UNIVERSITE DE LORRAINE

École Nationale Supérieure d'Agronomie et des Industries Alimentaires

Laboratoire d'Ingénierie des Biomolécules (LIBio)

THESE

AKHTAR Muhammad Javeed

Pour obtenir le grade de

Docteur de l'UL

Spécialité : Procédés Biotechnologiques et Alimentaires

Fonctionnalisation et Caractérisation de Films Bioactifs à Base d'HPMC: Influence de l'introduction d'antioxydants sur les Propriétés des Films et la Conservation des Aliments

Functionalization and Characterization of Bioactive Films Based on HPMC: Influence of Antioxidants Inclusion on Films Properties and Food Preservation

---

Soutenue publiquement le 13 Septembre 2012 devant la commission d'examen

Rapporteurs :

Mme. Violette DUCRUET	Ingénieur de Recherche, HDR, INRA, Massy, France
M. Jean-Charles BENEZET	Maître de Recherche, école de mines, Alès, France

Examinateurs :

Mme. Andrée VOILLEY	Professeur, Agrosup (Université de Bourgogne), Dijon, France
M. Michel LINDER	Professeur, LIBio, UL, Nancy, France
Mme. Muriel JACQUOT	MC (Co-directeur de thèse), LIBio, UL, Nancy, France
M. Stéphane DESOBRY	Professeur (Directeur de thèse), LIBio, UL, Nancy, France

Je dédie ce travail

A Mon défunt papa et ma chère maman

A ma chère femme et mon fils que j'aime

A ma famille entière

REMERCIEMENT

Je souhaite tout d'abord remercier le 'Higher Education Commission (HEC)' du Pakistan pour ses encouragements aux études supérieurs et la recherche scientifique au Pakistan et d'avoir accepté de financer ce travail de thèse qui a été réalisé au sein du Laboratoire d'Ingénierie des Biomolécules de l'Ecole Nationale Supérieur d'Agronomie et des Industries Alimentaires de Nancy, sous la direction de Professeur Stéphane Desobry, et la co-direction de Madame Muriel Jacquot, Maître de conférences HDR à l'ENSAIA.

Ma reconnaissance la plus sincère au Professeur Stéphane Desobry pour son encadrement, sa compréhension, son soutien et la grande confiance qu'il m'a témoigné tout au long de la thèse. Son concept à être optimiste et plein d'espoir, son aptitude à la neutralisation de l'énergie négative en bonne volonté, son approche utopique d'apporter le bonheur à tous, ses gestes de routine et d'humeur portant les ondes magnétiques de positivité, et ses manières simples d' « être humain » m'aideront tout au long de ma vie, professionnellement et personnellement.

Je remercie très chaleureusement Docteur Muriel Jacquot pour son encadrement, sa disponibilité, sa sympathie, ses conseils précieux ainsi que pour avoir patiemment corrigé ce document et les articles. Je l'apprécie beaucoup pour m'avoir fait apprendre le sens du mot «organisation» à la fois pour le travail expérimental et la transformation des idées, et son approche réaliste, tout en traitant de la science.

Je remercie également les membres de jury qui ont accepté de juger ce travail: Mme. Andrée Voilley (Professeur, Agrosup, Université de Bourgogne, Dijon, France), Mme. Violette Ducruet (Ingénieur de Recherche, HDR, INRA, Massy, France), M. Jean-Charles Benezet (Maître de Recherche, école de mines, Alès, France) et M. Michel Linder (Professeur, LIBio, UL, Nancy, France).

Mes sincères remerciements vont également à Professeur Joël Scher, Professeur Ghoul Mohamed, Professeur Jacques Fanni, Docteur Elmira Arab Tehrany, Docteur Claire Gaïani et Docteur Jordane Jasniewski pour leur aide, leurs conseils et leur patience.

Mes remerciements s'adressent également aux responsables des différents laboratoires pour m'avoir rendu accessible les différentes techniques, surtout Cedric

Paris (LCMS), Jaafar Ghanbaja et Jacquline Chanél (TEM et SEM), et Céline Charbonnel (Techniques biochimie)… Un grand merci pour m'avoir communiqué votre passion, vos discussions enrichissantes et vos conseils précieux.

Je tiens à remercier l'ensemble du personnel de l'ENSAIA qui me connaît et particulièrement celui du LIBio, et surtout Marie-Noële Maucourt (pour ses Tic-Tac), Anne-Laplace, Cécile Mangavel, Carole Jeandel, Carole Perroud, Mlle Turban, Delphine, Myriam et Paul.

Je remercie l'ensemble des thésards: Agnieszka, Majid, Guillaume, Smail, Lynn, Charbel, Imran, Charlotte (l'équipe emballage) ; Behnoush, Nabila, Pascale, Kassem, Ange, (l'équipe lipide) ; Abdur-Rehman, Inam, Amira, Thibaut, Mireille, (l'équipe microbiologie) ; Laetitia, Leila, Jennifer, Céleste, Chancelle, Nadia, Abdulhadi, Mohammed, Mahmoud, Citlalli et des stagiaires du LIBio notamment Marjolène et Warchol CUNY Diane qui ont contribué, de près ou de loin, à la réalisation de cette étude, dans une ambiance toujours amicale et stimulante. Je remercie aussi les jeunes chercheurs du laboratoire, venus de France ou d'ailleurs, avec qui j'ai eu l'occasion de partager de bons moments inoubliables.

Je voudrais aussi remercier ma petite famille (Hassnaa Aouinate et Rayane Akhtar) qui m'ont accompagné tout au long de cette aventure et ont constitué ma deuxième famille en France.

What actions are most excellent to gladden the heart

of human beings?

To feed the hungry, to help the afflicted, to lighten the

sorrow of the sorrowful and to remove the sufferings of

List of Abbreviation

AC	Anthocyanins compound
CA	Cellulose acetate
ASTM	American society for testing and material
B	Betanin
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
D	Diffusion coefficient
DHA	Docosahexaenoic acid
DP	Degree of polymerization
DSC	Differential scanning calorimetry
DVS	Dynamic vapor sorption
EC	Ethyl cellulose
EFSA	European food safety authority
EPA	Eicosapentaenoic acid
ERRMA	European renewable resource materials association
FAME	Fatty acid methyl esters
FDA	Food and drug administration
FFA	Film forming solution
FRAP	Fluorescence recovery after photobleaching
FTIR	Fourier transform infrared spectroscopy
G	Glycerin
GAB	Guggenheim–anderson–de boer
GAE	Gallic acid equivalents
GC	Gas chromatography
HDPE	High density polyethylene
HEC	Hydroxyethylcellulose
HPC	Hydroxypropyl cellulose
HPLC–MS	High performance liquid chromatography mass spectroscopy
HPMC	Hydroxypropyl methylcellulose
IBAW	International biodegradable polymers association and work group
IENICA	Interactive european network for industrial crops application
K	Partition coefficient
MC	Methylcellulose
MSW	Municipal solid waste
NNFCC	National non-food crops centre

NRC	Natural red colour
OP	Oxygen permeability
OTR	Oxygen transmission rate
PG	Propyl gallate
PHAs	Poly-hydroxylalkanoates
PHB	Poly-hydroxylbutyrate
PHV	Poly(b-hydroxyvalerate)
PLA	Polylactic acid
PTC	Contenu phénolique total
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
RH	Relative humidity
SEM	Scanning electron microscopy
SP	Soya protein
TBA	Thiobarbituic acid
TBARS	Thiobarbuthuric reactive substances
TBHQ	*Tert*-butylhydroquinone
TEAC	Trolox equivalent antioxidant capacity
TPC	Total phenolic content
TS	Tensile strength
TVBN	Total volatile basic nitrogen
UE	Ultimate elongation
USDA	United states department of agriculture
WVP	Water vapor permeability
YM	Young's modulus
ΔEab	Total color difference

Table de Matière

Interest and justification of the work

Food packaging, an important discipline in the area of food technology, concerns preservation and protection of all types of foods and their raw materials from oxidative and microbial spoilage (Tharanathan, 2003). Edible films and coatings are thin layers of edible materials applied on food products that can play an important role on their preservation, distribution and marketing (Falguera et al., 2011). These are produced from edible biopolymers and food-grade additives. Film-forming biopolymers can be polysaccharides, proteins or lipids. Edible films and coatings may enhance the quality of food products, functioning as barriers against oils, gases or vapours. Moreover, they can act as carriers of active substances, such as antioxidant, antimicrobial or colouring/flavouring compounds, resulting in shelf-life extension and safety improvement (Han and Gennadios, 2005). At present, the consumer demand has shifted to eco-friendly biodegradable materials that come from agro-food industry wastes and renewable low cost natural resources. In this sense, cellulose derivative such as hydroxy propyl methyl cellulose (HPMC) was chosen to develop biodegrable edible films.

Cellulose based materials are widely used due to their biocompatibility, edibility, barrier properties, non-polluting and being more economical material (Vasconez et al., 2009). The use of HPMC is attractive because it is a readily available non-ionic edible plant derivative shown to form transparent, odourless, tasteless, oil resistant, and water soluble edible films (Akhtar et al., 2010). In food industries it is used as a gelling and stabilizing agent. HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC 1995); its safety in food use has been affirmed by the JECFA (Burdock, 2007). The tensile strength of HPMC films is high and flexibility neither too high nor too fragile, which make them suitable for edible film and/or coating purposes (Brindle & Krochta, 2008). The functionality of edible films can be improved by inclusion of inert materials and/or reactive compounds in the polymer matrix (Rhim et al., 2006).

Recently, fruit and vegetable extracts have gained a considerable market in food industries (Stintzing & Carle, 2004). To consider the natural bioactive colors as the colorants, stability, yield and price are mostly constrains. The natural coloring agents in comparison with artificial colors show less stability against light, oxidation, temperature or pH change and other factors (Fabre et al., 1993; Laleh et al., 2006). In spite of such factors, these natural colorants are gaining importance due to their coloring potential, hygiene, nutrition, pharmaceutical activities, bioactivity and environmental consciousness, which indicates relative dependence on natural products (Hari et al., 1994; Frank et al., 2005). Anthocyanins are flavonoid derivatives which exhibit anti-oxidant activity. Although they are less stable in

various environmental conditions, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Markakis, 1982). Moreover, anthocyanins have many health benefits, including reduced risk of cardiovascular diseases (Bell & Gochenaur, 2006) and decreased risk of cancer (Dai, Patel & Mumper, 2007).

Betalains, known for a long time as safe colorants for food or other industrial purposes (von Elbe et al., 1974 & von Elbe and Schwartz, 1981), are phytochemicals. Several studies on the antioxidant and antiradical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been reported (Escribano et al., 1998; Pedreño & Escribano, 2000; Kanner, Harel, & Granit, 2001). In addition to their coloring properties, they are supposed to provide protection against oxidation stress related disorders in humans when being part of the regular diet (Kanner, Harel, & Granit, 2001). Betalains are reported to exhibit anti-inflammatory effects (Gentile et al., 2004).

The shelf life and storage-keeping quality of food products are influenced by a number of interrelated factors: temperature, oxygen pressure, endogenous enzymes, dehydration, light microbial load, etc. These factors determined the extension and kinetics of detrimental changes, affecting the colour, odour, texture and flavour of food products. Edible films containing bioactive compounds can extend shelf-life of packed food at lower active agents concentration than that applied directly onto product surfaces (Lee et al., 2010). These bioactive color compounds can be added into edible films to give them additional properties such as colour, antioxidant and gas barrier capacity. As previously reported, the red color of HPMC films allows very good control against photo-oxidation of polyunsaturated fatty acids (PUFA) in salmon oil (Akhtar et al., 2010). Such edible films would also provide additional benefits to traditional edible film forming materials by providing unique sensory and antioxidant capacity, thus attracting more potential applications as localizing functional effect at the food surface. In fact, the diffusion rate of the bioactive compounds added to the film matrix can be slowed down, and a high content of the active agent remains on the product surface (Sánchez-González et al., 2011).

In this work HPMC polymer based edible films were functionalized with natural red color compounds (anthocyanins & betalains) to give them additional properties. Likewise, the effects of the incorporation of these bioactive compounds to HPMC polymer films have been investigated through the induced changes in the film physico-chemical properties and the antioxidant/antimicrobial activity of the film applied to salmon oil preservation.

I. Introduction et objectifs de l'étude

I.1. Introduction et objectifs de l'étude

Les huiles de poissons contiennent des niveaux importants d'acide gras polyinsaturés (AGPI) et plus spécifiquement de l'acide eicosapentaenoique (EPA, omega-3) et docosahexaenoique (DHA, omega-3). La majorité des bénéfices liés à la consommation de poissons ou de produits de la mer sont dus à la présence de ces AGPI. Par exemple, ils ont des effets bénéfiques contre les problèmes coronariens, inflammatoires, les thromboses, les carcinomes et des syndromes métaboliques (Miller et al., 2008; Tsuzuki et al., 2004; Mori & Beilin, 2001). La connaissance de ces nombreux intérêts ont été à l'origine du développement de nouvelles méthodes de protection de ces huiles contre la dégradation oxydative (Hamilton et al., 1998).

L'oxydation des lipides polyinsaturés est le résultat d'un mécanisme bien connu entrainant de nombreuses modifications chimiques indésirables telles que l'apparition de faux goûts, la perte des qualités nutritionnelles et l'apparition de produits d'oxydation dont certains sont suspectés être dangereux pour la santé. De façon naturelle, les huiles de poissons contiennent des antioxydants comme l'astaxantine, le coenzyme-Q10, le selenium, la taurine et les vitamines (A, D, and E) pouvant ainsi retarder l'apparition des phénomènes d'oxydation (Rita Mattei et al., 2011). Cependant, la formulation et le stockage que subissent ces huiles, entrainent la dégradation de ces molécules et en réduisent d'autant leur capacité de protection des AGPI. Les antioxydants sont souvent directement mélangés aux aliments en tant qu'additifs ou les aliments sont plongés dans des solutions permettant la réalisation d'un coating protecteur. Les limites de ces méthodes d'introduction directe des antioxydants aux aliments sont une activité réduite suite à la non-sélectivité des réactions chimiques auxquelles elles participent et une absence de spécificité pour la surface de l'aliment, lieu où ces réactions sont les plus présentes.

L'oxydation des lipides est aussi directement corrélée à la présence de la lumière, à la longueur d'onde de cette lumière, son intensité, le temps d'exposition ainsi qu'à la température de stockage. C'est pourquoi, le choix des caractéristiques du matériau d'emballage utilisé telles que sa couleur, ses propriétés barrières à l'oxygène et la présence d'antioxydants sont aussi à prendre en considération pour contrôler ces phénomènes.

La protection contre la lumière obtenue par les emballages va dépendre de plusieurs facteurs comme l'absorption de la lumière par le matériau, son épaisseur et sa couleur, ces éléments pouvant se combiner afin d'optimiser la protection du produit alimentaire contre la photo-oxydation. Nelson et Cathcart en 1983 ont étudié l'impact de la coloration de surface sur la transmission de la lumière et ils ont montré que le rouge permettait de réduire la transmission

des longueurs d'onde inférieures à 550 nm. Une autre étude a montré une très bonne efficacité des pots en verre ou en polystyrène colorés en rouge pour la conservation de yaourts Bosset & Flückiger, 1989. De façon générale, les matériaux colorés offrent une meilleure protection que ceux qui sont imprimés. Les matériaux transparents quant à eux offrent une très faible protection.

La perméabilité à l'oxygène des emballages dépend principalement du choix du matériau mais aussi de la concentration en oxygène à l'intérieur de l'emballage (pression partielle), de l'humidité relative et de la température de stockage.

L'incorporation d'antioxydants naturels directement dans le matériau d'emballage est aussi une méthode prometteuse pour améliorer la durée de vie des produits alimentaires. Différents antioxydants naturels ou synthétiques ont été incorporés à différent films de polymères et leur migration a été étudiée soit dans l'aliment soit dans un stimulant. Parmi les antioxydants naturels, les extraits de fruits ou de végétaux prennent de plus en plus d'importance du fait de leur activité, leur pouvoir colorant, anti-radicalaire et anti-inflammatoire (Frank et al., 2005). Les anthocyanines, composés phénoliques largement présents dans ces extraits, couvrent une grande gamme de couleurs qui va du rouge, à l'orange jusqu'au bleu, ce qui en fait une bonne alternative aux colorants alimentaires synthétiques. De plus, ces molécules présentent de nombreux bénéfices pour la santé comme la réduction du risque d'apparition de maladies cardio-vasculaires. Les bétalaïnes, autre composé phénolique naturellement présent dans ces extraits, est utilisé comme colorant depuis le début du 20ème siècle. Ces bétalaïnes consistent surtout en un mélange de bétanines (betanidin 5-O-beta-glucoside). Plusieurs études ont souligné leur potentiel antioxydant, anti-radicalaire et anti-inflammatoire mais elles n'ont pas été étudiées en tant que composés bioactifs dans des films d'emballage.

Nous avons donc décidé de travailler avec ces deux molécules (antocyanine et bétanine) de façon à mettre à profit leurs caractéristiques, de tester leur capacité à se substituer à un colorant artificiel et enfin d'étudier leur relargage à partir d'un film de biopolymère vers un aliment afin d'en contrôler l'oxydation lipidique. Nous nous sommes aussi intéressé à l'impact de l'intégration de ces molécules sur les propriétés du film car il est bien connu que les antioxydants protègent aussi le polymère de la dégradation et peuvent ainsi éviter l'apparition de produits d'oxydation indésirables, de faible poids moléculaire, qui pourraient migrer vers l'aliment et entrainer un risque pour la santé et une dégradation de la qualité du produit. L'hydroxypropyl methylcellulose (HPMC) a été choisi comme matrice polymérique. L'HPMC est un dérivé de cellulose non-ionique et comestible. Il a des propriétés filmogènes permettant la production de films transparents, sans odeur ni goût,

résistants à l'huile, solubles dans l'eau et présentant de bonnes propriétés barrières à l'oxygène et à la vapeur d'eau. Il a aussi la capacité de retenir les pigments. La capacité d'élongation des films est élevée avec une flexibilité intermédiaire ce qui le place correctement pour des applications d'enrobage comestible. Il est approuvé pour des applications alimentaires par la FDA (21 CFR 172.874) et l'UE (EC, 1995); sa non-toxicité a été confirmée par l'ECFA "Joint expert committee on food additives".

Le second chapitre est une revue de la littérature consacrée aux polymères biodégradables, à la chimie de cette dégradation, à leur fonctionnalisation par des antioxydants et aux études de libération de ces molécules.

La première phase de l'étude a été de tester des films d'HPMC contenant différentes couleurs (bleu V (E131), jaune (FFA 200%), rouge (aqua color 60056), blanc (aqua color 60672) et vert) pour mesurer leur capacité à contrôler la photo-oxydation de l'huile de saumon. Nous avons ainsi pu choisir la couleur la plus efficace comme filtre à la lumière pour le reste de l'étude.

Dans un second temps, les films d'HPMC ont été fonctionnalisés avec un extrait naturel rouge (natural red color, NRC) commercialisé comme un mélange d'extrait de betteraves rouges (E162) et de carottes pourpres (E163). L'objectif était de remplacer les colorants synthétiques et d'étudier les effets sur les propriétés des films (optiques, mécaniques, thermiques, structurales et barrières). L'effet d'un photo-vieillissement sur ces mêmes propriétés a aussi été étudié. Différentes techniques ont été utilisées telles que la chromatographie haute performance en phase liquide à spectrométrie de masse (HPLC-MS) pour la caractérisation du NRC et la spectroscopie infrarouge à transformée de Fourier (FTIR) pour étude d'interaction HPMC-NRC.

Des films d'HPMC sont ensuite fonctionnalisés avec soit d'extrait de betterave (B - E 162), d'un extrait d'anthocyane rouge (amarante CA, E163) soit de leur mélange (B + CA / 50:50) à 2% (v/v) et ont été comparés avec des films fonctionnalisés avec du NRC. Les propriétés antioxydantes, le photo-vieillissement et la stabilité des films ont été étudiés. De plus, le transfert de ces molécules dans un simulant alimentaire (éthanol 95%) a été étudié à 20 ° C et 4 ° C.

Le coefficient de diffusion (D) et le coefficient de partage (K) a été calculé pour chaque antioxidant. Les modifications structurales des films sont évaluées avant et après le relargage des antioxydants par spectroiscopie infrarouge à transformé de Fourier en mode de réflexion atténué (ATR-FTIR).

Enfin, les films contenant une formulation d'anthocyanines optimisée en terme de propriétés barrière à la lumière et à l'oxygène ont été testés comme films d'emballage pour la conservation d'huile de saumon. La composition lipidique de l'huile, les diènes conjugués, l'indice de polyène, la couleur et la teneur en oxygène de l'espace de tête de l'emballage ont été mesurés afin d'évaluer la stabilité oxydative de l'huile de saumon.

I.2. Introduction and objectives of the study

Fish oil contains high levels of polyunsaturated fatty acids (PUFA) particularly eicosapentaenoic acid (EPA, omega-3) and docosahexaenoic acid (DHA, omega-3). Most of beneficial attributes of fish and fish products are due to these polyunsaturated fatty acids. For instance, they have protective effects against coronary heart disease, inflammatory processes, thrombosis, carcinomatosis, and metabolic syndrome (Miller et al., 2008; Tsuzuki et al., 2004; Mori & Beilin, 2001). Such nutritional benefits have promoted significant research into methods of stabilizing unhydrogenated fish oil against oxidative deterioration (Hamilton et al., 1998).

Oxidation reactions in polyunsaturated lipids are common mechanisms causing a number of unfavorable chemical changes including off-flavors formation, nutritional quality decrease, economic losses and oxidation products formation, some of which are supposed to be against human health. Naturally occurring antioxidants in fish oil, such as astaxanthin, coenzyme-Q10, selenium, taurine, and vitamins (A, D, and E) can delay the onset of lipids oxidation (Rita Mattei et al., 2011). However, processing and storage deplete these natural resources, resulting in decreased protection against lipid oxidation. Antioxidants are conventionally mixed as food additives or alternatively foods are dipped into solutions containing antioxidants to protect food against lipid oxidation. Limitations of antioxidants direct addition to foods include specific limit of activity due to their participation in complex reactions in food systems and lack of selectivity to target the food surface where most oxidative reactions occur. Lipids oxidation is directly related to light source, wavelength, intensity, exposure time, and temperature, as well as packaging material characteristics such as color, oxygen barrier properties and fictionalization as antioxidant.

Most appropriate way to control lipid oxidation may be decrease of light exposure and oxygen removal by the application of a suitable antioxidant-active packaging. Light protection offered by the packaging materials depends on several factors, including inherent absorption characteristics of the material, thickness and coloration of the material, which may all be combined to optimize photo-oxidative protection of specific foods. Incorporation of pigments into packaging materials is a way of improving the light barrier. Nelson and Cathcart in 1983 investigated the effect of surface coloration and found that red color was a very effective measure to reduce light transmission at wavelengths below 550 nm. Another investigation of red-pigmented glass and polystyrene jars pointed out the good photoprotective properties for several yogurt varieties (Bosset & Flückiger, 1989). In general,

colored materials offer better protection than do printed materials. Literature shows that black laminates offer the best protection against photo-oxidation of Havarti cheese, followed by a white laminate. The transparent materials provided little protection. Oxygen permeability of the packaging material depends first and foremost on the materials used and also on the concentration of oxygen inside and outside the packaging material (partial pressures), the relative humidity, and the storage temperature.

Controlled release of natural antioxidants from packaging materials to the foods is another promising technique for extending food shelf life. Different natural and synthetic antioxidants have been incorporated in several polymer films and their migration in real foods and simulants have been studied. Among natural antioxidants fruit or vegetable extracts (betalains from red beetroot extract) are gaining more importance because of their coloring potential antioxidant, antiradical and anti-inflammatory activities (Frank et al., 2005). Anthocyanins present in these phenolic compounds cover a large variety of colors such as red, orange and blue which make them an attractive alternative to synthetic food dyes. Moreover, anthocyanins have many health benefits, including reduced risk of cardiovascular diseases. The use of betalains from red beetroot extracts (*Beta vulgaris* L.) as separate food colours date from the early 20th century. Betalain consists mainly of betanin (betanidin 5-O-beta-glucoside). Several studies have indicated their potential as antioxidant, antiradical and anti-inflammatory pigments but they have not been much explored as bioactive compounds in edible food packaging.

We choose these natural antioxidants because of their dual functional properties; firstly, for their natural color replacing synthetic colorants and secondly, for their release providing a continuous control of lipids oxidation. Antioxidants also protect the polymers against the formation of undesirable oxidative and low-molecular compounds which can migrate to foods causing a decrease in food quality. We used natural antioxidant compounds which are usually consumed in foods to ensure the safety concerns regarding to their migration from active packaging to the food.

Hydroxypropyl methylcellulose (HPMC) was chosen as polymeric matrix carrying natural antioxidants. HPMC is a readily available non-ionic edible plant derivative forming transparent, odorless, tasteless, oil resistant, and water soluble edible films acting as a barrier to oxygen and water vapours. It has also the ability to absorb and retain the colour pigments. The tensile strength of HPMC films is high with medium flexibility, which makes them suitable for edible coating purposes. It has been approved for food uses by the FDA (21 CFR

172.874) and the EU (EC, 1995); its safety in food use has been confirmed by the (ECFA) "Joint expert committee on food additives".

In second chapter of this study biodegradable polymers, chemistry of biodegradation, biopolymer fictionalization, mechanism of antioxidants and release studies etc, are described as review of literature.

Then at the first step of our research work, HPMC films containing different synthetic colours such as blue patent V (E131), yellow (FFA 200%), red (aqua color 60056), white (aqua color 60672) and green were tested for their ability to avoid photo-oxidation in salmon oil. The main objective was to select a suitable color packaging as a light barrier.

At the second step of our research study, the HPMC films were functionalized with natural red colour (NRC) a commercial blend of beetroot juice (E162) and purple carrot extract (E163). The main objective was to replace the synthetic colors with natural pigments and to investigate their effect on HPMC films properties, such as optical, mechanical, barrier, thermal and structural properties. Effect of fluorescent light-ageing on films properties was also investigated. In the next step these HPMC films functionalized with NRC were further studied for their antioxidant and photo-ageing stability. The highly precise techniques such as High Performance Liquid Chromatography Mass Spectroscopy (HPLC–MS) for NRC characterization and Fourier Transform Infrared Spectroscopy (FTIR) for HPMC-NRC interaction study were used.

HPMC films were then functionalized with 2% (v/v) of each, beetroot extract (E 162), a red liquid extract of anthocyanin (amaranthine, E163) and their mixture (B+AC/50:50) and were compared with NRC films for their antioxidant and photo-ageing stability. The release behaviour of incorporated natural antioxidants from HPMC films into food simulating liquid (95%) ethanol at 20°C and 4°C was accomplished. The diffusion coefficient (D) and partition coefficient (K) of each antioxidant were calculated. Structural changes of films before and after antioxidants release were followed by Fourier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR).

Finally, HPMC films containing anthocyanin (amaranthine, E163) with their improved light barrier and oxygen barrier properties were tested as packaging materials for salmon oil preservation. Lipids quality parameters including oil color, headspace oxygen uptake, conjugated dienes, fatty acid composition and polyene index were tested to determine the oxidative stability of salmon oil.

The overall objective of the present study was to optimize and develop a biodegradable active packaging with improved physico-chemical and functional properties to continuously control the oxidation processes in packed foods. The scientific tasks were related to the packaging functionalization by the inclusion of natural antioxidants and/or antimicrobial agents, packaging physico-chemical properties, controlled release of these natural agents in a biological matrix (simulated food and biomaterials). Hence, the topic has covered the areas of chemistry, biochemistry, physics and basics of food microbiology.

II. Synthèse bibliographique

LITERATURE REVIEWED

II.1. FOOD PACKAGING DEVELOPMENT

A wide range of materials are used for packaging applications including metals, glass, wood, paper or pulp, plastics or combinations of more than one material as composites. They are applied in three broad categories of packaging:

- **Primary packaging**, which is normally in contact with the foods and taken home by consumers.
- **Secondary packaging**, which covers the larger packaging such as boxes, used to carry quantities of primary packaged foods.
- **Tertiary packaging**, which refers to the packaging that is used to assist transport of large quantities of foods, such as wooden pallets and plastic wrapping.

Secondary and tertiary packaging materials are normally in larger quantities and have less material variation and thus are relatively easier to collect and sort for recycling or reuse purposes. Primary packaging materials are not only more dispersed into households, they are also largely mixed, contaminated and often damaged and thus create problems in recycling or reuse of the materials. This has caused increasing environmental and health concerns. In a survey conducted by the Environment Agency in 1998 (Wasteline, 2002), paper and board packaging made up approximately 5wt% of the domestic waste bin, whilst non-packaging paper (mainly printed matters) and board accounted forover 29%. Generally, over 67 million tonnes of packaging waste is generated annually in the EU, comprising about one-third of all municipal solid waste (MSW) (Klingbeil, 2000). Plastics contribute 18 percent of the 10.4 million tonnes of packaging wastes produced annually in the UK (DEFRA 2007). Discarded packaging is also a very obvious source of litter, posing a major waste management challenge (Barnes et al., 2009; Gregory, 2009; Ryan et al., 2009; Thompson et al., 2009a,b). This has been resulted in the development of biodegradable packaging materials from renewable natural resources. Many national and international organizations have been established to facilitate the development in this area. These include the European Renewable Resource Materials Association (ERRMA), the National Non-Food Crops Centre (NNFCC) in the UK, the International Biodegradable Polymers Association and Work Group (IBAW) based in Germany and the Interactive European Network for Industrial Crops Application (IENICA)

(Davis & Song, 2006). The UK Government-Industry Forum has strongly recommended greater use of non food crops, particularly for biodegradable packaging applications (DEFRA, 2004). American Society for Testing and Material (ASTM) declared that any product claiming to be biodegradable must completely decompose into CO_2 and water with in a 180 days period.

