored films for 5 weeks (final). Samples A to D: non-microfluidized. Samples E to H: microfluidized. Mean values (standard deviation).

		WVP (g· mm· kPa^{-1}· h^{-1}·m^{-2})	
Sample	Starch:HPMC ratio	Initial	Final
A	100:0	7.9 (0.2)^{a1}	7.7 (0.9)^{ab1}
B	75:25	7.13 (0.36)^{b1}	8.6 (0.3)^{a2}
C	50:50	8.5 (0.4)^{a1}	7.6 (0.4)^{ab1}
D	0:100	8.32 (0.43)^{a1}	8.5 (0.5)^{a1}
E	100:0	6.24 (0.43)^{c1}	5.2 (0.3)^{c2}
F	75:25	7.16 (0.64)^{b1}	10.3 (1.0)^{d2}
G	50:50	5.95 (0.28)^{c1}	7.9 (0.8)^{ab2}
H	0:100	6.95 (0.23)^{b1}	7.3 (0.6)^{b1}

		O_2 perm. · 10^{14} (cm^3·m^{-1}·s^{-1}·Pa^{-1})	
Sample	Starch:HPMC ratio	Initial	Final
A	100:0	4 (4)^{ab1}	4.2 (0.4)^{a1}
B	75:25	10.67 (0.06)^{b1}	17.7 (0.3)^{a2}
C	50:50	27.5 (0.5)^{c1}	31.1 (2.5)^{a1}
D	0:100	285 (4)^{d1}	740 (88)^{b2}
E	100:0	2.22 (0.05)^{a1}	4.15 (0.18)^{a2}
F	75:25	7.9 (1.5)^{ab1}	17.4 (0.8)^{a2}
G	50:50	8 (1)^{ab1}	21 (2)^{a2}
H	0:100	566 (7)^{e1}	573 (40)^{c1}

a-e: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences due to storage time ($p < 0.05$).

shear stress forces (Carvalho, 2008; Myllymäki, Eerikäinen, Suortti, Forssell, Linko & Poutanen, 1997), such as those applied in microfluidization. Likewise, some authors (Courgneau, Domenek, Guinault, Avérous & Ducruet, 2011) reported that polyols (such as glycerol) as long as they plasticize the biopolymer matrix, they favour crystallization because of polymer mobility increase and, in some biopolymer matrices such as PLA, also induce chain length reduction (measured by size exclusion chromatography) in parallel to crystallization. The reduction of the polymer chain length will induce a lower permeability.

In order to evaluate the ability of films to limit oxygen transfer, the oxygen permeability (OP) was analyzed at 53 % HR and 25 ºC. Obtained values for OP are shown in Table 3 as mentioned above. Starch films have better oxygen barrier properties than HPMC films, so oxygen permeability greatly increased when HPMC ratio increased in the films. In newly prepared films, microfluidization gave rise to an increase in oxygen permeability of pure HPMC films and a decrease in the 50:50 polymer ratio films, but no changes in other cases. The obtained values for films containing were lower in comparison with those reported for sodium caseinate-starch films (Arvanitoyannis & Biliaderis, 1998) and for corn starch:cellulose:lignin composites (Wu, Wang, Li, Li, Wang, 2009).

The effect of storage time on oxygen permeability depended on the film composition and the homogenization process. In most of the cases an increase in the OP values was observed, mainly in microfluidized composite films and in non-microfluidized pure HPMC films. This must be explained by the microstructural rearrangement of the polymer chains during storage.

4. CONCLUSIONS

The addition of HPMC into the corn starch matrix gave rise to a more amorphous structure as was observed by X-ray diffraction. However, SEM micrographs revealed a polymer phase separation which provoked a loss of gloss in the films. Elastic modulus of newly prepared composite films decreased as compared to pure starch and HPMC films although tensile strength and deformation at break are slightly improved with respect to pure starch film. Composite films showed similar WVP to pure starch films but slightly higher oxygen permeability due to the contribution of the HPMC which showed high OP values. Microfluidization did not allow the homogenous mixture of both polymers, which showed phase separation in the films, but contributed to the inhibition of starch re-crystallization during storage, giving rise to slightly less rigid films with similar resistance to break and stretchability. Inhibition of the starch re-crystallization during film formation and storage was also reached in both microfluidized and non-microfluidized films when HPMC was incorporated to starch films in a 50:50 ratio.

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EFFECT OF SODIUM CASEINATE ON PROPERTIES AND AGEING BEHAVIOUR OF CORN STARCH BASED FILMS

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ABSTRACT

The effect of sodium caseinate addition on corn starch films was studied in terms of microstructural, mechanical, optical and water and oxygen barrier properties and the changes they underwent during their 5 weeks' storage under controlled conditions. The influence of the polymer mixture on the degree of crystallization of the films was also analysed. The following starch: protein ratios were considered. 100:0, 75:50, 50:50 and 0:100. SEM Microstructure analysis revealed the compatibility of both hydrocolloids since no phase separation was observed. The addition of sodium caseinate to the starch films provided films that were less stiff and resistant to fracture but more flexible and deformable than pure starch films, with similar water vapour permeability values. The films became more permeable to oxygen as their sodium caseinate content increased in line with the higher permeability values of the protein film. Incorporating sodium caseinate to starch films provoked a slight increase in transparency, but a loss of gloss, which also decreased in composite films during storage. The re-arrangement of polymer chains during storage caused a loss of mechanical resistance, stretchability and gloss in composite films.

Keywords: Starch, storage, re-crystallization, films, physical properties.

1. INTRODUCTION

In recent years, more and more research has been carried out into the substitution of petro-based plastic materials for biodegradable ones in order to solve the problems generated by plastic waste. So, there is increasing interest in biodegradable films made from renewable and natural materials, such as starch. Corn starch edible films exhibit appropriate physical properties since these films are isotropic, odourless, tasteless, non-toxic and biodegradable (Krochta, Baldwin & Nisperos-Carriedo, 1994). However, starch films have several drawbacks, such as brittleness, due to starch crystallization, and poor mechanical properties, which limit their uses or applications. The main method for reducing the brittleness of starch films is to add plasticizers which are able to reduce the intermolecular forces by increasing the chain mobility and improving the flexibility and extensibility of the films (Parra, Tadini, Ponce & Lugão, 2004). Another approach to overcome this aspect is to blend starch with other polymers or additives (Walia, Lawton & Shogren, 2000; Wang, Yang & Wang, 2004; Bourtoom & Chinnan, 2008, Ghanmbarzadeh, Almasi, & Entezami, 2010). Otherwise, the application of hydrophilic films, such as starch-based films, is limited by the poor water vapour barrier properties and water solubility of these films. In a first approach to solve the hygroscopicity and crystallization problems of starch based films, Jiménez, Fabra, Talens & Chiralt (2012) studied the effect of adding fatty acids (saturated and unsaturated) on the physico-chemical properties, and their changes during storage, in starch films. Nevertheless, they found that fatty acid addition did not notably improve the water vapour permeability of the films and, in all cases, the degree of starch crystallization increased during storage time, thus increasing the film’s brittleness and decreasing its stretchability and transparency. So, it was concluded that other biodegradable materials must be tested to improve the functional properties of starch films and their ageing problems.

The capacity of sodium caseinate to form films with appropriate properties is well-known (Fabra, Talens, Chiralt, 2008ab, 2010; Audic & Chaufer, 2005; Chen, 2002) and some works have revealed the improvement of the film properties when sodium caseinate is incorporated into other polymer matrices (Pereda, Amica, Rácz & Marcovich, 2011; Monedero, Fabra, Talens & Chiralt, 2010; Arvanitoyannis & Biliaderis, 1998). Nevertheless, only a few studies were found into the effect of sodium caseinate on the physico-chemical properties of corn starch films.

In this work, the effect of adding sodium caseinate on the microstructural, mechanical, optical and oxygen and water vapour barrier properties of corn starch matrices was analyzed for films in different storage conditions (non-stored and stored films for five weeks). X-ray diffraction was studied to evaluate the crystallization of film components in stored and non-stored films.

2. MATERIALS AND METHODS

2.1. Materials

Corn starch was purchased from Roquette (Roquette Laisa España, Benifaió, Spain) and sodium caseinate (NaCas) was supplied by Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). Glycerol, chosen as plasticizer, was provided by Panreac Química, S.A. (Castellar Del Vallés, Barcelona, Spain).

2.2. Preparation and characterization of films

Four different dispersions were obtained using corn starch, sodium caseinate and glycerol as plasticizer. Corn starch was dispersed in cool water to obtain 2 % (w/w) polysaccharide suspensions. Then, these dispersions were maintained at 95 ºC for 30 min under continuous stirring to promote starch gelatinization. Sodium caseinate was dissolved directly in cool distilled water (2 % w/w). Afterwards, both hydrocolloids were mixed to obtain four dispersions with starch:protein ratios of

1:0, 0.75:0.25, 0.5:0.5 and 0:1. Once the mixes were prepared, a controlled amount of glycerol was added (ratio hydrocolloid(es) : glycerol was 1:0.25). Afterwards, dispersions were homogenized for 1 min at 13,500 rpm and for 5 min at 20,500 rpm at 95 ºC, under vacuum, using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany). Film-forming dispersions of hydrocolloids and glycerol, containing 1.5 g of total solids, were gently spread over a Teflon plate (150 mm diameter) resting on a leveled surface. The dispersions were allowed to dry for approximately 48 hours at 45 % relative humidity (RH) and 20 ºC. Under these conditions, flawless films were peeled intact from the casting surface.

2.2.1. Film conditioning and storage

Before the tests, all the samples were conditioned in a desiccator at 25 ºC and 53 % RH, by using magnesium nitrate-6-hydrate saturated solutions (Panreac Quimica, SA, Castellar del Vallés, Barcelona) for one week when the first series of analyses was carried out. One part of the samples was stored under the same conditions for five weeks in order to perform the second series of analyses of stored films.

Film thickness was measured with a Palmer digital micrometer to the nearest 0.0025 mm at 6 random positions, previous to analysis

2.2.2. Tensile Properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, according to ASTM standard method D882 (ASTM, 2001) EM, TS, and E were determined from the stress-strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 10 cm long). Equilibrated samples were mounted in the film-extension grips of the testing machine and stretched at 50 mm min^{-1} until breaking.

The relative humidity of the environment was held constant at 53 (±2) % during the tests, which were performed at 25 (±1) °C. At least six replicates were obtained from each sample variation.

2.2.2. X-ray diffraction

X-ray diffraction patterns were recorded using a Rigaku Ultima IV multipurpose X-ray diffraction system (Rigaku Corporation, Tokyo, Japan). All the samples were analyzed between $2\theta = 5^{\circ}$ and $2\theta = 50^{\circ}$ using Kα Cu radiation ($\lambda = 1.542$ Å), 40 kV and 40 mA with a step size of 0.02 º. For this analysis, samples were cut into 2 cm squares, prior to storage, in order to avoid breakage during handling. Relative humidity and temperature during analyses were 53 % and 25 ºC, respectively.

2.2.3. Barrier properties

2.2.3.1. Water Vapour Permeability (WVP)

WVP was measured in film discs (ϕ=3.5 cm) according to a modification of the ASTM E96-95 (1995) gravimetric method (McHugh, Avena-Bustillos & Krochta, 1993), using Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle /s Argenteau, Belgium). Distilled water was placed inside the cup to expose the film to 100 % RH on one side. Once the films were secured, each cup was placed in a relative humidity equilibrated cabinet at 25 ºC, with a fan placed on the top of the cup in order to reduce resistance to water vapour transport. The RH of the cabinets (53 %) was held constant using oversaturated solutions of magnesium nitrate-6-hydrate (Panreac Quimica, SA, Castellar del Vallés, Barcelona). The free film surface during film formation was exposed to the highest RH value. The cups were weighed periodically (± 0.0001 g). WVP was determined from the slope obtained from the regression analysis of weight loss data *vs*. time, once the steady state was reached. The method proposed by McHugh *et al*. (1993)

to correct the effect established by concentration gradients in the stagnant air gap inside the cup was used.

2.2.3.2. Oxygen permeability (PO$_2$)

The oxygen permeation rate of the sodium caseinate films was determined at 53 % RH and 25ºC using an OX-TRAN Model 2/21 ML Mocon (Lippke, Neuwied, Germany). Film samples were previously conditioned at the relative humidity level of the test in a desiccator using an over-saturated $Mg(NO_3)_2$ solution. Two samples were placed in the equipment for analysis and they were conditioned in the cells for 6h, then the transmission values were determined every 20 min until the equilibrium was reached. The exposure area during the tests was 50 cm^2 for every formulation. In order to obtain the oxygen permeability, film thickness was considered in each case.

2.2.4. Scanning Electron Microscopy (SEM)

The microstructural analysis of the films was carried out by SEM using a scanning electron microscope (JEOL JSM-5410, Japan). Film samples were maintained in a desiccator with P_2O_5 for two weeks to ensure that no water was present in the sample. Then, films were frozen in liquid N_2 and cryofractured to observe the cross-section of the samples. Films were fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

2.2.5. Optical Properties

The film transparency was determined by applying the Kubelka-Munk theory (Hutchings, 1999) for multiple scattering to the reflection spectra. The surface reflectance spectra of the films were determined from 400 to 700 nm with a spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) on both a white and a black background. As the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (*K*) and the scattering (*S*) coefficients. Internal transmittance (T_i) of the films was quantified using eq 1. In this equation, R_0 is the reflectance of the film on an ideal black background. Parameters *a* and *b* were calculated by eqs 2 and 3, where *R* is the reflectance of the sample layer backed by a known reflectance R_g. Measurements were taken in triplicate for each sample on the free film surface during its drying.

$$T_i = \sqrt{(a - R_0)^2 - b^2}$$ Equation 1

$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right)$$ Equation 2

$$b = (a^2 - 1)^{1/2}$$ Equation 3

The gloss was measured on the free film surface during its drying, at 60º incidence angles, according to the ASTM standard D523 method, (ASTM, 1999), using a flat surface gloss meter (Multi Gloss 268, Minolta, Germany). Measurements were taken in triplicate for each sample and three films of each formulation were considered. All the results were expressed as gloss units, relative to a highly polished surface of black glass standard with a value near to 100.

2.3. Statistical Analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used for carrying out statistical analyses of data through analysis of variance (ANOVA). Fisher′s least significant difference (LSD) was used at the 95% confidence level.

3. RESULTS

3.1. Microstructural properties

The compatibility and miscibility of both hydrocolloids (starch and sodium caseinate) can be evaluated qualitatively through the microstructural analysis. SEM images give information about how the film's components are arranged in the dried films, which has an impact on film properties. Depending on the nature of the hydrocolloids and the interactions developed in the film forming dispersions and during film-drying, the components arrange themselves in different ways in the film matrix (Fabra, Talens & Chiralt, 2009a, Jiménez *et al*., 2012).

Figure 1 shows the cross-section images of films prepared with different starch:sodium caseinate ratios.

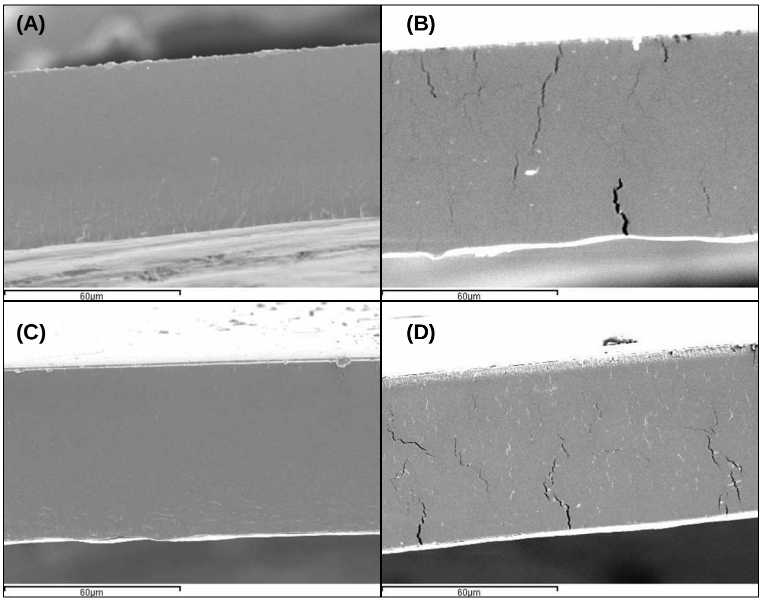

Figure 1. SEM micrographs of a cross-section of starch (A), 75:25 starch:sodium caseinate (B), 50:50 starch:sodium caseinate (C) and pure sodium caseinate (D) non-stored films.

Pure starch films (Figure 1A) and composite films with 50:50 polymer ratio (Figure 1C) show a continuous and smooth aspect, while several micro-cracks can be observed in the cross-section image of pure sodium caseinate (Figure 1D) and 75:25 polymer ratio films (Figure 1B). This indicates the formation of a more fragile structure in these two later cases. Both hydrocolloids are highly compatible as deduced from the SEM micrographs since no different phases are observed. So, we can postulate that interactions among film components (sodium caseinate, starch and glycerol) favoured the integration of hydrocolloids in a homogenous matrix. However, on a microstructural level, films containing pure sodium caseinate or those with a starch:caseinate ratio of 75:25 showed fragility probably due either to the differences in the distribution of glycerol molecules which does not allow it to perform an adequate plasticizer role or to not a high enough amount of plasticizer in these cases. In fact, Fabra *et al*., 2008a reported that sodium caseinate films required a minimum of 30% glycerol to show adequate mechanical properties and only 25% was used in this case, on the basis of the amount required for corn starch films (Jiménez *et al*., 2012).

In Figure 1B small white spots can be also observed which can be attributed to small particles produced during the film fracture prior observation which deposit on the cross section of the film. Small white enlarged formations are also observed in Figure 1D for pure caseinate film. Nevertheless, these seem the incipient crack formation in the insufficiently plasticized film. Probably did not occur in more wet films where water exerts a plasticizing role, thus diminishing the micro-fractures produced by the electron impact during observation.

3.3. Tensile properties

Table 1 shows tensile properties (EM: elastic modulus, TS: tensile strength and E (%): elongation at break) of studied films equilibrated at 25 ºC and 53% HR.

TS represents film resistance to elongation at break, EM the stiffness of the material and ε is a measure of the film's capacity for stretching.

Table 1. Tensile properties of starch-NaCas based films, both non-stored (initial) and stored for 5 weeks (final). Mean values (standard deviation).

	EM (MPa)		**TS (MPa)**	
Film	Initial	Final	Initial	Final
Starch:NaCas 100:0	806 (74)^{a1}	1474 (156)^{a2}	9.12 (0.63)^{a1}	20 (2)^{a2}
Starch:NaCas 75:25	442 (53)^{b1}	276 (65)^{bc2}	6.9 (0.4)^{b1}	5.03 (0.63)^{b2}
Starch:NaCas 50:50	376 (42)^{b1}	221 (32)^{b2}	6 (1)^{b1}	4.8 (0.5)^{b2}
Starch:NaCas 0:100	643 (109)^{c1}	329 (29)^{c2}	10.4 (1.5)^{c1}	6.5 (0.3)^{c2}

	E (%)	
Film	Initial	Final
Starch:NaCas 100:0	7.6 (4.4)^{a1}	2.16 (0.23)^{a2}
Starch:NaCas 75:25	16.7 (0.5)^{bc1}	8 (3)^{b2}
Starch:NaCas 50:50	19.14 (3.36)^{b1}	10.9 (1.6)^{b2}
Starch:NaCas 0:100	12.9 (1.8)^{c1}	23.7 (4.1)^{c2}

a-c: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences among formulations ($p < 0.05$).

Films prepared with only one of the hydrocolloids showed similar mechanical properties, although starch films were stiffer and more fragile than sodium caseinate films. Sodium caseinate addition to the starch matrix provoked a decrease in the elastic modulus and tensile strength and an increase in the elongation at break, in non-stored films. So, composite films prepared with mixtures of both hydrocolloids were less rigid (lower EM) and resistant to fracture (lower TS) and more flexible and stretchable (greater E) than films prepared with pure components. This indicates that, in composite films, attraction forces and bonds between polymer chains are less intense compared to the case when chains correspond to the same macromolecule. So, a decrease in the cohesive strength of the films occurs, thus increasing their stretchability and decreasing their hardness.