II.1.1. Bio-based food packaging materials

Bio-based food packaging polymers are the materials derived from renewable sources. These materials can be used for food applications" In addition, packaging materials recognized as biodegradable according to the standards outlined by the EU Standardization Committee. This amendment was included not to exclude materials which currently, of practical and economical reasons, are based on non-renewable resources, but at a later stage these materials may be produced based on renewable resources (Chandra & Rustgi, 1998; Weber et al., 2002).

II.1.1.1. Origin and description of biopolymers

Bio-based polymers may be divided into three main groups based on their origin and production (Petersen et al., 1999).

Group 1: Polymers directly extracted/removed from biomass. Examples are polysaccharides such as starch, cellulose and proteins like casein and gluten.

Group 2: These are the polymers produced by classical chemical synthesis using renewable bio-based monomers. A good example is polylactic acid, a bio-polyester polymerized from lactic acid monomers. The monomers themselves may be produced via fermentation of carbohydrate feedstock.

Group 3: Polymers produced by microorganisms or genetically modified bacteria. To date, this group of bio-based polymers consists mainly of the polyhydroxyalkonoates, but developments with bacterial cellulose are in progress.

These three groups are presented in schematic form in Figure 1. In general, compared to conventional plastics derived from mineral oil, bio-based polymers have more diverse

chemistry and architecture of the side chains giving the material scientist unique possibilities to tailor the properties of the final package.

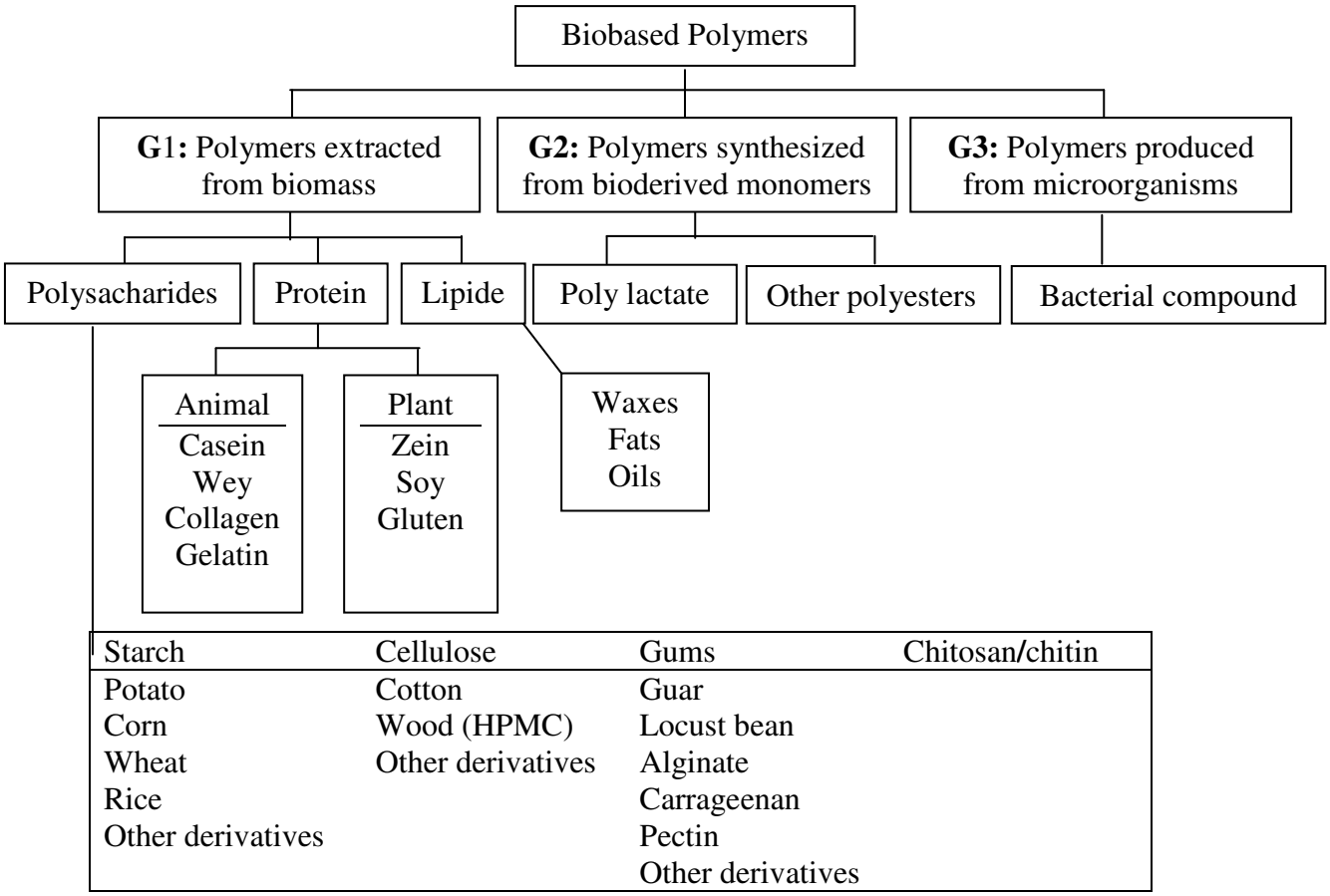


Figure 1. *Schematic presentation of bio-based polymers based on their origin and method of production (Weber et al., 2002).*

II.1.1.2. Group 1: Polymeric materials

Natural polymers belonging to this group are directly extracted from biomass. Most commonly available, are extracted from marine and agricultural products. Examples are polysaccharides such as cellulose, starch, and chitin and proteins (casein, whey, collagen & soy). All these polymers are, by nature, hydrophilic and somewhat crystalline, factors causing processing and performance problems, especially in relation to packaging of moist products. On the other hand, these polymers make materials with excellent gas barriers (Marron et al., 2000). The principal polysaccharides of interest for material production have been cellulose, starch, gums, and chitosan. Likely, the more complex polysaccharides produced by fungi and

bacteria (group 3 bio-based polymers) such as xanthan, curdlan, pullan and hyaluronic acid, will receive more interest in the future (Otles & Otles, 2004).

Chitin is a naturally occurring macromolecule present in the exoskeleton of invertebrates and represents the second most abundant polysaccharide resource after cellulose. In general, chitosan has numerous uses; flocculent, clarifier, thickener, gas selective membrane, plant disease resistance promoter, wound healing promoting agent and antimicrobial agent. Chitosan also readily forms films and, in general, produces materials with very high gas barrier, and it has been widely used for the production of edible coating. Furthermore, chitosan may very likely be used as coatings for other bio-based polymers lacking gas barrier properties (Kittur et al., 1998).

Proteins can be divided into proteins from animal origin (e.g. casein, whey, collagen & keratin) and proteins from plant origin (e.g. gluten, soy, pea and potato). A protein is considered to be a random copolymer of amino acids and the side chains are highly suitable for chemical modification which is helpful to the material engineer when tailoring the required properties of the packaging material (Graaf & Kolster, 1998).

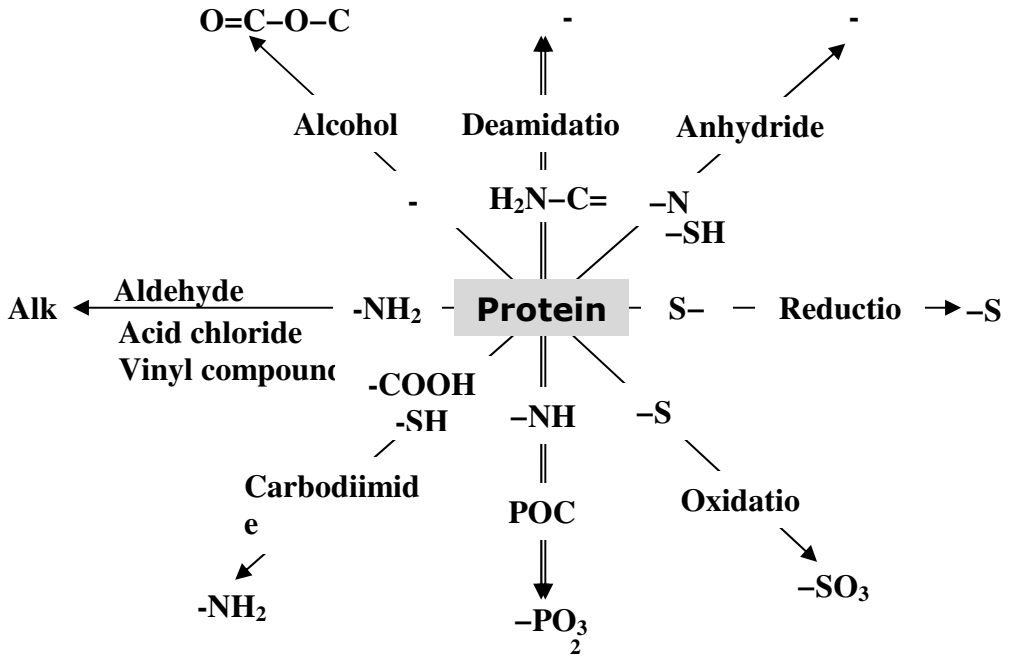


Fig 2. *The numerous and diverse side chains of proteins (Graaf and Kolster, 1998)*

Proteins based materials are highly suitable for packaging purposes due to their excellent gas barrier properties. However, like starch plastics mechanical and gas properties are influenced by the relative humidity due to their hydrophilic nature. One of the ways to modify protein properties is by chemical modification and, as seen in Figure 2, proteins contain a wide

variety of chemical moieties which may help tailoring protein properties towards specific applications (Graaf & Kolster, 1998).

Casein is a milk-derived protein. It is easily processable due to its random coil structure. Upon processing with suitable plasticizers at 80–100°C, materials can be made with mechanical performance varying from stiff and brittle to flexible and tough performance. Casein melts are highly stretchable making them suitable for film blowing. In general, casein films have an opaque appearance (Otles & Otles, 2004). Investigations of milk-protein-based films have focused on commercially available caseins, caseinates, and whey proteins. Little work has centered on individual casein fractions due to high manufacturing costs; however, Ward (1998) developed a cost-effective method for isolating β-casein. The β-casein is the most hydrophobic protein in milk, and edible β–casein films are expected to have lower water vapor permeability than other milk protein films (McHugh & Krochta, 1994).

Gluten is the main storage protein in wheat and corn. Wheat is an important cereal crop because of its ability to form viscoelastic dough. Mechanical treatment of gluten leads to disulfide bridge formation formed by the amino acid cysteine which is relative abundant in gluten. Processing temperatures are, depending on the plasticizer contents, in the range of 70-100°C. Mechanical properties may vary in the same range as those for caseins. Gluten plastics exhibit high gloss (polypropylene like) and show good resistance to water under certain conditions. They do not dissolve in water, but they do absorb water during immersion. Due to its abundance and low price, research on the use of gluten in edible films, adhesives, or for thermoplastic applications is currently being carried out (Otles & Otles, 2004). Soy proteins are commercially available as soy flour, soy concentrate and soy isolate, all differing in protein content. Soy protein consists of two major protein fractions referred to as the 7S (conglycinin, 35%) and 11S (glycinin, 52%) fraction. Both 7S and 11S contain cysteine residues leading to disulphide bridge formation and processing is, therefore, similar to gluten with similar mechanical properties. The most successful applications of soy proteins were the use in adhesives, inks and paper coatings (Fossen & Mulder, 1998). Keratin is by far the cheapest protein. It can be extracted from waste streams such as hair, nails and feathers. Due to its structure and a high content of cysteine groups, keratin is also the most difficult protein to process. After processing, a fully biodegradable, water-insoluble-plastic is obtained. However, mechanical properties are still poor compared to the proteins mentioned above (Shukla, 1992).

Starch, the storage polysaccharide of cereals, legumes and tubers, is a renewable and widely available raw material suitable for a variety of industrial uses. As a packaging material, starch alone does not form films with adequate mechanical properties (high percentage elongation, tensile and flexural strength) unless it is first treated by either plastization, blending with other materials, genetic or chemical modification or combinations of the above approaches (Otles & Otles, 2004). Starch based films can be formed by using its pure components such as 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 like other polysaccharide films are highly sensitive to moisture action. Furthermore, their mechanical behaviour can vary as a consequence of retrogradation phenomenon throughout time (Jiménez et al., 2012a). The hydrophilic character of starch films can be modified by different techniques such as surface sterification (Zhou et al., 2009), surface photocrosslinking (Zhou et al., 2008) or by adding hydrophobic compounds to film formulation (Averous et al., 2000; Fang & Fowler, 2003). On the other hand, starch retrogradation has been inhibited by mixing starch with other polymers such as hydroxypropyl methylcellulose (HPMC) or sodium caseinate (Jiménez et al., 2012b, 2012c).

Corn is the primary source of starch, although considerable amounts of starch are produced from potato, wheat and rice starch in Europe, the Orient and the United States. Starch is economically competitive with petroleum and has been used in several methods for preparing compostable plastics. However, a challenge to the development of starch materials is the brittle nature of blends with high concentrations of starch. Overcoming the brittleness of starch while achieving full biodegradability in blends can be accomplished by the addition of biodegradable plasticizers. Common plasticizers for hydrophilic polymers, such as starch are glycerol and other low molecular weight polyhydroxy–compounds, polyethers and urea. Plasticizers lower the water activity, thereby limiting microbial growth. When starch is treated in an extruder by application of both thermal and mechanical energy, it is converted to a thermoplastic material. Cellulose is the most abundantly occurring natural polymer on earth and is an almost linear polymer of anhydroglucose. Because of its regular structure and array of hydroxyl groups, it tends to form strongly hydrogen bonded crystalline microfibrils and fibres and is most familiar in the form of paper or cardboard in the packaging context. Waxed or polyethylene coated paper is used in some areas of primary food packaging. However, the bulk of paper is used for secondary packaging (Marron et al., 2000).

Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to form biofilms. Cellulose is the most common organic compound on the earth (Mandal et al., 2010). About 33% of all plant matter is cellulose (the cellulose content of cotton is 90% and that of wood is 40–50%). Cellulose consists of a linear chain of several hundred to over ten thousand d-glucose units which in contrast to starch are β-1, 4 linked. This β-1, 4 linkages make cellulose linear, highly crystalline and indigestible for humans (Al-Tabakha, 2010; Caraballo, 2010; Li et al., 2005).

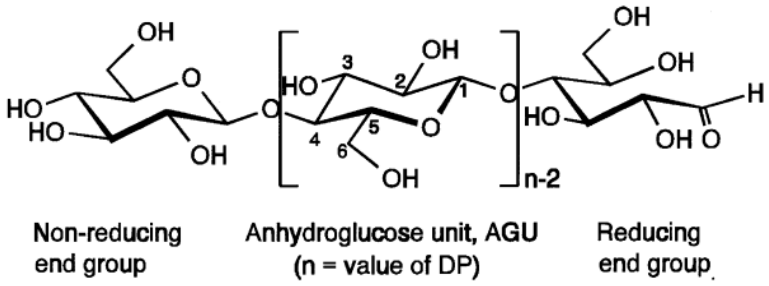


Figure 3. *Chemical structure of cellulose including C-atoms numbering*

Three hydroxyl groups with different polarities, secondary OH at the C-2, secondary OH at the C-3 and primary OH at the C-6 position are present, and the formation of strong various intermolecular and intramolecular hydrogen bonds play an important role in the cohesion and stability of macromolecular chains. Solubility in water decreases with a degree of polymerization (DP) of more than 6 (Klemm et al., 1998). The organization of the cellulose fibers leads to a porous structure. To elaborate derivatives with film forming properties, cellulose can be esterified or etherified to certain cellulose derivatives such as cellulose acetate (AC), methylcellulose (MC) or ethyl cellulose (EC), hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC) and hydroxypropyl methylcellulose (HPMC).

Hydroxypropyl methylcellulose (HPMC)

Hydroxypropyl methylcellulose is an odorless and tasteless, white to slightly off-white, fibrous or granular, free-flowing powder (CAS No. 9004-65-3) that is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC, 1995); its safety in food use has been affirmed by the JECFA (JECFA, 2004). HPMC is a synthetic modification of the natural polymer, cellulose. Specifically, it is a modification of alkali cellulose, which is produced when purified wood pulp is treated with 18% sodium hydroxide solution. Methyl and

hydroxypropyl ether groups are introduced into the molecule by reacting the alkali cellulose with methyl chloride and propylene oxide, respectively. These added groups confer on the molecule its unique properties of being cold-water soluble, while at the same time exhibiting reversible gelation when heated and recooled (Kibbe, 2000; Reilly, 2000).

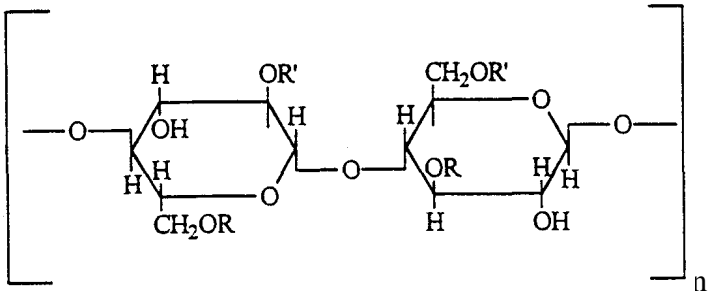


Figure 4. *Hydroxypropyl methylcellulose chemical structure (Al Rawaa, 2006)*

The cellulose ethers are manufactured by a reaction of purified cellulose with alkylating reagents (methyl chloride) in presence of a base, typically sodium hydroxide and an inert diluent. The addition of the base in combination with water activates the cellulose matrix by disrupting the crystalline structure and increasing the access for the alkylating agent and promotes the etherification reaction. This activated matrix is called alkali cellulose (Kirk Othmer, 1993). During the manufacture of HPMC alkali cellulose reacts with methyl chloride to produce methyl cellulose and sodium chloride. Side reactions of the methyl chloride and sodium hydroxide produce methanol and dimethyl ether by-products. The methylcellulose is then further reacted with the staged addition of an alkylene oxide, which in the case of HPMC is propylene oxide as shown in the following equation 1 (Kirk Othmer, 1993).

$$R_{cell}\,OH: NaOH + CH_3Cl \longrightarrow R_{cell}\,OCH_3 + NaCl$$

$$R_{cell}OCH_3 + NaCl + CH_3Cl + {}_XCH_3CH\text{-}CH_2 \longrightarrow R_{cell}\,OCH_3$$

$$(OCH_2\,CH)_XOH + NaCl \qquad \text{Eq. 1}$$

$$CH_3$$

After this reaction, HPMC is purified in hot water, dried and ground.

HPMC is widely used in the food, drug, and dietary supplement industries The physical and chemical properties of HPMC described above make these materials useful in the food industry as stabilizers of emulsions and foams, as a replacement for fat and as a non-caloric bulking agent in foods, as a barrier to oil and in moisture retention, and as a binder. HPMC imparts little or no flavor to food (Be Miller & Whistler, 1996).

Uses in other categories

HPMC is used in the drug industry and is considered as a 'pharmaceutical necessity' (Reilly, 2000). It finds use as a protective colloid by serving as a dispersing and thickening agent, as well as in ophthalmic solutions by providing the demulcent action and viscous properties essential for contact-lens use and in artificial-tear formulations (NLM, 2004; Murray, 2004a,b). The nonpyrogenic, viscoelastic properties of HPMC have also found use in ophthalmic surgery during procedures on the anterior segment of the eye, allowing for ''more efficient manipulation with less trauma to the corneal endothelium and other ocular tissues'' (Liesegang, 1993; Olin, 1995). In drug tablet formulations, HPMC is used for film-coating of tablets and as an extended-release tablet matrix (Kibbe, 2000). It has also been evaluated in humans as an alternative to gelatin as a source for two-piece hard capsules (Honkanen et al., 2001), including by the USDA as a potential organic source of these capsules for packaging some herbal dietary supplements (AMS/USDA, 2004). HPMC is also widely used in the cosmetics industry, primarily in hair shampoo, eye makeup, and skin care preparations, at concentrations ranging typically from 0.1% to 5% in product (CIR, 1986). The regulatory status of hydroxypropyl methylcellulose is provided in table 1.

Table 1. *Regulatory status of hydroxypropyl methylcellulose (George A. Burdock, 2007)*

Agency	Comments	Permitted Functionality	Use limits	Reference
FDA	21 CFR 172.874 Food additives permitted for direct addition to food for human consumption. Subpart I – multipurpose additives	Multipurpose	May be used in food, except in standardized foods which do not provide for such use if: (a) The additive complies with the definition and specifications prescribed in the National Formulary, 12th edition. (b) It is used or intended for use as an emulsifier, film former, protective colloid, stabilizer, suspending agent or thickener, in accordance with good manufacture practice. (c) To insure safe use of the additive, the container of the additive. (is subject to certain labeling requirements described in this section)	21CFR§172.874*
FDA	21 CFR 175.105 Indirect food additives: adhesives and components of coatings. Subpart B – substances for use only as components of adhesives	Adhesive	Defined in regulation	21CFR§175.105**
FDA	21 CFR 175.300 Indirect food additives: adhesives and components of coatings. Subpart C – substances for use as components of coatings	Resinous and polymeric coatings	Defined in regulation	21CFR§175.300***
EU	E464	Emulsifier, stabilizer, thickener, and gelling agent		FSA (UK) (2004)
NAS	534			Clydesdale (1997)
JECFA/ INS	464	Thickening agent; emulsifier; stabilizer	ADI: not specified; applies to the entire class of modified celluloses	JECFA (2004)

ADI = acceptable daily intake; CFR = Code of Federal Regulations; EU = European Union; FDA = US Food and Drug Administration; JECFA/ INS = Joint FAO/WHO Expert Committee on Food Additives/International Numbering System and NAS = US National Academy of Sciences.
*US Code of Federal Regulations (CFR), Title 21, Section 172.874.
**US Code of Federal Regulations (CFR), Title 21, Section 175.105.
***US Code of Federal Regulations (CFR), Title 21, Section 175.300.

II.1.1.3. Group 2: Polymeric materials

These polymers are produced by classical chemical synthesis using renewable bio-based monomers" by classical chemical synthesis for the production of polymers gives a wide spectrum of possible bio-polyesters. To date, polylactic acid (PLA) is the group 2 polymer with the highest potential for a commercial major scale production of renewable packaging materials (Weber et al., 2002). The PLA materials have a good water vapour barrier and have also relatively low gas transmittance. The feedstock can be agricultural resources, example corn or wheat, or alternatively agricultural waste products, such as whey or green juice, may be used (Garde et al., 2000). However, a wide range of other biopolyesters can be made. In theory, all the conventional packaging materials derived from mineral oil can be produced from renewable monomers gained by fermentation. Today, this approach is not economically feasible due to the cost of the production of the monomers (Garde et al., 2000). Lactic acid, the monomer of polylactic acid (PLA), may easily be produced by fermentation of carbohydrate feedstock. The carbohydrate feedstock may be agricultural products such as maize, wheat or alternatively may consist of waste products from agriculture or the food industry, such as molasses, whey, green juice, etc. Recent results point out that a cost-effective production of PLA can be based on the use of green juice, a waste product from the production of animal feeds. PLA is polyester with a high potential for packaging applications. The properties of the PLA material are highly related to the ratio between the two mesoforms (L or D) of the lactic acid monomer. Using 100% L-PLA results in a material with a very high melting point and high crystallinity. If a mixture of D-and LPLA is used instead of just the L-isomer, an amorphous polymer is obtained with a Tg of 60°C, which will be too low for some packaging purposes (Sinclair, 1996).

II.1.1.4. Group 3: Polymeric materials

In this Group the polymers "are produced directly by natural or genetically modified organisms" (Otles & Otles, 2004). Poly-hydroxylalkanoates (PHAs), of which poly-hydroxylbutyrate (PHB) is the most common, are accumulated by a large number of bacteria as energy and carbon reserves. Due to their biodegradability and biocompatibility these biopolyesters may easily find industrial applications. The properties of PHAs are dependent on their monomer composition and it is, therefore, of great interest that recent research has revealed that, in addition to PHB, a large variety of PHAs can be synthesized by microbial fermentation. The monomer composition of PHAs depends on the nature of the

carbon source and microorganisms used. PHB is a typical highly crystalline thermoplastic whereas the medium chain lengths PHAs are elastomers with low melting points and a relatively lower degree of crystallinity.

A very interesting property of PHAs with respect to food packaging applications is their low water vapour permeability which is close to that of LDPE. Recent application developments based on medium chain length PHAs range from high solid alkyd-like paints to pressure sensitive adhesives, biodegradable cheese coating sand biodegradable rubbers. Technically, the prospects for PHAs are very promising. When the price of these materials can be further reduced, application of biopolyesters will also become economically attractive (Walle et al., 2001). To date, bacterial cellulose is rather unexploited, but it represents a polymeric material with major potential. Bacterial strains of *Acetobacter xylinum* and *A. pasteurianus* are able to produce an almost pure form of cellulose (homo-beta-1, 4-glucan) (Iguchi et al., 2000). Its chemical and physical structure is identical to the cellulose formed in plants. Plant cellulose, however, has to undergo a harsh chemical treatment to remove lignin, hemicellulose and pectin. This treatment severely impairs the material characteristics of plant cellulose: the degree of polymerization decreases almost ten-fold and the form of crystallization changes.

Bacterial cellulose is processed under ambient conditions and the degree of polymerization is 15000, 15 times longer than cellulose from wood pulp. Bacterial cellulose is highly crystalline. In bacterial cellulose, 70% is in the form of cellulose I and the rest is amorphous. This composition results in outstanding material properties: a modulus as high as 15–30 GPa was determined across the plane of the film. Production costs of bacterial cellulose are high due to the low efficiency of the bacterial process; approximately 10% of the glucose used in the process is incorporated in the cellulose. The high price of bacterial cellulose of approximately 20 Euro/kg hampers its applicability in low-added-value bulk products. Several high-added-value specialty applications have been developed. The material has been used as an artificial skin, as a food grade on-digestible fiber, as an acoustic membrane, and as a separation membrane (Brown, 1996).

II.1.1.5. Concluding remarks

Cellulose-based materials are being widely used for food- packaging, but apart from these materials, very few other bio-based packaging materials have been commercially introduced. However, developments are taking place at a rapid and increasing speed and commercial trials with PLA-based pots for yoghurts (Germany) and starch based packaging for pasta (Italy) have already been performed. So, bio-based polymers have increasing importance. The main reason is they are produced from renewable resources and also they can be recycled. Bio-based polymers have different categories according to different production methods and different applications in food industry. The researches about applications of biobased polymers show that not only they have suitable properties for applications in food industry but also they have a low cost. If we compare them with petroleum products; having recycle option, products of renewable resources, having low cost and having suitable properties for packaging applications are going to make them the most preferable material in the near future.

II.2. Biopolymer based edible films and coatings

An edible coating is a thin layer of edible material formed as a coating on a food product, while an edible film is a preformed, thin layer, made of edible material, which once formed can be placed on or between food components (McHugh, 2000). Some of their functions are to protect the product from mechanical damage, physical, chemical and microbiological activities. Their use in food applications and especially highly perishable products such as horticultural ones, is based on some particular properties such as cost, availability, functional attributes, mechanical properties (flexibility, tension), optical properties (brightness and opacity), the barrier effect against gases flow, structural resistance to water and microorganisms and sensory acceptability (Falguera et al., 2011).

Edible films are usually classified according to their structural material. In this way, films are based on proteins, lipids, polysaccharides or their composites. For example, a composite film may consist of lipids and hydrocolloids combined to form a bilayer or a cluster (Krochta et al., 1994). In some recent studies the production of edible and biodegradable films by combining various polysaccharides, proteins and lipids is considered with the aim of taking advantage of the properties of each compound and the synergy between them. The mechanical and barrier properties of these films not only depend on the compounds used in the polymer matrix, but also on their compatibility, table 2 (Altenhofen et al., 2009).

Table 2 summarizes the main compounds used in edible films and coatings structural matrices. The optimization of edible films composition is one of the most important steps of the research in this field, since they must be formulated according to the properties of the fruits and vegetables to which they have to be applied (Rojas-Grau et al., 2009a). Thus, it is very important to characterize and test different films for fresh and minimally processed food, since each one of them has different quality attributes to be maintained and enhanced during the storage time. Carboxymethylcellulose, casein (Ponce et al., 2008) and its derivatives (Fabra et al., 2009), locust bean gum, guar gum, ethyl cellulose (Shrestha et al., 2003), gelatin supplemented with glycerol, sorbitol and sucrose as plasticizers (Sobral et al., 2001), composite edible films of gelatin casein cross-linked with transglutaminase (Chambi & Grosso, 2006), pectin (Maftoonazad et al., 2007), cassava starch with natural antimicrobial compounds (Kechichian et al., 2010), pre-gelatinized standard maize starch (Pagella et al., 2002), wheat gluten (Tanada et al., 2005) and mixtures of sodium alginate and pectin, with the addition of $CaCl_2$ as a crosslinker material affecting mechanical properties, water solubility, moisture content, film thickness and its ability to contain calcium (Altenhofen et al., 2009).

Table 2. *Summary of different compounds used in edible films and coatings*

Biopolymer packaging	**Reference**
HPMC with phenolic compound	Akhtar *et al.* 2012
HPMC with colorants	Akhtar *et al.* 2010
HPMC with fatty acids	Jiménez *et al.*2010
HPMC with tea tree essential oil	Sánchez-González *et al.*2010
HPMC with nisin, glycerol	Imran *et al.*2010
HPMC with carboxylic acid	Coma *et al.*2003
Carboxymethylcellulose, casein	Ponce *et al.*2008
Cassava starch	Kechichian *et al.*2010
Locust bean gum, guar gum, ethyl cellulose	Shrestha *et al.*2003
Mesquite gum	Bosquez-Molina *et al.* 2010
Gelatin with glycerol, sorbitol and sucrose	Sobral *et al.*2001
Gelatin-casein cross-linked with transglutaminase	Chambi & Grosso, 2006
Pectin	Maftoonazad *et al.*2007
Pre-gelatinized maize starch	Pagella *et al.* 2002
Beeswax	Morillon *et al.* 2002
Carnauba wax	Shellhammer & Krochta, 1997
Sodium alginate and pectin cross-linked with $CaCl_2$	Altenhofen *et al.* 2009
Maize starch-chitosan-glycerin	Liu *et al.* 2009
Cashew gum	Carneiro-da-Cunha *et al.* 2009 ; Souza *et al.* 2010
Galactomannans	Cerqueira *et al.* 2009a
Galactomannans-collagen-glycerol	Lima *et al.* 2010
Wheat gluten	Tanada-Palmu & Grosso, 2005
Casein derivates with beeswax and fatty acids	Fabra *et al.* 2009
Chitosan	Romanazzi *et al.* 2002; No *et al.* 2002; Devlieghere *et al.* 2004; Martínez-Camacho *et al.* 2010; Aider, 2010
Chitosan-gelatin	Arvanitoyannis *et al.* 1997

Hydroxypropyl methylcellulose (HPMC) has been used in combination with fatty acids to obtain composite films with lower water vapor permeability (WVP) and less transparency in comparison with the same film without lipids (Jiménez et al., 2010). In another study, HPMC films incorporated with nisin greatly affected the transparency, thickness and water sorption behaviour of active films. The presence of plasticizer substantially improved the stretch-ability and transparency but adversely altered the permeability and tensile strength (Imran et al, 2010). Similarly, HPMC has been used in combination with phenolic compounds to obtain composite films. Films containing phenolic compounds presented lower oxygen permeability, and high water vapor permeability (Akhtar et al., 2010).

Polysaccharides and proteins are great materials for the formation of edible films and coatings, as they show excellent mechanical and structural properties, but they have a poor barrier capacity against moisture transfer. This problem is not found in lipids due to their

hydrophobic properties, especially those with high melting points such as beeswax and carnauba wax (Morillon et al., 2002; Shellhammer & Krochta, 1997). To overcome the poor mechanical strength of lipid compounds, they can be used in combination with hydrophilic materials by means of the formation of an emulsion or through lamination with an hydrocolloid film lipid layer. The efficiency of an edible film against moisture transfer cannot be simply improved with the addition of hydrophobic materials in the formulation, unless the formation of a homogeneous and continuous lipid layer inside the hydrocolloid matrix is achieved (Karbowiak et al., 2007; Martin-Polo et al., 1992). In this way, it has been found that fatty acids can form stable layers in sodium caseinate or HPMC matrices, whose properties depend on their chain length: the lower the chain length, the greater the layers (Fabra et al., 2009; Jiménez et al., 2010). Emulsion based films are less efficient in controlling water transfer than bilayer films, as a homogeneous distribution of lipids is not achieved. However, they exhibit good mechanical strength and require a simple process for their manufacture and application, whereas multilayer films require a complex set of operations that depend on the number of coatings. It has been proved, in emulsion based films, that the smaller the particle size or lipid globules and the more homogeneously distributed, the lower WVP (Debeaufort & Voilley, 1995; McHugh & Krochta, 1994; Pérez-Gago & Krochta, 2001). However, its permeability to water vapor can be similar to the values presented by the films based on proteins or polysaccharides (Morillon et al., 2002).

Among polysaccharides, bioactive compounds such as chitosan and its derivatives show a great number of applications focused on active coating systems, in view of the increasing concern about the production of poorly biodegradable plastic materials. Chitosan has a vast potential that can be applied in the food industry because of its particular physicochemical properties such as biodegradability, biocompatibility with human tissues, null toxicity and especially its antimicrobial and antifungal properties (Aider, 2010). In addition to research based on its antimicrobial properties, some aspects such as mechanical and thermal properties and permeability to gases (O_2, CO_2) have been quantified, revealing that chitosan-gelatin films plasticized with water and polyols suffer an increase in permeability as the amount of plasticizers in their formulation is increased (Arvanitoyannis et al., 1997). Chitosan has been extensively used in films and coatings due to its ability to inhibit the growth of various bacterial and fungal pathogens (Romanazzi et al., 2002). Chitosan has also been studied in combination with other biopolymers.

II.3. FUNCTIONALIZATION OF EDIBLE FILMS

To broaden the range of potential applications where food packaging could be used, researchers are increasingly investigating additional functionalization of edible films and coatings by incorporating a variety of active food additives including antioxidants and antimicrobials.

II.3.1. Natural Antioxidants: Chemistry and Sources

Many different chemical compounds have shown some antioxidant activity through different mechanisms of action. According to Laguerre et al. (2007), these mechanisms result in antioxidant activity of a particular compound, such as UV filtration, singlet oxygen deactivation, peroxidant enzyme inhibition, chelation of transition metals, enzymatic detoxification of reactive oxygen species, and their stabilization through hydrogen radical transfer.

II.3.1.1. Tocopherols

Tocopherols are amongst the most commercially exploited antioxidants. The generalized chemical structure of tocopherol has been shown in figure 5 (EFSA Journal, 2008).

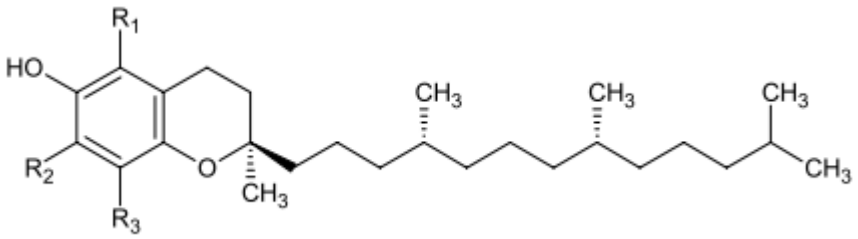


Figure 5. *Generalized chemical structure of tocopherol*

The schematic presentation of the chemical structures of alpha-, beta-, gamma- and delta-tocopherol is given in table 3. α, γ and δ-tocopherols are commonly added to food products, and are denoted by the E numbers E-307, E-308 and E-309, respectively. Extensive studies on the importance of tocopherols indicate that α -tocopherol is the most important lipid-soluble antioxidant, as it protects cell membranes from oxidation by scavenging oxygen free radicals, lipid peroxy radicals, and singlet oxygen (Moon & Shibamoto, 2009).

Table 3. *Properties of four homologues of tocopherol*

Homologue	formulae	Methyl groups			Molar mass g/mol
		R1	R2	R3	
α-tocopherol	$C_{29}H_{50}O_2$	CH_3	CH_3	CH_3	430
β-tocopherol	$C_{28}H_{48}O_2$	CH_3	H	CH_3	416
γ-tocopherol	$C_{28}H_{48}O_2$	H	CH_3	CH_3	416
δ-tocopherol	$C_{27}H_{46}O_2$	H	H	CH_3	402

Schematic presentation of the chemical structures of alpha-, beta-, gamma- and delta-tocopherol (EFSA Journal, 2008).

According to Podsedek, (2007) the predominant reaction responsible for the antioxidant activity of tocopherols is hydrogen atom donation, where a tocopheroxyl radical is formed. Vitamin E (α-tocopherol) is able to disrupt the chain reaction of lipid peroxidation (Christen et al., 1997) thus preventing free radical damage. Vitamin E works in conjunction with vitamin C, the latter regenerating α-tocopherol from the tocopherol radical formed by the reaction with radical oxygen species (Cemeli et al., 2009). The ability of vitamin E to trap peroxyl radicals and singlet O_2 has been reported by previous studies (Kaiser et al., 1990; Chaudiere & Ferrari-Iliou, 1999). According to Leopoldini et al. (2010), the radical scavenging ability of vitamin E is due to the OH group. Vitamin E is related to a series of health benefits such as coronary heart disease protection (Stampfer & Rimm, 1995). According to Greaves et al. (2005), tocopherols have a potent ability to inhibit lipid peroxidation in vivo by trapping peroxyradicals.

II.3.1.2. Phenolic Compounds

Phenolic compounds are a large group of antioxidants, widespread in the plant kingdom. Depending on their chemical structure, they are categorized into groups. The most diverse group is that of flavonoids, built upon a flavone skeleton (Fig. 6).

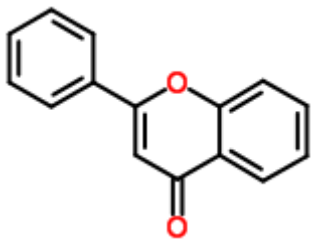


Figure 6. *Flavone (2-phenyl-1, 4-benzopyrone) skeleton*

Phenolics are able to scavenge reactive oxygen species due to their electron donating properties (Podsedek, 2007). Phenolic acids are also known to show transition metal chelation

capacity (Laguerre et al., 2007). Their stability in different systems, as well as the number and location of hydroxyl groups, determine their antioxidant effectiveness. According to Leopoldini et al. (2010) the planar structure of phenolics, which allows conjugation and electronic delocalization as well as resonance effects, is directly linked to a good radical-scavenging activity. The content of polyphenols in vegetables, as well as that of other phytochemicals, is affected by various factors: variety, cultural practices, climatic conditions, maturity at harvest and storage conditions. Colliver et al. (2007) patented a process for increasing the flavonoid content of plants by increasing the activity of chalcone synthase and flavonol synthase.

In recent years, a number of patents have dealt with extraction methods aimed at obtaining phenol-rich extracts from diverse vegetable sources. Cuomo & Rabowskiy (2002) invented several methods for extracting antioxidant compounds from olive-based starting materials, including olives, olive pulps, olive oil and wastewater from olive oil manufacturing. Romanczyk et al. (2004) claimed cocoa extracts (rich in polyphenols and procyanidins), the methods for preparing such extracts and their uses. King & Grabiel (2007) patented a relatively inexpensive extraction method to obtain anthocyanins, other flavonoids and related polyphenolic compounds from fruits and vegetables. Nair (2004) invented a method for isolating a mixture of anthocyanins, bioflavonoids, and phenolics from an edible berry using adsorbent, regenerable resins for reuse.

Resveratrol (trans-3, 5, 4'-trihydroxystilbene) is a natural product found in grapes, mulberries and peanuts. It is one of the main non-alcoholic components in red wines. Its structure is characterized by two phenolic rings, linked by a double bond (Fig. 7). The occurrence of multiple OH groups attached to an aromatic ring is directly linked to a good radical-scavenging activity (Leopoldini et al. (2010). Resveratrol has proved to be an effective antioxidant in different in vitro assays including: total antioxidant activity, reducing power, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$ and $O_2^{\bullet-}$ radical scavenging, hydrogen peroxide scavenging, and metal chelating activities, when compared to standard antioxidant compounds such as BHA, BHT, α-tocopherol, and trolox (Gülçin, 2010).

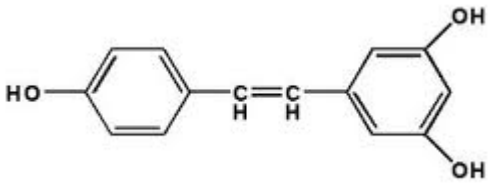


Figure 7. *Chemical structure of resveratrol*

The anthocyanins, anthocyanidins with sugar group(s), are mostly 3-glucosides of the anthocyanidins (Kong et al., 2008) (Fig. 8). These contribute greatly to the antioxidant properties of certain colourful foods, such as grapes and cranberries. As pigments, they are almost exclusively responsible for the red, blue and purple colours in fruits. Cyanidin is the most common anthocyanidin, and the 3-glucoside is the most active antioxidant anthocyanin (Eibond et al., 203).

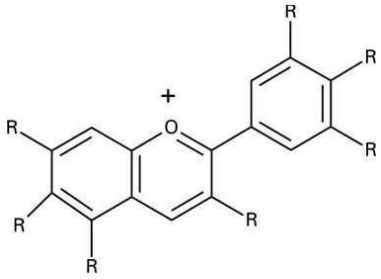
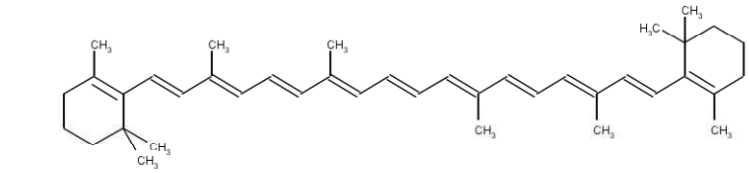


Figure 8. *Chemical structure of anthocyanins*

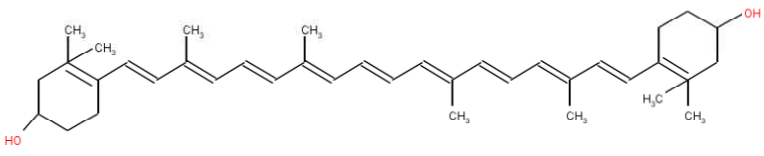
A method directed at efficient one or two step processes for producing phenol enriched compositions from dried and fresh plant material, particularly anthocyanins and proanthocyanins, was patented by Bailey et al. (2005). The method of preparation includes a novel column purification step using a brominated polystyrene resin.

II.3.1.3. Carotenoids

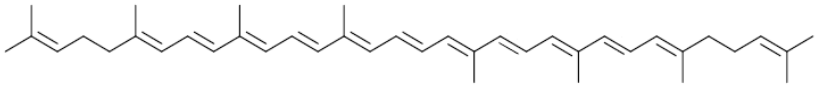
This group of compounds comprises both carotenes and xanthophylls, which are present in many fruits and vegetables. They are red, orange and yellow pigments, and act as vitamin A precursors. Some examples are β-carotene, cryptoxanthin and lycopene. Lycopene is a bright red carotenoid pigment, naturally found in red fruits. Because of its unsaturated nature, lycopene is considered as a potent antioxidant and an oxygen quencher (Rao & Rao, 2007).



β–caroten



Cryptoxanthin



Lycopene

Figure 9. *Chemical structure of some carotenoids: β-carotene, cryptoxanthin and lycopene*

Rice-Evans et al. (1997) found a correlation between low serum β-carotene levels and high rates of cancer, cardiovascular diseases and a high risk of myocardial infarction among smokers. Their conjugated double bonds (Fig. 9) provide carotenoids with a double mechanism of action: they are both quenchers of singlet oxygen and radical scavengers (Podsedek, 2007). It is commonly accepted that the presence of additional functional groups, such as carbon-carbon double bonds, is one of the main structural characteristics for good radical-scavenging activity (Leopoldini et al., 2010). Some recent patents have aimed at improving the characteristics of carotenoids. Takeda Chem. Ind. LTD (2000) obtained a water-dispersible carotenoid composition comprising a dissolving/suspending agent for the carotenoid and an emulsifier. This composition is useful as an additive for food, pharmaceuticals, cosmetics and feeds.

Table 4*. Some examples of naturally occurring colorants approved for use in contact with foods*

Coloring agent (E-number)	Color	Source	References
Anthocyanins (163)	Orange-red to red to blue	Berries, grapes, apples, roses, hibiscus, red cabbage, sweet potato	Schwarz & Winterhalter, 2003
Betacyanins (163)	Red	Red beets, red chard, cactus fruit, bougainvillea	Cai & Corke, 1991; Cai *et al.*1998
Caramel (150)	Beige to brown	Heated sugars	Galati *et al.* 2003;
Carmine (120)	Red	Cochineal insects	Greenfield, 2005
Carotenoids (150)	Yellow to orange to red	Saffron, tomatoes, paprika, corn, butter, palm oil, red salmon	Cai *et al.* 2001
Chlorophylls (141)	Green to olive green	Green plant leaves	Stintzing & Carle, 2004
Riboflavin (101)	Yellow	Vegetable leaves, milk, eggs, organ meats, malt	Galati *et al.* 2003; Stintzing *et al.* 2003
Turmeric (100)	Yellow	Curcuma longa rhizomes	Stintzing & Carle, 2004

Fullmer & Emmick (2005) formulated a stable nanodispersion of one or more carotenoids for use in supplementing aqueous systems, such as foods, beverages and dietary supplements. Hara et al. (1997) obtained a microcapsule including a natural high strength carotenoid, hardly undergoing oxidative deterioration, and useful in foods and medicines. These coloring active agents are usually included in edible films and coatings to enhance their physical and chemical properties. Some naturally occurring coloring active agents are given in table 4.

II.3.1.4. Essential Oils

Essential oils are volatile oils which constitute the aroma and flavor components of organic material (Greaves et al., 2005). They are used in a variety of products such as incense, aromatherapy oils, perfumes, cosmetics, pharmaceuticals, beverages, and foods. The market for these oils demands a consistently high quality and reliable supplies at competitive prices. Essential oils from aromatic and medicinal plants have been known to be biologically active, mainly possessing antibacterial, antifungal and antioxidant properties (Politeo et al., 2007). The main components can represent up to 85% of the total, while the remainder is present as traces. The concentration of the specific compound in the total mix of plant oils can be very variable, depending on factors such as the origin, species and plant organ, climatic conditions and growth, extraction and storage. Essential oils consist mainly of volatile terpenoids, consisting of linked isoprene units in structures of 10 carbons (monoterpenoids) and 15 carbons (sesquiterpenoids). The oil is composed of at least 100 different chemical compounds classified as aldehydes, phenols, oxides, esters, ketones, alcohols and terpenes (Fasseas et al., 2007). Different methods of essential oil extraction have been reported and patented. Some of them generally relate a method for the simultaneous extraction of essential oils and antioxidants from organic material, more particularly organic material from the Lamiaceae family, including rosemary, using solvent blends and which yields a liquid, oily extract containing antioxidants and a liquid extract containing essential oils. The extract containing antioxidants is readily mixed with edible oil to be added to animal feeds and human food.

Some extraction methods produce water soluble antioxidants that can be used in a wide range of food products. Nahas et al. (2010) patented a metal-chelating or sequestering antioxidant composition, derived from edible herbs and spices (mace, thyme, oregano, nutmeg, ginger, cinnamon, clove, basil, marjoram, mustard, savory, laurel and anise) that are useful for incorporating into food, beverages, and nutritional supplements to enhance their stability. In fact, these water soluble antioxidants were disclosed to be useful in fruit juices, processed meat products, such as ham and sausages, processed seafood, butter, margarine, mayonnaise, salad dressings, and essential oils (lemon, lime, grapefruit and orange).

II.3.1.5. Chitosan

Chitosan is a cationic biopolymer that is considered a secondary antioxidant, since it has the ability to chelate the metal ions involved in the catalysis of an oxidative reaction (Tharanathan & Kittur, 2003). Chitosan of differing degrees of N-deacetylation obtained from crab shells showed antioxidant activity, scavenging ability on hydroxyl radicals and chelating ability on

ferrous ions. It was more effective as an antioxidant agent when the deacetylation degree increased (Yen et al., 2008). Similar effects were observed in fungal chitosan obtained from shiitake stipes (Yen et al., 2008). The origin of the scavenging ability of chitosan is related with the presence of active hydroxyl and amino groups in the polymer chains (Fig. 10). The hydroxyl groups in the polysaccharide units can react with free radicals and, according to free radical theory, the amino groups in chitosan can react with free radicals to form additional stable macroradicals. As regards the effect of the molecular weight of chitosan on its antioxidant properties, Xing et al. (2007) showed that low molecular weight chitosan had a stronger scavenging activity on oxygen and hydroxyl groups than high molecular weight chitosan in an in vitro study. The same effect was observed by Feng et al. (2007) by reducing chitosan molecular weight by means of irradiation treatments.

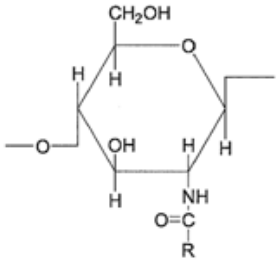


Figure 10. *Chemical structure of chitosan*

II.3.1.6. Synergistic effects

Numerous studies have reported the synergistic effects found in antioxidant mixtures (Eberhardt et al., 2000). Indeed, combinations of antioxidants may be more effective at reducing reactive oxygen species than pure compounds, especially if the mixture includes both water-soluble and lipid-soluble antioxidants. If this is the case, the mixture will be capable of quenching free radicals in both aqueous and lipid phases (Chen & Tappel, 1996). The application of antioxidants (pure, mixtures and extracts) to food products is described in the following section. The synergistic effect in the antioxidant mixtures is a common phenomenon that takes place in the final products.

II.3.1.7. Patents on the use of antioxidant

The number of patents on the application of antioxidants to food products has progressively increased over the last few years. Fukumoto et al. (2007) invented an antioxidant material containing flavonoid aglycon (derived from lemons, limes or sudachis) and vitamin C, a combination in which a synergistic effect is observed. This extract would be added to food products or beverages. Tan et al. (2010) reported the invention of a method for extracting the

antioxidant compounds (phenolics and flavonoids) from the palm tree. These compounds are potent antioxidants to be applied in foods and edible oils. Ahotupa et al. (2006) claimed a food product containing a phenolic compound (hydroxymatairesinol), and found that the administration of this could increase the level of enterolactone, thereby causing cancer prevention.

II.3.1.8. Meat and Fish Products

Meat, poultry and fish products are very prone to lipid oxidation both because of their high content in unsaturated fatty acids and also due to intrinsic factors to processing and storage conditions. In these products, oxidative reactions can lead to undesirable changes in taste, flavor, and color. The addition of antioxidants can reduce the rate of lipid oxidation and hydrolysis by sequestering and stabilizing free radicals. In this sense, Montenegro (2009) optimized a natural antioxidant composition prepared from phenolic extracts of monofloral honey that prevents the oxidation of meat products, especially poultry. The honey extract contained gallic acid, rutin, ferulic acid, salicylic acid, naringenin, kaempferol and a pH of 4.2-5.0. Kolar et al. (2009) patented a natural mixture for the antioxidative protection of fats and foodstuffs containing fats, such as fish, fresh meat, fresh spiced meat, fresh and cooked sausages, salami, dry cured and cooked cured products, and pastrami. The mixture was prepared with an extract of at least one plant selected from the Labiatae family and green tea extract and comprises carnosic acid, rosmarinic acid, and epigallocatechingallate. Fellenberg Plaza et al. (2008) formulated a liquid extract from soapbark tree comprising 1.4-5.4% total phenols. The extract was applied to marinated chicken leg and chicken breast. Results showed that the greater concentration of polyphenols in this extract showed a prooxidant effect after 2 days of refrigeration. Sandoval et al. (2006) succeeded in preventing the discoloration of fresh beef slices, which were vacuum packed and refrigerated, by means of the injection of a vegetable protein composition containing antioxidants (alkali metal salt of isoascorbic acid). Gaynor et al. (2004) incorporated ascorbate or erythrobate as antioxidants in the formulation of emulsified casings prepared with cellulose and nisin and applied to meat products (e.g. frankfurter).

II.3.1.9. Edible Oils

Off-flavors development in edible oils represents a serious problem in the food industry. Lipid oxidation, apart from producing rancid odors and flavours, decreases the nutritional quality and the safety of the product which can be controlled by the addition of antioxidants. Breivik

et al. (2010) provided a composition comprising marine oil and ascorbic acid and/or an ascorbic acid derivative (such as ascorbyl palmitate) that would slow down lipid oxidation in marine oil. This formulation does not include lecithin, which has to be declared as a potential allergen. Antioxidants can also be incorporated in the frying oil. Gertz (2003) obtained a water-in-oil emulsion that can be used as an additive for roasting, simmering or frying fat, preventing lipid oxidation. The emulsion contained antioxidants (tocopherol and/or ascorbyl 92 palmitate) in combination with water-soluble carboxylic acids (citric acid) which are present in the aqueous phase. Cholli et al. (2004) invented a substitute benzene antioxidant polymer to prevent rancidity. This antioxidant polymer can be blended with or mixed with the packaging material, present as a thin film, or be sprayed on the packaging material to form a coating.

II.3.1.10. Fruits and Vegetables

In plant products, enzymatic browning represents the main deteriorative process. This is caused by the enzyme polyphenol oxidase which, in the presence of oxygen, converts phenolic compounds into dark colored pigments. Sardo and Bompeix (2002) patented a process for treating fruits and vegetables with a composition containing one or more tocopherol salts and a terpene in an aqueous solution at 40-60ºC. This process was particularly suitable for treating lettuce, apples and pears after harvesting. Selleck (2004) used a flavonoid for the preservation of minimally processed fruits and vegetables. The products, previously cut and peeled, were sprayed or dipped in a flavonoid solution containing some components such as ascorbic acid, erythorbic acid or alpha lipoic acid. They can also be preserved by the addition of flavonoid and ascorbic acid into juices (if they are not present). Lee and Ryu (2004) patented a tocopherol containing milk rice and a method for its preparation. The method comprised one step where tocopherol was incorporated into the product.

II.3.2. Active food packaging

Active packaging is an innovative concept that can be defined as a mode of packaging in which the package, the product, and the environment interact to prolong shelf life or enhance safety or sensory properties, while maintaining the quality of the product. This is particularly important in the area of fresh and extended shelf-life foods as originally described by Labuza & Breene (1989). Two processes to produce antimicrobial or antioxidant biopackaging can be used in food preservation as illustrated in Fig. 11 (Coma, 2008).

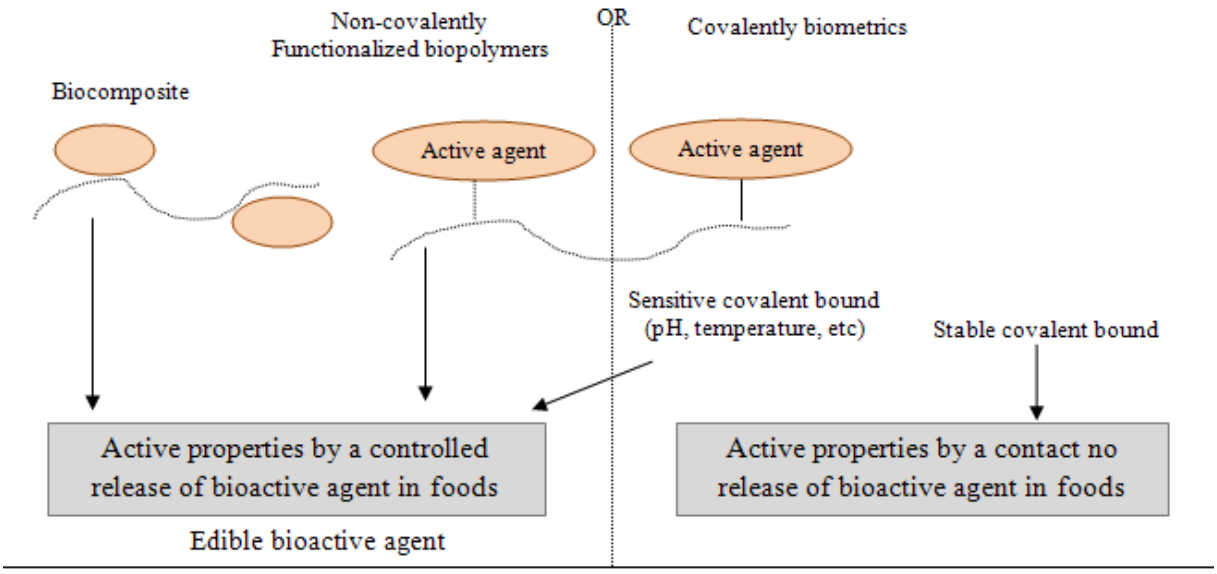


Figure 11. *Processes to produce antimicrobial or antioxidant biopackaging, from biopolymers which are not inherently active*

- Direct incorporation of the active agent into the biomatrix: elaboration of biocomposites
- Utilization of biopolymers or biomatrices which are chemically modified in order to produce bioactive properties or use inherently active biopolymers exhibiting film-forming properties, such as antimicrobial cationic amino-polysaccharides.

II.3.2.1. Fonctionnalization as antimicrobial packaging

The term antimicrobial packaging encompasses any packaging technique used to control microbial growth in a food product. These include packaging materials and edible films and coatings containing antimicrobial agents. In recent years, antimicrobial packaging has attracted much attention from the food industry because of the increase in consumer demand for minimally processed, preservative-free products. Reflecting this demand, the preservative agents must be applied to packaging in such a way that only low levels of preservatives comes into contact with the food. The film or coating technique is considered to be more effective, although more complicated to apply. New antimicrobial packaging materials are continually being developed. Many of them exploit natural agents to control common food-borne microorganisms. Current trends suggest that, in due course, packaging will generally incorporate antimicrobial agents, and the sealing systems will continue to improve.

The focus of packaging in the past has been on the appearance, size, and integrity of the package. A greater emphasis on safety feature associated with the addition of

antimicrobial agents is perhaps the next area for development in packaging technology. Table 4 represents some studies reporting the fonctionalization of edible films with different antimicrobial agents.