These effects were more marked when the polymer ratio was 50:50, when films showed the greatest stretchability and the lowest stiffness.

In agreement with the results found, the addition of lambda-carragenaan to sodium caseinate films provided more flexible and stretchable films than those made of pure sodium caseinate (Fabra *et al*, 2008b) Likewise, whey protein, soy protein and caseinate films combined with alginate, pectin and/or carboxymethylcellulose respectively and also showed greater flexibility than pure hydrocolloid films (Parris, Coffin, Joubran, & Pessen, 1995; Sabato, Ouattara, Yu, D'Aprano, Le Tien, & Mateescu, 2001; Yu, Sabato, D'Aprano, & Lacroix, 2004). Chen & Lai (2008) also observed that the mechanical properties of tapioca starch are strongly improved by the addition of leaf gum. However, Phan The, Debeaufort, Voilley & Luu (2009) reported that mechanical properties could become undesirable when the additive or the added hydrocolloid leads to a dispersion or to a two-phase separated film structure. This was observed when alginate was added to the sodium caseinate matrix in which the thermodynamic incompatibility of sodium caseinate-alginate systems usually leads to the separation of two phases, resulting in films that are less flexible and stretchable (Fabra *et al*., 2008b, Simeone, Alfani & Guido, 2004).

Ageing the films for five weeks induced changes in the starch film's tensile properties. Whereas TS and EM significantly increased in pure starch films, they decreased in films containing sodium caseinate at any ratio (75:25, 50:50 and 0:100, S:NaCas ratios). As previously reported (Jiménez *et al*., 2012), starch films tend to crystallize during ageing which gives rise to an increase in the film's stiffness. However, this seems not to occur, at least to the same extent, when sodium caseinate is added to the film matrix, probably due to the fact that the presence of sodium caseinate chains in the matrix makes the reorganization of the starch molecules difficult, avoiding the development of stiffness in the films. The elongation at break always decreased with ageing, except in films prepared with pure sodium caseinate, which could be, in part, attributed to glycerol migration and separation. This phenomenon, which reduces the effectiveness of the plasticizer and contributes to the modification of the film properties, has previously observed been with glycerol (Godbillot, Dole, Joly, Rogé & Mathlouthi, 2006; Rodríguez, Osés, Ziani & Maté, 2006; Talja, Helén, Roos & Jouppila, 2008), xylitol and sorbitol (Talja, Helén, Roos & Jouppila, 2007). In pure starch films, the re-crystallization process will also contribute to reducing film stretchability at the same time as the film's stiffness and hardness increase. In pure sodium caseinate films, EM and TS greatly decreased during storage whereas deformation capacity increased. This seems to indicate that the polymer chain attraction forces were reduced in line with the polymer matrix reorganization during storage. In this case, no phase separation of glycerol seems to occur and interactions of the macromolecules with the polyalcohol molecules could be promoted, thus increasing the chain mobility and giving rise to a more open matrix. The great water retention capacity of glycerol molecules in the matrix can also contribute to the mentioned effects.

In order to analyze the degree of crystallinity in the non-stored and stored films and to understand their mechanical behaviour, the X-ray diffraction patterns of different samples were characterized. Figure 2 shows the X-ray diffraction spectra of the non-stored and stored films. While pure starch films (Figure 2A) show a crystalline pattern, pure sodium caseinate films (Figure 2D) are completely amorphous. The crystalline region of starch is located in the range of 20º, as previously reported (Famá, Rojas, Goyanes & Gerschenson, 2005; Chen, Kuo & Lai, 2009, Jiménez *et al*., 2012). X-ray diffraction spectra of films prepared with mixtures of both hydrocolloids showed crystalline and amorphous zones in which crystalline peaks were more evident as the starch ratio increased in the film. In this sense, these were barely appreciable in 50:50 polymer ratio films, either in non-stored or stored films.

The most important changes in the crystalline zones brought about the ageing effect were found in pure starch films, which contribute to strengthening the films. However, in films containing sodium caseinate no significant differences were observed between X-Ray diffraction patterns of stored and non-stored films, due to the fact that the rearrangement of starch chains during crystallization is more difficult in the presence of another polymer which interacts with them. An indirect influence of different effects such as changes in the availability of free water molecules, associated to the distribution of sodium ions in the matrix, could also contribute to the inhibition of the starch re-crystallization.

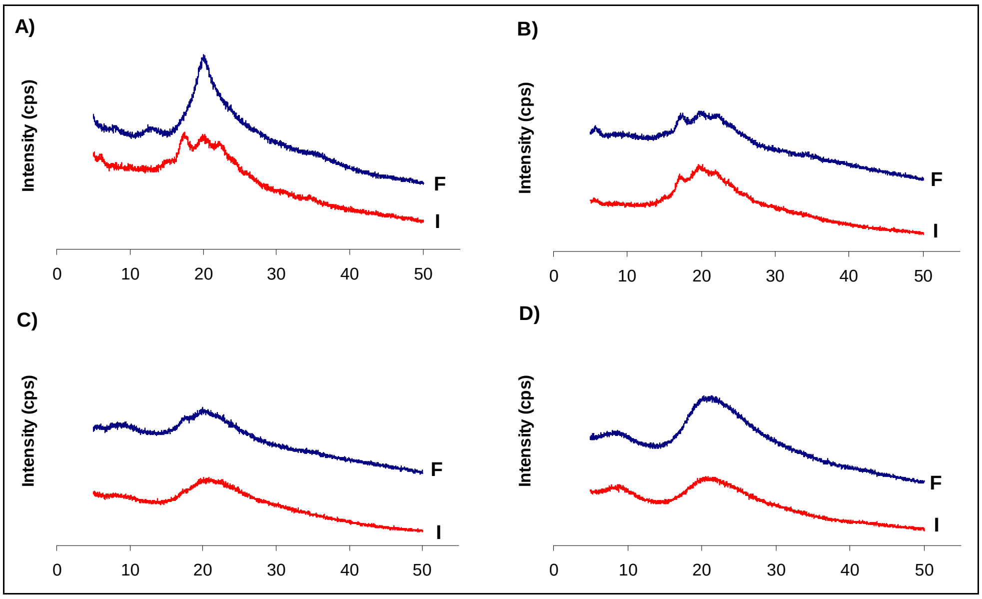

Figure 2. X-Ray diffraction spectra of starch (A), 75:25 starch:sodium caseinate (B), 50:50 starch:sodium caseinate (C) and pure sodium caseinate (D) stored and non-stored films. X-axis: 2θ (º). I: initial time; F: final time.

From these results, it is possible to conclude that sodium caseinate inhibited starch re-crystallization during film storage and hindered starch crystallization during film formation when it is present in the film in the same ratio as starch.

3.4. Barrier properties

3.4.1. Water vapour permeability

Table 2 shows water vapour permeability values of non-stored and stored films analyzed at 25 ºC and 53-100% RH gradient.

Table 2. Water vapour and oxygen permeability of starch-NaCas based films, both non-stored (initial) and stored for 5 weeks (final). Mean values (standard deviation).

	O_2 perm. · 10^{14} ($cm^3 \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$)	
Film	Initial	Final
Starch:NaCas 100:0	4 (1)^{a1}	4.22 (0.39)^{a1}
Starch:NaCas 75:25	7.36 (0.03)^{b1}	11.8 (0.5)^{b2}
Starch:NaCas 50:50	12.5 (0.4)^{c1}	14.1 (0.7)^{c1}
Starch:NaCas 0:100	18.23 (0.18)^{d1}	19.6 (0.2)^{d2}
	WVP (g· mm· kPa^{-1}· $h^{-1} \cdot m^{-2}$)	
Film	Initial	Final
Starch:NaCas 100:0	7.9 (0.2)^{a1}	7.72 (0.97)^{a1}
Starch:NaCas 75:25	7.65 (0.25)^{a1}	7.65 (0.24)^{a1}
Starch:NaCas 50:50	9.05 (0.18)^{b1}	7.32 (0.53)^{a2}
Starch:NaCas 0:100	7.8 (0.4)^{a1}	7.52 (0.36)^{a1}

a-d: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences among formulations ($p < 0.05$).

No significant differences were observed between the WVP values of the films, except in films prepared with a 50:50 S:NaCas ratio which showed the highest permeability values. Although some authors claim that protein based films have more interesting mechanical and barrier properties than polysaccharides (Cuq, Aymard, Cuq & Guilbert, 1995; Ou, Kwok & Kang, 2004; Cao, Fu & He, 2007), no differences were found for starch and sodium caseinate films containing the same ratio of glycerol. Proteins have a specific structure which confers a wide range of functional properties, especially high inter and intra-molecular binding potential. Polysaccharide-based films are more readily disintegrated by absorbing water and, in general, have poorer barrier properties than protein based films. In starch films, the more complex structure formed by the amylose and amylopectin could contribute to decreasing WVP. In fact, although comparisons are difficult because each author used different test conditions (temperature and relative humidity gradient), starch films appear to have lower WVP than other polysaccharides, such as HPMC (Jiménez, Fabra, Talens & Chiralt, 2010), iota-carrageenan (Hambleton, Debeaufort, Beney, Karbowiak & Voilley, 2008; Fabra, Hambleton, Talens, Debeaufort, Chiralt & Voilley, 2009b) or agar (Phan The *et al*., 2009) and similar values than that reported for sodium caseinate (Fabra *et al*, 2010) or soy protein (Monedero *et al*., 2010).

The higher WVP values observed for films with 50:50 starch:sodium caseinate ratio indicate that a more open matrix structure was formed for this formulation where the transport of water molecules was favoured. The coexistence at the same ratio of starch and caseinate polymer could imply an increase of the interchain space of the hydrocolloids (high-molecular-weight water-soluble macromolecules) with wider hydration layers where glycerol molecules interact, and where permeation of water molecules can occur more efficiently. Nevertheless, during the ageing of these films, their capacity for water transport decreased, diminishing the WVP values, which seems to indicate that a progressive rearrangement of the polymer chains occurs, promoted by the attraction forces (Van der Waals interactions and possible hydrogen bonds between amino groups of protein and hydroxyl groups of starch), giving rise to a more compact structure, with smaller inter-chain spaces, in which the transport of water molecules is more difficult. The high water binding capacity of glycerol could contribute to progressively reduce the hydrocolloid hydration layer, thus enhancing the action of the interchain attractive forces.

Ageing of the other films for five weeks did not induce changes in the water vapour barrier properties, which suggest that no relevant modification in the polymer chain arrangement in the matrix occurred in these cases.

3.4.2. Oxygen permeability

Oxygen permeability values of non-stored and stored films are given in Table 2. The oxygen permeability of starch films was more than four times lower than that of the sodium caseinate films. So, the incorporation of sodium caseinate into starch matrices provoked an increase in the oxygen permeability of the films; the greater the ratio, the higher the increase. The effect of storage on the oxygen permeability of films was not very appreciable and only a slight increase was observed for pure sodium caseinate films and those containing a 75:25 polymer ratio.

The obtained values are lower than those reported by Arvanitoyannis & Biliaderis (1998) for soluble starch-sodium caseinate films and by García, Martino & Zaritzky (2000) for corn starch containing a higher ratio of plasticizer.

So, whereas the addition of sodium caseinate to corn starch matrices did not modify water vapour permeability, it slightly increased oxygen permeability, proportionally to the ratio of incorporated caseinate.

3.5. Optical properties (transparency and gloss)

The internal transmittance (Ti) spectra of the films were obtained and the value at 450 nm was selected to compare samples because the greatest differences between the different films were found within this wavelength range. Table 3 shows the Ti values together with gloss values at 60 º. According to the Kubelka-Munk theory, high values of Ti are associated with greater film homogeneity and transparency. The highest Ti values were found in pure starch films, whereas the addition of sodium caseinate only slightly reduced internal transmittance, which reached its lowest value in films prepared with pure sodium caseinate. Nevertheless, differences were very small and all the films can be considered as highly transparent. Moreover, film transparency did not change significantly during storage time, except the film with a 50:50 polymer ratio where a slight decrease was observed. This could be attributed to the increase in the matrix compactness deduced from the changes in the WVP and stretchability values.

Table 3. Optical properties of starch-NaCas based films, both non-stored (initial) and stored for 5 weeks (final). Mean values (standard deviation).

	T_i (450 nm)	
Film	Initial	Final
Starch:NaCas 100:0	85.54 (0.36)^{a1}	85.21 (0.09)^{a1}
Starch:NaCas 75:25	84.4 (0.1)^{b1}	84.0 (0.8)^{b1}
Starch:NaCas 50:50	84.7 (0.3)^{b1}	83.11 (0.17)^{b2}
Starch:NaCas 0:100	83.47 (0.11)^{c1}	83.01 (0.46)^{b1}
	Gloss 60º	
Film	Initial	Final
Starch:NaCas 100:0	71 (5)^{ab1}	67 (9)^{a1}
Starch:NaCas 75:25	68.2 (5.6)^{b1}	55 (11)^{a2}
Starch:NaCas 50:50	76.3 (7.5)^{a1}	44 (11)^{b2}
Starch:NaCas 0:100	86.6 (6.6)^{c1}	84.45 (3.11)^{1}

a-c: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences among formulations ($p < 0.05$).

The gloss of non-stored films was greatly affected by film composition. Pure sodium caseinate films were glossier than those of pure starch, probably due to the film's structure after drying. Starch films tend to crystallize during film formation and the crystals near the film surface could reduce its smoothness, decreasing the gloss. Nevertheless, despite the fact that sodium caseinate was observed to inhibit crystallization, composite films showed lower gloss values. This indicates that, at surface level, the packaging of the different polymer in composite films promotes a rougher surface, thus decreasing gloss. After 5 storage weeks, only composite films showed a decrease of gloss, in agreement with the changes in surface topography which occurred due to the progressive polymer re-arrangement commented on above. It is remarkable that this did not affect the film transparency to the same extent.

4. CONCLUSIONS

The addition of sodium caseinate to starch matrices reduced the degree of crystallinity of starch films and inhibited starch re-crystallization during film storage. Mixtures of both hydrocolloids provide films which are less stiff and resistant to fracture but more flexible and deformable than pure starch films, with similar water vapour permeability values. As the sodium caseinate content in the film increased, so did oxygen permeability, in line with the higher permeability values of the protein film. The incorporation of sodium caseinate to starch films provoked a slight increase in transparency, but a loss of gloss, which also decreased during storage in composite films. The re-arrangement of polymer chains during storage caused a loss of mechanical resistance, stretchability and gloss in composite films. In general, films prepared using mixtures of both hydrocolloids showed better potential applications than pure starch films, mainly due to the inhibition of starch crystallization which implies undesirable changes in the film's mechanical behaviour.

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Capítulo III

Análisis de la influencia del α-tocoferol y/o ácido oleico sobre las propiedades de films a base de almidón y caseinato sódico

PHYSICAL PROPERTIES AND ANTIOXIDANT CAPACITY OF STARCH-SODIUM CASEINATE FILMS CONTAINING LIPIDS

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ABSTRACT

Biodegradable films based on starch, sodium caseinate, glycerol and lipids (oleic acid and/or α-tocopherol) were obtained and evaluated in terms of microstructure, mechanical behaviour, barrier and optical properties and antioxidant capacity. The effect of film storage time on these properties was also analysed. The lipid incorporation provoked phase separation due to the different interaction between each polymer and lipids, although structural rearrangement of components during storage could be deduced from the change in mechanical behaviour. After storage, all films showed similar mechanical properties, but lipid containing films were more stretchable. Lipid addition did not induce a notable decreased in water vapour permeability of the films, but oxygen permeability highly increased when they contained oleic acid. All films were highly transparent, with very small differences among formulations, although their gloss values increased when lipids were added. The incorporation of α-tocopherol greatly increased the antioxidant capacity of the films which affected oxygen permeability.

Keywords: Starch, sodium caseinate, α-tocopherol, barrier properties, antioxidant.

1. INTRODUCTION

Due to the severe environmental pollution caused by plastic food packaging, there has been a growing amount of interest in the production of edible and biodegradable films (Yan, Hou, Guo & Dong, 2012). In this sense, there are a great number of studies focussing on solving the problems produced by plastic waste and obtaining environmentally friendly materials (Jiménez, Fabra, Talens & Chiralt, 2012a). Several works study the possibility of substituting the petro-based plastics for biodegradable, low cost materials with similar properties (Psomiadou, Arvanitoyannis & Yamamoto, 1996; Mali, Grossmann, García, Martino & Zaritzky, 2002, 2006; Famá, Goyanes & Gerschenson, 2007). In this sense, starch is the most abundant natural polysaccharide and it is described as a renewable resource, inexpensive and widely available (Lourdin, Della Valle & Colonna, 1995). Starch-based films exhibit appropriate physical characteristics, since these films are isotropic, odourless, non-toxic, biodegradable, tasteless, colourless and constitute a good barrier against oxygen transfer (Krochta, Baldwin, & Nisperos-Carriedo, 1994; Yan *et al.*, 2012). Nevertheless, starch films exhibit several drawbacks which it would be beneficial to overcome to increase their potential use. One of the main disadvantages of starch is that the mechanical behaviour of films can be negatively affected by retrogradation phenomena. Jiménez, Fabra, Talens & Chiralt (2012b) found that starch re-crystallizes during film formation and storage, increasing the elastic modulus and decreasing the flexibility of films. In a recent work, they found that recrystallization of starch was also influenced by the amylose-lipid interactions, thus giving rise to V-type crystalline forms for amylose (Jiménez, Fabra, Talens & Chiralt, 2013). Composite films of starch with other biopolymers showed a lower degree of re-crystallization, thus minimizing mechanical changes associated to starch retrogradation. This was observed in corn starch-sodium caseinate and corn starch-hydroxypropylmethylcellulose (HPMC) films (Jiménez, Fabra, Talens & Chiralt, 2012ac).

Sodium caseinate has a satisfactory thermal stability and can easily form films from aqueous solutions, due to its random coil nature and ability to form extensive intermolecular hydrogen, electrostatic and hydrophobic bonds (Arvanitoyannis & Biliaderis, 1998). Different aspects of this polymer as a matrix of edible and biodegradable films have been extensively studied (Siew, Heilmann, Easteal & Cooney, 1999; Kristo, Koursoumanis & Biliaderis, 2008; Patzsch, Riedel & Pietzsch, 2010).

The addition of sodium caseinate to starch matrices reduced the degree of crystallinity of starch films and inhibited starch recrystallization during film storage (Jimenez *et al.* 2012a). Mixtures of both hydrocolloids provide films which are less stiff and resistant to fracture but more flexible and deformable than pure starch films, but with similar water vapour permeability values as starch films (Jimenez *et al.* 2012a). In the case of starch, this disadvantage has been avoided by different methods such as surface esterification (Ren, Jiang, Tong, Bai, Dong & Zhou, 2010), surface photocrosslinking (Zhou, Zhang, Ma & Tong, 2008) or blending starch with other hydrophobic polymers (Averous, Moro, Dole, Fringant, 2000; Fang & Fowler, 2003). For sodium caseinate, hydrophobic additives, such as fatty acids, waxes or oils, were incorporated in the films in order to improve the resistance to water vapour transfer (Morillon, Debeaufort, Blond, Capelle & Voilley, 2002; Pereda, Aranguren & Marcovich, 2010).

Fatty acids have been tested widely in film formulation to improve their water vapour barrier ability (Hagenmaier & Shaw, 1990; Koelsch & Labuza, 1992; Ayranci & Tunc, 2001; Fernández, Díaz de Apodaca, Cebrián, Villarán & Maté, 2006). In sodium caseinate matrices, oleic acid ($C_{18:1}$) acts as a plasticizer, greatly increasing the film extensibility (Fabra, Talens & Chiralt, 2008).