Table 4. *Summary of films functionalized as antimicrobial packaging*

Film matrix	Antimicrobial agents	Antimicrobial activity	Reference
HPMC-glycerol	Nisin	+	Imran *et al.* 2010
HPMC	Chitosan	+	Sebti *et al.* 2007
HPMC	Nisin	+	Sebti *et al.* 2002
HPMC	Nisin	-	Sebti *et al.* 2003
HPMC	Nisin	+	Sebti *et al.* 2007
HPMC-lipid	Sodium bicarbonate	+	Valencia-Chamorro *et al.* 2008
Chitosan-HPMC	Potassium sorbate, sodium benzoate	+	Möller *et al.* 2004
Chitosan-MC	--	+	Chen *et al.* 1996
Chitosan	Nisin	+	Sebti *et al.* 2007
Chitosan	Garlic oil	+	Pranoto *et al.* 2005a
Tapioca-starch	Potassium sorbate	+	Flores *et al.* 2007b
Sago starch	Lemongrass oil	+	Maizura *et al.* 2007
Pea starch	Grape seed extracts	+	Corrales *et al.* 2009
Sodium alginate	Garlic oil	+	Pranoto *et al.* 2005b
Sodium alginate	Lactoperoxidase	+	Yener *et al.* 2009
Alginate-apple puree	Oregano oil/ carvacrol	+	Rojas-Graü *et al.* 2007a
Apple puree	Oregano oil	+	Rojas-Graü *et al.* 2006
Tomato puree	Carvacrol	+	Du *et al.* 2008

The addition of 15% stearic acid to HPMC films decreased film inhibitory activity by 70 and 40% for *L. monocytogenes* and *S. aureus*, respectively. This phenomenon was explained by electrostatic interactions between the cationic nisin and the anionic fatty acid, which decreased nisin desorption from the film (Sebti et al., 2002). Similarly, a 3-fold reduction of film antimicrobial activity against K. rhizophila was observed when 18% milkfat was added to the HPMC film (Sebti et al., 2007).

Incorporation of chitosan to HPMC films at concentrations as low as 0.1% (w/v) showed a complete inhibition of the fungus A. Niger (Sebti et al., 2007). On the other hand, when nisin was added to cross-linked HPMC film (98% cross-linking level with citric acid), no antimicrobial activity against the bacterial strain *Micrococcus luteus* 270 was observed (Sebti et al., 2003). The authors concluded that HPMC could potentially graft nisin via ester bonds from the nisin C-terminal carboxylic acid group and cellulosic hydroxyl group. In addition, the primary amine group from the N-terminal position and from the lysine residues could react on the carboxylic function available on citric acid to form amine bonds. In both cases, nisin desorption could be strongly reduced, limiting film antimicrobial activity.

II.3.2.2. Fonctionnalization as antioxidant packaging

Generally, edible films and coatings have as additive lipids to reduce water vapour transfer due to its hydrophobic character. Therefore, incorporation of antioxidants in edible films-forming preparations to increase product shelf life by protecting foods against oxidative rancidity, degradation, and discoloration is became very popular (Baldwin et al., 1995). Most antimicrobial compounds have antioxidant properties. Natural antioxidants such as phenolic compounds, vitamins E and C in place of synthetic antioxidants are extensively used in edible films. For example, the antioxidants citric and ascorbic acid were incorporated into methylcellulose-based edible coatings in order to control oxygen permeability and reduce Vitamin C losses in apricots during storag (Ayranci & Tunc, 1997). Xanthan gum coatings mixed with vitamin E enhanced nutritional quality and improved the surface color of peeled baby carrots (Mei et al., 2002).

Carrageenan or whey protein coatings with added antibrowning agents, ascorbic acid or citric acid, effectively maintained color of apple slices during storage and extended the shelf-life of minimally processed apple slices by 2 week when stored in packed trays (Lee et al., 2003). Recently, banana slices coated with calcium chloride, ascorbic acid and cysteine prevented product weight loss and increase of polyphenol oxidase activity during the 5 days of storage (Bico et al., 2009). Table 5 represents some studies reporting the functionalization of edible films with different antioxidant agents.

Table 5. Summary of films functionalized as antioxidant packaging

Film matrix	Antioxydant agents	Food application	References
HPMC	Ascorbic acid, citric, ginger essential oil	Toasted almonds	Atarés *et al.*2011
HPMC	Colorants	Salmon oil	Akhtar *et al.* 2010
HPMC	Anthocyanins, betalains	--	Akhtar *et al.* 2012
HPMC-Chitosan	Bergamot essential oil	Table grapes	Sánchez-González *et al.* 2010
HPMC	Propolis extract	Table grapes	Pastor *et al.* 2011
CMC	Jujube extract, pomegranate extract	Peanuts	Wambura *et al.* 2008
MC, PEG	Stearic acid, citric acid, ascorbic acid	Apricot	Ayranci & Tunc, 2004
Soya protein	Malic acid, lactic acid	Apple	Eswaranandam *et al.* 2006
Fish skin gelatin	Lignosulphonate	--	Núñez-Flores *et al.* 2012
Chitosan	Garlic oil	--	Pranoto *et al.* 2005a
Polylactic acid	BHA, BHT, PG, TBHQ	--	Jamshidian *et al.* 2012
Sago starch	Lemongrass oil	--	Maizura *et al.* 2007
Pea starch	Grape seed extracts	Pork loins	Corrales *et al.* 2009
Sodium alginate	Garlic oil	--	Pranoto *et al.* 2005b

Essential oils exhibit a wide range of biological effects, including antioxidant and antimicrobial properties. In particular they exhibit antibacterial activity against food borne pathogens. The phenolic components, such as carvacrol, camphor, eugenol, linalool and thymol are most active and appear to act principally as membrane permeabilisers (Burt, 2004). Oregano or garlic essential oil added whey protein isolate edible films exhibited larger inhibitory zones on Staphylococcus aureus, Salmonella enteritidis, Listeria monocytogenes, Escherichia coli O157:H7 and Lactobacillus plantarum as compared to rosemary essential oil incorporated into film-forming preparations (Seydim & Sarikus, 2006). Furthermore, antimicrobial films prepared by incorporating oregano oil into sorbitol-plasticized whey protein isolate films was effective in increasing the beef's shelf life by a factor of 2, while minimizing changes in color (Zinoviadou et al., 2009).

Maizura et al. (2007) reported that starch-alginate edible films containing lemon grass oil are effective in inhibiting the growth of Escherichia coli O157:H7 at all levels. However, generally all these studies showed their efficacy in vitro against various microorganisms but they were not tested with real foods. Moreover, there is a lack of available information about their possible impact on the aroma and flavor of the coated products.

II.3.2.3. Antioxidant capacity of edible films

The antioxidant efficiency of edible films has been tested using different approaches. In some studies, the film was disintegrated and different tests (such as radical scavenging assays) were performed to the resulting formulation. In these cases, the disintegration procedure depended on the material and its solubility properties. For instance, HPMC films can be dissolved in distilled water (Akhtar et al., 2010), whereas a more elaborated procedure freezing, grinding and extraction with methanol) was essential for alginate films (Norajit et al., 2010).

Table 6. *Measurement of the antioxidant capacity of disintegrated edible films*

Film composition	**Antioxidant / additive**	**Measurement method**	**References**
Calcium caseinate & whey protein isolate)	Oregano and/or pimento essential oils	DPD and total phenolic content	Oussalah *et al.* 2004
Squid skin gelatin	Hydrolysates from squid gelatin	FRAP, ABTS	Giménez *et al.* 2009
Tuna-skin and bovine-hide gelatin	Oregano and rosemary extracts	FRAP, ABTS	Gómez-Estaca *et al.* 2009a
Sole skin gelatin/commercial fish gelatin	Borage extract	FRAP, ABTS, iron chelation activity	Gómez-Estaca *et al.* 2009b
Alginate	Ginseng extract	DPPH radical scavenging and reducing power activity	Norajit *et al.* 2010
Chitosan	Green tea extract	DPPH radical scavenging and total phenolic content	Siripatrawan & Harte 2010
Pumpkin oil cake	-	ABTS	Popovic *et al.* 2011
Hydroxypropyl methylcellulose (HPMC)	Beetroot extract	ABTS	Akhtar *et al.* 2012

Some of the reported studies including the following antioxidant tests: 2,2- diphenyl-1-picryhydrazyl radical (DPPH), N-diethyl- phenylenediamine (DPD) radical scavenging assay, ferric reducing antioxidant power (FRAP), 2,2'-azinobis(3- ethylbenzothiazoline- 6-sulphonate) (ABTS) assay and total phenolic content have been summarized in Table 6. Testing the activity of an antioxidant by more than one assay is desirable, because different methods approach this measurement in different ways (Erkan et al., 2008). Very often, radical trapping methods have been applied. These methods - DPPH, DPD, ABTS, FRAP, amongst others (Frankel & Meyer, 2000), measure the ability of an antioxidant agent to intercept free radicals. Additionally, the quantification of specific chemicals of recognized antioxidant activity (such as phenolic compounds) may be performed.

II.4. Application of antioxidant films and coatings on food products

In the last decade, many edible materials have been tested for protection against the harmful effect of oxygen, both on low moisture (nuts) and high moisture (meat, fish, fruit and vegetables) products. Some examples from studies performed on these foodstuffs are given in Tables 7, 8 and 9.

II.4.1. Application on nuts

Nuts are rich in unsaturated fatty acids, which make them very prone to lipid oxidation. Being low moisture products, they should be stored under dry conditions to keep their crispness, which would, in turn, reduce the kinetics of lipid oxidation. Some authors have demonstrated the efficiency of several coatings in prolonging the shelf life of this type of products (Table 7).

Table 7. *Application of antioxidant edible films to nuts*

Film or coating	Antioxidant compound	Application	Analyses	References
Hydroxypropyl cellulose & carboxymethyl cellulose	α -tocopherol BHA, BH	Pecans	Sensory analysis Hexanal	Baldwin and Wood (2006)
Native or heat denatured whey protein isolate, glycerol, lecithin, methyl paraben	Vitamin E	Peanuts	Hexanal content	Lee and Krochta (2002)
Whey protein, glycerol, lecithin, methyl paraben	Vitamin E	Peanuts	Sensory evaluation, hexanal content	Lee *et al.* (2002)
Whey protein, glycerol (60:40 and 50:50), distilled acetylated monoglycerides	(none)	Peanuts	Peroxide value, hexanal content	Maté *et al.* (1996)

The most common indicators of lipid rancidity in nuts are the peroxide value (PV) and hexanal levels. In this sense, Maté et al. (1996) measured the PV of nuts coated with whey protein and acetylated monoglycerides, as compared to non-coated nuts. They observed that non-coated samples showed values above the acceptance limit before 20 days of storage, those coated remained below this limit during most of the storage period (70 days).

The effect of coating thickness and storage relative humidity was also evaluated. The fact that the increase in coating thickness and the decrease in environmental relative humidity led to a decrease in lipid rancidity pointed out that the mechanism of protection of the coatings relies on its oxygen barrier properties. Moreover, the continuity and homogeneity of the coatings was found to be a critical factor to achieve an effective delay in the oxidative reactions. Lee et al. (2002) tested the effect of vitamin E addition into the formulation of whey protein isolate edible coatings by determining the hexanal content of non-coated and coated roasted peanuts. Vitamin E reduced hexanal levels, although the differences were not significant. As the storage time increased, the hexanal content was significantly correlated with the sensory score for rancid attribute, which corroborated that the hexanal measurement is a good indicator of rancidity in this type of products.

II.4.2. Application on meat and fish products

Likewise nuts, meat and fish products contain oxidized fat. Additionally, oxygen could have a very negative effect on the color of meat products, due to the spontaneous oxidation of myoglobin to form metmyoglobin, which imparts brownish color (Bekhit & Faustman, 2005). For this reason, the application of antioxidant films and coatings to meat products may be beneficial. In these products, the level of lipid oxidation is frequently evaluated by measuring thiobarbuthuric reactive substances (TBARS), which are expressed as malonaldehyde content. In this way, Gómez-Estaca *et al.* (2007) monitored the malonaldehyde content of cold smoked sardine during storage at 5ºC, to test the antioxidant efficiency of gelatine coatings incorporated with essential oils. In this case, the incorporation of these plant extracts reduced the lipid oxidation.

***Table 8**. Application of antioxidant edible films to meat and fish products*

Film or coating	Antioxidant compound	Application	Analyses	References
Milk protein-based films	Oregano and/or pimento essential oils	Beef muscle	TBA	Oussalah *et al.* (2004)
Gelatin-based films with chitosan.	Rosemary or oregano essential oils	Cold-smoked Sardine	PV, TBA, total phenol, FRAP method	Gómez-Estaca et al. (2007)
Alginate and glycerol coating	Sodium ascorbate and citric acid	Buffalo meat Patties	TBA, tyrosine value, sensory quality	Chidanandaiah and Sanyal (2009)
Chitosan coatings	Fish oil, vitamin E	Lingcod fillets	TBA	Duan *et al.* (2010)
Chitosan and gellatin	(none)	Fish patties	Colour, viscoelastic properties, TBA, TVBN	Lopez-Caballero *et al.* (2005)
Soy protein	Ferulic acid	Lard	PV	Ou *et al.* (2005)

In other study, Ojagh et al. (2010) developed chitosan coatings enriched with cinnamon oil with the aim of increasing the shelf life of cold-stored trout fillets. Their results revealed that chitosan coatings were effective in protecting lipids from oxidation. Moreover, these results coincide with the trend previously described by Jeon *et al*., (2002) when applying chitosan-based coatings to herring and cod fillets. In this case, both the inherent antioxidant activity and the oxygen barrier properties of chitosan films may have contributed to the control of lipid oxidation in the fish fillets. The influence of chitosan-based coatings in terms of lipid oxidation and colour stability has been also examined in meat products such as ground beef. Suman et al. (2010) showed that coating ground beef patties with chitosan reduced TBARS values and improved the surface red colour of patties as compared to non-coated samples. The antioxidant property of chitosan is attributed to its ability to chelate free iron, released by myoglobin degradation during meat storage (Kamil *et al.*, 2002).

II.4.3. Application on fruits and vegetables

Oxygen can also reduce the quality of plant products. Table 9 shows some examples of antioxidant film and coating applications on some fruits and vegetables. In these formulations, the films and coatings prevent the enzymatic browning, which is caused by the enzyme polyphenol oxidase that in presence of oxygen converts phenolic compounds into dark coloured pigments.

Pérez-Gago *et al.* (2006) studied the development of the browning index of coated and non-coated apple slices to test the antioxidant effect of whey protein beeswax ascorbic acid coatings. Rather than direct antioxidant addition, the best results in terms of colour preservation were obtained by incorporating the antioxidants into the coatings. Lin *et al.*, (2011) treated litchi fruit with chitosan edible coatings, which resulted in a significant reduction in the activity of polyphenol oxidase during storage as compared with non coated samples.

Table 9. *Application of antioxidant edible films to fruits and vegetable products*

Film or coating	**Antioxidant compound**	**Application**	**Analyses**	**References**
Chitosan coatings	Oleoresins: rosemary, onion cranberry, garlic and carvacrol	Butternut Squash	Peroxidase and polyphenoloxidase activities	Ponce *et al.* (2008)
Whey protein concentrate and beeswax	Ascorbic acid, cysteine and 4-hexylresorcinol (4-hexyl)	Apple	Weight loss, colour, sensory evaluation	Pérez Gago *et al.* (2006)
Alginate and gellan with glycerol	Ascorbic acid	Papaya	Water loss, respiration rate, ethylene production, firmness, ascorbic acid content	Tapia *et al.* (2008)
Methylcellulose-polyethylene glycol (3g:1ml) stearic acid	Ascorbic acid, citric acid	Mushroom and cauliflower	Water loss, colour, vitamin C, polyphenoloxidase activity, total phenol	Ayranci and Tunc (2003)
Methylcellulose-polyethylene glycol (3g:1ml) stearic acid	Ascorbic acid, citric acid	Apricots and green peppers	Water loss, vitamin C	Ayranci and Tunc (2004)

II.5. Effect of packaging on light-induced changes in pack foods

The interaction between product, packaging, light, and oxygen is schematically outlined in Fig.12 showing factors of importance for the light absorption by the product and the content of oxygen, respectively.

Factors influencing light absorbed ($I_{abs,p}$):

- Intensity of light (I_0)
- Spectrum of light source
- Absorption ($I_{abs,f}$) and reflection ($I_{r,m}$) of packaging depending on color
- Product reflection ($I_{r,p}$)
- Product absorption ($I_{abs,p}$)
- Contents of specific endogenous absorbers

Factors influencing oxygen (O_2) present:

- Initial gas composition ($O_{2,p}$+ $O_{2,h}$)
- Product-to-headspace volume ratio
- Product respiration
- Oxygen permeability of packaging material

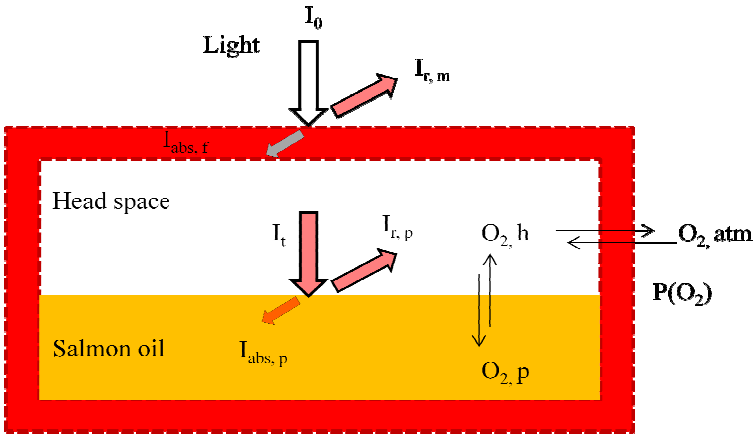


Figure 12. *Schematic overview of salmon product/packaging interactions related to film color and oxygen permeability*

Packaging material and product reflect part of the transmitted light, partly explaining some of the variations from one product or packaging material to another, when exposed to the same light source. Initial gas composition must be taken into account not only in respect of the oxygen present in headspace but also of that at the reaction site, i.e. enclosed in the food matrix.

II.5.1. Gas composition and product-to-headspace volume ratio

Initial oxygen composition must be considered not only in respect for oxygen amount in headspace but also for oxygen enclosed in the food matrix. Oxygen availability is a prerequisite for photo-oxidation. Hence, vacuum packaging limits oxygen content in headspace, thereby minimizing the rate of oxidation (Marsh et al., 1994; Hong et al., 1995b) which is a radical-chain process involving three stages of initiation, propagation, and termination (Gordon, 2001). However, critical oxygen concentration has not yet been determined and is likely to vary between products and to depend upon packaging and storage conditions.

According to Mortensen et al. (2003a, b) very low residual oxygen levels may be obtained by packaging in modified atmospheres with low residual oxygen levels and by using oxygen absorbers. Furthermore, the critical initial oxygen level may also be dependent on the product-to-headspace ratio, i.e. the total amount of oxygen molecules available compared to the amount of photooxidative substrate, i.e. cheese (Mortensen et al., 2003a). Reducing oxygen content of the headspace, e.g. by vacuum packaging or packaging in modified atmospheres with high carbon dioxide/nitrogen and low residual oxygen, and/or using oxygen impermeable packaging, may actually reduce light-induced oxidation by limiting the oxygen pool available for dissolution, and thereby the reaction in the aqueous phase.

It is evident from the above that the relationships between gas composition, product-to-headspace volume, and light-induced changes have not been thoroughly investigated and more research would be useful in this field.

II.5.2. Oxygen transmission of packaging material

The oxygen transmission rate of the packaging material depends first and foremost on the materials used and secondly on the concentration of oxygen inside and outside the packaging material (partial pressures), the relative humidity, and the storage temperature (Paine & Paine, 1992). Generally, a high oxygen transmission rate material in combination with low oxygen content in the headspace of the package leads to a net influx of oxygen molecules. The effect of oxygen transmission rate on product quality has been evaluated for cream cheese (Petterson, et al., 2002), Havarti cheese (Mortensen et al., 2002b), and for processed cheese (Alves et al., 2002).

II.5.3. Light transmission of packaging material

Light protection offered by the packaging depends on numerous factors, including absorption by the material, thickness and coloration, which may be combined to optimize photo-oxidative protection. Different packaging material offer varying degrees of protection against light-induced changes. Generally, metals and metalized films offer the best protection, followed by paper/paperboard, plastics, and finally glass, through which up to about 90% of the light is transmitted (Bosset et al., 1994; Lennersten, 1998). Nelson and Cathcart (1983) examined effects of wall thickness of polyethylene bottles on light transmission and found that increasing thickness led to lower light transmission rates. Similarly, Nelson and Cathcart (1983) and Lennersten (1998) concluded that heavier paperboard resulted in lower overall transmittance. However, increasing wall thickness is not a generally feasible approach, as e.g. present European legislation mandates minimized use of packaging materials (Anon, 1994). Incorporation of pigments into packaging is another way of improving barrier against light transmission. Recently, we investigated that red pigmented packaging pointed out good photo-protective properties for salmon oil (Akhtar et al., 2010), in accordance with findings of Bosset & Flückiger (1989) for yogurts. Mottar (1984) reported the order for efficient protection: black > brown > green > blue > red > yellow > colorless, in accordance with the results of Nelson and Cathcart (1983). The effect of coloring materials may vary somewhat, since different color concentrations are used. In a study by Mortensen et al. (2002b), black laminates offered the best protection against photo-oxidation of Havarti cheese, followed by a white laminate. Mortensen et al. (2003c) also noted that monochromatic UV-light at 366nm was less detrimental than visible light of 405 and 436nm.

II.5.4. Effect of storage conditions

II.5.4.1. Light exposure

Any light source may be characterized by its emission spectrum, i.e. the intensity of radiation at different wavelengths. Light with high quantum energy, i.e. lower wavelength light in the visible/UV-spectrum, has the potential for the most severe effects (Bekbölet, 1990; Lennersten, 1998), as sunlight can be absorbed by a variety of molecular structures. This may be partially prevented when using the so-called warm fluorescent light, which is rich in yellow, orange, and red components, and poorer in the more energy rich violet, blue, and green components (Borle et al., 2001). For obvious reasons, light sensitive food products should never be exposed to direct sunlight or UV-light during manufacturing. Exposure to both ultraviolet radiation and visible light causes oxidative deterioration of lipids, vitamins,

proteins and colorants in foods, leading to the formation of off-flavors, nutrient losses and discoloration. Studies of the effect of fluorescent light on quality changes in milk and milk products (DeMan, 1978; Bosset et al., 1994), oils (Sattar et al., 1976) and other foods (Lennersten & Lingnert, 1998) have shown that factors influencing the deteriorative effect of light include the intensity and spectrum of the light source, the duration of light exposure, the composition of the food product and the light transmittance of the packaging material.

Pesek & Warthesen (1987) studied the effect of fluorescent light on the colour and content of α-carotene, β-carotene and lycopene in a vegetable juice system. After 4 days of light exposure at 2,475 lux, only around 25% of the initial α- and β-carotene remained while 75% of the lycopene was still present. They found that the loss of α- and β-carotene correlated well with a corresponding decrease in yellowness, expressed as Gardner b-value. It was concluded that a slight increase in Gardner L-value (lightness) correlated with the photo-degradation of all pigments, especially lycopene (Lee & Min, 1990).

Studies on Havarti cheese have confirmed that even short light exposure times (<12h) lead to photooxidative quality changes in these cheeses (Mortensen et al., 2002a,b, 2003a,b,c). Fresh cheeses appear to be not as light sensitive as e.g. milk, cream, and butter, but it is still necessary to protect the product from light transmission by proper packaging (Goursaud, 1996). Another study on goat cream cheese concluded that the product was affected by light exposure, and that these changes could be determined by sensory evaluations and fluorescence measurements (Wold et al., 2002). Petterson et al. (2002) also noted that light-exposure affected the quality of cream cheese. In contrast to fresh cheeses, mold-ripened cheeses seem to require only limited light protection. Recently, we (Akhtar et al., 2010; Akhtar et al., 2012) studied the effect of fluorescent light exposure on the physico-chemical properties of salmon oil. After 20 days of light exposure under controlled temperature and relative humidity, salmon oil samples covered by transparent packaging showed maximum photooxidation as compared to that covered by colored packaging acting as light filters.

II.5.4.2. Light Intensity

Increasing the light intensity, i.e. the photon flux, accelerates light-induced oxidation (Deger & Ashoor, 1987; Hong et al., 1995a; Alves et al., 2002). However, a recent study on Havarti cheese, evaluating the effect of light intensity, revealed that 1200 lx, as compared to 600 lx warm fluorescent light, did not accelerate photo-oxidation (Mortensen et al., 2003a). This may be attributable to the relatively low intensities evaluated in the experiment. Light intensities in cheese display cabinets in US retail stores were determined to be in the range of

1100–1900 lx (Deger & Ashoor, 1987), whereas Chapman et al. (2002) noted that light intensities in dairy cases ranged from 750 to 6460 lx. DeMan (1978) concluded that light intensities ranged from 550 to 5500 lx in Canadian dairy display cabinets, whereas in many supermarkets, values in the 1000–3000 lx range were common. Haisman et al. (1992) measured light intensities throughout production (packaging line and cold store), display (three supermarkets), and during transport to the consumers (shaded daylight). Light intensities varied significantly. At the dairy plant, light intensities ranged from 220 to 320 lx at the packaging line to 80–220 lx at the cold store. Unfortunately, measurement of total light intensity cannot be used for prediction of damage potential, unless the light sources measured have identical spectral distribution of radiation; hence, results from different studies can most frequently be compared on a qualitative basis only.

II.5.4.3. Wavelengths

Packaging materials absorb most of the energy-rich UV-light, which, although having the potential, is generally not as harmful to the packaged product as is light in the blue-violet region (400–500 nm) of the spectrum. Light in this wavelength range is absorbed by the two major colorants of the product, riboflavin and β-carotene. Riboflavin may act as a photosensitizer initiating oxidative changes, whereas β -carotene may screen part of this effect off by absorbing photons, which could otherwise have excited riboflavin. The effect of specific wavelengths present in commercial fluorescent light has been insufficiently examined. Sattar and deMan, (1976) were among the first to use sharp-cut filters to evaluate the wavelength effect on light-induced oxidation of lipids, vitamin A and β-carotene. Sattar et al. (1976) concluded by monitoring peroxide values, that milk fat oxidation was affected by wavelengths shorter than 455 nm, indicating riboflavin-sensitizing, and by wavelengths longer than 595 nm, suggesting sensitizing by unidentified green–blue components. Hansen, (1996) noted that monochromatic light at 405 and 448 nm was more detrimental to the examined dairy spread model than was monochromatic light at 460 nm.

II.5.4.4. Storage temperature and exposure time

Temperature changes in the 5–25°C interval had no significant effects on the rate of light-induced lipid oxidation (Sattar et al., 1977; Mortensen et al., 2003a), which is in agreement with the limited temperature dependency expected for photochemical processes (Turro, 1991). However, Kristensen et al. (2001) noted that increasing the storage temperature from 5°C to 37°C for processed cheese resulted in distinct differences in lipid oxidation lready after a few

days of light-exposed storage. Hong et al. (1995a) noted that the effect of storage temperature was difficult to determine with respect to discoloration of annatto-colored Monterey Jack cheese. However, this may be partly due to autoxidative processes. Evidently, prolonged exposure time increases the light-induced damage (Mortensen et al., 2002a,b, 2003a,b,c; Wold et al., 2002; Kim).

II.5.4.5. Concluding remarks

Obviously, detailed knowledge of the food products as well as the packaging methods/materials enables the food scientist to cope with some of the obstacles presented by light-induced oxidation of dairy products. Unfortunately, it is evident that integration of results and conclusions reported on the effects of light on the sensory characteristics of packaged food is very complicated due to different detail levels of reporting experimental set-up and varied methods applied to evaluate the effects. Hence, there is an urgent need for a more systematic approach to shelf-life testing, not only with respect to exposure time and temperature, but also with respect to light sources and their intensity. Summarizing present knowledge, the food manufacturer may reduce photooxidation by (1) minimizing light exposure, (2) optimizing packaging barrier and (3) improving headspace conditions. Light-induced reactions are minimized by storing products in the dark whenever possible, e.g. by the use of non-transparent transport packaging materials, or at least by avoiding exposure to visible light (especially 400–500 nm) by altering the light sources, or by packaging in non transparent materials. A feasible way of reducing light exposure is through innovative packaging design, e.g. using non-transparent materials (Mortensen et al., 2002b; Akhtar et al., 2010; 2012) or applying packaging materials with transparent windows, allowing the consumer to view part of the product. Optimization of the packaging conditions is attained by reducing the surface-to-volume ratio, because photooxidation takes place primarily at the surface of the food product, and by increasing the product-to-headspace volume ratio, thus minimizing the oxygen-to-product ratio. In both cases, residual oxygen levels should be kept as low as possible, preferably <1%, since increased levels will result in more pronounced oxidation. In order to maintain such oxygen levels, low oxygen transmission characteristics of the packaging materials are a prerequisite. Future research will hopefully result in predictive safe modeling of photooxidative quality changes based on measurements of early effects. Combining predictive modeling with product and packaging expertise will ultimately prevent light-deteriorated food from reaching the consumers.

III. Matériel et Méthodes

III.1. Materials

Colors like yellow FFA 200% (Direct yellow 28, water solubility at 90 °C: 20-30 g/l), white aqua color 60672 (PW6, Cl 77891, pH at 20 °C: 8.5), blue patent V (E131) and red pigment aqua color 60056 (PR5 = Cl 12439, pH at 20 °C: 9) were purchased from "Viskase" (France) while green color was prepared by mixing equal quantities of direct yellow 28 and blue patent V (E131) edible colors. Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark). Height and diameter of Petri-dishes were 1cm and 8.5cm, respectively. Salmon oil was extracted from the head of salmon, by means of enzymatic extraction in low temperature (Linder, Matouba, Fanni & Parmentier, 2002).

A viscous red juice of beetroot (*Beta vulgaris*), standardized for color (E 162) with water solubility 100% and pH 4.9 was provided by Naturex, France. A red liquid extract of anthocyanin (amaranthine, E163) with water solubility 100% and pH 3.3 was also provided by Naturex, France. Natural red color (NRC) mixture of beetroot juice (E162) and purple carrot juice (E163) was obtained from ColorMaker (California, USA). HPMC powder (Fluka-Biochemika, Japan) is a biochemical product containing 9% hydroxylpropoxyl and 28% methyl radicals. It had a viscosity of 15mPas and a water solubility of 2% at 25°C. Ethanol (purity > 99%) (Pharmaceutics Carlo, Erba) was used to improve HPMC solublization. The Folin-Ciocalteu reagent (Merck, Darmstadt, France) and sodium carbonate (Sigma-Aldrich, Steinheim, France) were employed for the measurement of the Folin-Ciocalteu total phenolic content. The calibration curve was constructed with gallic acid (Sigma-Aldrich, France). Trolox (6-hydroxy-2, 5, 7, 8-tetramethychroman-2-carboxylic acid; Fluka, 56510) was used as antioxidant standard. For antioxidant capacity measurements ABTS (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and potassium persulfate were sourced from Sigma-Aldrich (France). All organic solvents were analytical grade reagents. Phosphorus pentoxide (P_2O_5) was purchased from Sigma–Aldrich (France). Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark).

III.2. Methods

III.2.1. HPLC analysis

The HPLC equipment was a Shimadzu (Tokyo, Japan) with auto sampler (SIL-20AC), communication bus module (CBM-20A), pump (LC-20AD), column-oven (CTO-20AC) with ULFC (Shimadzu) cooling module in series with a diode array detector (SPD-M20A). Optimum separation of anthocyanins and betalains was achieved on an analytical scale (250 ×

4.6mm i.d.) Agilent C18 (5µM) reversed phase column with a particle size of 5µm (Phenomenex, Torrance, CA), fitted with a security guard C18 ODS (4 × 3.0mm i.d.) at a flow rate of 0.5mL/min and a constant temperature of 25°C. Eluent A was 5% formic acid and B was MeCN/H_2O (60/40, v/v). Separation was accomplished starting with 3%B, followed by a linear gradient to 20%B for 30min and then to 50%B for 40min. Maximum absorption of betalains tended to be higher than those of the anthocyanins. Therefore, an intermediate monitoring wavelength of 530nm was chosen for both pigment groups. Aliquots mixed samples of 20µL were injected for analyses. Duplicate determinations were performed throughout.

III.2.2. Mass spectrometric conditions

The LC–MS equipment includes a binary solvent delivery pump and a linear ion trap mass spectrometer (LTQ-MS, Thermo Finnigan, San Jose, CA, USA). LC analysis parameters were the same as described above except use of a specific LCMS C18 column (150 * 2.1 and 5µm – Alltima, Alltech, France) at a smaller flow rate of 0.2ml/min. LTQ equipped with an atmospheric pressure ionization interface operating in electro spray positive mode (ESI positive). Data were processed using Xcalibur 2.1 software. The operational parameters of mass spectrometer were as follows.

Spray voltage was 4.20kV and the temperature of heated capillary was set at 300°C. Flow rates of sheath gas, auxiliary gas, and sweep gas were set (in arbitrary units min^{-1}) to 35, 10, and 10, respectively. Capillary voltage was -48V, tube lens was -13V, split lens was -38V and the front lens was -4.25V. All parameters were optimized by using a standard rutin solution as representative glycosylated flavonoid (0.1g/L) in mobile phase (A/B: 50/50) at a flow rate of 5µL/min. The compounds of interest were monitored through specific MS2 scans in addition of MS full scan (50 to 1000m/z): MS2 (743), MS2 (581), MS2 (949), MS2 (919), MS2 (889) for the screening of anthocyanins compounds and MS2 (551), MS2 (507), MS2 (389), MS2 (549) for screening of betanin compounds.

III.2.3. Preparation of HPMC films

Film solutions were prepared according to Khwaldia, Banon, Desobry, & Hardy, (2004) by dissolving 6 g of HPMC in a solution of 35 ml ethanol, 65 ml of distilled water and an optimised quantity (1%) of edible color. The solutions were mixed for 40 min at 65 °C with a heating magnetic Stirrer (Fisher Bio-block scientific). After mixing, the solutions were degassed at 50-60°C under vacuum (YAMATO®) for 30min. Film solutions with the same

composition were prepared by incorporating the different edible colors like blue, green, yellow, red and white. After cooling, films were made by pouring 5 ml of each solution (optimised solution composition as in fig. 1), in the lids of the Petri-dishes and then left in darkness at room temperature (20°C) and 50% relative humidity for drying on a levelled surface for 48 h.

III.2.4. Preparation of antioxidant-HPMC composite solutions

Initially, HPMC solutions were prepared by dissolving 6g of HPMC powder in ethanol solution (35% v/v) for 40min at 65°C. Secondly, 2% (v/v) of betalain extract, anthocyanin extract, their mixture (B+AC/50:50) and commercial natural red color (NRC) were dissolved separately in ethanol solution (35% v/v) at 20°C because of their higher light and temperature sensitivity. All antioxidant solutions were then mixed separately with HPMC solutions and stirred for 30min at 20°C to obtain homogeneous solutions. The pH was adjusted at 3.17±0.08 for NRC, 3.30±0.08 for anthocyanin and 4.90±0.08 for betanin solutions with HCl (0.1M). After mixing, the solutions were degassed at room temperature under vacuum "Handy Aspirator WP-15 (Yamato®)" for 30min (Akhtar et al. 2010).

III.2.5. Fabrication of antioxidant films

6g of each film forming solution were put in the Petri-dishes and dried in a dark room at ambient temperature for 48h. For complete evaporation of solvent, films were then incubated at 30°C for one week in a hermetic container containing P_2O_5 powder before each analysis. Finally, antioxidant films were obtained with thickness 48±3µm measured by a mechanical micrometer (Messmer, London, UK) according to ASTM D374–99.

III.2.6. Film conditioning for photo-aging

For photo-aging, the films were conditioned under the fluorescent light (OSRAM L36W/640) or darkness for 20 days in an experimental chamber with controlled conditions of temperature (20°C) and relative humidity (50%). The distance of fluorescent tube from the films was 14cm.

III.3. HPMC FILM CHARACTERIZATION

III.3.1. Film thickness measurement

Thickness was measured according to the standard NF Q 03-016 with a manual micrometer (Messmer, London, England) equipped with a measuring head of 1cm in diameter and a sensitivity of 2µm. The thickness was measured in 8 randomly selected points on each film and then an average value was calculated.

III.3.2. Film color measurements

Color measurements were carried out with a Minolta CM, CR-210 colorimeter (Minolta, Colombes, France) using the Hunter and CIE scale. A black standard color plate (L* = 24.60, a* = 0.16, b* = -0.28) was used as a background for color measurements. Value L*describes lightness (0=black to 100=white). Value a* describes the amount of redness (positive) or greenness (negative) present in the specimen, while Value b* describes the amount of yellowness (positive) or blueness (negative) present in the specimen. Combined values a* and b* define the hue and intensity (saturation) of the color (Moslemi, 1967).The L, a, and b values of each film were taken as the average of at least five points. Color difference (ΔE) is the magnitude of the resultant vector of three component differences. Total color difference (ΔEab), was calculated by following equation:

Eq. 1 $$\Delta Eab = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

where Δa = ai - a0, Δb = bi - b0 and ΔL = Li - L0. The index i, indicates the values observed after storage period and index 0, indicates initial values observed before samples storage (Jutaporn, Suphitchaya, & Thawien, 2011).

III.3.3. Light transparency

UV–visible light barrier properties of films (1cm×3cm) were measured using a UV–visible recording spectrophotometer (Ultro-Spec 4000 UV/visible, Pharmacia Biotech, UK) at selected wavelengths from 200 to 900nm following the ASTM method D 1746-92 with slight modifications (Fang, Tung, Britt, Yada, & Dalgleish, 2002; Hamaguchi, Weng, & Tanaka, 2007). The transparency was calculated from Han & Floros, (1997) equation:

Eq. 2 $$\text{Transparency } (T) = -log(T_{600}/X)$$

where T_{600} is the transmittance (%) at 600nm and X is film thickness in mm. Three replicates of each treatment were tested.

III.3.4. Mechanical properties

A universal testing machine Lloyd instrument (AMETEK, United Kingdom) was used to determine mechanical properties, i.e. tensile strength (TS, MPa), ultimate elongation (UE, percent at break point) and Young's modulus (YM, MPa) according to ASTM D882. Tests were performed on 6 specimens previously stored for 48h at 50±2% relative humidity (RH) in a container using magnesium nitrate saturated solution at 20±1°C. Equilibrated film samples of 6×2cm were stretched at a rate of 10mm/min until breaking. The RH and temperature of the testing environment was held at 50±2% and 20±2°C, respectively. The stress–strain curves were recorded and exploited with Nexygen software.

III.3.5. Water vapor permeability

Films water vapor permeability (WVP) was determined by using a gravimetric method described in the AFNOR NFH00-030 standard (1974), at 38°C and 90% RH gradient. The film was sealed in a permeation cell containing a desiccant (silica gel). The plastic permeation cells used had an exposed film area of 26.42cm^2. The permeation cells were placed in a close chamber having controlled conditions of temperature, (38°C) and RH, (90%). The water vapor transport was determined from the weight gain of the permeation cell that was determined each hor of experiment up to 10 hors. At least three replicates were made for each film. WVP was calculated as follows (Khwaldia, Banon, Perez, & Desobry, 2004):

Eq. 3 $$WVTR = \frac{\Delta M}{\Delta t} \times \frac{1}{A} \ (gh^{-1}\, m^{-2})$$

Eq. 4 $$P = \frac{WVTR}{\Delta p\ 3600} \ (gs^{-1}\, m^{-2}\ Pa^{-1})$$

Eq. 5 $$WVP = P \times X \ (g\ m^{-1}\ s^{-1}\, Pa^{-1})$$

where (ΔM) is the weight gain of the permeation cell over time (Δt), (A*)* is the exposed film area, (Δp) is the differential vapor pressure across the film and (X*)* is the film thickness.

III.3.6. Oxygen permeability

Control and NRC films were conditioned under controlled relative humidity (50%) and temperature (20°C) for one week. Gas chromatography system (Shimadzu GC-4A; Shimadzu Corp., Kyoto, Japan) was used to measure films oxygen permeability by directly injecting

samples with a gas sampling syringe (Dynatec Pressure Lok, Baton Rouge, LA, USA) into a gas chromatograph equipped with a thermal conductivity detector and molecular sieve columns (Desobry & Hardy, 1997). Helium gas at flow rate of 25ml/min was used as carrier gas and column temperature was 50°C. Method was based on measurement of oxygen diffusing through film over time. The film was first sealed into a test cell of 26.42cm^2 exposed area and 0.8 bar oxygen pressure gradient across the film, which was filled with ~100% oxygen. At suitable intervals, gas samples were withdrawn from the cell via a sampling stopper and analyzed by gas chromatography. Oxygen transmission rate (OTR) and then oxygen permeability were determined according to Khwaldia, Banon, Perez, & Desobry (2004).

Eq. 6 $$OTR = \frac{\Delta C \times V}{A \Delta t} \ (cm^3\ m^{-2}\ h^{-1})$$

Eq. 7 $$PO_2 = \frac{OTR \times X}{\Delta p} \ (cm^3\ \ m^{-1}\ s^{-1}\ Pa^{-1})$$

where $\Delta C/\Delta t$ is the slope of O_2 concentration loss over time (t), V is the volume of cup containing ~100% oxygen, A is the area of exposed film sample, X is the film thickness and Δp is the partial pressure difference of O_2 across the film.

III.3.7. Water sorption isotherms

For the water adsorption experiments, films were dried for about 3 weeks at 20°C in a vacuum chamber containing phosphorus pentoxide (P_2O_5). Measurements were obtained on film samples of 30mg using a dynamic vapor sorption system (DVS, SMS Ltd., UK). The samples were equilibrated at 20°C for different relative humidity values. During experiments, RH was increased from 0 up to 90% with a 10% increment. The samples were considered to be at equilibrium when the value $\Delta m/\Delta t$ (slope of mass change with time) was <0.002 mass%/min. The Guggenheim–Anderson–de Boer (GAB) model was used for sorption isotherms modeling (Eq. 7). Non-linear regression (curve fitting), procedure was used to estimate the parameters for this mode with Origin 6.1 software (Origin Lab Corporation, USA).

Eq. 8 $$X = \frac{X_m .C_{GAB} .K .a_w}{(1-k .a_w)(1-K .a_w + C .K .a_w)}$$

where X is adsorbed water at a given a_w (g.g^{-1}), X_m is the monolayer value, and a_w is the water activity (a_w = RH%/100). Constant C_{GAB} is related to the bonding energy of water molecules on the matrix primary interactions sites (monolayer). K is a temperature-dependent constant related to heat of multilayer sorption.

III.3.8. Differential scanning calorimetry analysis

A thermal analysis of the films was performed with a differential scanning calorimeter (DS-7, Netzsch, Germany). 10mg of film sample was weighed and sealed into a DSC aluminum pan. A tiny hole was made in each lid of aluminum pan to allow samples dehydration. Samples were then heated under protective nitrogen (40ml/min) at the rate of 10°C/min from 25 to 130°C and equilibrated at this temperature for 5min then cooled down to 25°C at 10°C/min and reheated up to 300°C at the same rate.

III.3.9. Scanning electron microscopy (SEM)

Structural morphology of dry films (preconditioned at 20°C in P_2O_5 desiccators) was carried out by cryofracturing. Film cross-sections were prepared by dipping a film into liquid nitrogen followed by fracturing with a pre-chilled razor. Freshly fractured film specimens were retrieved from the liquid nitrogen bath and placed as quickly as possible into the Petri dishes containing filter papers. These Petri dishes were then placed in a desiccator to dry and warm to room temperature. Fractured film pieces were than mounted on a SEM tube. All samples were analyzed and photographed in a Hitachi S-4800 scanning electron microscope (Hitachi, Japan) at 0.5 to 2kV. Topographic analysis of film surfaces was carried out by using previously conditioned films stuck onto a cylindrical aluminum stub by a double-sided tape to observe surfaces.

III.4. Films Application on Salmon Oil

5ml of salmon oil sample was taken in each Petri-dish. Firstly, Petri dishes containing salmon oil were covered from the top with transparent lids along with films inside to study the effect of film color alone (shown below in Fig. 1a). Secondly, they were covered directly with films (shown below in Fig. 1b) to study color and PO_2 effects on light induced oxidation of salmon oil. Each side of Petri-dish was covered with black scotch to control oxygen and light permeation. Analyses to study light-induced oxidation of salmon oil were done at 3^{rd}, 6^{th} and 12^{th} days of storage.

III.4.1. Determination of total phenolic contents

Pure extracts and film samples total phenolic content (TPC) was determined using slightly modified Folin-Ciocalteu method (Singelton, Orthifer, & Lamuela-Raventos, 1999). Briefly, 500µL of sample was mixed with 500µL of 100g/L sodium carbonate (Na_2CO_3, Labosi, Paris,

France) and was left for 10min in a water bath at 38°C. 500µL of Folin-Ciocalteu reagent were added and after stirring solution was stored for 15min in darkness at 20°C. Absorbance at 660 nm was measured using a Shimadzu UV-2101 spectrophotometer

III.4.2. Release of antioxidant agents from films

Release test was performed in an environmental incubator at 20°C and 4°C. The total phenolic content (TPC) of samples was evaluated by adapting the elution technique described by Zhang and Kosaraju, (2007). A film (0.541mg) was placed in a beaker containing 100 ml of ethanol (95%) as food simulant. The solution sample was taken out at 10min intervals until 1h and every 1h afterward. Solutions were than diluted and examined for total phenolic content released from the films. Mean of three readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/L of sample solution.

III.4.3. Estimation of Diffusion and Partition coefficient

To determine diffusion coefficient (D) of antioxidants, Fick's second law was used which describes changes of antioxidant agents concentration in the film with respect to time and position. Fick's second law of diffusion is presented in Crank (1975). The total amount of active compound desorbed from film at any time t (M_t) normalized with respect to amount desorbed at equilibrium (M∞) is given below:

Eq. 11 $$\frac{M_t}{M\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[\frac{(2n+1)^2\pi^2}{L^2}Dt\right]$$

where $\frac{M_t}{M\infty}$ is the concentration of natural antioxidants released from the film at time t, divided by the concentration of antioxidants released at equilibrium and L is the film thickness. For short times ($M_t/M\infty < 2/3$), equation 1 can be simplified to equation 2 and then equation 3 for easy calculation of diffusion coefficient (D) as given below:

Eq. 12 $$\frac{M_t}{M\infty} = 4(Dt/4L^2\pi)^{0.5}$$

Eq. 13 $$D = (S.L/2)^2\pi$$

where S is the slope of a plot representing $M_t/M\infty$ against $t^{0.5}$.

Partition coefficient (K) is defined as the ratio of migrant equilibrium concentration in the polymeric material, (C_p) to its equilibrium concentration, in food simulant (C_s) (Tehrany & Desobry, 2004). It can be determined by the following equation.

Eq. 14 $$K = C_p/C_s$$

III.4.4. ABTS radicals scavenging activity

Evaluation of 2,-2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical scavenging activity was based on the ability of antioxidants to inhibit long-life ABTS radical cation, a blue/green chromophore with characteristic absorption at 734nm, in comparison with that of Trolox.

The scavenging of $ABTS^{+\bullet}$ is assumed to be an electron transfer process (Xican, Wang, Chen & Chen, 2011):

Eq. 15 $$ABTS^{+\bullet} + e \rightarrow ABTS$$

$ABTS^{+\bullet}$ was previously produced by the reaction between ABTS diammonium salt and potassium persulfate:

Eq. 16 $$2(NH_4)_2ABTS + S_2O_8{}^{2-} \rightarrow 2SO_4{}^{2-} + 2ABTS^{+\bullet} + 2NH_4{}^{+}$$

Yellow Green

ABTS radical cation was produced by reacting ABTS stock solution (7mM) with 2.45mM potassium persulfate and allowing the mixture to stand in darkness, at room temperature, for 12–16h before use. To study antiradical activity of phenolic compounds, $ABTS^{\bullet+}$ solution was diluted with ethanol at 30°C, to obtain an absorbance of 0.70±0.02 at 734nm. After addition of 1.0mL of diluted $ABTS^{\bullet+}$ solution to 10µL of sample or standard Trolox in ethanol (concentration between 0 and 16µM), the absorbance was measured at 30°C exactly 6min after initial mixing. Appropriate solvent blanks were run with each assay. All experiments were performed in triplicate. A standard curve was obtained by using Trolox standard solution at various concentrations. The absorbance of reaction samples was compared to that of Trolox standard and results were expressed in terms of Trolox equivalents (Re et al., 1999).

III.4.5. FTIR analysis of antioxidant films

Changes in structure of HPMC composite films before and after antioxidants release were followed by Forier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25°C with 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 was set at 4mm. The scanning rate was 10 kHz, and 80 scans were performed both for the reference and the sample from 4000 to 800cm^{-1} with 4cm^{-1} resolution. All data treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using

a nine-point Savitsky-Golay smoothing functions. Elastic baseline correction was applied to spectra, which were further cut between 1800 and 800cm^{-1}, centered and normalized.

III.5. SALMON OIL QUALITY PARAMETERS

III.5.1. Oil Color Measurement

Color measurements were carried out with a Minolta CM, CR-210 colorimeter (Minolta, Colombes, France) using the Hunter and CIE-Lab scale. The L* value describes lightness (0 = black to 100 = white). The value a* describes the amount of redness (positive) or greenness (negative), while the b* value describes the amount of yellowness (positive) or blueness (negative). Combined a* and b* values define the hue and intensity (saturation) of the color (Moslemi, 1967). The L, a, b values of each oil sample were taken as the average of at least 3 replications. Color difference (ΔEab) is the magnitude of the resultant vector of three component differences which was calculated by following equation:

Eq. 9 $$\Delta Eab = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

where Δa = ai - a0, Δb = bi - b0 and ΔL = Li - L0. The index i, indicates the values observed after a given storage period and index 0, indicates initial values observed before samples storage (Jutaporn et al., 2011).

III.5.2. Headspace Oxygen Uptake

The analytical method described above to measure O_2 concentration in the PO_2 cell was also used for headspace oxygen uptake measurement. The Petri-dishes containing oil samples to be studied for oxidation were sealed with their lids containing inside edible films and were placed under fluorescent light. At 3rd, 6th and 12th day, gas samples were withdrawn from the lower compartment of Petri-dish via a sampling valve air tightened with glue (UHU® Patafix) and analyzed by gas chromatography. Tests were performed in controlled conditions of temperature (20°C) and relative humidity (50%). Oxygen content is reported as percent of detected peaks (O_2 and N_2). Analyses were made in triplicate.

III.5.3. Conjugated Dienes Determination of Salmon Oil

For conjugated dienes determination, solutions were prepared according to Pazos et al. (2005) by sampling 0.002g of oil in 4ml of hexane (purity = 97%) in a sterilise test tube. After

mixing (1min), solutions were analysed for conjugated dienes by spectrophotometer (Ultrospec 4000 UV/visible, Pharmacia Biotech, Orsay, France) at a wavelength of 233nm. Absorbance values obtained are averages ± standard deviations of at least 3 replicates.

III.5.4. Fatty Acid Composition of Salmon Oil

The fatty acid composition of salmon oil was determined using a Perichrom^{TM} 2000 gas chromatograph (Perichrom, Saulx-lès-Chartreux, France), equipped with a flame-ionization detector and a fused silica capillary column (50m, 0.25mm i.d. × 0.25µm film thicknesses, CP 7419 Varian, Middelburg, Netherlands). Injector and detector temperatures were set at 260°C. A temperature programme of column initially set at 145°C for 5min, then rising up to 210°C at a rate of 2°C/min and held at 210°C for 10min was used.

Fatty acid methyl esters (FAMEs) were prepared by transmethylation of lipid aliquots as described by Ackman (1998). 100mg of salmon oil was dissolved in 5ml hexane in test tubes in which 200µl KOH methanol solutions (2M) were added. Tubes were then strewed for 1min at nitrogen. After mixing, the solutions were kept at rest for 30min, when both phases were clearly separated. The upper phase was evaporated under a nitrogen stream in order to obtain 100mg/mL FAMEs hexane. The analysis was done by the injection of 1µl FAMEs in gas chromatograph device. Standard mixtures (PUFA 1 from marine sorce and PUFA 2 from animal sorce (Supelco, Sigma–Aldrich, Bellfonte, PA, USA) were used to identify fatty acids (internal standard C21:0). The results were presented from triplicate analyses. To evaluate oxidation extent, polyene index was determined, based on the following formula (Rodriguez et al., 2007):

Eq. 10 $$\text{Polyene index} = \frac{\%\ \text{EPA} + \%\ \text{DHA}}{\%\ \text{C16:0}}$$

III.5.5. FTIR Analysis of Salmon Oil

Salmon oil oxidative stability after 12 days of continuous light exposure was followed by Forier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25°C with 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 was set at 4mm. The scanning rate was 10 kHz, and 80 scans were performed both for reference and sample from 4000 to 800cm^{-1} with 4cm^{-1} of resolution. All data treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-point

Savitsky-Golay smoothing functions. Elastic baseline correction was applied to spectra, which were further cut in required regions, centered and normalized.

IV. Résultats et Discussion

Chapitre IV.1: Contrôle de la photo-oxydation de l'huile de saumon au cours du stockage dans un film d'HPMC : influence de la couleur du film

Transition: Les performances des emballages polymériques sont fortement influencées par les propriétés physico-chimiques des polymères utilisés. Les caractéristiques physiques requises pour un emballage dépendent autant du produit emballé que de l'environnement dans lequel il sera stocké (Nazan, Turhan & Sahbaz, 2004).L'utilisation de coating ou de nouveaux matériaux est devenu un sujet de recherche d'intérêt croissant notamment pour l'industrie alimentaire, car ces nouveaux biopolymères ont généralement une forte capacité à accroître la durée de vie de nombreux produits (Sorrentino et al., 2007). Le challenge aujourd'hui reste leur industrialisation. Les biopolymères ont généralement des perméabilités à la vapeur d'eau et à l'oxygène élevées, des propriétés mécaniques assez faibles et des taux de transmission de la lumière élevés. En effet, l'énergie apportée par la lumière, notamment par les petites longueurs d'ondes du spectre visible, peut entraîner de nombreux dommages à l'aliment car ces ondes sont captées par les structures moléculaires des produits (Lennersten, 1998). Les ondes lumineuses causent de nombreuses modifications qui entraînent une perte de qualité sensorielle et nutritionnelle, des risques sanitaires et économiques (Drusch & Berg, 2008). Cela peut être partiellement contrôlé par l'utilisation de lumière fluorescente chaude qui émettent principalement dans les longueurs d'onde jaune, orange et rouge et peu dans les violets, bleus et verts (Borle et al., 2001). Mais filtrer la lumière peut aussi être un moyen intéressant par exemple en introduisant dans les emballages des pigments colorés (Mottar, 1984; Lennersten, 1998; Bosset & Flückiger, 1989). C'est cet objectif que c'est fixé ce travail. Les produits gras contiennent de nombreux acides gras polyinsaturés (AGPI) très sensibles à la lumière. *Ce travail se propose de contrôler les phénomènes de photo-oxydation de ces produits, en prenant comme modèle l'huile de saumon, lors de leur conservation dans des films d'HPMC (hydroxypropyl methylcellulose) fonctionnalisés par différents pigments colorés.* Les dérivés de cellulose comme l'HPMC sont des biopolymères prometteurs pour le secteur de l'emballage. L'HPMC est soluble dans l'eau et est déjà utilisé dans l'industrie alimentaire comme gélifiant, stabilisateur et émulsifiant. Il permet de former des films qui présentent une perméabilité à l'oxygène intéressante (Tharanathan, 2003; Burdock, 2007; Turowski et al., 2007). Il est inodore et ne modifie pas le goût des produits (BeMiller & Whistler, 1996). Il permet de réaliser de la gélification thermique (Chen, 2007).

Chapter IV.1: Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film color

Transition: The performance of polymeric packaging is greatly influenced by the physico-chemical properties of the polymers used. The physical characteristics required in packaging depend on what item will be packaged as well as on the environment in which the package will be stored (Nazan, Turhan & Sahbaz, 2004). The use of protective coating and suitable packaging by the food industry has become a topic of great interest because of its potential for increasing the shelf life of many food products (Sorrentino et al., 2007). The current challenge for composite films to be used in the packaging industry is their relatively high water vapor and oxygen permeability, poor mechanical behavior and high light transmission rate. Light with high quantum energy, i.e. lower wavelength light in the visible/UV- spectrum, has the potential for the most severe effects (Lennersten, 1998), as sunlight can be absorbed by a variety of molecular structures. Light induced lipid oxidation causes a number of unfavourable changes like decrease in nutritious quality, health risk and economic losses (Drusch & Berg, 2008). This may be partially prevented when using the so-called warm fluorescent light, which is rich in yellow, orange, and red components, and poorer in the more energy-rich violet, blue, and green components (Borle et al., 2001). Light barrier may also be improved by incorporating suitable color pigments into packaging films (Lennersten, 1998; Bosset & Flückiger, 1989). Similar results are expected for products such as lipids containing polyunsaturated fatty acids (PUFA) packed in hydroxypropyl methylcellulose (HPMC) films incorporated with suitable colors. Cellulose derivatives such as HPMC are promising materials for edible coatings or films for packaging. HPMC is a water-soluble polymer used in the food industry as a gelling, stabilizing and suspending agent, emulsifier, protective colloid, film former and as a barrier to oxygen and water vapour (Tharanathan, 2003; Burdock, 2007; Turowski et al., 2007). It gives little or no flavour to food (BeMiller & Whistler, 1996). In addition, HPMC also has a unique property of thermal gelation at high temperature (Chen, 2007). The aim of the present study was to select the suitable bio-degradable film to increase the time of conservation of salmon oil by limiting the happening of photo-oxidation. For this purpose, HPMC films incorporated with different color pigments were tested.

Chapitre IV.1

Contrôle de la photo-oxydation d'huile de saumon conservée dans des films d'emballage à base d'HPMC: effet de la couleur du film

Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film colour

Akhtar, M. J., Jacquot, M., Tehrany, E. A., Gaiani, C., Linder, M., & Desobry, S.

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France.

(Food Chemistry, 2010, 120, 395–401)

Résumé

L'objectif de cette étude est de développer des films d'hydroxypropyle méthylcellulose (HPMC couleur) afin d'éviter la photo-oxydation des graisses et des huiles alimentaires. Dans la présente étude, des films d'HPMC de différentes couleurs (bleu, vert, jaune, rouge et blanc) ont été testés pour leur capacité à contrôler la photo-oxydation de l'huile de saumon. Les échantillons d'huile conservés dans des boites de Pétri sont recouverts d'un film d'HPMC de 40 µm d'épaisseur. Ils sont ensuite placés sous une lumière fluorescente à 20°C. Pendant le stockage, les paramètres chimiques de la qualité de l'huile tels que l'oxydation des graisses ont été suivi pendant 8 jours. La consommation d'oxygène est mesurée par chromatographie en phase gazeuse, les valeurs de dienes conjugués par spectrophotométrie et la composition en acides gras par chromatographie en phase gazeuse (GC). Les résultats de notre étude montrent que les films d'HPMC transparents et colorésde façon appropriée agissent comme une barrière à la lumière suffisante pour éviter la photo-oxydation de l'huile de saumon pendant un stockage prolongé. Les films blanc, rouge et jaune sont les plus efficaces et maîtrisent la photo-oxydation de l'huile de façon comparable à un stockage à l'obscurité. Les échantillons d'huile conservés avec les films bleu et vert montrent une augmentation progressive de l'oxydation de l'huile lors de l'augmentation du temps d'exposition à la lumière. Ce comportement est identique à celui des échantillons conservés avec des films transparents non colorés.