Another lipid that has been used in film formulation is α-tocopherol, which improves water barrier properties while confering antioxidant capacity to the film (Fabra, Hambleton, Talens, Debeaufort & Chiralt, 2011). This component is a lipid-soluble antioxidant whose antioxidant activity has been clearly documented (Rupérez, Martín, Herrera & Barbas, 2001, Manzanarez-López, Soto-Valdez, Auras & Peralta, 2011). Like other tocols (tocopherols and tocotrienols), α-tocopherol acts as a free radical scavenger specifically within cell membranes, by preventing the oxidation of polyunsaturated lipids by free radicals, especially hydroxyl radical OH (Hejtmánková, Lachman, Hejtmánková, Pivec & Janovská, 2010). This antioxidant has been used in film formulation, by using both synthetic (Lee, An, Lee, Park & Lee, 2004; Granda-Restrepo, Soto-Valdez, Peralta, Troncoso-Rojas, Vallejo-Córdoba, Gámez-Meza & Graciano-Verdugo, 2009) and natural polymers (Martins, Cerqueira & Vicente, 2012).

The aim of this work is to evaluate the effect of the addition of oleic acid and/or α-tocopherol on the properties of corn starch-sodium caseinate composite films, through the analyses of the microstructural, mechanical, barrier and optical properties of films, as well as their antioxidant capacity. This was carried out in newly prepared/conditioned films and in those stored for 5 weeks.

2. MATERIALS AND METHODS

2.1. Materials

Corn starch and sodium caseinate (NaCas) were purchased from Roquette (Roquette Laisa España, Benifaió, Spain) and Sigma (Sigma–Aldrich Chemie, Steinheim, Germany), respectively. Oleic acid (OA) and α-tocopherol (TOC), selected as the hydrophobic dispersed phase, were also supplied by Sigma. Furthermore, glycerol was provided by Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain) as plasticizer.

2.2. Preparation and characterization of films

Four different formulations based on corn starch, sodium caseinate, glycerol as plasticizer, and lipids were prepared. Corn starch was dispersed in cold water to obtain 2 % (w/w) polysaccharide dispersions. These were maintained, under stirring, at 95 ºC for 30 minutes to induce starch gelatinization. Sodium caseinate was dissolved directly in cool distilled water (2 % w/w). Afterwards, both hydrocolloids were mixed to obtain dispersions with a starch:protein ratio of 1:1. Once the mixtures were prepared, a controlled amount of glycerol was added (hydrocolloid:glycerol ratio was 1:0.25). In the case of emulsions containing lipids, these were incorporated prior to the homogenization step. The hydrocolloid:lipid ratios were 1:0.15 and 1:0.10 for oleic acid and α-tocopherol, respectively. Dispersions were homogenized for 1 min at 13,500 rpm and for 5 min at 20,500 rpm at 95 ºC, under vacuum, using a rotor-stator homogenizer (Ultraturrax T25, Janke and Kunkel, Germany). High temperature homogenization favoured the denaturation of the protein and polymer interaction as well as the lipid dispersion in the system.

Film-forming dispersions, containing 1.5 g of total solids, were gently spread over a Teflon plate (150 mm diameter) resting on a leveled surface. The dispersions were allowed to dry for approximately 48 hours at 45 % RH and 20 ºC. Dry films could be peeled intact from the casting surface. Film thickness, used in different analyses, was measured with a Palmer digital micrometer to the nearest 0.0025 mm at 6 random positions. Four kinds of films were prepared: without lipids (control), with only oleic acid (OA) or α-tocopherol (Toc) and with both of them (OA-Toc).

2.2.1. Film equilibration and storage

Before characterizing the films, samples were equilibrated in dessicators at 25 ºC and 53 % RH, by using magnesium nitrate-6-hydrate saturated solutions

(Panreac Química, SA, Castellar del Vallés, Barcelona) for one week when the first analyses were carried out. One part of the samples was stored under the same conditions for five weeks in order to perform the second series of analyses.

2.2.2. Tensile Properties

A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to determine the film elastic modulus (EM), the tensile strength (TS), and elongation at break (E) of the films, according to ASTM standard method D882 (ASTM, 2001). EM, TS and E parameters were determined from stress–strain curves, estimated from force-deformation data. After drying, flawless films were selected to determine their mechanical behaviour. At least eight replicates of each formulation were tested. The cut film samples had a rectangular section of 2.5 cm wide and 10 cm long. Before testing, all samples were equilibrated as explained previously at 53 % RH and 25 ºC. Equilibrated film specimens were mounted in the film-extension grips of the testing machine and stretched at a rate of 50 $mm \cdot min^{-1}$ until breaking.

2.2.3. Barrier properties

2.2.3.1. Water Vapour Permeability (WVP)

WVP of films was determined by using the ASTM E96-95 gravimetric method, taking into account the modification proposed by McHugh, Avena-Bustillos & Krochta (1993). Films were selected for WVP tests based on the lack of physical defects such as cracks, bubbles, or pinholes. Distilled water was placed in Payne permeability cups (3.5 cm diameter, Elcometer SPRL, Hermelle /s Argenteau, Belgium) to expose the film to 100 % RH on one side. Once the films were secured, each cup was placed in a relative humidity equilibrated cabinet at 25 ºC, with a fan placed on the top of the cup in order to reduce resistance to water vapour transport. RH of the cabinets (53 %) was held constant using oversaturated solutions of magnesium nitrate-6-hydrate (Panreac Química, SA, Castellar del Vallés, Barcelona). The cups were weighed periodically (0.0001 g) and water vapour transmission rate was determined from the slope obtained from the regression analysis of weight loss data versus time, once the steady state had been reached, divided by the film area. From this data, water vapour permeability values were obtained, taking into account the average film thickness in each case. The equation proposed by McHugh *et al*. (1993) was used to correct the effect of concentration gradients established in the stagnant air gap inside the cup.

2.2.3.2. Oxygen permeability (OP)

The oxygen barrier capacity of the films was evaluated by measuring oxygen permeability (OP) by means of an Ox-Tran 1/50 system (Mocon, Minneapolis, USA) at 25 ºC (ASTM Standard Method D3985-95, 2002). Measurements were taken at 53% in films previously equilibrated at the same RH. Films were exposed to pure nitrogen flow on one side and pure oxygen flow on the other side. The OP was calculated by dividing the oxygen transmission rate by the difference in the oxygen partial pressure on the two sides of the film, and multiplying by the average film thickness. At least three replicates per formulation were taken into account.

2.2.4. Scanning Electron Microscopy (SEM)

Microstructural analysis of the films was carried out using a Scanning Electron Microscope (JEOL JSM-5410, Japan). Film samples were maintained in a desiccator with P_2O_5 for 15 days. Then films were frozen in liquid N_2 and gently and randomly broken to investigate the cross-section of the samples. Films were fixed on copper stubs, gold coated, and observed using an accelerating voltage of 10 kV.

2.2.5. Optical Properties

The transparency of the films was determined by applying the Kubelka-Munk theory (Hutchings, 1999) for multiple scattering to the reflection spectra. The surface reflectance spectra of the films were determined from 400 to 700 nm with a spectrocolorimeter CM-3600d (Minolta Co., Tokyo, Japan) on both a white and a black background. As the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the scattering (S) coefficients. Internal transmittance (T_i) of the films was quantified using eq 1. In this equation R_0 is the reflectance of the film on an ideal black background. Parameters a and b were calculated by eqs 2 and 3, where R is the reflectance of the sample layer backed by a known reflectance R_g. Measurements were taken in triplicate for each sample on the free film surface during its drying.

$$T_i = \sqrt{(a - R_0)^2 - b^2} \qquad \text{Equation 1}$$

$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \qquad \text{Equation 2}$$

$$b = (a^2 - 1)^{1/2} \qquad \text{Equation 3}$$

The gloss was measured on the free film surface during film formation at 60° angle from the normal to the surface, according to the ASTM standard D523 method, (ASTM, 1999) using a flat surface gloss meter (Multi.Gloss 268, Minolta, Germany). Measurements were taken in triplicate for each sample and three films of each formulation were considered. All results are expressed as gloss units, relative to a highly polished surface of black glass standard with a value near to 100.

2.2.6. Antioxidant capacity of films

The antioxidant capacity of starch-NaCas films with and without lipids conditioned for one week, and stored for 5 weeks under the same conditions, were evaluated by means of the Trolox equivalent antioxidant capacity (TEAC), using a modification of the original TEAC method (Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, 1999). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analogue, was used as an antioxidant standard. ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt) was dissolved in water to a concentration of 7 mM and allowed to react with a 2.45 mM potassium persulfate solution (final concentrations) for 16 h. To this end, ABTS solution was stored overnight in the dark. All three reagents were provided by Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). ABTS radical cation ($ABTS^+$), a blue chromophore, was produced in the reaction. The $ABTS^+$ solution was diluted with a water:methanol (2:8) solution to an absorbance of 0.70 (± 0.02) at 734 nm. All the determinations were carried out in a Beckman Coulter DU 730 spectrophotometer, using a water:methanol (2:8) solution as blank. 0.1 g of each film sample was cut into small pieces and hydrated with 2 ml of distilled water for one hour, when 8 ml of methanol were added in order to favour the extraction of the antioxidant components. These samples were maintained under stirring overnight and centrifuged at 4ºC, 5,000 rpm for 10 min. The supernatant (film extract) obtained was analyzed for ABTS radical scavenging activity. To this end 10 μl of the film extracts were added to 1 ml of the $ABTS^+$ solution and absorbance at 734 nm was registered every minute for 6 min. For calibration, Trolox standards of different concentrations (between 3.12 and 500 mg/l) were prepared and the same procedure was followed. The TEAC of the film samples was determined by comparing the corresponding percentage of absorbance reduction at 6 min with the Trolox concentration–response curve. The molarity of trolox which gives the same absorbance reduction as the film extract was considered as the TEAC value. At least three determinations were considered for each formulation.

2.3. Statistical analysis.

Statistical analyses of data were performed through analysis of variance (ANOVA) using Statgraphics Centurion XVI (Manugistics Corp., Rockville, MD). Fisher′s least significant difference (LSD) procedure was used at the 95% confidence level.

3. RESULTS

3.1. Microstructural properties of films

Film components can be arranged in different ways in the dried film, depending on their interactions in the film-forming dispersions and during film drying. The microstructure of the starch-NaCas based films, with and without lipids, was qualitatively studied using scanning electron microscopy (SEM). Figure 1 shows SEM micrographs obtained from the cross-sections of the starch-NaCas based films, with and without oleic acid and/or α-tocopherol. Control film exhibited a homogeneous structure as can be observed in Fig. 1A. However, the incorporation of oleic acid and/or α-tocopherol promoted relevant changes in the hydrocolloid matrix. In films containing oleic acid (Fig. 1B), no visible droplets of oleic acid were observed, which indicates that the lipid is well integrated in the starch-NaCas matrix. However, it is noteworthy that two separate layers can be distinguished in the film cross-section. The bottom phase shows a homogeneous aspect, while a coarser structure can be observed in the upper part of the film, which indicates that lipid is mainly located in this phase. This reflects an irregular distribution of the lipid between the two hydrocolloids, promoting phase separation. Oleic acid has greater affinity with sodium caseinate due to the anphiphilic nature of protein (Chen, 2002). In previous studies, oleic acid did not form visible droplets in sodium caseinate films and interactions between these components have been proven (Fabra, Talens & Chiralt, 2010). However, Jiménez *et al*. (2012b) reported visible, well-distributed droplets of oleic acid in the structure of the oleic acid-starch films while the formation of V type crystalline forms of amylose was revealed by X-Ray difractograms. (Jiménez *et al*., 2013). So, differences in oleic acid interactions with both polymers in sodium caseinate-starch blend films seem to affect the polymer compatibility, promoting phase separation: one caseinate-rich phase, containing most of the lipid, and one starch-rich phase in the lower part of the film where no lipid droplets can be observed.

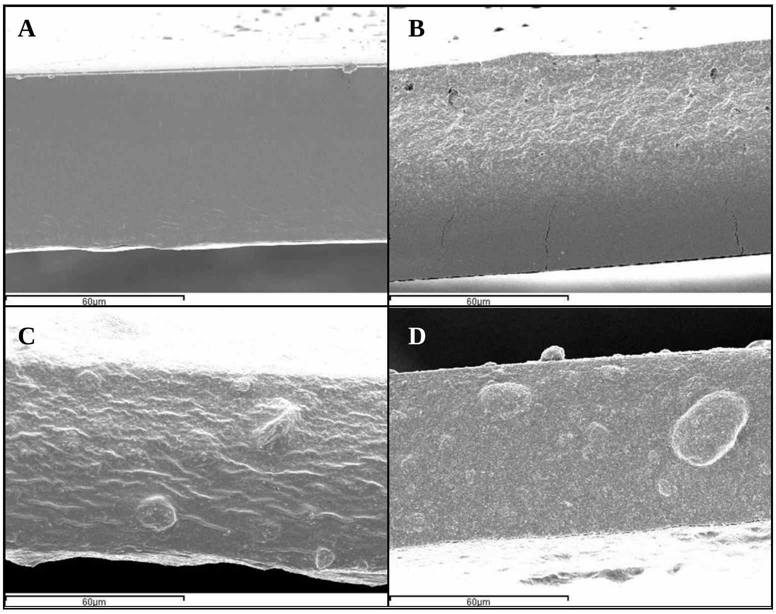


Figure 1. SEM micrographs of the cross-sections of the films. (A: Control, B: OA, C: OA-Toc, D: Toc.).

When α-tocopherol was incorporated to the film without oleic acid, a phase separation was also induced (Fig. 1D) although these phases were interpenetrated (big droplets of one appear in the other) and both of them show a coarser aspect than the blended film without lipids. This indicates that a distribution of the lipid between the two phases occurs and that the different interactions of the lipid with each hydrocolloid modify the polymer chain interactions, reducing their compatibility. In the case of films prepared with mixtures of both lipids, the two phenomena described for oleic acid and α-tocopherol occur simultaneously (Fig. 1C): an oleic acid-rich phase can be distinguished in the upper part of the film, attributable to caseinate-oleic acid association, at the same time as the interpenetrated phases, associated with the α-tocopherol interactions, could also be observed. So, SEM analysis reveals that both lipids interact with sodium caseinate and starch polymers to a different extent, modifying their compatibility and producing phase separation with a different distribution of components in each phase according with their respective partition coefficients.

Film microstructure affects optical properties of the films, which are greatly influenced by the internal and surface heterogeneity of the matrix. Table 1 shows the gloss values of the studied films, measured at 60º and their internal transmittance (Ti) obtained at 450 nm (the wavelength at which the highest differences were observed).

High values of Ti are associated with greater film homogeneity and transparency. Unlike what was observed in a previous work, where oleic acid was incorporated into starch films (Jiménez *et al.*, 2012b), the incorporation of this component in the studied blend films did not significantly ($p > 0.05$) modify their transparency. Similarly, Fabra, Jiménez, Atarés, Talens & Chiralt (2009) observed that oleic acid did not significantly decrease Ti values of sodium caseinate based films. The obtained result can be explained by the good integration of oleic acid in the caseinate rich phase, and with the phase separation in two layers.

Table 1. Optical properties: Transparency (internal transmittance: Ti) and gloss at 60º of the films before and after storage for 5 weeks. Mean values (standard deviation).

	T_i (450 nm)		Gloss 60º	
Film	Initial	Final	Initial	Final
Control	84.7 (0.3)^{a1}	83.1 (0.2)^{a2}	76 (8)^{a1}	44 (11)^{a2}
OA	84.8 (0.5)^{a1}	84.6 (0.5)^{b1}	90 (3)^{b1}	61 (8)^{b2}
OA-Toc	84.2 (0.5)^{ab1}	83.2 (0.3)^{a1}	78 (2)^{a1}	44 (12)^{a2}
Toc	83.2 (0.7)^{b1}	83.1 (0.5)^{a1}	92 (1)^{b1}	83 (3)^{c2}

a-c: Different superscripts within the same column indicate significant differences among formulations ($p < 0.05$).
1-2: Different superscripts within the same line indicate significant differences among formulations ($p < 0.05$).

The incorporation of α-tocopherol significantly decreased the transparency of the films as a consequence of the presence of a dispersed phase in a continuous matrix, as observed in the cross-section image, which favours light dispersion.

No significant changes in transparency were observed during storage for films containing lipids, although a slight decrease was observed for lipid-free films. This decrease was associated to the increase in the matrix compactness (Jiménez *et al*., 2012a).

The gloss of non-stored films was greatly affected by the film composition. Films containing oleic acid or α-tocopherol were glossier than those of lipid-free films. However, gloss values of films prepared with oleic acid-α tocopherol blend did not vary significantly with respect to the control film. The increase in gloss observed when pure oleic acid is added is coherent with the observed microstructural features: the induced phase separation and the migration of the caseinate-lipid rich phase to the upper part of the film. Caseinate-glycerol films were glossier than starch-glycerol films according to Jiménez *et al*. (2012a) and so, the blend film showed greater gloss when caseinate was predominant at the film surface. This did not occur when film contained oleic acid and α-tocopherol, since the latter compound greatly contributed to reduce gloss of caseinate films (Fabra *et* *al*., 2011). In films containing only α-tocopherol, no phase separation in layers occurs, but one dispersed in the other with α-tocopherol distributed in both of them. Nevertheless, the incorporation of the lipid (oleic acid or α-tocopherol) implied an increase in gloss with respect to the lipid-free films. The partial migration of this compound to the film surface could be responsible for this behaviour. It is remarkable that all films lose gloss during storage, which can be attributed to the changes in the film surface topography which occurred due to the progressive rearrangement of the polymer chains during storage, giving rise to a rougher surface (Jiménez *et al*. 2012a).

3.2. Mechanical properties

Figure 2 shows mechanical behaviour of the films before and after the storage. In all cases, the stress values decreased during storage, which indicates that polymer chains rearrange throughout time, giving rise to a more deformable material. This is especially marked in films containing α-tocopherol as the only lipid. On the other hand, whereas films without lipids became less extensible during storage, plastic deformability greatly increased for all lipid-containing films. This behaviour suggests that when no lipids are present in the film, polysaccharide and protein chains progressively aggregate though hydrogen bonds, which makes the chain slippage during the tensile test more difficult and films become less extensible. On the contrary, the presence of lipids limits the bond formation between the macromolecules due to their interaction with the lipid molecules, which contributes to increase the film extensibility, evidencing the plasticizing role of lipids after a reasonable storage time when functional groups of components are adequately re-oriented. Figure 3 shows the values of elastic modulus (EM) and tensile strength (TS) and elongation (E: %) at break of the non-stored and stored films. . In non stored films, the addition of oleic acid did not significantly modify EM and TS values, although it provoked a decrease in the film extensibility, despite the fact that the opposite effect was observed in films of pure sodium caseinate (Fabra *et al*., 2008) and starch based films (Jiménez *et al*., 2012b).