Food Chemistry 120 (2010) 395–401

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film colour

Muhammad Javeed Akhtar, Muriel Jacquot, Elmira Arab-Tehrany *, Claire Gaïani, Michel Linder, Stéphane Desobry

Institut National Polytechnique de Lorraine (INPL), Laboratoire d'Ingénierie des biomolécules (LIBio), ENSAIA-2.av.de la Forêt de Haye, Boîte postale 172-54505, Vandoeuvre les Nancy Cedex, France

ARTICLE INFO

Article history:
Received 24 March 2009
Received in revised form 1 September 2009
Accepted 6 October 2009

Keywords:
Edible packaging
Edible colours
PUFA
Light-oxidation
Fish oil

ABSTRACT

The efforts are being made to design protective hydroxypropyl methylcellulose (HPMC) colour packages to avoid photo-oxidation of edible fats and oils. In the present study, edible films of HPMC containing different edible colours like blue, green, yellow, red and white were tested for their ability to avoid photo-oxidation in salmon oil. The samples taken in petri-dishes and covered with coloured HPMC films of thickness 40 μm were placed under fluorescent light at 20 °C. During storage, chemical parameter of oil quality such as fat oxidation was monitored during 8 days of storage. Oxygen consumption by gas chromatography, conjugated diene values by spectrophotometery and fatty acid composition by gas chromatography (GC) was measured. The results of our study show that HPMC films with suitable edible colours act as adequate light barrier to avoid photo-oxidation of salmon oil during extended storage. HPMC films containing white, red and yellow edible colours show good control of oil photo-oxidation almost similar to the control samples stored in darkness. Oil samples treated with blue and green edible films show gradual increase in oil oxidation with increasing time of light exposure. Oxidation behaviour of samples treated with blue and green films was almost similar to the samples stored in transparent films.

1. Introduction

Non-bio-degradable materials used in packaging are responsible to cause serious environmental problems. As a consequence, in recent years research attention has turned to develop bio-degradable packaging of natural polymers which may be protein, lipid, or polysaccharide-based. Selection of bio-polymer packaging depends on study of its main permeants like oxygen, water vapours and light because they can transfer through the polymer package wall to cause adverse affect on product quality and its shelf life (Nazan Turhan & Sahbaz, 2004). Polysaccharide-based polymer such as HPMC is used in food Industries as an emulsifier, protective colloid, suspending agent, film former and as a barrier to oxygen and water vapour (Tharanathan, 2003). It gives little or no flavour to food (BeMiller & Whistler, 1996). In addition, HPMC also has a unique property of thermal gelation at high temperature (Chen, 2007). Edible films of bio-degradable polymers, in general, have good barrier properties to oxygen especially in the food components susceptible to oxidation, such as unsaturated lipids (Cuq, Gontard, & Guilbert, 1998). Light barrier properties of these films may be enhanced by the addition of suitable edible colours. The absorption of light by naturally occurring or synthetic pigments is principally related in food products, directly exposed to the light. Certain food colourants have been studied in relation with oxidation of lipids (Kajimoto, Yamaguchi, Kasutani, Yoshida, & Shibahara, 1994; Pan, Ushio, & Ohshima, 2005). The most appropriate way to protect a fat rich product is to remove all kinds of light exposure (Moyssiadi et al., 2004) which is very difficult, but one can try to avoid the most harmful wavelength of light. Several studies show that the 400–500 nm regions are the most harmful part of the visible spectral region with regard to photo-oxidation (Bosset, Gallmann, & Siebar, 1993; Mortensen, Sørensen, & Stapelfeldt, 2003). Lipids containing polyunsaturated fatty acids (PUFA) are more sensitive to oxidation than saturated ones which help to predict lipid susceptibility to oxidation processes (Frankel, 1985). PUFA oxidation causes a number of unfavourable changes like decrease in nutritious quality, health risk and economic losses (Drusch & Berg, 2008). So, scientific objective of the present study was to select the suitable bio-degradable edible film to increase the time of conservation of salmon oil by limiting the happening of photo-oxidation. For this purpose, HPMC films with different edible colours were tested.

* Corresponding author.
E-mail address: elmira.arab-tehrany@ensaia.inpl-nancy.fr (E. Arab-Tehrany).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.10.010

2. Materials and methods

2.1. Materials

HPMC (Fluka–Sigma–Aldrich Biochemika) used during the experiments is a biochemical product containing 8.8% hydroxylpropoxyl and 28.18% methyl contents. It has viscosity as ~15 mPa s and moisture contents about 2% at 25 °C. Ethanol of 96.2% (pharmaceutics CARLO, Erba) was used. It plays a very important role to improve hydration of the HPMC, helps to dry the film and also reduces the formation of air bubbles in film solution. Hexane (HPLC Quality) was provided by Carlo Erba (Val de Reuil, France) and is concentrated at 97%. Edible colours like yellow FFA 200% ("Direct yellow 28", water solubility at 90 °C: 20–30 g/l), white aqua colour 60672 (PW6, Cl 77891, pH at 20 °C: 8.5), blue patent V (E131) and red pigment aqua colour 60056 (PR5 = Cl 12439, pH at 20 °C: 9) were purchased from "Viskase" (France) while green colour was prepared by mixing equal quantities of direct yellow 28 and blue patent V (E131) edible colours.

Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark). Height and diameter of petri-dishes were 1 and 8.5 cm, respectively. Salmon oil was extracted from the head of salmon, by means of enzymatic extraction in low temperature (Linder, Matouba, Fanni, & Parmentier, 2002).

2.2. Film preparation

Film solutions were prepared according to Khwaldia, Banon, Desobry, and Hardy (2004) by dissolving 6 g of HPMC in a solution of 35 ml ethanol, 65 ml of distilled water and an optimised quantity (1%) of edible colour. The solutions were mixed for 40 min at 65 °C with a heating magnetic Stirrer (Fisher Bio-block scientific). After mixing, the solutions were degassed at 50–60 °C under vacuum (YAMATO®) for 30 min. Film solutions with the same composition were prepared by incorporating the different edible colours like blue, green, yellow, red and white. After cooling, films were mad by pouring 5 ml of each solution (optimised solution composition as in Fig. 1), in the lids of the petri-dishes and than left in darkness at room temperature (20 °C) and 50% relative humidity for drying on a levelled surface for 48 h.

2.3. Film thickness measurement

The thickness of films was determined according to the standard NF Q 03-016 with a manual micrometre (Messmer, London, England) equipped with a head measuring 5 mm in diameter a sensitivity of 2 μm. The thickness was measured in 10 randomly selected points on each film and then an average value was calculated.

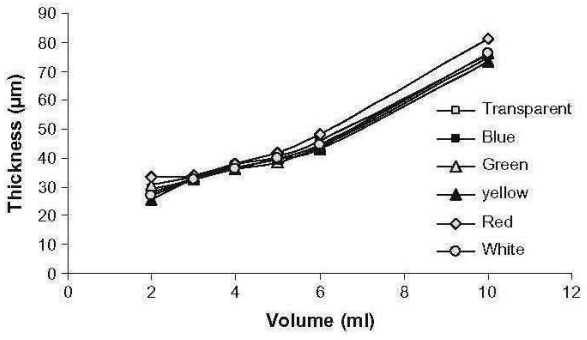


Fig. 1. Comparison between volume of solution (ml) and thickness (μm) of HPMC films with different colours.

2.4. Film colour measurement

Measurements were carried out with a Minolta CM, CR-210 colourimeter (Minolta, Colombes, France) employing the Hunter and CIE scale. Measurements were taken as the average of at least four points of each film (sample) to be tested. Colour difference (ΔC) is the magnitude of the resultant vector of three component differences: lightness difference, ΔL; red–green chromaticity difference, Δa; and yellow–blue chromaticity difference, Δb (Valencia Rodrıguez, 2001). Colour differences were calculated as

$$\Delta C = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

where L, represents lightness; a, redness; b, yellowness; and $\Delta a = ai - a0$, $\Delta b = bi - b0$ and $\Delta L = Li - L0$. The index i, indicates the values observed for each storage time (2, 4 and 8 days) and index 0, indicates the references used given in Table 1.

2.5. Light transmission and transparency

The barrier properties of HPMC films against ultraviolet (UV) and visible light were measured at selected wavelengths between 200 and 900 nm, using a UV–visible recording spectrophotometer (Ultrospec 4000 UV/visible, PHARMACIA BIOTECH, Orsay, France) according to the procedure given by the Fang, Tung, Britt, Yada, and Dalgleish (2002). The transparency of the films was calculated by the equation: transparency = A_{600}/x or log (T_{600}/x), where A_{600} is the absorbance at 600 nm, T_{600} is the % transmittance at 600 nm and x is the film thickness in millimetres (Han & Floros, 1997).

Fig. 2 shows all the phenomena involved in the interaction between light and coloured filter in the case of a real interface between two media. The light passing through the blue filter, for instance, is blue because all the other colours are filtered out, or absorbed, by the blue filter.

2.6. Method of conditioning the samples

Salmon oil (5 ml) sample was taken in each petri-dish covered from the top with HPMC films. Each petri-dish was covered along the sides with black scotch in order to avoid of oxygen and light permeation and were placed under the fluorescent light (OSRAM L36w/640) for 8 days. Analyses to study the light-induced oxidation of salmon oil kept under the coloured edible films, were done on 2nd, 4th and 8th day of storage. While conditioning the samples, distance of fluorescent tube from the sample was 14 cm, temperature of room was 20 °C and number of petri-dishes was 18.

2.7. Oxygen contents

Oxygen content in each petri-dish was determined using a gas chromatographic system (Shimadzu GC-4A; Shimadzu Corp., Kyo-

Table 1
The colours with their standard reference values of L^*, a^* and b^* (AFNOR, 2007).

Colours	L	a	b
Blue	53.81	−0.65	−38.66
Green	48.89	−45.59	8.39
Yellow	81.31	17.72	83.32
Red	42.72	47.70	10.68
White	96.46	−0.16	2.35
Black	24.60	0.16	−0.28

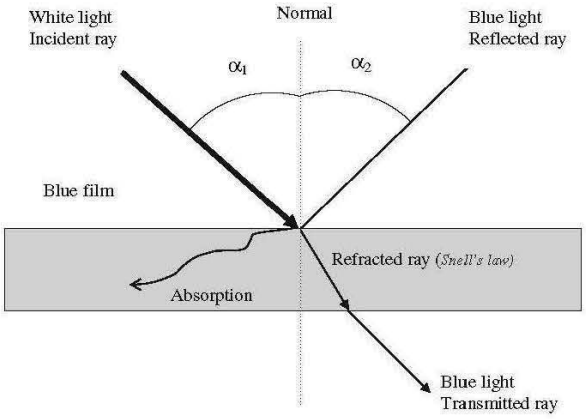


Fig. 2. Fluorescent light in air incident on blue film where blue light is partly reflected at the interface and partly transmitted through the blue film. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to, Japan). Gas concentrations were measured by directly injecting samples with a gas sampling syringe (Dynatec Pressure Lok, Baton Rouge, LA, USA) into a gas chromatograph equipped with a thermal conductivity detector and two columns (Hayesep for CO_2 and molecular sieve for O_2). The flow rate of the helium carrier gas was 25 mL/min and column temperature was 50 °C. This method was based on measurement of the amount of oxygen consumed during photo-oxidation over time. The petri-dishes containing oil samples to be studied for oxidation were sealed with their lids containing inside edible films and were placed under fluorescent light. At 2nd, 4th and 8th day, gas samples were withdrawn from the lower compartment of petri-dish via a sampling valve air tightened with glue (UHU® Patafix) and analysed by gas chromatography (Khwaldia et al., 2004). Testing was performed in controlled conditions (50% R.H., 20 °C). Oxygen content is reported as percent of detected peaks (O_2 and CO_2).

2.8. Conjugated dienes

The solutions were prepared according to Pazos, Gallardo, Torres, and Medina (2005) by taking oil sample of 0.002 g/4 ml of 97% hexane in the sterilised test tube. After mixing of one minute, these solutions were analysed for conjugated dienes by spectrophotometer (Ultrospec 4000 UV/visible, PHARMACIA BIOTECH, Orsay, France) at a wavelength of 233 nm.

2.9. Fatty acid composition of salmon oil

The fatty acid composition of salmon oil was determined by using gas chromatography (GC). Fatty acid methyl esters (FAME) were obtained by transmethylation of lipid aliquots (100 mg) according to Ackman (1998). Oil (100 mg) was dissolved in 5 ml hexane which was added in 200 µl (2 M) KOH methanol solution (1.29 g of potassium hydroxide/10 ml methanol). The tubes were then strewed for 1 min at nitrogen. After mixing, the solutions were kept at rest for 30 min, when both phases were clearly separated; one got the upper stage and was focused on evaporation under a stream of nitrogen in order to obtain 100 mg/1 ml FAME hexane. The analysis was done by the injection of 1 µl FAMEs in the device Perichrom™ 2000 gas chromatograph (Perichrom, Saulx-les-chartreux, France), which was separated on a fused capillary column of silica 50 m in length and 0.25 mm of internal diameter (CP7419 VARIAN, North America). The temperature was set as follows: 2 min initial period at 120°, then increasing at 39.9°/min to reach a second step at 180° during 8 min, and flowing out at 3°/min to the final period (220°, 45 min). Injection and detector ports were both maintained at 260°.

Polyene index was calculated by the following formula (Lin, Lin, & Hwang, 1995):

$$\text{Polyene index} = (\%\text{EPA} + \%\text{DHA}) \times 100/\%\text{C16} : 0) \quad (2)$$

2.10. Statistical analysis

Data were subjected to analysis of variance using the Excel 97 software programme (Microsoft, CA UAS) and where statistical differences were noted, Analysis of variance (ANOVA) was used to compare means of different variables.

3. Results and discussion

3.1. Formation of HPMC films

Several films based on bio-polymer (HPMC) were prepared with different edible colours like white aqua colour, blue patent V (E131), direct yellow 28, red aqua colour and green colour (prepared by mixing blue and yellow). During this work, conditions were optimised for solution preparation, like HPMC 6 g, distilled water 65 ml, ethanol 35 ml and edible colour 1%. Addition of glycerol as plasticiser makes the film more flexible and increases its vapour permeability. Same results have been shown by Navarro-Tarazaga, Sothornvit, and Perez-Gago (2008). Observations showed that addition of glycerol along with edible colours has adverse affect on surface smoothness of edible films. Many air bubbles in the solution and pinholes on films surfaces were found. It was noted that once the temperature is exceeded of 65 °C, the molecules of HPMC flocculate and turn into gel form due to an increase in the viscosity of solution. This phenomenon is due to an increase in hydrophobic interactions caused by lower layers of moisture around the polymer chains.

3.2. Thickness optimisation of different colour HPMC films

Film thickness is an important factor for the optimal functional performance. This factor is highly dependent on the concentration of dry matter and film preparation methods. By way of comparison, Mallikarjunan, Chinnan, Balasubramaniam, and Phillips (1997) have prepared solutions of HPMC film with a concentration of 2 g/100 ml of solvent. These films have a mass/volume less than our films produced from the solutions at a concentration of 6 g HPMC/100 ml solvent. We prepared different HPMC films by the incorporation of different edible colours and found that edible colours with same quantity have no effect on the thickness of HPMC film. A slight increase in film thickness was observed due to high density of red colour.

If the composition and conditions (solution preparation temperature and duration and density of colours) of solutions are similar, the edible colours having almost same quantities have no effect on film thickness. So, we can form different coloured films of same thickness by using same volume of solution. We used 5 ml solution of each colour to form films of thickness 40 µm.

3.3. Colour measurement of HPMC films

Normally, the films based on HPMC are transparent. They are flexible and visually smooth without defects while the films containing glycerol are bright and more flexible than those without glycerol (Khwaldia et al., 2004). The transparency of HPMC films shows that HPMC has an ability to be solubilised completely in sol-

vent composed of 35% ethanol and 65% water (Tarvainen et al., 2003). Addition of suitable colours has changed the transparency of the films. Table 2 shows the values obtained for colour parameters (a, b and L) of different films. ΔC values of different coloured HPMC films were measured at 2nd, 4th and 8th day. No significant difference was found in colour of HPMC films at 2nd, 4th and 8th days during the phenomena of photo-oxidation. So, we can consider that each film is identical within a series.

3.4. Light transmission and transparency

Lighting conditions directly influence degree of lipid oxidation (Papachristou et al., 2006). Table 3 below shows the transparency of coloured HPMC films in the range of 0.81–3.29 and indicates a prominent difference of light transparency with suitable colours. The light transparency of edible films with respect to different colours is in the order of transparent 3.29 > yellow 3.26 > green 3.03 > blue 2.84 > red 0.85 > white 0.81. Numerous studies have shown that greater the light transparency of edible films greater is the photo-oxidation of the product packed in it (Fang et al., 2002; Shiku, Hamaguchi, & Tanaka, 2003). Same observations have been found from the results, we observed more photo-oxidation of salmon oil in transparent edible films exposed to the fluorescent light. Similarly white edible films have shown minimum light transparency (0.81) and given significant control of photo-oxidation, very close to that of samples stored in darkness. The yellow films have transparency very close to that of transparent films but have good control against photo-oxidation similarly green films have transparency more than that of blue films but they are more effective against photo-oxidation. So, the results showed that not only the transparency but also colour of edible films plays very important role to protect stored product from photo-oxidation. Edible films with different colours transmit light of different wavelengths, i.e. blue (410–470 nm), green (510 nm), yellow (570 nm) and red (650 nm) and photo-oxidation is a wavelength dependent phenomenon (Lennersten & Lingnert, 2000).

Light transmission properties of coloured edible films at selected wavelength of 200–900 nm are shown in Fig. 3. These properties can be discussed in characteristic visible spectral region (400–700 nm).

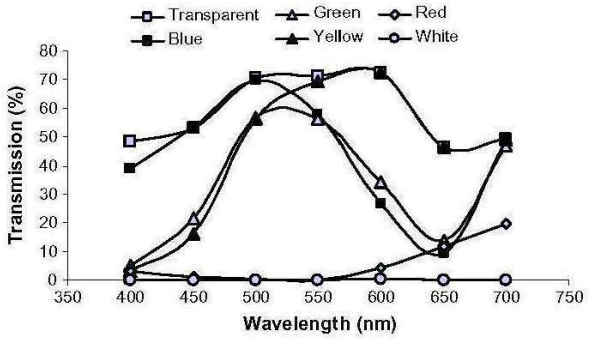


Fig. 3. Light transmission (% T_{600}) of edible HPMC films like transparent and of different colours blue, green, yellow, red and white. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Light transmission rate of all the films was variable in all the different wavelength regions and it was due to colour difference of the films. We have found maximum transmission of transparent and blue lights in the visible spectral region ranging from 400 to 500 nm which is responsible of photo-oxidation in samples treated with transparent and blue films. Similarly red and white, edible films showed no more transmission in visible region of 400–700 nm so, they are more protective against light-oxidation (Fig. 3). Lennersten and Lingnert (2000) indicate that the 400–500 nm range is the most harmful part of the visible spectral region with regard to photo-oxidation. We found that blue and green lights were more harmful than yellow and red lights in the visible spectral region same results have been shown by Lennersten and Lingnert (2000). Similarly, according to Sattar, Tavanger, and Ahmad (1983), homogenised milk samples were treated with clear glass bottles, green bottles, amber glass bottles and Tetra-Pack waxed packaging at 16–24 °C. Statistical treatment of data obtained by them revealed that amber glass bottles gave the best protection to milk from flavour changes; tetra-pack and green glass bottles were intermediate and clear glass bottles were least effective, just like the exposed control.

Table 2
ΔC values of HPMC films of different colours measured with data colour International (D65/10) at 2nd, 4th and 8th days.

Films	ΔC		
	2nd Day	4th Day	8th Day
Transparent	64.29 ± 0.02	64.38 ± 0.15	64.32 ± 0.09
Blue	68.94 ± 0.10	68.70 ± 0.07	68.61 ± 0.07
Green	68.87 ± 0.32	68.46 ± 0.69	68.43 ± 0.39
Yellow	65.70 ± 0.16	65.50 ± 0.03	65.57 ± 0.10
Red	71.35 ± 0.36	71.53 ± 0.09	71.59 ± 0.44
White	07.11 ± 0.47	07.41 ± 0.77	07.32 ± 0.50

Table 3
Light transparency (log (T_{600}/x)) of edible HPMC films, transparent and of different colours (white, blue, yellow, red, and green).

Nos.	Edible films	Film thickness = x (mm)	% Transmission at 600 nm (T_{600})	Transparency = log (T_{600}/x)
1	Transparent	0.038 ± 0.009	73.51	3.29
2	Blue	0.039 ± 0.008	26.73	2.84
3	Green	0.037 ± 0.008	39.2	3.03
4	Yellow	0.039 ± 0.009	71.28	3.26
5	Red	0.040 ± 0.007	0.28	0.85
6	White	0.040 ± 0.008	0.26	0.81

3.5. Oxygen contents

The dissolved oxygen concentration at the beginning of experiment was almost similar, slightly high concentration may be due to sufficient amount of oxygen present in the headspace of the petri-dishes covered with different colour HPMC films. Upon continuous light exposure, oxygen concentration was decreased as a function of the storage time in the petri-dishes covered with transparent, blue, green and yellow edible films while remained constant in those covered with red and white edible films. This decline was most remarkable for green, blue and transparent films, respectively. The difference in dissolved oxygen concentration between petri-dishes can be considered as a measure for the O_2 consumption in light-induced oxidation that differs in different coloured edible films (Fig. 4).

The results show that light passes easily through transparent HPMC film and causes photo-oxidation, so, more oxygen has been consumed with the passage of time. With blue HPMC films, there was a gradual decrease in the oxygen percentage while with green HPMC films; the oxygen percentage was decreased suddenly after 4 days. The samples covered with other coloured films like red, yellow and white showed very slight decrease in O_2 percentage which

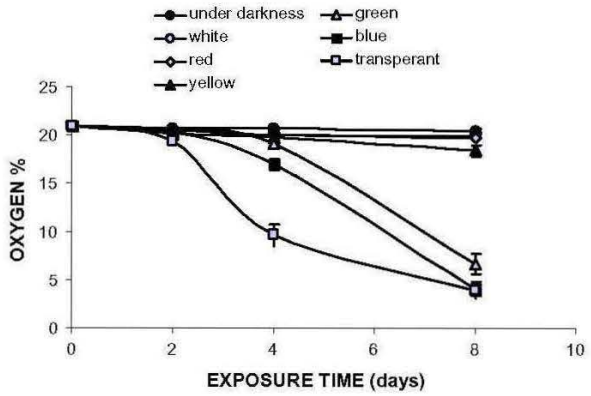


Fig. 4. Quantity of oxygen in petri-dishes covered with HPMC films of different colours and kept under light.

means that these colours cause hindrance to the light to pass through the films (Fig. 4). Blue light was more effective to cause photo-oxidation because it has short wavelength in the maximum range of 450 nm and more oxygen was consumed, similarly green light also has short wavelength in maximum range of 510 nm but it was less effective than blue light.

3.6. Conjugated dienes

The conjugated dienes are the primary products of oxidation; in fact it is the re-arrangement occurring to the positions of double bonds of fatty acids. Conjugated dienes have conjugated double bonds separated by one single bond. These compounds absorb at a wavelength of 233 nm. Their values increased over time for different colours of edible films (Fig. 5). Oil samples covered with transparent HPMC films showed a rapid increase in formation of conjugated dienes. Samples covered with blue and green HPMC films showed gradual increase in formation of conjugated dienes in relation with time. Under darkness the conjugated diene formation in control sample was not significantly more different from those of samples treated with red and white films.

During oxidation of PUFAs containing methylene, interrupted dienes and polyenes, there is a shift in the double bond positions due to isomerisation and conjugate bond formation. This is accompanied by increased UV absorption at 234 nm for diene unsaturation and 268 nm for triene unsaturation (Wanasundara & Shahidi, 1994). Red films have ability to transmit red spectral light (Chandrasekaran, 2001) which has a specific wavelength of 510 nm (Lennersten & Lingnert, 2000) and was more effective against

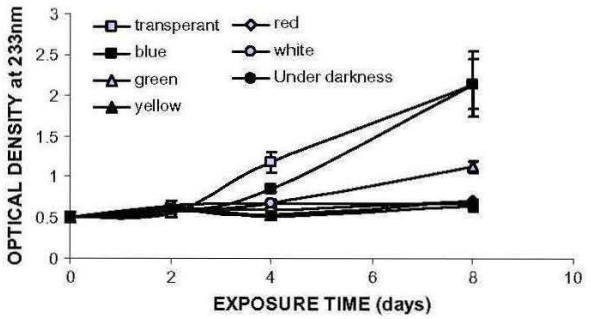


Fig. 5. Evaluation of the absorbance of conjugated dienes of salmon oil covered with HPMC films of different colours at 233 nm for a retention period of 8 days under fluorescent light.

photo-oxidation than blue light having its short wavelength of 475 nm. The transparent films have ability to transmit all the wavelengths of light and it was more prone to form conjugated bonds. Similarly all other films having their ability to absorb specific wavelength of light have specific effect to form conjugated bonds. For instance, blue film showed high values of conjugated dienes due to their ability to absorb short wavelength of 410–475 nm, while red films showed low values of conjugated dienes due to their ability to absorb wavelength of 510 nm. So, it was noted that shorter the wavelength absorbed by the samples more was the formation of conjugated dienes.

3.7. Fatty acids composition

It can be concluded that there is a gradual increase in the photo-oxidation of samples treated with coloured edible films and exposed to the fluorescent light.

According to the literature, increase in the quantity of fatty acid (C16) and decrease in docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) with the time represents oxidation in the samples. Quantity of (C16) increases from 13.56 to 16.54 while the quantities of EPA (C20:5*n*3) and DHA (C22:6*n*3) decreases from 9.20 to 7.59 and 14.25 to 10.32, respectively, within the samples treated with blue films. Similar results have been shown by the sample treated with green edible films. This is an evidence of photo-oxidation process. Salmon oil exposed to blue radiation oxidised very quickly. Just after 2 days the concentration of C16 started to increase and after 4 days of storage the increase was very rapid. The samples treated with blue light were found to be decoloured at 8th day of storage. This may be due to the presence of astaxanthin as the major carotenoid orange pigment in salmon oil (Mendes-Pinto, Choubert, & Morais, 2004). Orange colour is the complimentary to blue colour and has ability to absorb blue light (Chandrasekaran, 2001). So, it can be conclude that change of sample colour may be due to the absorption of blue light.

Polyene index has been reported as an excellent index for the idea of monitoring degradation of polyunsaturated fatty acids during extended storage (Lin et al., 1995). To characterise evolution of this index during storage, the fatty acid profile was determined and the calculated values are presented in Table 4. Polyene index values of the entire sample were calculated using the percentages of major polyunsaturated fatty acids EPA (20:5*n*-3) and DHA (22:6*n*-3) and the percentage of C16, according to Eq. (2) and a graph was plotted between exposure time (days) and polyene index as follows.

Samples treated with different coloured edible films showed the variable polyene index at the end of storage period. Polyene index decreases in the samples covered with transparent, blue and green HPMC films, while the samples with other coloured films have slight change in values (Fig. 6). This index does not seem to be the most adequate to monitor the oxidation of fish oil because only a small decrease is observed during all the storage period. It is obvious that in the transparent and blue films, the effect of light was more pronounced than in other coloured films. The higher degree of lipid oxidation recorded for the blue HPMC films as compared to red HPMC films, maybe attributed to its complimentary colour (orange) and the colour of the salmon oil (orange) (Chandrasekaran, 2001).

The transparent films can transmit the wavelength in the range of 250–800 nm and all other colours have their own specific absorption range, the shorter wavelengths have more energy (Lennersten & Lingnert, 2000). Obviously, the coloured HPMC films have provided better protection to the product with respect to light than transparent HPMC films but blue colour has an extremely low protective effect on the photo-oxidation. To obtain a significant protection effect, it is necessary to use the complementary colour

Table 4
Fatty acid composition (% of total fatty acids) of salmon oil in petri-dishes (covered with different coloured HPMC films and kept under light), measured by gas chromatography at 2nd, 4th and 8th days.

Edible films	Fatty acids	Light exposure time			
		0 Reading	2nd Day	4th Day	8th Day
Transparent	C16	13.6 ± 0.64	15.5 ± 0.43	15.8 ± 0.32	17.3 ± 0.63
	C20:5n3 (EPA)	9.20 ± 0.18	9.02 ± 0.20	8.69 ± 0.15	7.50 ± 0.25
	C22:6n3 (DHA)	14.3 ± 0.93	12.9 ± 0.35	12.0 ± 0.12	10.3 ± 0.47
Blue	C16	13.6 ± 0.64	13.9 ± 0.15	14.5 ± 0.43	16.5 ± 0.17
	C20:5n3 (EPA)	9.20 ± 0.18	9.23 ± 0.040	9.11 ± 0.14	7.59 ± 0.150
	C22:6n3 (DHA)	14.3 ± 0.93	14.3 ± 0.18	13.3 ± 0.19	10.3 ± 0.29
Green	C16	13.6 ± 0.64	16.1 ± 0.17	16.2 ± 0.35	17.1 ± 0.26
	C20:5n3 (EPA)	9.20 ± 0.18	8.82 ± 0.14	8.81 ± 0.07	7.10 ± 0.25
	C22:6n3 (DHA)	14.3 ± 0.93	12.2 ± 0.22	12.5 ± 0.50	10.7 ± 0.47
Yellow	C16	13.6 ± 0.64	14.6 ± 0.14	15.1 ± 0.30	15.9 ± 0.43
	C20:5n3 (EPA)	9.20 ± 0.18	9.27 ± 0.13	8.84 ± 0.11	8.77 ± 0.05
	C22:6n3 (DHA	14.3 ± 0.93	13.5 ± 0.15	12.5 ± 0.30	12.2 ± 0.29
Red	C16	13.6 ± 0.64	15.1 ± 0.11	15.9 ± 0.26	16.0 ± 1.17
	C20:5n3 (EPA)	9.20 ± 0.18	8.83 ± 0.05	8.83 ± 0.10	9.08 ± 0.25
	C22:6n3 (DHA	14.3 ± 0.93	12.4 ± 0.19	12.2 ± 0.07	12.5 ± 0.46
White	C16	13.6 ± 0.64	14.2 ± 0.64	14.6 ± 0.36	15.8 ± 0.33
	C20:5n3 (EPA)	9.20 ± 0.18	9.31 ± 0.09	9.13 ± 0.09	8.87 ± 0.04
	C22:6n3 (DHA	14.3 ± 0.93	14.0 ± 0.49	13.5 ± 0.29	12.4 ± 0.22
Under darkness	C16	13.6 ± 0.64	14.0 ± 0.09	15.0 ± 0.20	14.8 ± 0.10
	C20:5n3 (EPA)	9.20 ± 0.18	9.23 ± 0.05	9.25 ± 0.05	8.91 ± 0.05
	C22:6n3 (DHA	14.3 ± 0.93	14.3 ± 0.02	13.2 ± 0.29	12.8 ± 0.23

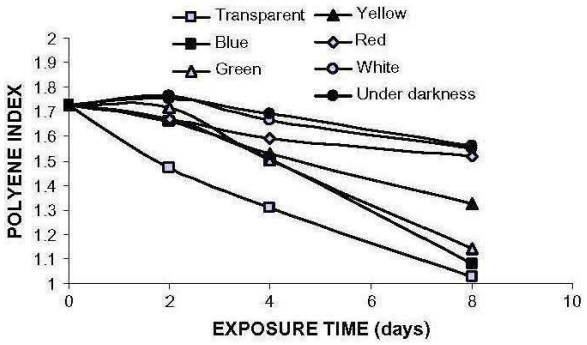


Fig. 6. Evolution of polyene index with respect to the extended light exposure time (days).

of the blue–green one, i.e. the red–brown (Borle, Sieber, & Bosset, 2001).

The higher rate of fat oxidation was recorded for oil in transparent HPMC films after 8 days of storage may be compared to other samples. Similar results have been shown by Cladman, Scheffer, Goodrich, and Griffiths (1998) for milk packaged in various plastics containers after a storage period of 6 days. Also, Vassila, Badeka, Kondyli, Savvaidis, and Kontominas (2002) have reported that photo-oxidation of milk packaged in various HDPE (high density polyethylene) pouch materials and paper board cartons increases with the storage time. Alexander Saffert (2006) has reported that the use of highly pigmented PET bottles to reduce the light transmittance around 10–15%, or better below 10%, at a wavelength of 450 nm appear to protect the milk fat from photo-oxidation.

4. Conclusion

HPMC films containing suitable edible colours provided an attractive and convenient form of packaging, offering protection against photo-oxidation in PUFA rich salmon oil stored under fluorescent light (OSRAM L36w/640) at 25 °C. Observed lipid oxidation was higher in samples treated with transparent, blue and green films. The yellow edible films provided almost equivalent protection with regard to photo-degradation as compared to the samples stored under darkness while white and red films provided maximum amount of protection against photo-oxidation. The shelf life of salmon oil tested in the present study was 8 days. Based on analytical evaluation the loss in O_2 percentage measured by gas chromatography (GC) was maximum in samples treated with transparent and blue films while minimum in that treated with white and red films. The conjugated dienes values measured by spectrophotometer were high in samples treated with transparent and blue films while low in that treated with red and white films. Based on the fact of specific wavelength absorption, as well as spectral transmission curves of edible films tested by spectrophotometer, it is suggested that the use of white and red HPMC films in salmon oil packaging will provide a better protection to its light-sensitivity. Keeping in view the consumer demands, use of yellow HPMC films would be more suitable because of its transparency and protection behaviour against photo-oxidation. In general, such kind of bio-degradable films fulfil the environmental concerns and are able to protect food from qualitative and quantitative deteriorations. In addition, the use of such edible films with suitable colours may also provide a unique opportunity to attract the consumers and might increase the consumption of fat rich commodities.

Acknowledgements

The authors are very grateful to both of Carole Jeandel and Carole Perrood, "Laboratoire d'Ingénierie des biomolécules" (LIBio), ENSAIA, France, for their availability and maximum help in this favour. We would like to thank the Higher Education Commission (HEC), Government of Pakistan for funding and its kind responsibility.

References

Ackman, R. G. (1998). Laboratory preparation of conjugated linoleic acids. *Journal of the American Oil Chemists Society, 75*, 541–545.

AFNOR (2007). Association Française de Normalisation, FD X08-012-2. <www.afnor.org>.

Alexander Saffert, G. P. J. J. (2006). Effect of package light transmittance on the vitamin content of pasteurized whole milk. *Packaging Technology and Science, 19*, 211–218.

BeMiller, J. N., & Whistler, R. L. (1996). Carbohydrates. In O. R. Fennema (Ed.), *Food chemistry* (3rd ed., pp. 205–207). New York, NY: Marcel Dekker Inc..

Borle, F., Sieber, R., & Bosset, J. O. (2001). Photo-oxidation and photoprotection of foods, with particular reference to diary products: An update of a review article (1993–2000). *Sciences des Aliments, 21*, 571–590.

Bosset, J. O., Gallmann, P. U., & Sieber, R. (1993). Influence of light transmittance of packaging materials on the shelf-life of milk and dairy products, a review. *Le Lait, 73*(1), 3–49.

Chandrasekaran, J. (2001). Chemistry of colours. *Resonance, 6*(3), 66–75.

Chen, H. H. (2007). Rheological properties of HPMC enhanced Surimi analyzed by small- and large-strain tests: I. The effect of concentration and temperature on HPMC flow properties. *Food Hydrocolloids, 21*(7), 1201–1208.

Cladman, W., Scheffer, S., Goodrich, N., & Griffiths, M. W. (1998). Shelf-life of milk packaged in plastic containers with and without treatment to reduce light transmission. *International Dairy Journal, 8*, 629–636.

Cuq, B., Gontard, N., & Guilbert, S. (1998). Proteins as agricultural polymers for packaging production. *Cereal Chemistry, 75*, 1–9.

Drusch, S., & Berg, S. (2008). Extractable oil in microcapsules prepared by spray-drying: Localisation, determination and impact on oxidative stability. *Food Chemistry, 109*, 17–24.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science, 67*, 188–193.

Frankel, E. N. (1985). Chemistry of autoxidation: Mechanism, products and flavor significance. In D. B. Min & T. H. Smouse (Eds.), *Flavor chemistry of fats and oils* (pp. 1–37). Champaign: AOCS.

Han, J. H., & Floros, J. D. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film and Sheet, 13*, 287–298.

Kajimoto, G., Yamaguchi, M., Kasutani, S., Yoshida, H., & Shibahara, A. (1994). Influence of synthetic food colorants on oxidative deterioration of oil. *Nippon Shokuhin Kogyo Gakkaishi, 41*(11), 793–796.

Khwaldia, K., Banon, S., Desobry, S., & Hardy, J. (2004). Mechanical and barrier properties of sodium caseinate-anhydrous milk fat edible films. *International Journal Food Science and Technology, 39*, 403–411.

Lennersten, M., & Lingnert, H. (2000). Influence of wavelength and packaging material on lipid oxidation and colour changes in low-fat mayonnaise. *LWT – Food Science and Technology, 33*(4), 253–260.

Lin, C. C., Lin, S. Y., & Hwang, L. S. (1995). Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. *Journal of Food Science, 60*, 36–39.

Linder, M., Matouba, E., Fanni, J., & Parmentier, M. (2002). Enrichment of salmon oil with *n* 3 PUFA by lipolysis, filtration and enzymatic re-esterification. *European Journal of Lipid Science and Technology, 104*, 455–462.

Mallikarjunan, P., Chinnan, M. S., Balasubramaniam, V. M., & Phillips, R. D. (1997). Edible coatings for deep-fat frying of starchy products. *Lebensmittel-Wissenschaft und Technologie, 30*, 709–714.

Mendes-Pinto, M. M., Choubert, G., & Morais, R. (2004). Effect of dietary bile extracts on serum response of astaxanthin in rainbow trout (*Oncorhynchus mykiss*): A preliminary study. *Aquaculture Nutrition, 10*, 353–357.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2003). Response surface models used for prediction of photooxidative quality changes in havarti cheese. *European Food Research and Technology, 216*(2), 93–98.

Moyssiadi, T., Badeka, A., Kondyli, E., Vakirtzi, T., Savvaidis, I., & Kontominas, M. G. (2004). Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: Chemical and sensorial aspects. *International Dairy Journal, 14*(5), 429–436.

Navarro-Tarazaga, M. L., Sothornvit, R., & Perez-Gago, M. B. (2008). Effect of plasticizer type and amount on hydroxypropyl methylcellulose-beeswax edible film properties and postharvest quality of coated plums (Cv. Angeleno). *Journal of Agricultural and Food Chemistry, 56*(20), 9502–9509.

Nazan Turhan, K., & Sahbaz, F. (2004). Water vapor permeability, tensile properties and solubility of methylcellulose-based edible films. *Journal of Food Engineering, 61*(3), 459–466.

Pan, X., Ushio, H., & Ohshima, T. (2005). Effects of molecular configurations of foo colorants on their efficacies as photosensitizers in lipid oxidation. *Food Chemistry, 92*(1), 37–44.

Papachristou, C., Badeka, A., Chouliara, I., Kondyli, E., Kourtis, L., & Kontominas, M. G. (2006). Evaluation of polyethylene terephthalate as a packaging material for premium quality whole pasteurized milk in Greece. Part II. Storage under fluorescent light. *European Food Research and Technology, 224*(2), 237–247.

Pazos, M., Gallardo, J., Torres, J., & Medina, I. (2005). Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food Chemistry, 92*, 547–557.

Sattar, A., Tavanger, R., & Ahmad, S. (1983). Effect of packaging materials on photochemical changes in buffalo milk. *Zeitschrift Fur Lebensmittel-untersuchung Und-forschung A Food Research, 177*, 121–123.

Shiku, Y., Hamaguchi, P. Y., & Tanaka, M. (2003). Effect of pH on the preparation of edible films based on fish myofibrillar proteins. *Fisheries Science, 69*, 1026–1032.

Tarvainen, M., Sutinen, R., Peltonen, S., Mikkonen, H., Maunus, J., Vähä-Heikkilä, K., et al. (2003). Enhanced film-forming properties for ethyl cellulose and starch acetate using *n*-alkenyl succinic anhydrides as novel plasticizers. *European Journal of Pharmaceutical Sciences, 19*(5), 363–371.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: Past, present and future. *Trends in Food Science and Technology, 14*(3), 71–78.

Valencia Rodriguez, M. T. (2001). Efecto del tratamiento de preservacion por depresion e la actividad acuosa en la calidad del alga. Tesis de Magister en Bromatologia y Tecnologia de la Industrializacion de Alimentos. FCEN-UBA. Argentina.

Vassila, E., Badeka, A., Kondyli, E., Savvaidis, I., & Kontominas, M. G. (2002). Chemical and microbiological changes in fluid milk as affected by packaging conditions. *International Dairy Journal, 12*, 715–722.

Wanasundara, U. N., & Shahidi, F. (1994). Canola extract as an alternative natural antioxidant for canola oil. *Journal of the American Oil Chemists' Society, 71*(8), 817–822.

Chapitre IV.2 : Fabrication et caractérisation physico-chimique de films d'HPMC fonctionnalisés par des extraits de plantes commerciaux : influence de la lumière et de la composition du film

Transition: Suite aux problèmes sanitaires et environnementaux causés par les additifs synthétiques, on peut observer un accroissement de l'intérêt pour les produits naturels. C'est pourquoi les biopolymères à base de polysaccharides, gommes, protéines et leurs complexes combinaisons avec des antioxydants naturels se développent afin d'encore mieux protéger naturellement les produits (Ray & Bousmina, 2005). Depuis plusieurs années, le marché propose de plus en plus d'extraits naturels de plantes pas seulement pour leur pouvoir colorant mais aussi pour le fort potentiel des pigments qu'ils contiennent (Frank et al., 2005). En effet, ce sont généralement des composés phénoliques très actifs. De plus, leurs sources sont abondantes : les fruits, les légumes, les déchets de l'agro-industrie (Ali et al., 2008; Liu, Qiu, Ding, & Yao, 2008; Bonilla, Mayen, Merida, & Medina, 1999).

Plusieurs études ont été publiées sur l'activité anti-inflammatoire, antioxydante et antiradicalaire des bétanines issues d'extraits de betteraves (Beta vulgaris L.) (Gentile, Tesoriere, Allegra, Livrea, & Alessio, 2004; Kanner, Harel, & Granit, 2001; Pedreño & Escribano, 2000). D'autres composés d'intérêt présents dans ces extraits sont les anthocyanines. Elles sont très utilisées dans l'industrie comme substitut des colorants synthétiques et ont de nombreux bénéfices pour la santé comme par exemple la réduction du risque de cancer et de maladies cardiaques (Bell & Gochenaur, 2006; Dai, Patel & Mumper, 2007). Plutôt que d'incorporer directement ces composés dans les aliments, leur utilisation pour fonctionnaliser des films d'emballage semble très prometteuse car ils peuvent ainsi améliorer les propriétés antioxydantes, antimicrobiennes, mécaniques, barrières, thermiques et la couleur de ces films d'emballage (Bifani et al., 2007). La structure moléculaires de ces molécules présente de nombreux groupements hydroxyl qui peuvent interagir avec les groupements OH des dérivés de la cellulose (Bifani et al., 2007). L'objectif de cette étude est d'étudier la capacité d'un extrait commercial de couleur rouge (NRC) à améliorer les propriétés de nos films d'HPMC. La stabilité des films fonctionnalisés et de l'extrait est étudiée lors d'un stockage prolongé sous lumière fluorescente.

Chapter IV.2: Fabrication and physicochemical characterization of HPMC films with commercial plant extract: Influence of light and film composition

Transition: Due to increased health and environmental concerns replacement of synthetic chemical additives with natural edible compounds in packaging is a modern concept in the food industry. In response to this consumer requirement, bioactive films based on natural biodegradable polymers, such as structural polysaccharides, gums, proteins, lipids and their complexes (Ray & Bousmina, 2005), combined with natural antioxidants is one of the most promising technologies. Development of such bioactive films can protect food against chemical and physical damages and reduce food preservatives. Recently, the use of plant natural products such as fruit or vegetable extracts has gained a considerable market, not only because of their coloring potential but also the positive physiological attributes of their pigments (Frank et al., 2005). These phenolic compounds are among the most effective and abundant bioactive compounds from different fruits and vegetables, agro-industrial wastes, and by-products (Ali et al., 2008; Liu, Qiu, Ding, & Yao, 2008; Bonilla, Mayen, Merida, & Medina, 1999).

Several studies on the anti-inflammatory, antioxidant and antiradical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Gentile, Tesoriere, Allegra, Livrea, & Alessio, 2004; Kanner, Harel, & Granit, 2001; Pedreño & Escribano, 2000). Anthocyanins play a role in industry as synthetic colorant replacer and have health benefits, including reduced risk of cancer and heart diseases (Bell & Gochenaur, 2006; Dai, Patel & Mumper, 2007). As opposed to mixing these phenolic compounds directly in foods, their incorporation into edible packaging is particularly encouraged since they can improve antioxidant, antimicrobial, mechanical, barrier, thermal and coloring properties of edible packaging (Bifani et al., 2007). The molecular weight and structure of these phenolic compounds show great variations and contain different numbers of hydroxyl groups capable to interact with cellulosic OH groups (Bifani et al., 2007). The aim of this work was to investigate the effect of natural red color (NRC) addition on HPMC films properties, such as optical, mechanical, barrier, thermal and structural properties. Films and NRC stability during storage under fluorescent light were also investigated.

Chapitre IV.2

Fabrication et caractérisation physico-chimique de films d'HPMC fonctionnalisés par des extraits de plantes commerciaux : influence de la lumière et de la composition du film

Fabrication and physicochemical characterization of HPMC films with commercial plant extract: Influence of light and film composition

Muhammad-Javeed Akhtar, Muriel Jacquot, Majid Jamshidian, Muhammad Imran, Elmira-Arab Tehrany, Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France.

(Food Hydrocolloides, 2013, 31, 420-427)

Résumé

Un extrait naturel rouge constitué principalement de bétacyanines (NRC) est reconnu pour son activité anti-oxydante. Cette étude a porté sur l'effet de son incorporation sur les propriétés physico-chimiques de films d'hydroxypropyle méthylcellulose (HPMC). Des films d'HPMC témoins avec et sans glycérine ont été préparés afin de comparer leur comportement avec celui des films contenant du NRC à différents niveaux (1%, 2%, 3% ou 4% v / v). L'effet du photo-vieillissement de ces films a été évalué en suivant leurs propriétés optiques, mécaniques, barrières, thermiques ainsi que les propriétés structurales des films. La résistance à la traction et le module de Young des films contenant du NRC a diminué, tandis que l'allongement à rupture a sensiblement augmenté par rapport aux films témoins. Les données de sorption dynamique à la vapeur d'eau modélisées par le modèle de Guggenheim-Anderson-de Boer (GAB) ont montré des valeurs inférieures de d'énergie de sorption pour les films composés de NRC. Ces Films présentent aussi une baisse de la perméabilité à l'oxygène accentuée après 20 jours de photo-vieillissement. Inversement, une augmentation significative de la perméabilité à la vapeur d'eau des films a été observée lorsque la concentration en NRC augmente. Les films composés de 4% de NRC (v/v) ont montré la plus haute perméabilité à la vapeur d'eau et la plus basse perméabilité à l'oxygène. Enfin, la transparence des films a diminué lorsque la quantité de NRC augmente.

Food Hydrocolloids 31 (2013) 420–427

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Fabrication and physicochemical characterization of HPMC films with commercial plant extract: Influence of light and film composition

Muhammad-Javeed Akhtar, Muriel Jacquot*, Majid Jamshidian, Muhammad Imran, Elmira Arab-Tehrany, Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France

ARTICLE INFO

Article history:
Received 15 May 2012
Accepted 9 October 2012

Keywords:
Hydroxypropyl methylcellulose
Bioactive films
Natural red color
Photo-aging
Physicochemical properties

ABSTRACT

Betacyanins used as natural red color (NRC) are known as antioxidants. The present paper was focused on their effect on physicochemical properties of hydroxypropyl methylcellulose (HPMC) films. All the films were evaluated for their photo-aging stability on optical, mechanical, barrier, thermal and structural properties. Both, tensile strength and Young's modules of NRC composite films decreased, while elongation significantly increased compared to control films. Dynamic vapor sorption data fitted by Guggenheim–Anderson–de Boer (GAB) model showed lower values of sorption energy for NRC composite films. NRC films showed an initial decrease in oxygen permeability that was more decreased after 20 days of photo-aging. Inversely, a significant increase in water vapor permeability of films by increasing NRC was observed. The films composed of 4% NRC (v/v) showed the highest WVP and lowest oxygen permeability. HPMC films transparency decreased with NRC contents.

1. Introduction

Face to increased health and environmental concerns replacement of synthetic chemical additives with natural edible compounds in packaging is a modern concept in the food industry. In response to this consumer requirement, bioactive films based on natural biodegradable polymers, such as structural polysaccharides, gums, proteins, lipids and their complexes (Ray & Bousmina, 2005), combined with natural antioxidants are one of the most promising technologies. Development of such bioactive films can protect food against chemical and physical damages and reduce food preservatives.

Selection of bio-polymers depends on their barrier properties like oxygen, water vapor and light because they cause adverse affect on product quality and limit shelf life (Turhan & Sahbaz, 2004). Polysaccharides are important biopolymers used to prepare edible films and coatings (Gontard, Guilbert, & Cuq, 1993; Mali, Grossmann, Garcia, Martino, & Zaritzky, 2006; Peressini, Bravin, Lapasin, Rizzotti, & Sensidoni, 2003). Cellulose-based materials are widely used because of their biocompatibility, edibility and barrier properties. Moreover, they are non-polluting and economical materials (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Use of hydroxypropyl methylcellulose is attractive because, it is a readily available non-ionic edible plant derivative forming transparent, odorless, tasteless, oil resistant, and water soluble edible films. It has also the ability to absorb and retain the color pigments (Akhtar et al., 2010). HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC, 1995); its safety in food use has been confirmed by the (ECFA) "Joint expert committee on food additives" (Burdock, 2007). Food grade HPMC is listed as suitable for use in applications falling under the provisions of the regulation as additive or polymer production aid with no specific migration limit (Annex I UE N. 10/2011). The tensile strength of HPMC films is high with medium flexibility, which makes them suitable for edible coating purposes (Brindle & Krochta, 2008).

Recently, the use of plant natural products such as fruit or vegetable extracts has gained a considerable market, not only because of their coloring potential but also the positive physiological attributes of their pigments (Frank et al., 2005). These phenolic compounds are among the most effective and abundant bioactive compounds from different fruits and vegetables, agro-industrial wastes, and by-products (Ali et al., 2008; Bonilla, Mayen, Merida, & Medina, 1999; Liu, Qiu, Ding, & Yao, 2008). Several studies on the anti-inflammatory, antioxidant and anti-radical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Gentile, Tesoriere, Allegra, Livrea, & Alessio, 2004; Kanner, Harel, & Granit, 2001; Pedreño & Escribano, 2000). Anthocyanins play a role in industry as synthetic colorant replacer and have health benefits, including

* Corresponding author.
E-mail address: muriel.jacquot@univ-lorraine.fr (M. Jacquot).

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2012.10.008

reduced risk of cancer and heart diseases (Bell & Gochenaur, 2006; Dai, Patel, & Mumper, 2007).

As opposed to mixing these phenolic compounds directly in foods, their incorporation into edible packaging is particularly encouraged since they can improve antioxidant, antimicrobial, mechanical, barrier, thermal and coloring properties of edible packaging (Akhtar et al., 2010; Bifani et al., 2007). The molecular weight and structure of these phenolic compounds show great variations and contain different numbers of hydroxyl groups capable to interact with cellulosic OH groups (Bifani et al., 2007).

The aim of this work was to investigate the effect of natural red color (NRC) addition on HPMC films properties, such as optical, mechanical, barrier, thermal and structural properties. Films and NRC stability during storage under fluorescent light were also investigated.

2. Materials and methods

2.1. Raw materials

The HPMC (Fluka-Biochemika, Japan) contained 9% of hydroxylpropoxyl and 28% of methyl radicals and had a viscosity of 15 mPa s and a water solubility of 2% at 25 °C. Ethanol 96.2% (Pharmaceutics Carlo Erba) was used to improve HPMC solubilization. Phosphorus pentoxide (P_2O_5) was purchased from Sigma–Aldrich (France). Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark). A liquid (NRC) "Natural Red Color blend" of beetroot juice (E162) and purple carrot extract (E163) containing 20% glycerin was obtained from Color-Maker, California, USA, and used as coloring agent to investigate changes in physico-chemical properties of HPMC films.

2.2. HPMC solution making and films casting

Film forming solutions (FFS) were prepared according to Akhtar et al. (2010) by dissolving 6 g of HPMC in 35% solution of ethanol for 40 min at 65 °C using a heating magnetic stirrer (Fisher Bio-block Scientific). NRC was dissolved separately in 35% solution of ethanol at room temperature to avoid oxidation. Both, HPMC and NRC solutions were then centrifuged together at 4000 rpm for 30 min at 20 °C to obtain homogeneous solution. The pH of NRC solutions was controlled with HCl (0.1 M) and fixed at 3.2 ± 0.1. After mixing, solutions were degassed at room temperature under vacuum "Handy Aspirator WP-15 (YAMATO®)" for 30 min. Films were made by pouring 6 g of each FFS in the lids of Petri-dishes and then left to dry at 20 °C and 50% relative humidity for 48 h, in a dark room.

2.3. Film conditioning for photo-aging

Films were placed under the fluorescent light (Osram L36w/640) for 20 days in an experimental chamber with controlled conditions of temperature (20 ± 1 °C) and relative humidity (50 ± 2%). The distance from fluorescent tube to films was 14 cm.

2.4. Thickness measurement

Thickness was measured according to the standard NF Q 03-016 with a manual micrometer (Messmer, London, England) equipped with a measuring head of 1 cm in diameter and a sensitivity of 2 μm. The thickness was measured in 8 randomly selected points on each film and then an average value was calculated.

2.5. Mechanical properties

A universal testing machine Lloyd instrument (AMETEK, United Kingdom) was used to determine mechanical properties, i.e. tensile strength (TS, MPa), ultimate elongation (UE, percent at break point) and Young's modulus (YM, MPa) according to ASTM D882. Tests were performed on 6 specimens previously stored for 48 h at 50 ± 2% relative humidity (RH) in a container using magnesium nitrate saturated solution at 20 ± 1 °C. Equilibrated film samples of 6 × 2 cm were stretched at a rate of 10 mm/min until breaking. The RH and temperature of the testing environment was held at 50 ± 2% and 20 ± 2 °C, respectively. The stress–strain curves were recorded and exploited with Nexygen software.

2.6. Light transparency

UV–visible light barrier properties of films (1 cm × 3 cm) were measured using a UV–visible recording spectrophotometer (Ultro-Spec 4000 UV/visible, Pharmacia Biotech, UK) at selected wavelengths from 200 to 900 nm following the ASTM method D 1746-92 with slight modifications (Fang, Tung, Britt, Yada, & Dalgleish, 2002; Hamaguchi, Weng, & Tanaka, 2007). The transparency was calculated from Han and Floros (1997) equation:

$$\text{Transparency } (T) = -\log(T_{600}/X) \quad (1)$$

where T_{600} is the transmittance (%) at 600 nm and X is film thickness in mm. Three replicates of each treatment were tested.

2.7. Water vapor permeability

Films water vapor permeability (WVP) was determined by using a gravimetric method described in the AFNOR NFH00-030 standard (1974), at 38 °C and 90% RH gradient. The film was sealed in a permeation cell containing a desiccant (silica gel). The plastic permeation cells used had an exposed film area of 26.42 cm^2. The permeation cells were placed in a close chamber having controlled conditions of temperature, (38 °C) and RH, (90%). The water vapor transport was determined from the weight gain of the permeation cell that was determined each hour of experiment up to 10 h. At least three replicates were made for each film. WVP was calculated as follows (Khwaldia, Banon, Perez, & Desobry, 2004):

$$\text{WVTR} = \Delta M/\Delta T \times 1/A \left(\text{gh}^{-1}\text{m}^{-2}\right) \quad (2)$$

$$P = \text{WVTR}/\Delta P \times 3600 \left(\text{gs}^{-1}\text{m}^{-2}\text{Pa}^{-1}\right) \quad (3)$$

$$\text{WVP} = P \times X \left(\text{gm}^{-1}\text{s}^{-1}\text{Pa}^{-1}\right) \quad (4)$$

where (ΔM) is the weight gain of the permeation cell over time (Δt), (A) is the exposed film area, (Δp) is the differential vapor pressure across the film and (X) is the film thickness.

2.8. Oxygen permeability

Control and NRC films were conditioned under controlled relative humidity (50%) and temperature (20 °C) for one week. Gas chromatography system (Shimadzu GC-4A; Shimadzu Corp., Kyoto, Japan) was used to measure films oxygen permeability by directly injecting samples with a gas sampling syringe (Dynatec Pressure Lok, Baton Rouge, LA, USA) into a gas chromatograph equipped with a thermal conductivity detector and molecular sieve columns (Desobry & Hardy, 1997). Helium gas at flow rate of 25 mL/min was used as carrier gas and column temperature was 50 °C. Method was based on measurement of oxygen diffusing through film over time. The film was first sealed into a test cell of 26.42 cm^2 exposed area and 0.8 bar oxygen pressure gradient across the film, which was

filled with ~100% oxygen. At suitable intervals, gas samples were withdrawn from the cell via a sampling stopper and analyzed by gas chromatography. Oxygen transmission rate (OTR) and then oxygen permeability were determined according to Khwaldia et al. (2004).

$$\mathrm{OTR} = \Delta C \times V/A\Delta T\left(\mathrm{cm^3 m^{-2} h^{-1}}\right) \quad (5)$$

$$\mathrm{OP} = \mathrm{OTR} \times X/\Delta P\left(\mathrm{cm^3 m^{-1} s^{-1} Pa^{-1}}\right) \quad (6)$$

where $\Delta C/\Delta t$ is the slope of O_2 concentration loss over time (t), V is the volume of cup containing ~100% oxygen, A is the area of exposed film sample, X is the film thickness and Δp is the partial pressure difference of O_2 across the film.

2.9. Water sorption isotherms

For the water adsorption experiments, films were dried for about 3 weeks at 20 °C in a vacuum chamber containing phosphorus pentoxide (P_2O_5). Measurements were obtained on film samples of 30 mg using a dynamic vapor sorption system (DVS, SMS Ltd., UK). The samples were equilibrated at 20 °C for different relative humidity values. During experiments, RH was increased from 0 up to 90% with a 10% increment. The samples were considered to be at equilibrium when the value $\Delta m/\Delta t$ (slope of mass change with time) was <0.002 mass%/min. The Guggenheim–Anderson–de Boer (GAB) model was used for sorption isotherms modeling (Eq. (7)). Non-linear regression (curve fitting), procedure was used to estimate the parameters for this mode with Origin 6.1 software (Origin Lab Corporation, USA).

$$X = X_m \cdot C_{GAB} \cdot K \cdot a_w/(1 - K \cdot a_w)(1 - K \cdot a_w + C \cdot K \cdot a_w) \quad (7)$$

where X is adsorbed water at a given a_w (g g^{-1}), X_m is the monolayer value, and a_w is the water activity (a_w = RH%/100). Constant C_{GAB} is related to the bonding energy of water molecules on the matrix primary interactions sites (monolayer). K is a temperature-dependent constant related to heat of multilayer sorption.