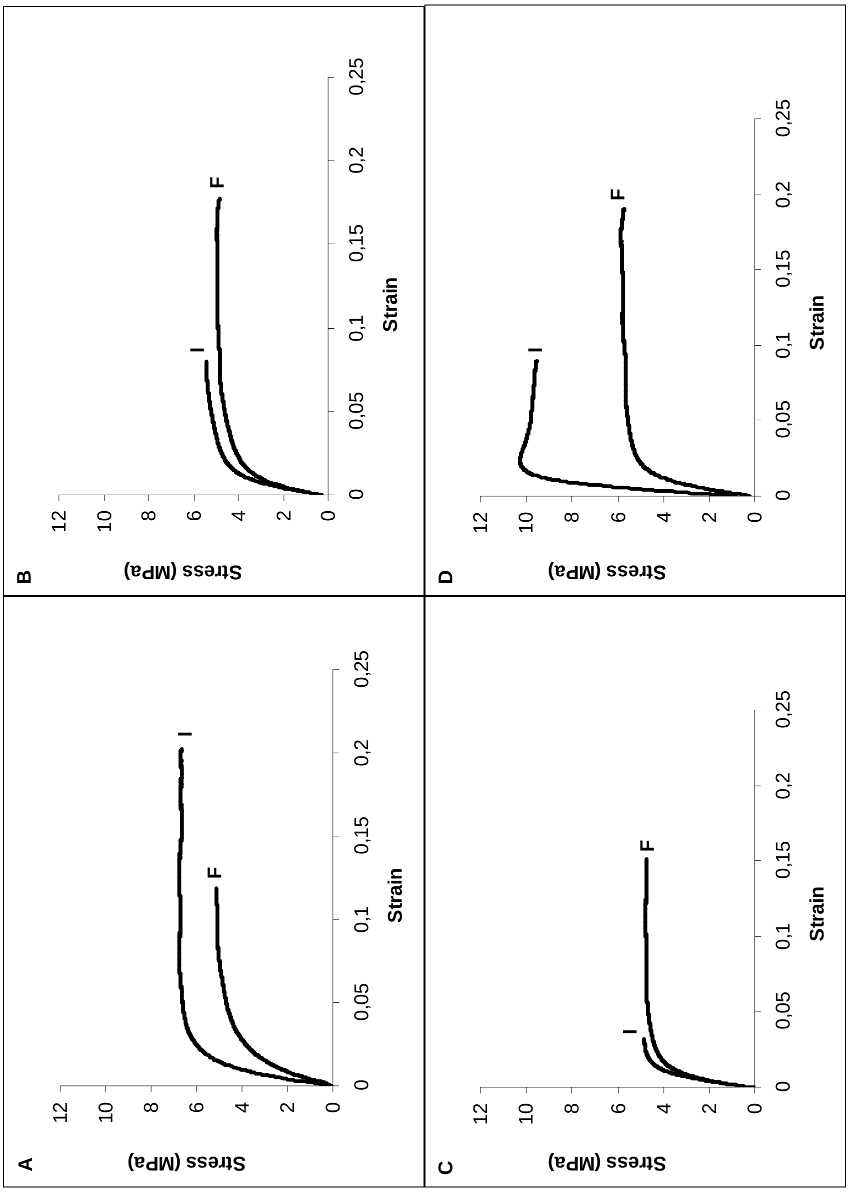
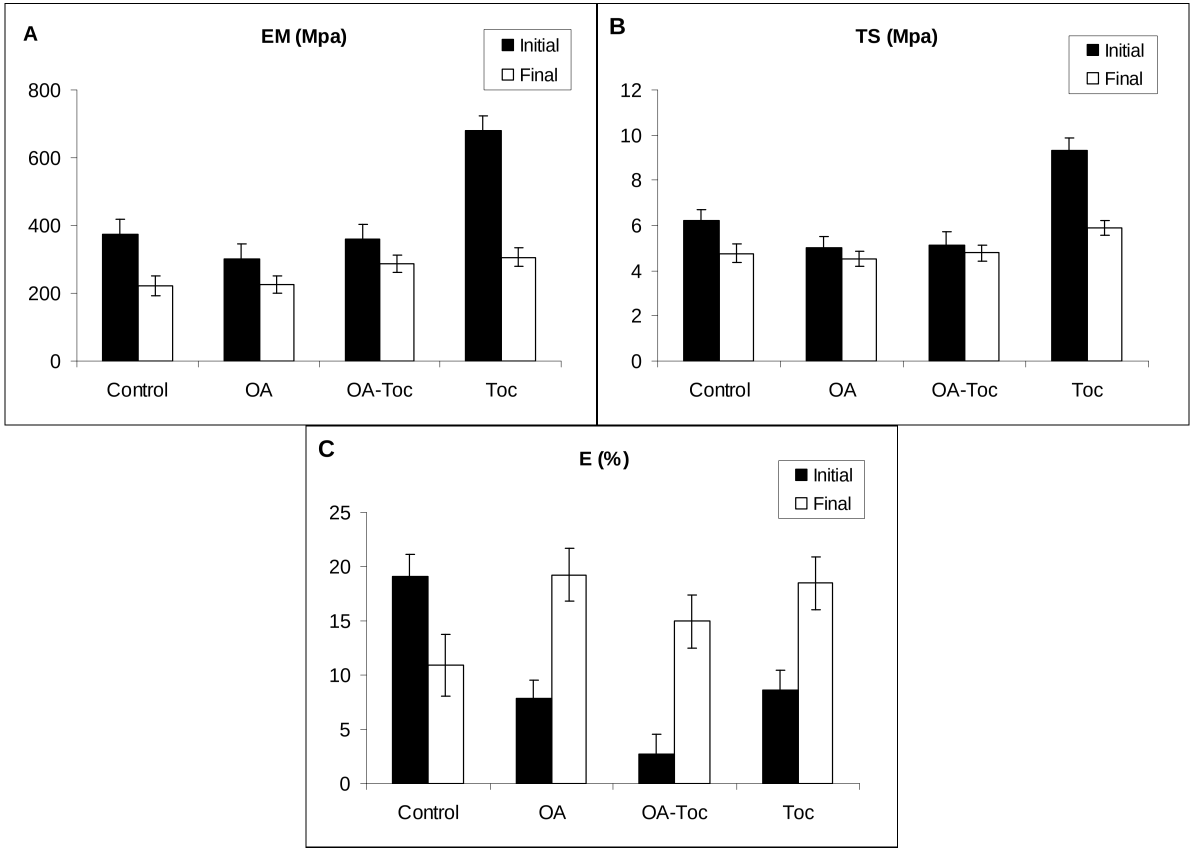


Figure 2. Typical stress-Hencky strain curves of the films before (I) and after (F) storage for 5 weeks. (A: Control, B: OA, C: OA-Toc, D: Toc).

Figure 3. Mechanical properties (elastic modulus (EM), tensile strength (TS) and elongation at break (E)) of the films before and after storage for 5 weeks.



It was in sodium caseinate films that a strong plasticizing effect was particularly observed for oleic acid (Fabra *et al.*, 2010). This opposite effect in the blend film could be attributed to the formation of two separate ensemble layers in the film with different stretchability which does not permit the extension of the possibly plasticized caseinate phase which contains most of the oleic acid. The film extensibility will be limited by the less extensible layer in films with layer-separated phases. This reduction in the film stretchability occured in all lipid containing films and was especially marked in the film containing both oleic acid and α-tocopherol, where the layer-separated phases coexist with a dispersed phase. Nevertheless, it is remarkable that, in non-stored films, when only α-tocopherol was added to the matrix, film stiffness is doubled and resistance to break markedly increased. This indicates that the generated film microstructure leads to a network which is more resistant to break.

After 5 weeks' storage, the films' mechanical behaviour notably changed, which is coherent with the structural rearrangements occurring in the matrix, as commented on above, thus implying a different response to the tensile stress. EM values significantly decreased in all films, mainly in those containing α-tocopherol where a reduction of about 50% was observed, all of which reached very similar values. A slight decrease in resistance to break was also observed for all films, whereas lipid-containing films showed a highly marked increase in stretchability. On the contrary, lipid-free films became less extensible.

The obtained results suggest that lipids had, in fact, a plasticizing effect in caseinate-starch films, but this was only observed at long storage times, probably due to a slow kinetics of orientation of the functional groups in the matrix which favours lipid-polymer interactions, weakening the polymer chain attraction forces. This effect implies a softening of the polymer network and facilitates the slippage of the chains during the film stretching, increasing the extensibility. In fact, the plasticizing effect of oleic acid on hydrocolloid matrices has been previously reported by other authors for sodium caseinate (Fabra *et al*., 2008), soy protein isolate (Rhim, Weller & Schnepf, 1999) or zein (Santosa y Padua, 1999). So, it can be concluded that, after a relatively short storage period (5 weeks), blend lipid-containing films showed very similar mechanical behaviour, with similar resistance to break to lipid-free films, but more stretchable.

3.3. Barrier properties

Table 2 shows water vapour permeability values of non-stored and stored films analyzed at 25 ºC and 53-100% RH gradient.

Table 2. Barrier properties (WVP: water vapour permeability and OP: oxygen permeability) and antioxidant capacity (TEAC: trolox equivalent antioxidant capacity:) of the films before and after storage for 5 weeks.

	WVP (g· mm· kPa^{-1}· h^{-1}·m^{-2})		**O$_2$ perm. · 10^{14} (cm^3·m^{-1}·s^{-1}·Pa^{-1})**	
Film	Initial	Final	Initial	Final
Control	9.05 (0.18)^{a1}	7.3 (0.5)^{a2}	12.5 (0.4)^{a1}	14.1 (0.7)^{a1}
OA	7.0 (0.2)^{b1}	8.4 (0.2)^{b2}	99 (32)^{b1}	104 (10)^{b1}
OA-Toc	8 (1)^{ac1}	9.1 (0.2)^{c1}	39 (9)^{a1}	118 (22)^{b2}
Toc	7.2 (0.7)^{cb1}	8.2 (0.4)^{b1}	16.53 (0.03)^{a1}	15.8 (0.4)^{a1}

	TEAC (mM)	
Film	Initial	Final
Control	0.249 (0.002)^{a1}	0.25 (0.02)^{a1}
OA	0.171 (0.010)^{a1}	0.084 (0.006)^{a2}
OA-Toc	1.40 (0.07)^{b1}	1.73 (0.05)^{b2}
Toc	1.37 (0.06)^{b1}	1.5 (0.2)^{b1}

In non-stored films, the addition of oleic acid and/or α-tocopherol slightly reduced water vapour permeability values because of the increase in the films' hydrophobicity. The increase in the water barrier efficiency of protein or polysaccharide films when lipids were incorporated has been reported in previous studies by using hydroxypropylmethylcellulose (HPMC) and stearic acid

(Hagenmaier & Shaw, 1990), methylcellulose and fatty acids (FA) (Koelsch & Labuza, 1992), whey protein isolate and beeswax (BW) (Pérez-Gago & Krochta, 2001), fish protein and BW or FA (Tanaka, Ishizaki, Suzuki, & Takai, 2001), HPMC and FA (Jiménez, Fabra, Talens, & Chiralt, 2010), pistacho protein and FA (Zahedi, Ghanbarzadeh, & Sedaghat, 2010) and hake protein in combination with thyme oil (Pires, Ramos, Teixeira, Batista, Mendes, Nunes & Marques, 2011. The most important reduction in WVP occurred when pure oleic acid (22 % reduction with respect to the lipid-free film) or α-tocopherol (20 % reduction with respect to the lipid-free film) was added. Not only does the presence of lipids affect barrier properties but also the final structural distribution of film components. In fact, storage time, which provokes changes in the component arrangement in the film (as deduced from mechanical behaviour), led to a slight increase in the WVP of lipid-containing films and a notable decrease in lipid-free films. The progressive aggregation of polymer chains in the lipid-free films could lead to a more compact network where the transfer of water molecules is more difficult. In lipid containing films, this aggregation phenomenon is inhibited by the lipid interactions with the macromolecules which limit the hydrogen bond formation between them.

Oxygen permeability values of non-stored and stored films are given in Table 2. The obtained values for the control film are lower than those reported by Arvanitoyannis & Biliaderis (1998) for soluble starch-sodium caseinate films and by García, Martino & Zaritzky (2000) for corn starch containing a higher ratio of plasticizer. The addition of sodium caseinate to corn starch matrices slightly increased oxygen permeability, proportionally to the ratio of incorporated caseinate (Jimenez *et al.*, 2012a). The incorporation of the oleic acid in the films provoked a lower increase in the oxygen permeability values than that promoted in sodium caseinate-glycreol films (Fabra *et al.* 2012).

At the initial time, films without oleic acid showed the lowest oxygen permeability and no significant differences were observed between control films and those prepared with α-tocopherol. This has been related with the fact that oleic acid is in liquid state in the film and with the increase of the oxygen solubility in the matrix when liquid lipids are present (Fabra *et al*., 2008). The same trend was observed in pure sodium caseinate films containing oleic acid-beeswax mixtures, where oxygen permeability exponentially increased in line with the greater ratio of oleic acid in the lipid blend (Fabra, Talens, Gavara & Chiralt, 2012). Although the same effect could be expected for α-tocopherol, a chemical blocking of oxygen, due to the antioxidant action of the lipid, might contribute to increase the oxygen barrier properties.

Storing films for five weeks did not induce significant changes in the oxygen barrier properties of the films, except for that containing both lipids, where a slight increase in the oxygen permeability was observed.

3.4. Antioxidant capacity

The study of the radical-scavenging capacity of films using ABTS is a technique which has been frequently used by researchers in the last few years (Arcan & Yemenicioğlu, 2011; Akhtar, Jacquot, Jasniewski, Jacquot, Imran, Jamshidian, Paris & Desobry, 2012; Helal, Tagliazucchi, Conte & Desobry, 2012). Values of the Trolox equivalent antioxidant capacity (TEAC), corresponding to both newly prepared films and those stored for 5 weeks are shown in Table 2.

At initial time, control film showed antioxidant activity, probably due to the action of some peptides from sodium caseinate. The antioxidant capacity of casein and its derivates has been reported (Rossini, Noreña, Cladera-Olivera & Brandeli, 2009; Ries, Ye, Haisman & Singh, 2010). In this sense, different authors have demonstrated that there is greater casein antioxidant activity when the hydrolysis degree increases because of the release of peptides with antioxidant properties (Gómez-Ruiz, López-Expósito, Pihlanto, Ramos & Recio, 2008; Mao, Cheng, Wang, Wu, 2011).

When oleic acid was incorporated, the film antioxidant capacity decreased, in part due to the reduction of the mass fraction of the active compounds, coming from caseinate, in the matrix when oleic acid was incorporated to the film. Nevertheless, a greater decrease than that expected from the mass fraction reduction of caseinate in the film solids was obtained. This suggests a loss of the film antioxidant capacity provoked by the oleic acid addition, which is more accentuated in stored films. This result could be explained by the inhibition of the extractability of active compounds when there is oleic acid in the film or by the promotion of oxidation reactions, due to the increase in the oxygen solubility in the matrix, which consume antioxidant compounds.

When α-tocopherol, which has proven to be a good antioxidant in different food products, such as sardine (Vicetti, Ishitani, Salas & Ayala, 2005) or sunflower oil (Marinova, Toneva & Yanishlieva, 2008), was present in the films, their antioxidant activity greatly increased. Nevertheless, the obtained TEAC values were slightly lower than those corresponding to the amount of this compound in the analysed film, taking into account that the antioxidant capacity of this pure compound is similar to that of TROLOX. This indicates that a part of the lipid molecules were oxidized during the film preparation or storage or that the compound was not completely released during the film extraction.

The effect of storage time on films' antioxidant activity was dependent on the lipids included in the blend matrix. Whereas for control film (without lipid) the radical scavenging activity did not vary significantly with time ($p < 0.05$), films containing oleic acid showed a significant decrease. This could be due to the promotion of the consumption of antioxidant compounds by the increase of the oxygen solubility in the films, as previously commented on.

Nevertheless, the antioxidant activity of the film containing both oleic acid and α-tocopherol increased ($p < 0.05$) during time, which could only be explained by an increase in the film compound extractability associated to the structural re-

arrangements in the film. In films containing only α-tocopherol, no significant differences were observed in their antioxidant capacity at different storage times.
In conclusion, the incorporation of α-tocopherol in the starch-sodium caseinate films greatly increased their antioxidant capacity, which indirectly affected oxygen permeability, which was significantly reduced with respect to that of the film containing oleic acid, where oxygen permeability significantly increased by the presence of a liquid lipid in the matrix, which enhance the oxygen solubility in the film.

4. CONCLUSIONS

The lipid incorporation provoked phase separation in sodium caseinate-starch films due to the different interaction with each polymer (anphiphilic protein and hydrophilic starch), although a structural rearrangement of components during storage can be deduced from the change in their mechanical behaviour. After storage, all films showed similar mechanical properties, but lipid-containing films were more stretchable. Lipids slightly affected water vapour barrier properties, depending on the film storage time, but the incorporation of oleic acid greatly increased oxygen permeability. This effect was also expected for α-tocopherol, but it was mitigated by the antioxidant activity of this compound, which greatly improved the antioxidant capacity of the films. All the films showed similar transparency, but their gloss values increased when lipids were incorporated.

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Capítulo IV

Desarrollo de films antimicrobianos a base de almidón y caseinato sódico. Influencia de la adición de nanoliposomas, como vehículos de compuestos bioactivos, en sus propiedades

INFLUENCE OF NANOLIPOSOMES INCORPORATION ON PROPERTIES OF FILM FORMING DISPERSIONS AND FILMS BASED ON CORN STARCH AND SODIUM CASEINATE

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ABSTRACT

The incorporation of potentially antimicrobial volatile compounds (orange essential oil and limonene) into rapeseed and soy nanoliposomes was carried out by encapsulating them trough sonication of their aqueous dispersions. Nanoliposomes were added to starch-sodium caseinate (50:50) film forming dispersions, which were dried to obtain films without losses of the volatile compounds. Structural, mechanical and optical properties of the films were analysed, as well as their antimicrobial activity against *Listeria monocytogenes*. The addition of liposomes in the polymeric matrix supposed a decrease of the mechanical resistance and extensibility of the films. The natural colour of lecithin conferred a loss of lightness, a chroma gain and a redder hue to the films, which were also less transparent than the control one, regardless the lecithin and volatile considered. The possible antimicrobial activity of the films containing orange essential oil or limonene was not observed, which could be due to their low antilisterial activity or to the inhibition effect of the encapsulation which difficult their release from the matrix.

Keywords: Starch-sodium caseinate films, nanoliposomes, antimicrobial, encapsulation.

1. INTRODUCTION

Nowadays, it is well known that edible and biodegradable films obtained from biopolymers are able to substitute, at least partially, conventional plastics. The biodegradable plastics, after their useful life, get assimilated by microorganisms and return to the natural ecosystem without causing any pollution or harm to the environment (Maran, Sivakumar, Sridhar & Immanuel, 2013). Polysaccharides and proteins are used in film formulations, since it is possible to obtain transparent, tasteless, odorless and isotropic films by using these polymers (Chick & Ustunol, 1998; Han, 2002; Soliva-Fortuny, Rojas-Graü & Martín-Belloso, 2012). In this sense, one of the most used polysaccharide to obtain films with adequate properties is starch. This biopolymer is a renewable resource, inexpensive (compared with other compounds) and widely available (Lourdin, Della Valle & Colonna, 1995). Starch based films can be formed by using its pure components (amylose and amylopectin; Paes, Yakiments, & Mitchell, 2008), native starch (López & García, 2012), modified starches (López, García & Zaritzky, 2008) and soluble or pregelatinized starch (Pagella, Spigno & De Faveri, 2002). Nevertheless, starch films, as other polysaccharide films, are highly sensitive to moisture action. Furthermore, their mechanical behaviour can vary as a consequence of retrogradation phenomenon throughout time (Famá, Goyanes & Gerschenson, 2007; Jiménez, Fabra, Talens, & Chiralt, 2012a)

The hydrophilic character of starch films can be modified by different techniques such as surface sterification (Zhou, Ren, Tong, Xie, & Liu, 2009), surface photocrosslinking (Zhou, Zhang, Ma, & Tong, 2008) or by adding hydrophobic compounds to film formulation (Averous, Moro, Dole, & Fringant, 2000; Fang & Fowler, 2003). On the other hand, starch retrogradation has been inhibited by mixing starch with other polymers such as hydroxypropylmethylcellulose (HPMC) or sodium caseinate (Jiménez, Fabra

Talens & Chiralt, 2012bc). Whereas starch-HPMC films showed phase separation in the film, starch-sodium caseinate films were completely homogeneous and showed good functional properties.

Biodegradable films are able to act as carriers of active compounds such as antioxidants or antimicrobials to enlarge the self life of food products where they are applied. Among these compounds, essential oils have a great relevance due to the fact that they can act as an antioxidants and antimicrobials at the same time (Ruiz-Navajas, Viuda-Martos, Sendra, Perez-Alvarez, Fernández-López, 2013; Ye, Dai & Hu, 2013). In general, essential oils are a mix of volatile (85-99 %) and non volatile compounds (1-15 %) (Sánchez-González, Vargas, González-Martínez, Cháfer & Chiralt, 2011a) in which the volatile fraction is composed by terpens, terpenoids and other aromatic and aliphatic components with low molecular weight (Smith-Palmer, Stewart, Fyfe, 2001; Bakkali, Averbeck, Averbeck & Idaomar, 2008). Previous studies reported antimicrobial activity of films containing different essential oils (Sánchez-González, Cháfer, Chiralt & González-Martínez, 2010a; Sánchez-González, González-Martínez, Chiralt & Cháfer, 2010b; Sánchez-González, Cháfer, Hernández, Chiralt & González-Martínez, 2011b; Iturriaga, Olabarrieta, Martínez de Marañón, 2012). However, isolate terpenes (limonene, geranyl acetate and alpha-pinene) have been found to promote the growth of *Listeria monocytogenes* in biofilms structures (Sandasi, Leonard & Viljoen, 2008), whereas the antimicrobial activity of essential oils has been attributed to the synergism between different terpenes, which would improve their activity against bacteria (Gallucci, Oliva, Casero, Dambolena, Luna, Zygadlo & Demo, 2009; Piccririllo, Demiray, Silva Ferreira, Pintado & Castro, 2013) and fungi (Edris & Farrag, 2003).

Due to its volatile nature, essential oils can evaporate from film forming dispersions during drying, thus reducing its effectiveness in dried films. The encapsulation of essential oils could be a solution to maintain their usefulness for a longer time, by a control release of the compounds. The encapsulation of a hydrophobic compound in an aqueous dispersion requires the utilization of amphiphilic substances such as lecithin. Recently Zhang *et al*. (Zhang, Arab Tehrany, Kahn, Ponçot, Linder & Cleymand, 2012) have obtained very stable lecithin nanoliposomes by means of by means of sonication, in order to incorporate them in chitosan films.

The aim of this work was the development of starch-sodium caseinate films containing nanoliposomes as carriers of antimicrobial compounds (orange essential oil and D-limonene). The influence of the nanoliposomes addition with and without antimicrobials in the properties of film forming dispersions (surface tension and rheological properties) and films (mechanical, optical and antimicrobial properties) was studied.

2. MATERIALS AND METHODS

2.1. Materials

Corn starch was purchased from Roquette (Roquette Laisa España, Benifaió, Spain) and sodium caseinate (NaCas) was supplied by Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). Glycerol (99.5 % AnalaR NORMAPUR), choosen as plasticizer, was provided by WVR International. To form nanoliposomes, rapeseed and soy lecithins were obtained from The Solae Company (Solae Europe, Geneva, Switzerland) and Novastell (Etrépagny, France), respectively. Furthermore, D-Limonene stabilized (purchased from Acros Organics, Geel, Belgium) and orange essential oil (supplied by Laboratoires Mathe, Maxeville, France) were choosen as antimicrobial compounds. BF_3(Boron trifluoride)/methanol (99 %) and chloroform (99.8%), used in gas chromatography, were obtained from Bellfonte-PA (USA) and Prolabo-VWR (Italy) respectively. Hexane (95%) and methanol (99.9%) were obtained from Carlo-Erab (France) meanwhile acetonitrile (99.9%) was obtained from Sigma (Sigma–Aldrich Chemie, Steinheim, Germany). These organic solvents were analytical grade reagents.

2.2. Preparation and characterization of nanoliposomes

Nanoliposomes were obtained by modifying the method of Zhang *et al*. (2012). 2 g of lecithin were added in 38 gr of distilled water and then stirred for 5 h. After this step, the mixture was sonicated at 40 kHz and 40% power for 300 s (1 s on and 1 s off). In the case of formulations containing antimicrobials, these compounds were added to the aqueous dispersion previously to sonicate.

2.2.1. Fatty acid composition

Fatty acid esters (FAMEs) were prepared as described by Ackman (Ackman, 1998). The separation of the FAMEs was carried out on a Shimadzu 2010 gas chromatograph Perichrom (Saulx-lès-Chartreux, France), equipped with a flame-ionization detector. A fused silica capillary column was used (60 m, 0.2 mm i.d. ×0.25 µm film thicknesses, SPTM2380, Supelco, Bellefonte, PA, USA). Injector and detector temperatures were set at 250 °C. A temperature program of column initially set at 120 °C for 3 min, then rising to 180 °C at a rate of 2 °C/min and held at 220 °C for 25 min. Standard mixtures (PUFA1, from marine source, and PUFA2, from vegetable source; Supelco, Sigma–Aldrich, Bellefonte, PA, USA) were used to identify fatty acids. The results were presented as triplicate analyses.

2.2.2. Lipid classes

The lipid classes of the different fractions were determined by Iatroscan MK-5 TLC-FID (Iatron Laboratories Inc., Tokyo, Japan). Each sample was spotted on ten Chromarod S-III silica coated quartz rods held in a frame. The rods were developed over 20 min in hexane/diethyl ether/formic acid (80:20:0.2, v:v:v), then oven dried for 1 min at 100 °C and finally scanned in the Iatroscan analyzer. The

Iatroscan was operated under the following conditions: flow rate of hydrogen, 160 ml/mn; flow rate of air, 2 L/min. A second migration using a polar eluant of chloroform, methanol, and ammoniac (65:35:5) made it possible to quantify polar lipids. The FID results were expressed as the mean value often separate samples. The following standards were used to identify the sample components:

-Neutral lipids: 1-monostearoyl-rac-glycerol, 1.2-dipalmitoyl-snglycerol, tripalmitin, cholesterol.

-Phospholipids: L-a-phosphatidylcholine, 3 sn-phosphatidylethanolamine, L-a-phosphatidyl-L-serine, L-a-phosphatidylinositol, lyso-phosphatidylcholine, sphingomyelin.

All standards were purchased from Sigma (Sigma–Aldrich Chemie, Steinheim, Germany. The recording and integration of the peaks were provided by the ChromStar internal software.

2.2.3. Nanoliposomes size measurement

Size of nanoliposomes was determined by using a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, U.K.) considering the method of Zhang *et al*. (2012). Samples were diluted in distilled water (1:100) and measured at 25 ºC. At least five replicates were considered for each formulation.

2.2.4. Electrophoretic mobility

Electrophoretic mobility of nanoliposomes was measured in the aqueous dispersion by means of a Malvern Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK) at 25 °C. Dispersions were diluted to a particle concentration of 0.01 % using deionised water.

2.2.5. Surface tension

Surface tension of nanoliposomes aqueous dispersions (and film forming dispersions) was measured by using a Krüss K100 tensiometer (Krüss GmbH; Hamburg, Germany) equipped with a platinum plate. All measures were taken in triplicate at 25 °C.

2.3. Preparation and characterization of film forming dispersions

Seven different film forming dispersions based on corn starch, sodium caseinate and glycerol as plasticizer were prepared. Corn starch was dispersed in cool water to obtain 2 % (w/w) polysaccharide dispersions. These were maintained, under stirring, at 95 ºC for 30 min to induce starch gelatinization. Sodium caseinate was dissolved directly in cool distilled water (2 % w/w). Afterwards, both hydrocolloids were mixed to obtain dispersions with a starch:protein ratio of 1:1. This ratio was used on the basis of a previous study carried out by Jiménez *et al.* (2012c) who observed no starch crystallization in this mixture. After this step, a controlled amount of glycerol was added (hydrocolloid:glycerol ratio was 1:0.25). In the case of dispersions containing nanoliposomes, 10 g of nanoliposome solution were added to 90 g of hydrocolloid dispersions. Then the mixtures were maintained 1 hour under stirring at 300 rpm to disperse nanoliposomes.

2.3.1. Rheological behaviour

The rheological behaviour of the film forming dispersions was analyzed in triplicate at 25 °C by means of a rheometer (Malvern Kinexus, Malvern Instruments, Worcestershire, U.K.) with a coaxial cylinder sensor. Flow curves were obtained after resting the sample in the sensor for 5 min at 25 °C. The shear stress (σ) was measured as a function of shear rate ($\dot{\gamma}$) from 0 to 1000 s^{-1} and up and down curves were obtained. When samples showed non-Newtonian behaviour, the power law model was applied to determine the consistency index (k) and the flow behaviour index (n).

2.4. Preparation and characterization of films

Films were obtained by casting. Film forming dispersions were gently poured (88.84 g of solids/m^2) over PET Petri dishes (85 or 140 mm diameter) resting on a leveled surface. The dispersions were allowed to dry for approximately 48 h at 45 % RH and 20 ºC. Dry films could be peeled intact from the casting surface. Seven kinds of films were prepared: without nanoliposomes (control), with lecithin nanoliposomes (Rap or Soy), with limonene-lecithin nanoliposomes (Rap-lim or Soy-lim) and with essential oil-lecithin nanoliposomes (Rap-oil or Soy-oil).

2.4.1. Film conditioning

Before tests, all samples were conditioned in a desiccator at 25 ºC and 53 % RH, by using magnesium nitrate-6-hydrate saturated solutions (Sigma–Aldrich Chemie, Steinheim, Germany) for one week, when the analyses were carried out.

2.4.2. Mechanical properties

A Lloyd instruments universal testing machine (AMETEK, LRX, U.K.) was used to determine the tensile strength (TS), elastic modulus (EM), and elongation (E) of the films, according to ASTM standard method D882 (2001). EM, TS, and E were determined from the stress-Hencky strain curves, estimated from force-distance data obtained for the different films (2.5 cm wide and 10 cm long). At least four replicates were obtained for each formulation. Equilibrated film specimens were mounted in the film-extending grips of the testing machine and stretched at a deformation rate of 50 mm/min until breaking. The relative humidity of the environment was held constant at 53 % during the tests, which were performed at 25 °C.

Measurements of film thickness were carried out by using an electronic digital micrometer (0–25 mm, 1 μm).

2.4.3. FTIR analysis of films

Fourier transform infrared spectroscopy was used to study the presence of interactions between components in conditioned films in total attenuated reflection mode (ATR-FTIR). Measurements were carried out at 25 ºC by using a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with a Platinum ATR optical cell and an RT-Dla TGS detector (Bruker, Karlsruhe, Germany). The diaphragm during analysis was set at 4 mm whereas the scanning rate was 10 kHz. For the reference (air) and each formulation 154 scans were considered from 4000 to 800 cm^{-1}, with a resolution of 4 cm^{-1}.

After measurements, data were treated by using OPUS software (Bruker, Karlsruhe, Germany). Initial absorbance spectra were smoothed using a nine-points Savitsky-Golay algorithm as well as elastic baseline correction (200 points) was applied to spectra. These were then centered and normalized using the mentioned software.

2.4.4. Optical Properties

To evaluate the films transparency, the Kubelka-Munk theory was considered for multiple scattering to the reflection spectra (Hutchings, 1999). When the light passes through the film, it is partially absorbed and scattered, which is quantified by the absorption (K) and the scattering (S) coefficients. Internal transmittance (Ti) of the films was quantified using Equation 1. In this equation R_0 is the reflectance of the film on an ideal black background. a and b parameters are calculated by Equations 2 and 3 where R is the reflectance of the sample layer backed by a known reflectance (R_g). The surface reflectance spectra of the films were determined from 400 to 700 nm with a spectrocolorimeter CM-5 (KonicaMinolta

Co., Tokyo, Japan) on both a white and a black background. All measurements were performed at least in triplicate for each sample on the free film surface during its drying.

$$T_i = \sqrt{(a - R_0)^2 - b^2} \qquad \text{Equation 1}$$

$$a = \frac{1}{2} \cdot \left(R + \frac{R_0 - R + R_g}{R_0 R_g} \right) \qquad \text{Equation 2}$$

$$b = (a^2 - 1)^{1/2} \qquad \text{Equation 3}$$

Colour coordinates of the films, L*, C_{ab}* (Equation 4) and h_{ab}* (Equation 5) from the CIELAB colour space were determined, using D65 illuminant and 10º observer and taking into account R_∞ (Equation 6) which correspond with the reflectance of an infinitely thick layer of the material.

$$C_{ab}{*} = \sqrt{a{*}^2 + b{*}^2} \qquad \text{Equation 4}$$

$$h_{ab}{*} = arctg\left(\frac{b*}{a*}\right) \qquad \text{Equation 5}$$

$$R_\infty = a - b \qquad \text{Equation 6}$$

Finally, to evaluate the colour differences between the different films and control film, Equation 7 was used.

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{Equation 7}$$

2.4.5. Scanning Electron Microscopy (SEM)

Microstructural analysis of the films was carried out by SEM using a scanning electron microscope (Hitachi S-4800, Japan). Film samples were maintained in a desiccator with P_2O_5 for two weeks to ensure that no water was present in the sample. Then, films were frozen in liquid N_2 and cryofractured with a pre-chilled razor to observe the cross-section of the samples. Fractured film pieces were then mounted on a SEM tube and observed using an accelerating voltage of 10 kV.

2.4.6. Microbiological analysis

To perform the microbiological analysis, a modification of the method proposed by Kristo, Koutsoumanis & Biliaderis (2008) was considered. *Listeria monocytogenes* (CIP 82110), supplied by the Collection Institut Pasteur (CIP, France), was regenerated (from a culture stored at -80 ºC) by transferring a loopful of bacteria into 10 ml of Tryptone Soy Broth-Yeast Extract, (TSB-YE, Biokar Diagnostics, Beauvais, France) and incubating at 37 °C overnight. Subsequently, a 10 µl aliquot from the overnight culture was then transferred to 10 ml of TSB-YE and grown at 37 °C until the end of the exponential phase of growth. This culture, appropriately diluted, was then used for inoculation of the agar plates in order to obtain a target inoculum of aproximately 10^3 CFU/cm^2. Tryptone soy agar (TSA, Biokar Diagnostics, Beauvais, France) was used as a model solid food system. Aliquots of TSA (20 g) were poured into Petri dishes. After the culture medium solidified, diluted overnight culture was inoculated on the surface.

The different test films of the same diameter as the Petri dishes (containing or not nanoliposomes) were placed on the inoculated surface. Inoculated and uncoated

TSA Petri dishes were used as control. Petri dishes were then covered with parafilm to avoid dehydration and stored for 7 days at 10 °C.

Microbial counts on Palcam agar base (Biokar Diagnostics, Beauvais, France) plates were examined immediately after the inoculation and after 1, 4 and 7 days of storage. To this end, the agar was removed aseptically from Petri dishes and placed in a sterile plastic bag with 100 ml of Tryptone salt broth (Biokar Diagnostics, Beauvais, France). The bag was then homogenized for 150 s in a Stomacher blender 400 (Interscience, Saint-Nom-La-Breteche, France). Serial dilutions were made and poured onto Palcam agar base. The dishes were incubated during 24 h at 37 °C before colonies were counted. All tests were performed in duplicate.

2.5. Statistical Analysis

Statgraphics Plus for Windows 5.1 (Manugistics Corp., Rockville, MD) was used for carrying out statistical analyses of data through analysis of variance (ANOVA). Fisher′s least significant difference (LSD) was used at the 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Characteristics of nanoliposomes

3.1.1. Fatty acids analyses

The main fatty acid composition is shown in Table 1. The percentage of total polyunsaturated fatty acids was the highest in soy lecithin. The high proportions of fatty acids were C18:2 n-6 (52.27 %), found in the polyunsaturated fatty acids class, C18:1 n-9 (21.49 %) in the monounsaturated fatty acids class and C16:0 (17.07 %) in the saturated fatty acids class for soy lecithin. The largest amount of fatty acid was a monounsaturated fatty acid, in regards to rapeseed lecithin, the percentage of C18:3n-3 (6.60 %) was important in the polyunsaturated fatty acids class. The fatty acid most present was C18:1n-9 (56.51 %) found in the monounsaturated fatty acids class.

Table 1. Main fatty acids composition of rapeseed and soy lecithins.

Fatty acids	**Rapeseed lecithin**		**Soy lecithin**	
	%	**SD**	**%**	**SD**
C14	-	-	-	-
C15	-	-	-	-
C16	7.41	0.01	17.07	0.48
C17	-	-	-	-
C18	1.31	0.00	3.32	0.16
C20	0.36	0.01	-	-
C21	-	-	-	-
C22	0.21	0.02	0.43	0.04
C23	-	-	-	-
SFA	**9.29**		**20.82**	
C15:1	-	-	-	-
C16:1	0.33	0.01	-	-
C17:1	-	-	-	-
C18:1n9	56.51	0.04	21.49	0.47
C20:1n11	0.72	0.04	-	-
C22:1n9	0.25	0.03	-	-
MUFA	**57.81**		**21.49**	
C18:2n6	26.32	0.04	52.27	0.36
C18:2n3	6.60	0.01	5.41	0.04
C20:2n6	-	-	-	-
C20:3n6	-	-	-	-
C20:3n3	-	-	-	-
C20:4n6	-	-	-	-
C20:5n3 (EPA)	-	-	-	-
C22:4n6	-	-	-	-
C22:5n3	-	-	-	-
C22:6n3 (DHA)	-	-	-	-
PUFA	**32.92**		**57.68**	

3.1.2. Lipid classes

The lipid classes of lecithins were separated by thin-layer chromatography (Iatroscan). Moreover, the percentage of triacylglycerols (TAG) contained in lecithins were respectively 37.75±0.1 and 18.15±0.2% for rapeseed and soy lecithins. However, the percentage of polar fraction showed that soy lecithin was richer in polar lipids with 84.76 ±0.6% which was 62.26±0.8% for rapeseed lecithin.

3.1.3. Size of nanoliposomes

The size analysis of nanoliposomes is of interest because of its impact on different properties of the films and its stability and capacity to release the entrapped compounds in the liposome core. Different authors (Pérez-Gago & Krochta, 2001; Bravin, Peressini & Sensidoni, 2004) have related the particle size of lipids in the film forming emulsions with different properties of the films such as mechanical or barrier properties. Low particle size is generally desired since small particles increase the tortuosity of the structure thus improving the barrier capacity and provides a more homogeneous structure. The particle size of nanoparticles (or nanoliposomes) has to be controlled since they can be toxic for humans and for the environment. In this sense, different authors estimated the toxicity of different nanoparticles such as silver nanoparticles (Lankveld, Oomen, Krystek, Neigh, Troost-de Jong, Noorlander, Van Eijkeren, Geertsma, De Jong, 2010), TiO_2 nanoparticles (Clément, Hurel, Marmier, 2013) or ZnO nanoparticles (Hsiao & Huang, 2011). Unfortunately, there are no works concerning the toxicity of active compounds loaded-nanoliposomes.

The mean particle diameter of rapeseed and soy nanoliposomes without and with antimicrobial compounds is showed in Table 2. The obtained values are in the same order as those found by Zhang *et al*. (2012) and differences may be related with the different time of sonication. They obtained stable nanoliposomes by using

180 s of sonication whereas 300 s of sonication were necessary to incorporate completely the antimicrobials into the nanoliposomes in this case.

Table 2. Particle size, electrophoretic mobility and surface tension of aqueous nanoliposomes solutions.

	Particle size (nm)	**µE (µm·cm/V·s)**	**ST (mN·m^{-1})**
Rap	146 (1)^{a}	-3.21 (0.01)^{a}	26.8 (0.5)^{a}
Rap-lim	150 (3)^{a}	-3.36 (0.03)^{b}	27.7 (0.2)^{ab}
Rap-oil	148 (2)^{a}	-3.31 (0.02)^{b}	27.9 (0.4)^{b}
Soy	188 (5)^{1}	-3.89 (0.07)^{1}	31.6 (0.6)^{1}
Soy-lim	175 (5)^{2}	-3.98 (0.03)^{12}	28.6 (0.7)^{2}
Soy-oil	159 (5)^{3}	-3.99 (0.02)^{2}	28 (1)^{2}

a-c: Different superscripts within the same column indicate significant differences among formulations containing rapeseed nanoliposomes ($p < 0.05$).
1-3: Different superscripts within the same column indicate significant differences among formulations containing soy nanoliposomes ($p < 0.05$).

Size of rapeseed nanoliposomes ranged between 146 and 150 nm without significant differences between them. However, soy nanoliposomes showed different sizes depending on the core compounds. Antimicrobial loaded-nanoliposomes showed lower sizes. The addition of hydrophobic compounds seemed to favour the compactness of soy nanoliposomes by improving the orientation of amphiphilic molecules of soy lecithin through the interactions with the oil compounds. Previous studies (Zhang *et al.*, 2012) showed the formation of vesicles for the major part of lecithin molecules (soy and rapeseed) with some remanent droplets, when applying sonication in water dispersion in similar conditions. So, the formation of vesicles can be expected in this case, although the incorporation of the essential oil or limonene could imply the formation of a different structure due to the change in the balance of the interaction forces in the mixture. Spherical micelles could entrap in their core the incorporated non-polar compounds and a reduction in their size can occur.

3.1.4. Electrophoretic mobility

The electrophoretic mobililty values of soy and rapeseed nanoliposomes containing solutions are shown in Table 2. The study of the surface charge of the particles is of interest since it affects the stability of the nanoliposomes, specially in the studied solutions in which the viscosity is too low. The electrophoretic mobility of nanoliposomes containing solutions ranged between -3.21 and -3.36 $\mu m \cdot cm \cdot V^{1} \cdot s^{-1}$ for rapeseed nanoliposomes and between -3.89 and 3.99 $\mu m \cdot cm \cdot V^{1} \cdot s^{-1}$ for soy nanoliposomes. These values are in agreement with values reported by Zhang *et al*. (2012). According to Arab Tehrany, Kahn, Baravian, Maherani, Belhaj, Wang & Linder (2012), rapeseed and soy lecithins contain different type of phospholipids such as phosphatidylserine, phosphatidic acid, phosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and phosphatidylethanolamine. These components are negatively charged at neutral pH (except phospatidylcholine which is not charged) thus being responsible of the negative electrophoretic mobility of liposomes (Chansiri, Lyons, Patel & Hem, 1999). The incorporation of the essential oil and limonene slightly increase the particle charge which agrees with the induced changes in the micellar structure.

3.1.5. Surface tension of nanoliposomes dispersions

Lecithins were choosen since its amphiphilic nature allows to incorporate the hidrophobic antimicrobials into the hydrophilic starch-sodium caseinate dispersions. Due to its low molecular weight, these surfactants migrate rapidly to the small terpen droplets that are formed during sonication thus preventing coalescence and flocculation (McSweeney, Healy & Mulvihill, 2008). Surface tension values of rapeseed and soy nanoliposomes containing solutions are showed in Table 2. As expected, the surface tension of nanoliposomes solutions was lower in comparison with pure water whose surface tension is 72 $mN \cdot m^{-1}$ (Walstra, 2003). The obtained values demonstrate the ability of lecithins to form stable nanoliposomes with and without antimicrobials in the aqueous media. Differences in the surface tension values of the dispersions of rapeseed and soy nanoliposomes were found. The surface tension of rapeseed nanoliposomes was lower than for soy nanoliposomes containing solution. This fact can be associated to the total content of polyunsaturated fatty acids (PUFA) with different surface activity. In this sense, Zhang *et al*. (2012) found that the content of PUFA was higher in soy lecithin than in rapeseed lecithin and Leshem, Landau & Deutsch (1988) related the presence of unsaturations with an important effect on the surface tension. They explained that for a fixed surfactant monolayer area in a completely expanded state, an increase in the number of cis-double bonds cause an increase in the surface tension, in agreement with results found in this work.

The inclusion of orange essential oil and D-limonene did not produce any difference in surface tension for rapeseed nanoliposomes. However, the addition of these compounds significantly reduced the surface tension of soy nanoliposomes containing solutions. This indicates that the addition of these compounds affects the critical micellar concentration and the corresponding minimal surface tension of the soy lecithin. The lower value of the surface tension of rapeseed lecithin with respect to the soy lecithin could be explained, as commented on above, by its lower concentration of PUFA

3.2. Characterization of the film forming dispersions

3.2.1. Surface tension

Table 3 shows the values of the surface tension of all film forming dispersions under study. The presence of the protein in combination with starch remarkably reduced the surface tension of water (72 mN·m^{-1} at 25 °C; Walstra, 2003) to 51.1 mN·m^{-1} as it can be observed for control formulation. This effect is due to the amphiphilic nature of caseinate and is in agreement with the results found by Fabra, Jiménez, Atarés, Talens & Chiralt (2009). The analysis of the surface tension of film forming dispersions are of interest, specially in the food industry, since low values of surface tension would favour the coating of products (Fernández, Díaz de Apodaca, Cebrían, Villarán & Maté, 2006). In the case of film forming dispersions containing nanoliposomes, the surface tension was always lower as compared with the control sample as it was expected by the action of surfactants. Nevertheless, the values did not reach those obtained in the aqueous nanoliposome dispersions, which indicates that protein is present to a great extent in the water-air interface and no total substitution of this occurred when nanoliposomes were added. No notable differences were found between the different formulations with and without antimicrobials, except for the film forming dispersion with soy-orange oil nanoliposomes where the lowest surface tension was obtained. The greater migration of surfactant to the sample surface seems to occur, thus decreasing the surface tension to a greater extent. This fact could be related with a lower stability of liposomes in this case.

Table 3. Surface tension and rheological properties of film forming dispersions.

	Newtonian Viscosity (·10^3, Pa·s)	**n**	**K (·10^3, Pa·s^n)**	**ST (mN·m^{-1})**
Control	5.7 (0.1)^{ab1}	1.27 (0.01)^{a1}	1.47 (0.07)^{a1}	51.1 (0.1)^{a1}
Rap	5.8 (0.2)^{a}	1.24 (0.01)^{b}	1.75 (0.14)^{b}	44.9 (0.7)^{b}
Rap-lim	5.40 (0.2)^{b}	1.25 (0.01)^{ab}	1.5 (0.1)^{a}	43.8 (1.7)^{b}
Rap-oil	5.7 (0.1)^{ab}	1.24 (0.01)^{b}	1.75 (0.06)^{b}	43.6 (1.4)^{b}
Soy	5.62 (0.04)^{12}	1.26 (0.01)^{1}	1.52 (0.07)^{1}	44.6 (1.2)^{2}
Soy-lim	5.5 (0.1)^{12}	1.25 (0.01)^{1}	1.5 (0.1)^{1}	46 (1)^{2}
Soy-oil	5.4 (0.2)^{2}	1.25 (0.01)^{1}	1.6 (0.2)^{1}	42.0 (0.3)^{3}

a-b: Different superscripts within the same column indicate significant differences among formulations containing rapeseed nanoliposomes ($p < 0.05$).

1-3: Different superscripts within the same column indicate significant differences among formulations containing soy nanoliposomes ($p < 0.05$).

3.2.2. Rheological behaviour

The study of the rheological behaviour of film forming dispersions is of interest to have information about the fluid structure and interactions between particles during flow. The analyses of the rheological behaviour of the film forming dispersions were carried out at 25 ºC with a shear rate between 0 and 1000 s^{-1}. All formulations showed newtonian behaviour at low shear rates and a shear thickening or dilatant character from a determined shear rate. Furthermore, all samples showed non-time dependent behaviour since up and down curves coincided. The change in the rheological behaviour as the shear rate increases can be related with particles (starch and sodium caseinate chains and vesicles) aggregation due to orthokinetic flocculation (Peker & Helvaci, 2007). The aggregates would present sufficient cohesive forces to withstand the shear stress, thus producing shear thickening behaviour (Christianson & Bagley, 1983). The water content entrapped in these aggregates would increase, leading to a greater flow resistance.

The viscosity of studied film forming dispersions in the newtonian domain are showed in Table 3. The obtained viscosities are low, in agreement with the polymer concentrations used, thus indicating that no gels were formed during the film forming dispersions preparation. Although starch-sodium caseinate interactions can take place in determined conditions (Jiménez *et al*., 2012c), in this case these not lead to a gel formation. No significant differences were found among the Newtonian viscosity values of the different formulations, despite the different total solid contents. In this sense, it is remarkable that the composition of continuous phase is the same in all cases and the volume concentration of the nanoliposomes is relatively low to affect notably the sample viscosity.

The change from newtonian to shear thickening behaviour took place at a shear rate ranging between 238-291 s^{-1}, regardless the type of sample. From these shear rate values, the experimental data were fitted to the Ostwal-de-Waele model (power law). The flow behaviour index (n) and the consistency index (k) of film forming dispersions are shown in Table 3. No signficant differences in n values, were found for the different samples; these values being higher than 1, as corresponds to dilatant fluids and the consistency index was also similar for all formulations.

In conclusion, small differences were found between the different film forming dispersions concerning their rheological behaviour and only a decrease of their surface tension was observed for those containing lipids due to the surfactant action of lecithin.

3.3. Characterization of the films

3.3.1. Structural and mechanical properties

Despite the small size of liposomes obtained in aqueous dispersion, when they are incorporated into the film forming dispersions, the changes in the aqueous environment and the establishment of interactions, mainly between surfactants and proteins (Erickson, 1990), can promote significant changes in the structure of lipid particles. In fact the mean size of particles increased when liposomes were incorporated in the film forming dispersions and it could not be measured with the available equipment because they were out the measure range.

A positive aspect of the essential oil incorporation as nanoliposomes was the inhibition of the oil evaporation during the film drying step, which supposes a decrease of the film thickness by the loss of solids, when a constant of solids per surface area was poured in the plate to obtain de film. This has been previously observed in previous works (Sánchez-González *et al*. 2010ab) and supposes the loss of potentially active compounds of the film. Table 4 shows the values of the film’s thickness, where the increase of this parameter when nanoliposomes were incorporated can be observed, on the contrary that occurs when free essential oil was incorporated in the film. This increase confirms that, not only essential oil was not evaporated but also that the arrangement of the polymer chains with lipids is more open probably due to a different coupling of the components on the basis of the developed interactions.

Table 4. Mechanical properties and colour of the obtained films.

	EM (MPa)	TS (MPa)	E (%)	Thickness (µm)
Control	1930 (212)^{a1}	27.6 (1.5)^{a1}	2.9 (0.5)^{a1}	55 (8)^{a1}
Rap	1309 (211)^{b}	8.0 (0.7)^{b}	0.8 (0.2)^{b}	63 (8)^{a}
Rap-lim	657 (105)^{c}	7.8 (0.4)^{b}	2.0 (0.2)^{c}	76 (11)^{b}
Rap-oil	909 (193)^{c}	7.1 (1.8)^{b}	1.0 (0.3)^{b}	89 (12)^{c}
Soy	875 (141)^{2}	9.6 (1.9)^{2}	1.7 (0.6)^{2}	66 (7)^{2}
Soy-lim	996 (133)^{2}	11 (2)^{2}	1.8 (0.6)^{2}	71 (11)^{2}
Soy-oil	863 (77)^{2}	9.6 (0.3)^{2}	1.7 (0.1)^{2}	81 (9)^{3}

	L*	C_{ab}*	h_{ab}*	ΔE
Control	88 (2)^{a1}	8.8 (1.3)a^{1}	95.4 (1.2)^{a1}	--
Rap	74.5 (1.0)^{b}	32.5 (0.6)^{b}	82.6 (0.2)^{b}	27.4
Rap-lim	74.6 (1.1)^{b}	32 (1)^{b}	83 (1)^{b}	26.9
Rap-oil	74 (2)^{b}	33 (1)^{b}	82.7 (1.3)^{b}	28.9
Soy	75 (1)^{2}	33.0 (0.5)^{2}	79.4 (0.2)^{2}	27.8
Soy-lim	77.8 (0.5)^{2}	30.8 (0.8)^{3}	81 (1)^{23}	24.5
Soy-oil	75.9 (0.8)^{2}	31 (1)^{23}	82 (1)^{3}	25.6

EM: Elastic modulus; TS: Tensile strength; E: Elongation at break. L*: Lightness, Cab*: Chroma; hab*: Hue; ΔE: Colour difference in comparison with control film.

a-c: Different superscripts within the same column indicate significant differences among formulations containing rapeseed nanoliposomes ($p < 0.05$).

1-3: Different superscripts within the same column indicate significant differences among formulations containing soy nanoliposomes ($p < 0.05$).

Figure 1 shows the SEM micrographs of the cross section of control film and those containing liposomes. Control film showed a quite homogeneous structure, but coarser than that obtained by Jiménez *et al.* (2012c) for films with the same composition. In this work, the authors prepared the film forming dispersions by applying a homogenization step, using a rotor-stator equipment, at 95ºC.

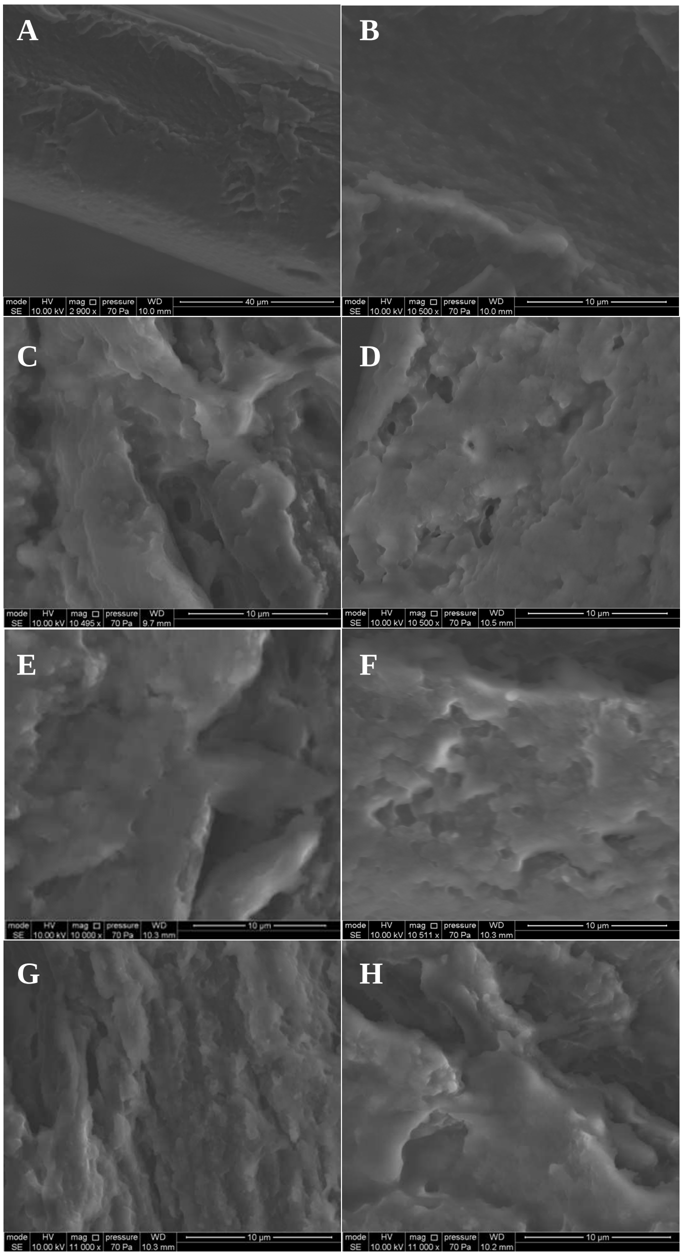

Figure 1. SEM micrographs of the cross-sections of the films. (A-B: Control, C:Rap, D: Rap-lim, E: Rap-oil, F: Soy, G: Soy-lim, H: Soy-oil).

The high temperature and the shear stress promoted denaturation of the protein and the interaction between polymers, which favours the formation of a more homogenous blend.

When control samples are compared with those containing lipids, a much coarser structure is observed for the latter, which agrees with the increase of size of nanoliposomes when incorporated to the film forming dispersions (data not showed) and the possibly progress of this increase during the film drying step. During this step, as the water of the system is being removed phase transitions occurs in the lipid association structures which may promote the break of liposomes and the re-restructuration of the molecule association, even giving rise to inverted structures (Krog, 1990; Larsson, K., & Dejmek, 1990). In fact voids of different sizes can be observed in the matrix, which can be associated to the presence of the lipid droplets interrupting the matrix continuity in a size higher than nano-scale.

From the analysis of the stress-Hencky strain curves, elasticity modulus (EM), tensile strength (TS) and elongation at break (E) were determined for each film sample. According to McHugh & Krochta (1994), these parameters are very useful for describing the mechanical properties of a film, and are closely related with its internal structure. Table 4 shows the obtained values for each sample. Film without liposomes presented the highest EM value, in comparison with nanoliposome containing films. The addition of nanoliposomes introduces discontinuities in the matrix, as commented on above, which affects significantly the mechanical resistance of films. The same behaviour is observed for TS values. Considering the EM and TS values of control film it is remarkable that there is a great difference between these values and those obtained obtained by Jiménez *et al*. (2012c) using the same formulation starch-sodium caseinte, but by applying a heat-homogenization step before the film casting. This could provoke a reduction the mechanical resistance and extensibility of the obtained structure.

Among films containing nanoliposomes, for rapeseed liposomes, elastic modulus significantly decreased when essential oil or limonene are present in the film which could be due to a different release of this compounds in the matrix from liposomes.

As concerns extensibility of the films (E), these can be considered few extensible, in comparison with other films in which starch were blended with other polymers (Phan The, Debeaufort, Voilley & Luu, 2009; Jiménez *et al*., 2012bc). This low extensiblity can be related with the kind of structure generated where the slippage of the chains during the film stretching is more difficult. When heat-homogenization is was applied to starch-sodium caseinate films with the same composition, extensibility is almost 2.5 times higher probably due to the heat induced unfolding of proteins and the more linear entanglement of the chains in the matrix. In this work the heat-homogenization step was not applied to avoid the rupture of nanoliposomes, thus losing the active compounds.

3.3.2. Optical properties

Spectral distribution curves of Ti parameters are plotted in Figure 2. In general, high values of Ti are associated with greater film homogeneity, which gives rise to more transparent films. On the contrary, lower values of Ti are related with a higher opacity of the films. As observed in Figure 2, control film was the most transparent with high values of Ti, in agreement with that reported in previous works for starch and sodium caseinate (Fabra *et al*., 2009; Jiménez *et al*., 2012a). The addition of nanoliposomes decreased the transparency of films regardless the type of lecithin and the antimicrobial mainly at low wavelength. This fact is due, in part, to the natural brown colour of lecithins. This produces the absorption of the blue and green light (low wavelength) thus giving rise to a yellow-brown colour in the films. The presence of a dispersed phase in the matrix also contributes to the decrease in the Ti values.

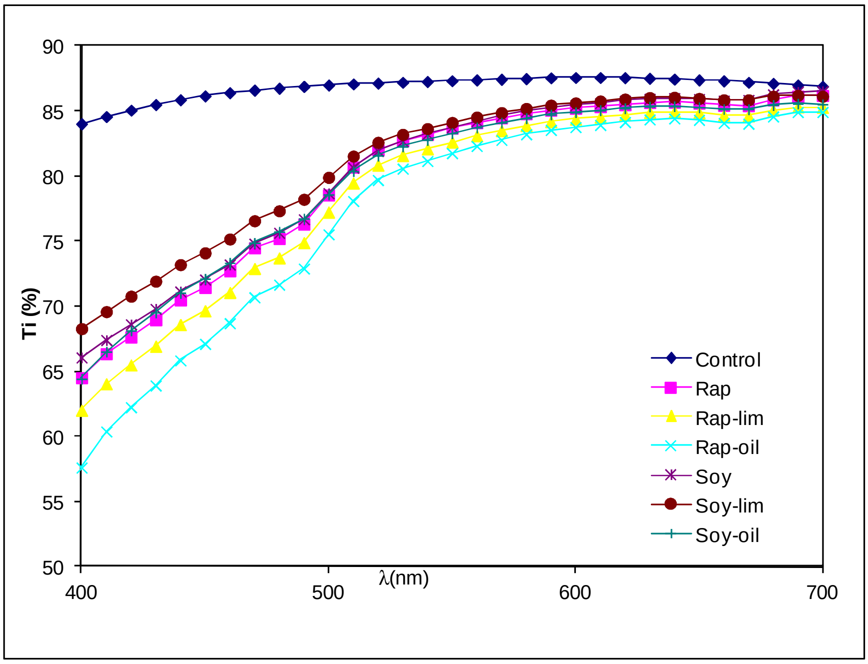

Figure 2. Spectra of internal transmitance (Ti) of obtained films.

The colour of films as a consequence of nanoliposomes addition, is shown in Table 4, in terms of lightness (L*), chroma (C_{ab}*) and hue (h_{ab}*) parameters, for each formulation. L* and C_{ab}* values for control film varied significantly by the incorporation of nanoliposomes. Incorporation of nanoliposomes provoked a decrease the lightness and the hue and an increase of chroma, due to the colour of lecithins. The film colour become more vivid a redder by the action of lecithin liposomes. To estimate colour differences, ΔE were calculated between control film and the films containing nanoliposomes. These values ranged between 25 and 29, thus indicating that there is a relevant difference of colour between films. Nevertheless no notable differences were found among in films containing nanoliposomes since there are no significant differences in their colour parameters.

3.3.3. Fourier transform infrared spectroscopy

Figure 3A shows the FTIR spectra of the films without and with nanoliposomes and Figure 3B the corresponding spectra of each one of film components, in order to compare the main characteristics peaks of the different components and films. The broad band located at 3300 cm^{-1} corresponds with vibration modes of OH-groups from the absorbed water (García, Famá, Dufresne, Aranguren & Goyanes, 2009) and from the polymers themselves (Bourtoom & Chinnan, 2008; Pereda, Amica, Rácz & Marcovich, 2011). The peaks located at 2854 and 2923 cm^{-1} are related with vibration of $–CH_2$ and $–CH_3$ groups (axial carbon-hydrogen bond) (Zhang *et al.*, 2012).

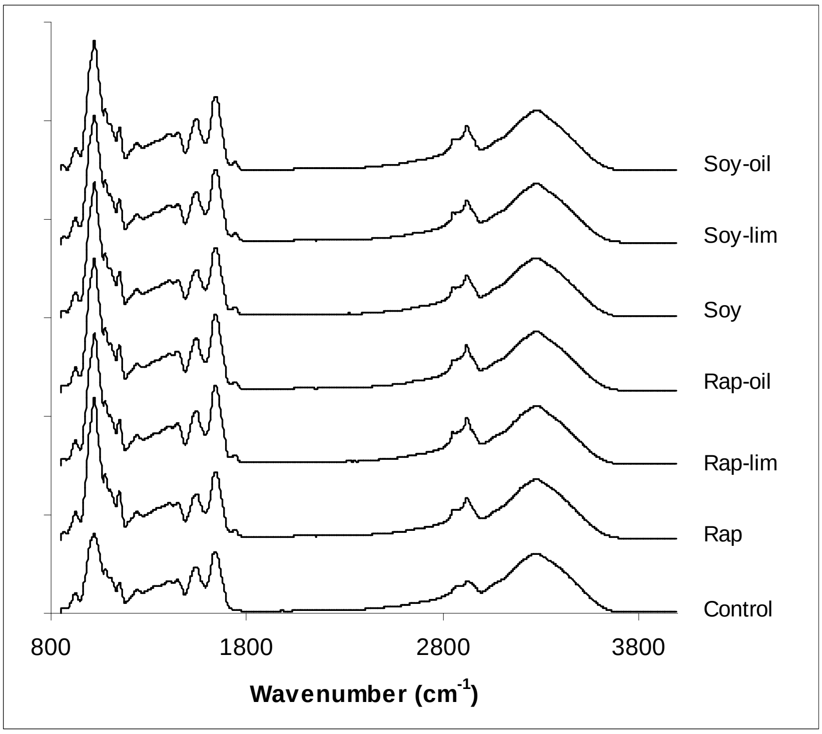

Figure 3A. FTIR spectra of the studied films.

The band at 1690-1590 cm^{-1} corresponds to the amide I vibrations, which is common to proteins (Pereda, Aranguren & Marcovich, 2008; Pereda *et al.*, 2011), as can be seen in Figure 3B for sodium caseinate. Other main peak observed in Figure 3A, which has been associated with C-O stretching vibrations (Zhang *et al.*, 2012), appeared at 1022 cm^{-1}.

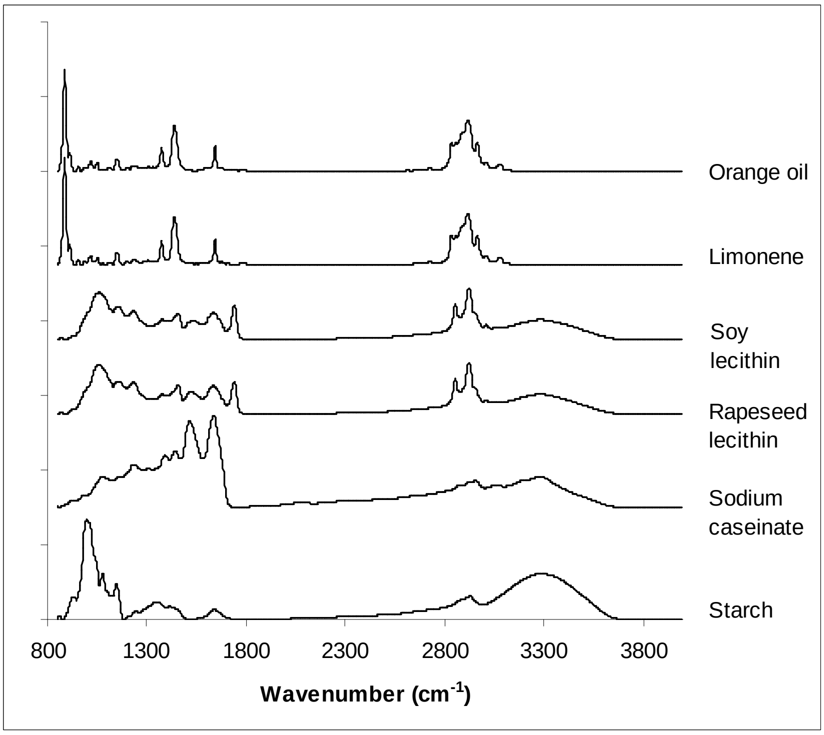

Figure 3B. FTIR spectra of isolated components of the films.

FTIR spectra of the films were very similar, as correspond to their similar composition. Nevertheless, some differences can be drawn. The main difference is the higher intensity of the peak at 1022 cm^{-1} in films containing nanoliposomes in comparison with the control. As can be obseved, in Figure 3B, lecithins presented a broad band around this wavenumber, thus explaining the greater intensity observed in Figure 3A for films containing nanoliposomes. The intensity of peaks at 2854 and 2923 cm^{-1} also increased with nanoliposomes addition, in agreement with spectra observed in Figure 3B for lecithins and antimicrobial compounds. For films with nanoliposomes, Figure 3A also shows a little peak at 1746 cm^{-1} which correspond to the C=O stretching (Tantipolphan, Rades, McQuillan & Medlicott, 2007). This group is located between hydrophobic tails and hydrophilic head group of the lecithin molecule. Nevertheless, no displacement of peaks in the film spectra with respect to the characteristic wavelength found for each isolated compound was observed, which indicates that no specific interactions among components can be detected from FTIR spectra.

3.3.4. Antimicrobial activity against *Listeria monocytogenes*

Figures 4A and 4B show the growth curves of *Listeria monocytogenes* on TSA medium without film and on those coated with the different films. Bacteria population increases from 3 to 8 logs CFU/cm^2 at the end of the storage period. The slightly greater microbial growth in plates coated with the different films than in uncoated one can be observed in Figures 4A and B. This indicates that they did not have antimicrobial activity, as expected for starch-NaCas film (control), while contribute to the bacteria nutrients as a consequence of its composition (protein and starch). The incorporation of nanoliposomes did not improve the antimicrobial capacity of films, regardless the type of lecithin and the potentially antimicrobial compound. Only orange oil-soy lecithin nanoliposomes containing film seemed to present a little activity at the end of the storage (day 7). In this case, some more days of analysis would be necessary to evaluate if there is a significant antimicrobial activity. These results could be attributed to the encapsulation of the active compounds in liposomes, which inhibit their release to the plate surface and to a low antimicrobial activity of limonene and orange essential oil.

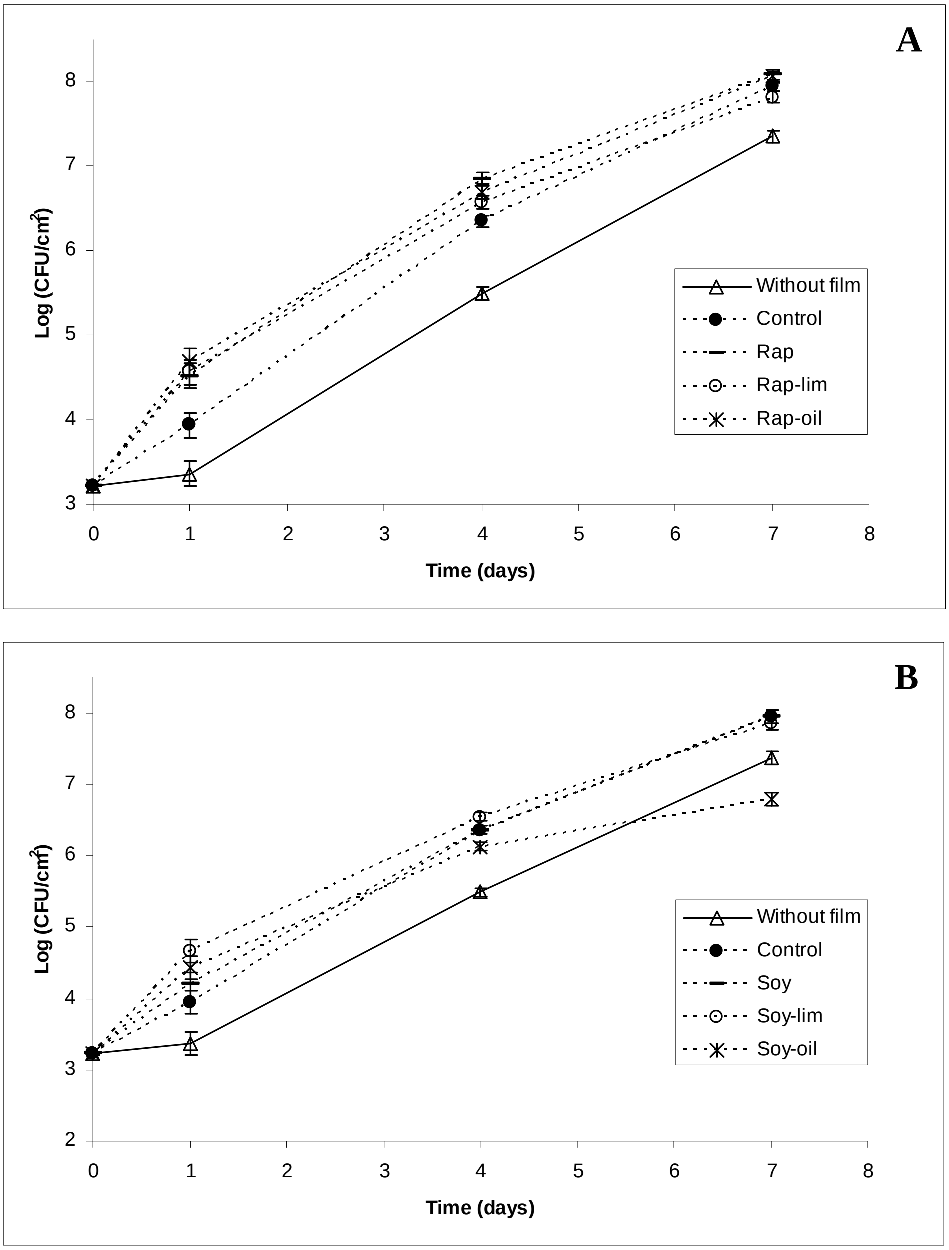

Figure 4. Microbial counts as a function of time for samples without films and coated with the different films containing rapeseed liposomes (A) and soy liposomes (B).

Similar results were reported by Imran (Imran, 2011) since he found that the release of bioactive compounds entrapped onto nanoliposomes is a relatively long process. Moreover this study revealed that nanoliposome composition is an important factor to take into account to control the release of active compounds. In addition, Imran, Revol-Junelles, René, Jamshidian, Akhtar, Arab-Tehrany, Jacquout & Desobry (2012) observed that the highest antilisterial activity corresponded with films containing both free and encapsulated antimicrobial compound. This result demonstrates that at initial time it is necessary a little amount of free bioactive compound to avoid microbial growth until it was released from the nanoliposomes. Concerning differences observed between antilisterial activity of films with limonene and orange oil nanoliposomes, previous studies reported also a greater effectiveness of the essential oils in terms of antimicrobial activity than the mix of the major components or pure terpens (Gill *et al*. 2002; Mourey & Canillac 2002). Minor components therefore play an important role, and synergism phenomena occur.

4. CONCLUSIONS

The incorporation of potentially antimicrobial volatile compounds (orange essential oil and limonene) to starch-sodium caseinate blend films was carried out in a effective way to avoid the losses of volatile compounds during the film drying step. Nanoliposomes of soy and rapeseed lecithins were obtained by sonication of their water dispersions. Incorporation of the essential oil and limonene to the liposomes was also effective by using the same method. The addition of lipids in the polymeric matrix supposed a decrease of the mechanical resistance and extensibility of the films. The natural colour of lecithin conferred a loss of lightness, a chroma gain and a redder hue to the films, which were also less transparent than the control one, regardless the lecithin and volatile lipid considered. The possible antimicrobial activity of the films containing orange essential oil or limonene was not observed, which could be due to their low antilisterial activity or to the inhibition effect of the encapsulation which difficult their release from the matrix.

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IV. CONCLUSIONES

- La incorporación de ácidos grasos en los films de almidón y glicerol no mejoró notablemente la capacidad barrera de los films frente a la transferencia de vapor de agua y solo los ácidos grasos saturados la mejoraron ligeramente, pero sólo cuando los films no fueron almacenados. El grado de cristalinidad se incrementó con el tiempo de almacenamiento, lo que se tradujo en un aumento de la rigidez y fragilidad de los films y una disminución de su extensibilidad, brillo y transparencia. La presencia de ácidos grasos influyó significativamente en este fenómeno, formándose estructuras cristalinas tipo V para la amilosa y cristales de los propios lípidos que afectaron también a las propiedades de los films. En consecuencia, no se obtuvo la mejora esperada en los films de almidón y se ve necesario probar otros compuestos orgánicos no polares u otros biopolímeros para este fin.

- La capacidad de adsorción de agua de los films de almidón se vio afectada por la cristalización, que depende de la temperatura y la humedad relativa de equilibrio. La cristalización del almidón estuvo promovida a baja temperatura y alta humedad relativa, modificando las tendencias esperadas en la capacidad de sorción de agua en función de la temperatura. La temperatura de transición vítrea de la matriz de almidón a baja humedad disminuyó con la adición de ácidos grasos saturados, pero no con la adición de ácido oleico. La presencia de ácidos grasos favoreció la formación de estructuras cristalinas tipo V, indicando la formación predominante de complejos amilosa-lípido e inhibiendo la formación de otro tipo de estructuras. La intensidad de los picos del espectro de difracción de rayos X disminuyó con el aumento de la humedad, observándose que la formación de estructuras tipo V tuvo lugar principalmente a bajas humedades relativas. El comportamiento mecánico de los films de almidón y ácidos grasos resultó coherente con los resultados de calorimetría diferencial de barrido y las observaciones microestructurales. El efecto plastificante del agua y las discontinuidades estructurales determinaron los valores de módulo de elasticidad y comportamiento en la rotura. La incorporación de ácidos grasos redujo en todos los casos la resistencia mecánica de los films, independientemente de la humedad de equilibrio, aunque los films con ácido oleico fueron más extensibles a baja humedad.

- La adición de hidroxipropilmetilcelulosa (HPMC) en matrices de almidón dio lugar a estructuras más amorfas tal y como se observó por difracción de rayos X. Sin embargo, las micrografías mostraron separación de fases en los films que provocó una disminución de su brillo. El módulo de elasticidad de los films *composite* no almacenados disminuyó en comparación con los films de almidón y HPMC puros, aunque la tensión y la deformación en la fractura mejoraron ligeramente en comparación con el film de almidón puro. Estos mostraron similar permeabilidad al vapor de agua que los film de almidón pero mayor permeabilidad al oxígeno, debido a la contribución negativa del HPMC. La microfluidización de las dispersiones formadoras de films no mejoró la integración de los dos polímeros, aunque si contribuyó a la inhibición de la retrogradación del almidón, dando lugar a films menos rígidos, sin afectar a su extensibilidad. La inhibición de la recristalización del almidón con el tiempo se consiguió en los films microfluidizados y no microfluidizados cuando la proporción almidón:HPMC fue de 1:1.

- La adición de caseinato sódico (NaCas) en matrices de almidón redujo el grado de cristalinidad de los films de almidón e inhibió su recristalización con el tiempo. Las mezclas de ambos polímeros dieron lugar a films menos rígidos y resistentes a la fractura pero más flexibles y deformables que los films de almidón, sin afectar a la permeabilidad al vapor de agua. La permeabilidad al oxígeno aumentó con cantidades crecientes de NaCas, de acuerdo con la mayor sensibilidad de la proteína a la transferencia de oxígeno. La incorporación de NaCas incrementó ligeramente la transparencia de los films y redujo el brillo, el cual disminuyó con el almacenamiento. La reorganización de las cadenas de polímero en la matriz durante el almacenamiento produjo un descenso de la resistencia mecánica, flexibilidad y brillo de los films *composite*. En general, los films preparados con mezclas de los dos polímeros mostraron mejores propiedades y aplicaciones potenciales que los films de almidón puro, debido a la inhibición de la cristalización del almidón, que implica cambios indeseables en el comportamiento mecánico de los films.

- La incorporación de lípidos (ácido oleico y α-tocoferol) en films *composite* de almidón y NaCas dio lugar a separación de fases debido a las diferentes interacciones de los lípidos con cada polímero (proteína anfifílica y almidón hidrofílico), aunque a partir del cambio en las propiedades mecánicas puede deducirse que tiene lugar una reorganización de los componentes en la matriz con el tiempo de almacenamiento. Después del almacenamiento, los films con y sin lípidos presentaron un comportamiento mecánico similar, aunque resultaron ser más extensibles los films con lípidos. La incorporación de lípidos afectó ligeramente a la permeabilidad al vapor de agua, dependiendo del tiempo de almacenamiento. Sin embargo, la adición de ácido oleico aumentó significativamente la permeabilidad al oxígeno. Este efecto era esperable también para el α-tocoferol, pero fue mitigado debido a la capacidad antioxidante de este compuesto, que afectó claramente a la actividad antioxidante de los films. Todos los films mostraron una transparencia similar siendo el brillo mayor cuando se incorporaron lípidos.

- La incorporación de agentes potencialmente anticrobianos (D-limoneno y aceite esencial de naranja) a films *composite* de almidón-NaCas se llevó a cabo eficazmente en forma de nanoliposomas para minimizar las pérdidas por evaporación durante el sacado del film. Se obtuvieron nanoliposomas de lecitina de colza y soja mediante sonicación de sus dispersiones acuosas. La incorporación de D-limoneno y aceite esencial de naranja en los nanoliposomas se llevó a cabo siguiendo la misma metodología. La adición de estos nanoliposomas en la matriz polimérica supuso un descenso de la resistencia mecánica y la extensibilidad de los films. El color natural de las lecitinas produjo una perdida de luminosidad y una ganancia de croma en los films, variando el tono de estos hacia anaranjados. Además disminuyó la transparencia en comparación con el control independientemente del tipo de lecitina y antimicrobiano considerado. La posible actividad antimicrobiana de los films con D-limoneno y aceite esencial de naranja no se observó, debido probablemente a su baja actividad antilisteria y la encapsulación, que podría afectar a su liberación.

V. ANEXOS

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REVIEW PAPER

Edible and Biodegradable Starch Films: A Review

Alberto Jiménez · María José Fabra · Pau Talens · Amparo Chiralt

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Abstract Mainly due to environmental aims, petroleum-based plastics are being replaced by natural polymers. In the last decades, starch has been evaluated in its film-forming ability for applications in the food packaging area. Characteristics of the starch film matrices, the film formation methods, and physicochemical properties of the starch films are reviewed in this paper. The influences of different components added in casting methods and thermoplastic processes have been also analyzed. Comparison of mechanical properties of newly prepared starch films and stored films reveals that the recrystallization phenomenon made the films more rigid and less stretchable. These effects can be inhibited by adding other polymers to the starch matrix. Other approaches to improve the starch films' properties are the reinforcement by adding organic or inorganic fillers to the starch matrix as well as the addition of functional compounds. In this way starch films have improved mechanical and barrier properties and can act as a bioactive packaging. Physicochemical properties of the starch films showed a great variability depending on the compounds added to the matrix and the processing method. Nevertheless, dry methods are more recommendable for film manufacturing because of the greater feasibility of the industrial process. In this sense, a better understanding of the nano and microstructural changes occurring in the matrices and their impact on the film properties is required.

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Introduction

Edible starch films and coatings are used for food protection to increase the shelf-life of the foodstuffs. Nowadays, a great number of research studies focus on solving the problems produced by plastic waste in order to obtain environmentally friendly material. To this end, several works study the possibility of substituting the petro-based plastics with biodegradable, low-cost materials with similar properties (Psomiadou et al. 1996; Mali et al. 2002, 2006; Famá et al. 2007; Jiménez et al. 2012). These biodegradable polymeric films offer an alternative packaging option which does not contribute to environmental pollution and is obtained from renewable sources (Lu et al. 2005; Tharanathan 2003; Yu and Chen 2009).

This review analyses the film-forming ability of starch and the physicochemical properties of these types of films, focusing on the way to obtain biodegradable starch films and the relationship between the structure and physicochemical properties. Other aspects related to the obtention of starch-based films or packages, such as the effect of the addition of other components (lipid, hydrocolloids, fillers, or active compounds) and applications of starch-based materials have been also reviewed.

Starch: a Biodegradable Polymer Matrix

Starch is one of the most abundant natural polysaccharide raw materials. It is a renewable resource, inexpensive, and

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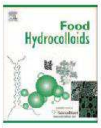

Effect of re-crystallization on tensile, optical and water vapour barrier properties of corn starch films containing fatty acids

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ABSTRACT

Starch films are very hygroscopic and undergo crystallization during processing and storage. In this work, fatty acids (SA, PA and OA) were incorporated into starch films containing glycerol as plasticizer, in order to reduce the hygroscopic character of the films and to improve water vapour permeability. Microstructural, mechanical, barrier and optical properties were studied in both non-stored films and those stored for 5 weeks. Fatty acid addition affected the main properties of films since mechanical resistance was decreased, water vapour permeability was reduced and gloss and transparency were reduced. The degree of starch and FA crystallinity increased with storage time and gave rise to changes in film properties: films became stiffer, less effective as water vapour barriers and less transparent and glossy. Saturated fatty acids were more efficient at reducing WVP as compared to oleic acid, but these differences decreased after storage due to the greater increase in crystallization of the former as it has been observed by X-ray diffraction.

1. Introduction

Starch is more and more commonly used to obtain polymer matrices for packaging due to its wide availability in many natural products and low cost (Mali & Grossmann, 2003; Rodríguez, Osés, Ziani, & Mate, 2006). Furthermore, it is known to be completely biodegradable in soil and water which is of great advantage from the environmental point of view (Kampeerapappun, Aht-ong, Pentrakoon, & Srikulkit, 2007). In starch biopolymers, physical and chemical changes, associated with retro-gradation, which occur during ageing have a great impact on the material properties (Delville, Joly, Dole, & Bliard, 2003) affecting the material functionality. Retro-gradation implies that starch molecules are re-associated into crystalline zones. Several works point out that initial crystallinity of starch films depends on the drying temperature and relative humidity (Liu, 2005) as well as the chain lengths, concentration of starch, pH and composition of the system. The development of the degree of crystallinity of starch films during storage (ageing) is greatly affected by the storage conditions, such as the temperature, relative humidity and time (Osés, Fernández-Pan, Mendoza, & Maté, 2009). For instance, if the storage temperature is higher than the glass transition temperature (T_g), starch matrix tends to crystallize. Thus, even though recently prepared starch films are almost amorphous (García, Martino, & Zaritzky, 2000a; Myllärinen, Buleon, Lahtinen, & Forssell, 2002; Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998), in time they develop crystallinity (García et al., 2000a). As a consequence, starch films usually become stronger, stiffer and less flexible (Forssell, Mikkilä, Moates, & Parker, 1997).

Similarly to many polysaccharide based films, pure starch films are too brittle and need the incorporation of plasticizers to make them easier to handle and achieve the optimum mechanical properties. Plasticizers reduce the cohesive forces in hydrocolloid films, through the limitation of intermolecular forces responsible for the chain-to-chain interactions, which allows this drawback to be overcome. So, plasticizers improve flexibility, elongation and toughness of films and they must be compatible with the film-forming polymer (Sothornvit & Krochta, 2005). In this sense, different polyols such as glycerol (Famá, Goyanes, & Gerschenson, 2007; Rodríguez et al., 2006; Talja, Helén, Roos, & Jouppila, 2007), xylitol (Talja et al., 2007) and sorbitol (Pagella, Spigno, & De Faveri, 2002; Talja et al., 2007) have been used in edible film formation. Nevertheless, given that polyols plasticize the polymer matrix, they can favour crystallization because of the increase in the polymer chain mobility. However, previous studies into yam starch films, did not report a significant effect of glycerol on the amylose re-crystallization throughout film storage (Mali, Grossmann, García, Martino, & Zaritzky, 2002). In thermoplastic starch (TPS), obtained by the extrusion of potato starch, an increase in crystallinity was obtained when the amount of glycerol was reduced in the

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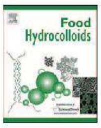

Phase transitions in starch based films containing fatty acids. Effect on water sorption and mechanical behaviour

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ABSTRACT

The water sorption capacity (WSC) of starch films containing, or not, glycerol (1:0.25 starch:glycerol ratio) and fatty (palmitic, stearic and oleic) acids (1:0.15 starch:fatty acid ratio) was analysed at 10, 25 and 40 °C in the entire range of a_w. Starch re-crystallization during the equilibration time was also analysed by X-ray diffraction. Likewise, the glass transition and lipid melting properties as a function of the film water activity were determined. Mechanical behaviour was also analysed at 25 °C for film samples equilibrated at 0, 53, 68 and 75% RH. Crystallization was promoted at low temperature and high relative humidity which modifies the expected tendencies in WSC as a function of the temperature. In relationship with this, V-type structures were formed during time, mainly in fatty acids containing films. Glass transition of the starch matrix at low moisture contents was affected by the presence of saturated fatty acids. Microstructural observations seem to corroborate the complex formation between these and the polymer chains. The mechanical behaviour of the films, as a function of the moisture content, is coherent with the results of the phase transition analysis and microstructural observations. The plasticization effects and the structural discontinuities in the polymer matrices determine the value of the elastic modulus and the behaviour at break.

1. Introduction

Starch alone cannot form films with satisfactory mechanical properties (high elongation at break and tensile strength), unless it is plasticized, blended with other materials, chemically modified or modified with a combination of these treatments (Liu, 2005). Generally, polyalcohols are the most common types of plasticizers for biodegradable films, including propylene glycol, glycerol, sorbitol and other polyols. There are many publications where various types of polyols were studied for their effects on the resulting films (Arvanitoyannis, Psomiadou, & Nakayama, 1996; Arvanitoyannis, Psomiadou, Nakayama, Aiba, & Yamamoto, 1997; Famá, Rojas, Goyanes, & Gerschenson, 2005; Parra, Tadini, Ponce, & Lugão, 2004; Psomiadou, Arvanitoyannis, & Yamamoto, 1996; Rodríguez, Oses, Ziani, & Maté, 2006). In most studies, starch based films are plasticized most effectively with glycerol, which has specific advantages. Moreover, the strength of the films has also been demonstrated to be dependent upon their moisture content, which is in turn influenced by the ambient relative humidity (Bertuzzi, Armada, & Gottifredi, 2003; Chang, Cheah, & Seow, 2000). So, knowing the water sorption equilibrium conditions of starch based films is useful to understand the performance of films under varying RH conditions. Different factors such as phase transition, like crystal formation, or phase separation can also affect the water sorption behaviour of polymers such as gelatinized starch.

Several works have focused on overcoming the inherent hydrophilicity of starch films, by surface esterification (Ren et al., 2010; Zhou, Ren, Tong, Xie, & Liu, 2009), surface photocrosslinking (Zhou, Zhang, Ma, & Tong, 2008) or blending starch with other hydrophobic materials, such as polycaprolactone and cellulose acetate (Averous, Moro, Dole, & Fringant, 2000; Fang & Fowler, 2003; Koenig & Huang, 1995). Other non-polar compounds, such as lipids, can also be combined with starch either by incorporating lipids in the hydrocolloid film-forming solution (emulsion technique) or by depositing lipid layers onto the surface of the pre-formed hydrocolloid film to obtain bilayers. Multi-component films have been extensively reviewed by Wu, Weller, Hamouz, Cuppett, and Schnepf (2002). Jiménez, Fabra, Talens, and Chiralt (2012) have recently studied the effect of fatty acid addition (by the emulsion technique) on physico-chemical properties and their changes during ageing, of starch based films. They observed that re-crystallization process in starch matrix during storage was not inhibited by the lipid addition which could modify water–starch relationships and phase transitions due to the crystal formation in the amorphous phase.

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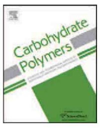

Influence of hydroxypropylmethylcellulose addition and homogenization conditions on properties and ageing of corn starch based films

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ABSTRACT

Edible films based on corn starch, hydroxypropyl methylcellulose (HPMC) and their mixtures were prepared by using two different procedures to homogenize the film forming dispersions (rotor-stator and rotor-stator plus microfluidizer). The influence of both HPMC-starch ratio and the homogenization method on the structural, optical, tensile and barrier properties of the films was analysed. The ageing of the films was also studied by characterizing them after 5 weeks' storage. Starch re-crystallization in newly prepared and stored films was analysed by means of X-ray diffraction. HPMC-corn starch films showed phase separation of polymers, which was enhanced when microfluidization was applied to the film forming dispersion. Nevertheless, HPMC addition inhibited starch re-crystallization during storage, giving rise to more flexible films at the end of the period. Water barrier properties of starch films were hardly affected by the addition of HPMC, although oxygen permeability increased due to its poorer oxygen barrier properties.

1. Introduction

Nowadays, many researchers are focusing their work on obtaining environmentally friendly materials which are able to protect food products from spoilage but also present an adequate biodegradability. In this way, traditional petroleum-based polymers, such as polyethylene or polypropylene, are being substituted by biopolymers obtained from natural and renewable sources. These polymers are mainly polysaccharides (starch, chitosan, cellulose and its derivatives) and proteins, such as gelatin, caseinates or zein, which are generally processed to obtain edible films or coatings. These structures are thin layers of edible materials applied to food products, which play an important role in their preservation, distribution and marketing (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). One of the most suitable polymers with which to substitute conventional plastics is starch due to the fact that it is able to present thermoplastic behaviour if an adequate amount of plasticizers is used. Starch is a well-known polysaccharide that presents different properties depending on its amylose/amylopectin ratio. Properties, such as glass transition temperature (Liu et al., 2010) or digestibility (resistant starch; Zhu, Liu, Wilson, Gu, & Shi, 2011), vary according to the amylose content. Their properties, as packaging material alone or in combination with other materials, have been widely studied (Bertuzzi, Armada, & Gottifredi, 2007; Chillo et al., 2008; Flores, Conte, Campos, Gerschenson, & Del Nobile, 2007; Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011; Phan The, Debeaufort, Luu, & Voilley, 2005; Tang, Alavi, & Herald, 2008). Although starch based films and coatings generally present adequate properties, it has been found that storage greatly increases the crystalline fraction in the starch matrix (Jiménez, Fabra, Talens, & Chiralt, 2012; Mali, Grossmann, García, Martino, & Zaritzky, 2006); a fact that may lead to a deterioration of the protective ability of starch-based packaging. Jiménez et al. (2012) related the increase in crystallinity with the changes in different properties (gloss, transparency, brittleness) of the films, which can affect the film functionality and consumer acceptance of coated products. One way to avoid the recrystallization of starch is by combining this polymer with others, preferably amorphous. One of the biopolymers with this characteristic is hydroxypropyl-methylcellulose (HPMC). Its ease of use, availability, water solubility, and non-toxicity makes HPMC the most extensively used cellulose derivative (Fahs, Brogly, Bistac, & Schmitt, 2010). The amorphous state of HPMC has been reported by Kou et al. (2011) through X-ray diffraction analysis. Huang, Chen, Lin, & Chen (2011) succeeded in reducing the crystallinity index of bacterial culture cellulose by adding HPMC to the bacterial culture medium, pointing to this polymer's capacity to inhibit crystallization.

HPMC has also been studied as a matrix of edible films in combination with different components, such as fatty acids (Jiménez, Fabra, Talens, & Chiralt, 2010) or cellulose nano-particles (Bilbao-Sainz, Bras, Williams, Sénechal, & Orts, 2011). Nevertheless, there is

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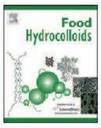

Effect of sodium caseinate on properties and ageing behaviour of corn starch based films

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ABSTRACT

The effect of sodium caseinate addition on corn starch films was studied in terms of microstructural, mechanical, optical and water and oxygen barrier properties and the changes they underwent during their 5 weeks' storage under controlled conditions. The influence of the polymer mixture on the degree of crystallization of the films was also analysed. The following starch: protein ratios were considered. 100:0, 75:50, 50:50 and 0:100. SEM Microstructure analysis revealed the compatibility of both hydrocolloids since no phase separation was observed. The addition of sodium caseinate to the starch films provided films that were less stiff and resistant to fracture but more flexible and deformable than pure starch films, with similar water vapour permeability values. The films became more permeable to oxygen as their sodium caseinate content increased in line with the higher permeability values of the protein film. Incorporating sodium caseinate to starch films provoked a slight increase in transparency, but a loss of gloss, which also decreased in composite films during storage. The re-arrangement of polymer chains during storage caused a loss of mechanical resistance, stretchability and gloss in composite films.

1. Introduction

In recent years, more and more research has been carried out into the substitution of petro-based plastic materials for biodegradable ones in order to solve the problems generated by plastic waste. So, there is increasing interest in biodegradable films made from renewable and natural materials, such as starch. Corn starch edible films exhibit appropriate physical properties since these films are isotropic, odourless, tasteless, non-toxic and biodegradable (Krochta, Baldwin, & Nisperos-Carriedo, 1994). However, starch films have several drawbacks, such as brittleness, due to starch crystallization, and poor mechanical properties, which limit their uses or applications. The main method for reducing the brittleness of starch films is to add plasticizers which are able to reduce the intermolecular forces by increasing the chain mobility and improving the flexibility and extensibility of the films (Parra, Tadini, Ponce, & Lugão, 2004). Another approach to overcome this aspect is to blend starch with other polymers or additives (Bourtoom & Chinnan, 2008; Ghanmbarzadeh, Almasi, & Entezami, 2010; Walia, Lawton, & Shogren, 2000; Wang, Yang, & Wang, 2004). Otherwise, the application of hydrophilic films, such as starch-based films, is limited by the poor water vapour barrier properties and water solubility of these films. In a first approach to solve the hygroscopicity and crystallization problems of starch based films, Jiménez, Fabra, Talens, and Chiralt (2012) studied the effect of adding fatty acids (saturated and unsaturated) on the physico-chemical properties, and their changes during storage, in starch films. Nevertheless, they found that fatty acid addition did not notably improve the water vapour permeability of the films and, in all cases, the degree of starch crystallization increased during storage time, thus increasing the film's brittleness and decreasing its stretchability and transparency. So, it was concluded that other biodegradable materials must be tested to improve the functional properties of starch films and their ageing problems.

The capacity of sodium caseinate to form films with appropriate properties is well-known (Audic & Chaufer, 2005; Chen, 2002; Fabra, Talens, & Chiralt, 2008a, 2008b; Fabra, Talens, & Chiralt, 2010) and some works have revealed the improvement of the film properties when sodium caseinate is incorporated into other polymer matrices (Arvanitoyannis & Biliaderis, 1998; Monedero, Fabra, Talens, & Chiralt, 2010; Pereda, Amica, Rácz, & Marcovich, 2011). Nevertheless, only a few studies were found into the effect of sodium caseinate on the physico-chemical properties of corn starch films.

In this work, the effect of adding sodium caseinate on the microstructural, mechanical, optical and oxygen and water vapour barrier properties of corn starch matrices was analyzed for films in different storage conditions (non-stored and stored films for five

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Physical properties and antioxidant capacity of starch–sodium caseinate films containing lipids

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Antioxidant

ABSTRACT

Biodegradable films based on starch, sodium caseinate, glycerol and lipids (oleic acid and/or α-tocopherol) were obtained and evaluated in terms of microstructure, mechanical behaviour, barrier and optical properties and antioxidant capacity. The effect of film storage time on these properties was also analysed. The lipid incorporation provoked phase separation due to the different interaction between each polymer and lipids, although structural rearrangement of components during storage could be deduced from the change in mechanical behaviour. After storage, all films showed similar mechanical properties, but lipid containing films were more stretchable. Lipid addition did not induce a notable decreased in water vapour permeability of the films, but oxygen permeability highly increased when they contained oleic acid. All films were highly transparent, with very small differences among formulations, although their gloss values increased when lipids were added. The incorporation of α-tocopherol greatly increased the antioxidant capacity of the films which affected oxygen permeability.

1. Introduction

Due to the severe environmental pollution caused by plastic food packaging, there has been a growing amount of interest in the production of edible and biodegradable films (Yan et al., 2012). In this sense, there are a great number of studies focusing on solving the problems produced by plastic waste and obtaining environmentally friendly materials (Jiménez et al., 2012a). Several works study the possibility of substituting the petro-based plastics for biodegradable, low cost materials with similar properties (Psomiadou et al., 1996; Mali et al., 2002, 2006; Famá et al., 2007). In this sense, starch is the most abundant natural polysaccharide and it is described as a renewable resource, inexpensive and widely available (Lourdin et al., 1995). Starch-based films exhibit appropriate physical characteristics, since these films are isotropic, odourless, non-toxic, biodegradable, tasteless, colourless and constitute a good barrier against oxygen transfer (Krochta et al., 1994; Yan et al., 2012). Nevertheless, starch films exhibit several drawbacks which it would be beneficial to overcome to increase their potential use. One of the main disadvantages of starch is that the mechanical behaviour of films can be negatively affected by retrogradation phenomena. Jiménez et al. (2012b) found that starch re-crystallizes during film formation and storage, increasing the elastic modulus and decreasing the flexibility of films. In a recent work, they found that recrystallization of starch was also influenced by the amylose–lipid interactions, thus giving rise to V-type crystalline forms for amylose (Jiménez et al., 2013). Composite films of starch with other biopolymers showed a lower degree of re-crystallization, thus minimising mechanical changes associated to starch retrogradation. This was observed in corn starch–sodium caseinate and corn starch–hydroxypropylmethylcellulose (HPMC) films (Jiménez et al., 2012a,c).

Sodium caseinate has a satisfactory thermal stability and can easily form films from aqueous solutions, due to its random coil nature and ability to form extensive intermolecular hydrogen, electrostatic and hydrophobic bonds (Arvanitoyannis and Biliaderis, 1998). Different aspects of this polymer as a matrix of edible and biodegradable films have been extensively studied (Siew et al., 1999; Kristo et al., 2008; Patzsch et al., 2010).

The addition of sodium caseinate to starch matrices reduced the degree of crystallinity of starch films and inhibited starch recrystallization during film storage (Jiménez et al., 2012a). Mixtures of both hydrocolloids provide films which are less stiff and resistant to fracture but more flexible and deformable than pure starch films, but with similar water vapour permeability values as starch films (Jiménez et al., 2012a). In the case of starch, this disadvantage has been avoided by different methods such as surface esterification (Ren et al., 2010), surface photocrosslinking (Zhou et al., 2008) or blending starch with other hydrophobic polymers (Averous et al., 2000; Fang and Fowler, 2003). For sodium caseinate, hydrophobic additives, such as fatty acids, waxes or oils, were incorporated in the films in order to improve the resistance to water vapour transfer (Morillon et al., 2002; Pereda et al., 2010).

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