2.10. Differential scanning calorimetry analysis

A thermal analysis of the films was performed with a differential scanning calorimeter (DS-7, Netzsch, Germany). 10 mg of film sample was weighed and sealed into a DSC aluminum pan. A tiny hole was made in each lid of aluminum pan to allow samples dehydration. Samples were then heated under protective nitrogen (40 mL/min) at the rate of 10 °C/min from 25 to 130 °C and equilibrated at this temperature for 5 min then cooled down to 25 °C at 10 °C/min and reheated up to 300 °C at the same rate.

2.11. Scanning electron microscopy (SEM)

Structural morphology of dry films (preconditioned at 20 °C in P_2O_5 desiccators) was carried out by cryofracturing. Film cross-sections were prepared by dipping a film into liquid nitrogen followed by fracturing with a pre-chilled razor. Freshly fractured film specimens were retrieved from the liquid nitrogen bath and placed as quickly as possible into the Petri dishes containing filter papers. These Petri dishes were then placed in a desiccator to dry and warm to room temperature. Fractured film pieces were than mounted on a SEM tube. All samples were analyzed and photographed in a Hitachi S-4800 scanning electron microscope (Hitachi, Japan) at 0.5–2 kV. Topographic analysis of film surfaces was carried out by using previously conditioned films stuck onto a cylindrical aluminum stub by a double-sided tape to observe surfaces.

3. Statistical analysis

Experimental values were given as means ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare mean differences of the samples. Differences at $P < 0.05$ were considered to be significant.

4. Results and discussion

4.1. Film mechanical properties

4.1.1. Effect of film composition

Mechanical properties of control HPMC films were in the range of those reported by other authors working with edible packaging based on pure HPMC (equilibrated at different conditions of temperature and relative humidity) as shown in Table 1. The capacity of HPMC films for preserving the integrity of food stuff was evaluated by measuring the tensile strength (TS), Young's modulus (YM) and ultimate elongation at break (UE).

In the present work (Table 2) by increasing glycerin content of films, YM and TS decreased and UE increased insignificantly. Glycerin added films were weaker, more stretchable and flexible. Results indicated that glycerin was more effective by weakening interactions among HPMC polymer chains and changing film mechanical properties. These results were in accordance with Maria, Carvalho, Sobral, Habitante, and Solorza-Feria (2008) and Imran, El-Fahmy, Revol-Junelles, and Desobry (2010). Increase in film flexibility with glycerin content was previously observed in protein films by Kim, Weller, Hanna, and Gennadios (2002).

An increase in ultimate elongation at break and decrease in films TS and YM was observed with increased NRC content. It is clear that phenolic compounds present in NRC affected films morphology and strength. NRC resulted in decreasing of film matrix density,

Table 1
Mechanical and water vapor permeability properties of HPMC films compared with previous studies (mean standard deviation of triplicate analysis).

Test conditions (Temperature °C; ΔRH, %)	Film thickness (μm)	TS (MPa)	UE (%)	Y (MPa)	WVP(×10 ^{10} g m ^{1} s ^{1} Pa ^{1})	Literature cited
20 ± 1; 50 ± 2	48.2 ± 3.5	64.5 ± 6.9	4.3 ± 1.0	2492 ± 51	6.13 ± 0.91	Present work
20 ± 0; 54.4	61.6 ± 0.6	56 ± 7	7.9 ± 0.6	643 ± 74	7.1 ± 0.7	Sánchez-González et al., 2011
24; 30	34 ± 2	28.3 ± 1	8.1 ± 0.7	900 ± 34	8.9 ± 0.3	De Moura, Mattoso, & Zucolotto, 2012
20 ± 1; 50 ± 2	47 ± 2	63 ± 8	13 ± 1	2334 ± 99	4.2 ± 0.1	Imran et al., 2010
21 ± 0; 33	26 ± 7	35.6 ± 3.3	4.9 ± 1.3	–	4.7 ± 0.4	Bilbao-Sainz, Wood, Avena-Bustillos, Williams, & Mchugh, 2010
10 ± 1; 58 ± 2	2.50	55 ± 5	7.0 ± 2	2550 ± 50	4.6 ± 0.1/6.2 ± 0.1	Jiménez, Fabra, Talens, & Chiralt, 2010
20 ± 0; 54.4	44 ± 8	59 ± 6	0.10 ± 0.06	1697 ± 80	8.0 ± 00	Sánchez-González et al., 2011
23; 50	2.54	61 ± 7.95	16 ± 5.35	1656 ± 357	–	Brindle & Krochta, 2008
20 ± 1; 50 ± 5	30 ± 4	34 ± 6	6.63 ± 1.28	1900 ± 60	–	Möller, Grelier, Pardon, & Coma, 2004

TS, tensile strength; UE, ultimate elongation; Y, young's modulus; WVP, water vapor permeability.

Table 2
Thickness and mechanical properties of HPMC–NRC–plasticizer composite films before and after 20 days of light aging (mean standard deviation of triplicate analysis).

Film type	Tensile strength, TS (MPa)		Ultimate elongation, UE (%)		Young's modulus, Y (MPa)	
	0 day light exposure	20 day light exposure	0 day light exposure	20 day light exposure	0 day light exposure	20 day light exposure
Control (C)	64.5 ± 6.9^{a}	60.3 ± 7.8^{a}	4.30 ± 1.03^{c}	4.88 ± 1.63^{b}	2492 ± 51^{a}	2828 ± 168^{a}
C + G1%	62.6 ± 4.4^{a}	62.1 ± 3.7^{a}	4.64 ± 0.12^{bc}	4.64 ± 0.32^{b}	2333 ± 95^{ab}	2217 ± 75^{b}
C + G4%	57.9 ± 5.6^{a}	56.9 ± 4.5^{ab}	5.64 ± 0.48^{bc}	5.64 ± 0.50^{ab}	2204 ± 99^{b}	2029 ± 43^{bc}
C + NRC1%	55.7 ± 1.3^{ab}	47.1 ± 2.7^{bc}	5.43 ± 0.87^{bc}	3.47 ± 0.16^{b}	1950 ± 166^{c}	1749 ± 19^{cd}
C + NRC2%	46.4 ± 3.5^{bc}	45.3 ± 1.7^{c}	6.05 ± 1.08^{bc}	5.05 ± 0.25^{ab}	1632 ± 96^{d}	1382 ± 99^{e}
C + NRC3%	42.3 ± 3.2^{c}	42.1 ± 2.7^{c}	7.18 ± 1.3^{ab}	6.08 ± 1.54^{ab}	1482 ± 44^{d}	1622 ± 13^{de}
C + NRC4%	39.9 ± 6.6^{c}	42.8 ± 5.3^{c}	8.72 ± 1.84^{a}	8.46 ± 2.78^{a}	1102 ± 141^{e}	1372 ± 11^{e}

HPMC, hydroxypropyl methylcellulose; NRC, natural red color.
Different letters in each segment of the column indicate significant difference at $P < 0.05$.

facilitating movements of polymer chains under stress, hence declining the film resistance. NRC (betacyanins) weakened the intermolecular forces between adjacent macromolecules, increased the free volume and caused mechanical resistance reduction (Sobral, Menegalli, Hubinguer, & Roques, 2001). HPMC films with 1, 2, 3 and 4% of NRC contained 0.2, 0.4, 0.6 and 0.8% of glycerin respectively. As a result, effect of NRC concentration on films mechanical properties was a combined effect of phenolic compounds and glycerin.

Comparing control films (C + G1%) with C + NRC4% films, both containing about same quantity of glycerin, NRC in particular showed a significant decrease in both TS (64.50 ± 6.9 to 39.92 ± 6.6 MPa) and YM (2492 ± 51 to 1102 ± 141 MPa). On the other hand, UE significantly increased from 4.30 ± 1.03 to 8.72 ± 1.84% (Table 2). These results were in the range of those reported by Arcan and Yemenicioğlu (2011) who studied the effect of different phenolic compounds on physicochemical properties of zein films.

4.1.2. Light-aging effect

Aging produced physico-chemical changes in the polymeric material after exposure to given environmental conditions (light, temperature, relative humidity). Light-oxidation may cause degradation of polymer matrix (Akhtar et al., 2012; Crompton, 1979; Petersen & Breindahl, 1998).

Light aging effect on films mechanical properties was studied (Table 2). All films were lightly affected in their mechanical properties. A slight decrease in TS and increase in both YM and UE of control HPMC films may be associated with chemical change such as photo-degradation of polymer matrix. Films containing glycerin and NRC were almost identical before and after aging due to their ability to retain moisture contents during aging (Anker, Stading, & Hermansson, 2001) or ability of NRC to protect polymer from light-oxidation.

4.2. Light transparency

Table 3 compares transparency for all films before and after 20 days of photo-aging. HPMC control films showed an excellent transparency characteristic (3.10 ± 0.09). Usually plasticizers increased films transparency (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006) but HPMC control films showed such an excellent transparency that glycerin addition resulted a slight decrease of transparency. NRC addition had an effect on films light transparency (Table 3). Film transparency slightly decreased with the addition of NRC at low concentration but drastically decreased at higher concentration since HPMC films containing NRC showed minimum transparency values from 3.03 ± 0.08 to 2.09 ± 0.21.

After 20 days of photo-aging HPMC films alone and with glycerin 1% or 4% were stable for their transparency characteristics. NRC films were sensitive to light because of their color. A significant increase in films transparency NRC3% and 4% was caused by light induced degradation of surface color. After 20 days at light, all films had the same transparency independently from glycerin or NRC content, showing NRC degradation.

4.3. Water sorption isotherms

Water sorption isotherm of HPMC based films showed an initial light increase in moisture content with a progressive increase in relative humidity up to 40% and then a rapid increase in films water adsorption for higher RH (Fig. 1). Such a negligible curves convexity at low RH was related with type III sorption isotherm (Villalobos, Hernandez-Munoz, & Chiralt, 2006), corresponding to hydrophilic matter. Similar behavior has already been observed for HPMC based films (Guiga et al., 2009; Kristo, Biliaderis, & Zampraka, 2007; Kristo, Koutsoumanis, & Biliaderis, 2008; Müller, Laurindo, & Yamashita, 2009; Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007). Incorporation of 1–4% glycerin as plasticizer led to slight increase of films water sorption capacity (Fig. 1). At higher RH levels, water sorption capacity of films plasticized with glycerin was enhanced. This could be attributed to the creation of hydrogen bonds between plasticizer and water (Enrione, Hill, & Mitchell, 2007; Lourdin, Coignard, Bizot, & Colonna, 1997).

Consequently, plasticizer–polymer interactions decreased at high moisture content (Gontard et al., 1993). Greater free volume was created, allowing matrix to absorb more water. This suggests that glycerin plasticized HPMC films could only serve at low RH level as good protective layer against moisture. Similar behavior was noticed for films functionalized with NRC. Water sorption capacity of HPMC films was increased with increasing NRC concentration at high RH level (Fig. 1) due to the presence of more -OH groups. There may be the creation of more hydrogen bonds (O–H) between OH active groups of NRC and water (Enrione et al., 2007; Lourdin et al., 1997) representing high water sorption capacity at high RH.

Table 3
Light transparency of edible HPMC films, affected by NRC concentration and fluorescent light exposure (mean values of triplicate analysis).

Film types	Film thickness (μm)	Film transparency = Log (T_{600}/X)	
		0 day light exposure	20 day light exposure
Control (C)	48.2 ± 3.5^{a}	3.10 ± 0.09^{Ac}	3.09 ± 0.09^{Aa}
C + G1%	49.0 ± 4.1^{a}	3.08 ± 0.01^{Aab}	3.08 ± 0.01^{Aa}
C + G4%	49.5 ± 5.5^{a}	3.07 ± 0.08^{Aab}	3.07 ± 0.06^{Aa}
C + NRC1%	49.3 ± 3.4^{a}	3.03 ± 0.08^{Aabc}	3.05 ± 0.08^{Aa}
C + NRC2%	52.3 ± 3.9^{a}	2.94 ± 0.15^{Aabc}	3.08 ± 0.11^{Aa}
C + NRC3%	53.1 ± 4.8^{a}	2.10 ± 0.12^{Abc}	3.14 ± 0.08^{Ba}
C + NRC4%	54.3 ± 5.6^{a}	2.09 ± 0.21^{Aa}	3.06 ± 0.13^{Ba}

The mean values having same letters within the columns are not significantly different and the mean values within the rows having same upper numbers are not significant. Significant difference at $P < 0.05$.

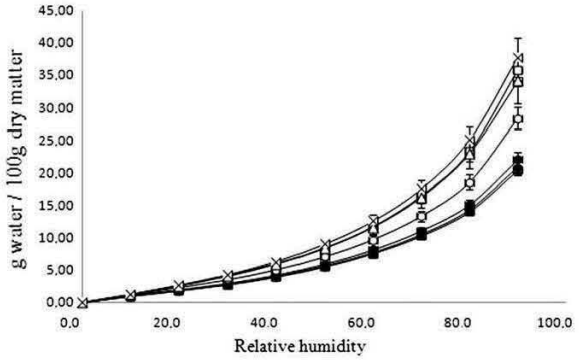


Fig. 1. Moisture sorption isotherms of HPMC composite films at 25 °C. Experimental DVS values were averaged and fitted by isotherm equation; lines represent the GAB model fitted to data. (▲) Control films, (◆) C + G1%, (●) C + G4%, (○) C + NRC1%, (□) C + NRC2%, (△) C + NRC3%, (-X-) C + NRC4%.

4.4. GAB model of sorption isotherms

The Guggenheim–Anderson–de Boer (GAB) model was used to fit water adsorption data in the whole RH range. Table 4 gives GAB equation constants. The GAB model fit very well with films adsorption data as previously reported (Müller et al., 2009). The value of monolayer water content (*Xm*) is of particular interest as it indicates the amount of water that is strongly adsorbed at specific hydrophilic sites. Monolayer moisture content (*Xm*) increased with NRC contents and may be associated with hydrophilic nature of phenolic compounds of NRC. The constant C, related to the water–substrate interaction energy, decreased with NRC incorporation and indicated that water molecules are adsorbed with less energy in the active sites as observed by Imran et al. (2010) and Villalobos et al. (2006) in HPMC based films. On the other hand, NRC concentration increase lowered K values suggesting higher water content in multilayer system (Quirijns, Van Boxtel, Van Loon, & Van Straten, 2005). Thus glycerin and NRC molecules reduced the interaction energies between water molecules, on the second and higher water layers.

4.5. Water vapor permeability

Water vapor permeability of films with different concentrations of glycerin and NRC was calculated (Table 5). This tendency could be explained by structural modifications of polymer network. The network may become less dense because of an increase in polymeric chains mobility and in film free volume. As shown above, NRC is favorable to water adsorption by contributing to extend molecular relaxation of films and enhancing moisture transport through films. A decrease in the polymer network interaction density due to glycerin and phenolic compounds in NRC was associated with this increase of solubility properties.

Effect of fluorescent light on control and composite films WVP was studied (Table 5). WVP was nearly constant for both types of films as before and after aging and showed a non-significant effect of HPMC oxidation on WVP barrier properties. Anker et al. (2001) observed a decrease in the moisture contents over time which decreased the WVP which compensated WVP increase due to pore size increase (Anker, Stading, & Hermansson, 2000). This agrees with the findings of Maté and Krochta (1996) who reported that WVP increases with increased moisture contents for hydrophilic films as a result of the plasticizing effect of water.

Table 4
Parameter values obtained from the curves fitted to various composite films with GAB model.

Film types	GAB model		
	Xm (g/100 g)	*C*	*K*
Control (C)	5.76 ± 0.46	3.35 ± 0.13	0.92 ± 0.01
C + G1%	6.56 ± 0.76	1.15 ± 0.41	0.84 ± 0.003
C + G4%	7.39 ± 0.27	1.93 ± 0.30	0.89 ± 0.01
C + NRC1%	7.68 ± 0.51	2.01 ± 0.30	0.90 ± 0.01
C + NRC2%	9.41 ± 0.26	1.37 ± 0.06	0.87 ± 0.004
C + NRC3%	10.89 ± 0.36	1.15 ± 0.05	0.83 ± 0.005
C + NRC4%	11.49 ± 0.17	1.13 ± 0.02	0.85 ± 0.002

G, glycerin; C, control HPMC film; GAB, Gggenheim–Anderson–de Boer.

Table 5
HPMC films water vapor permeability at 38 °C and 90% RH gradient and glass transition temperature (*Tg*), mean values of triplicate analysis.

Film type	*Tg* (°C)	Water vapor permeability ($\times 10^{-10}$ g m^{-1} s^{-1} Pa^{-1})	
		0 day light exposure	20 day light exposure
Control (C)	150.53 ± 1.59	6.13 ± 0.91	7.05 ± 0.81
C + G1%	150.35 ± 1.34	6.24 ± 0.02	6.21 ± 0.01
C + G4%	145.70 ± 1.25	6.59 ± 0.03	6.52 ± 0.02
C + NRC1%	146.33 ± 1.23	8.16 ± 1.11	8.27 ± 0.70
C + NRC2%	144.63 ± 1.53	9.86 ± 0.54	10.06 ± 0.5
C + NRC3%	144.20 ± 1.54	13.46 ± 1.56	13.68 ± 0.5
C + NRC4%	144.83 ± 1.62	16.68 ± 0.90	16.76 ± 0.4

G, glycerin; C, control HPMC film.

4.6. Oxygen permeability

The ability of films to modify gas transport is very important for applications to fresh fruits and vegetables, which are characterized by active metabolism even during refrigerated storage (Guilbert, Gontard, & Gorris, 1996). Results presented in Fig. 2 indicated a significant effect of NRC on films oxygen permeability.

NRC increased oxygen barrier properties of HPMC films which may be attributed to availability of free hydroxyl groups of NRC which took part in hydrogen bonding with HPMC matrix giving more compact structure of polymer matrix (De Moura et al., 2009). Films oxygen permeability after aging was further increased as shown in (Fig. 2). Due to temperature, some of the B-rings of phenolic compounds present in NRC could be hydrolyzed, and these smaller molecules, could act as filling part of pores in NRC films, reducing their sizes, avoiding some of the free passage of oxygen. Edible films and coatings with selective gas permeability could be very promising for controlling respiratory gas exchange and improving the preservation of fresh or minimally processed vegetables. NRC films act in this direction.

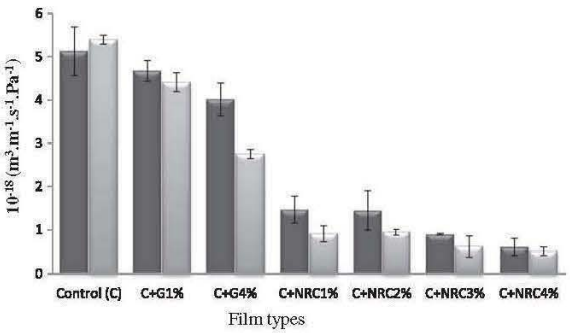


Fig. 2. Oxygen permeability of edible HPMC films as a function of NRC concentration and fluorescent light exposure, (■) 0 day light exposure, (■) 20 days light exposure.

4.7. Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) was used to measure glass transition temperature (*Tg*). *Tg* is defined as a physical change from the glassy to the rubbery state in amorphous materials promoted by heat (Roos & Karel, 1991). *Tg* values for control and NRC films are shown in Table 5. Glycerin plasticizing effect decreased glass transition temperatures of cellulose based films in agreement with the free volume theory of plasticization. The increase of glycerin concentration led to an increase of molecules free volume and mobility (Olivas Guadalupe & Barbosa-Canovas Gustavo, 2008). NRC incorporation in HPMC films increased the mobility of HPMC as confirmed by the decrease of *Tg* values (Table 5). Decrease in glass transition temperature of films containing NRC was due to hydrogen bonding between OH groups of NRC and those of HPMC. The results were in accordance with Hsu, Chen, and Tsai (2004) who reported that polymer containing phenolic hydroxyl groups had lower glass transition temperature through hydrogen bonding.

4.8. Scanning electron microscopy (SEM)

SEM images were obtained for cross-sections of control and NRC films before and after photo-aging (Fig. 3). Control HPMC films displayed relatively smooth and continuous surface (Fig. 3a). The incorporation of NRC reduced the smoothness and porosity of the films due homogeneous distribution within the polymer. This result explained the more hydrophilic nature of NRC films suggesting a relative increase in WVP. The micro holes represented by cross-section of control HPMC films were filled by NRC resulted in more compact films (Fig. 3c and e). This result explained the decrease in oxygen permeability of NRC composite films. The cross-sectional structures of control HPMC films were less compact after 20 days of photo-aging (Fig. 3b) which could be the effect of polymer matrix photo-degradation. On the other hand, cross-sectional structure of NRC composite films was more compact after photo-aging of 20 days (Fig. 3d and f). This result supported our observations about lower oxygen permeability of NRC films after aging.

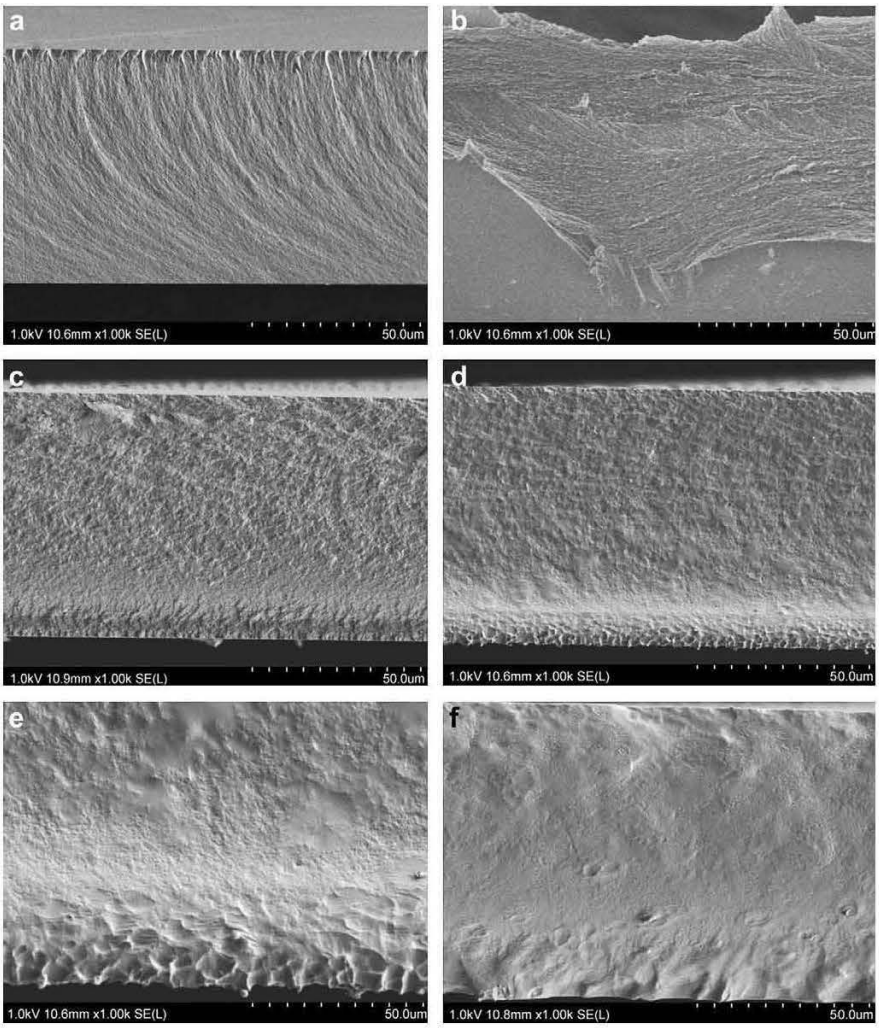


Fig. 3. Micrographs of scanning electron microscope (SEM); cross-section view, (a) fresh control HPMC film; (b) 20 day control HPMC film; (c) fresh C + 1%NRC film; (d) 20 day C + 1%NRC film; (e) fresh C + 4% NRC film; (f) 20 day C + 4% NRC.

5. Conclusion

The objective of this work was to develop and characterize the HPMC films by incorporating commercially available naturally active color molecules such as flavonoids. It was verified that addition of NRC containing phenolic compounds greatly affected the water sorption behavior, water vapor permeability, oxygen permeability, mechanical properties, and glass transition temperature of HPMC films. Films containing NRC presented lower oxygen permeability which gave an efficient food protection against oxidative deterioration. On the other hand, NRC active films showed high moisture sorption capacity and WVP which can be used to control product dehydration in packages. NRC films presented an increase of ultimate elongation at break and decrease of both, TS and YM. Results showed the high potential of using NRC phenolic compounds in developing flexible bioactive packaging materials.

Acknowledgments

The authors would like to thank Jordane Jasniewski, Carole Jeandel, Céline Charbonnel, Cedric Paris and Carole Perroud for their technical assistance.

References

Akhtar, M. J., Jacquot, M., Jasniewski, J., Jacquot, C., Imran, M., Jamshidian, M., et al. (2012). Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract. *Carbohydrate Polymers, 89*(4), 1150–1158.

Akhtar, M. J., Jacquot, M., Tehrany, E. A., Gaiani, C., Linder, M., & Desobry, S. (2010). Control of salmon oil photo-oxidation during storage in HPMC packaging film: influence of film color. *Food Chemistry, 120*, 395–401.

Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A., et al. (2008). Indian medicinal herbs as sources of antioxidants. *Food Research International, 41*, 1–15.

Anker, M., Stading, M., & Hermansson, A. M. (2000). Relationship between the microstructure and the mechanical and barrier properties of whey protein films. *Journal of Agricultural and Food Chemistry, 48*, 3806–3816.

Anker, M., Stading, M., & Hermansson, A. M. (2001). Aging of whey protein films and the effect on mechanical and barrier properties. *Journal of Agricultural and Food Chemistry, 49*, 989–995.

Arcan, I., & Yemenicioğlu, A. (2011). Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. *Food Research International, 44*, 550–556.

Bell, D. R., & Gochenaur, K. (2006). Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. *Journal of Applied Physiology, 100*, 1164–1170.

Bifani, V., Ramirez, C., Ihl, M., Rubilar, M., Garcia, A., & Zaritzky, N. (2007). Effects of murta (Ugni molinae Turcz) extract on gas and water vapor permeability of carboxymethylcellulose-based edible films. *LWT–Food Science and Technology, 40*, 1473–1481.

Bilbao-Sainz, C., Wood, R. J., Avena-Bustillos, D. F., Williams, T. G., & Mchugh, T. H. (2010). Composite edible films based on hydroxypropyl methylcellulose reinforced with microcrystalline cellulose nanoparticles. *Journal of Agricultural and Food Chemistry, 58*, 3753–3760.

Bonilla, F., Mayen, M., Merida, J., & Medina, M. (1999). Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chemistry, 66*, 209–215.

Brindle, L. P., & Krochta, J. M. (2008). Physical properties of whey protein hydroxypropyl methylcellulose blend edible films. *Journal of Food Science, 73*, 446–454.

Burdock, G. A. (2007). Safety assessment of hydroxypropyl methylcellulose as a food ingredient. *Food and Chemical Toxicology, 45*, 2341–2351.

Crompton, T. R. (1979). *Additive migration from plastics into food*. Oxford: Pergamon Press.

Dai, J., Patel, J. D., & Mumper, R. J. (2007). Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *Journal of Medicinal Food, 10*, 258–265.

De Moura, M. R., Fauze, F. A., Avena-Bustillos, R. J., McHugh, T. H., Krochta, J. M., & Mattoso, L. H. C. (2009). Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles. *Journal of Food Engineering, 92*, 448–553.

De Moura, M. R., Mattoso, L. H. C., & Zucolotto, V. (2012). Development of cellulose-based bactericidal nanocomposites containing silver nanoparticles and their use as active food packaging. *Journal of Food Engineering, 109*, 520–524.

Desobry, S., & Hardy, J. (1997). The increase of CO_2 permeability of paper packaging with increasing hydration. *International Journal of Food Science and Technology, 32*, 407–410.

Enrione, J. I., Hill, S. E., & Mitchell, J. R. (2007). Sorption behavior of mixtures of glycerol and starch. *Journal of Agricultural and Food Chemistry, 55*, 2956–2963.

EC. (1995). *European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colors and sweeteners*. Official Journal of the European Communities No. L61, 18.3.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science, 67*, 188–193.

Frank, T., Conrad, F., Carle, S. R., Bitsch, I., Daniela, Q., Gabriele, B., et al. (2005). Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. *Pharmacological Research, 52*, 290–297.

Gentile, C., Tesoriere, L., Allegra, M., Livrea, M. A., & Alessio, P. (2004). Antioxidant betalains from cactus pear (Opuntia ficus-indica) inhibits endothelial ICAM-expression. *Annals of the New York Academy of Sciences, 1028*, 481–486.

Gontard, N., Guilbert, S., & Cuq, J.-L. (1993). Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science, 58*, 206–211.

Guiga, W., Galland, S., Peyrol, E., Degraeve, P., Carnet-Pantiez, A., & Sebti, I. (2009). Antimicrobial plastic films: physico-chemical characterization and nisin desorption modeling. *Innovative Food Science and Emerging Technologies, 10*, 203–207.

Guilbert, S., Gontard, N., & Gorris, L. G. M. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. *Lebensmittel Wissenschaft und–Technologie, 29*, 10–17.

Hamaguchi, P. Y., Weng, W. Y., & Tanaka, M. (2007). Effect of pH on the formation of edible films made from the muscle proteins of blue marin (Makaira mazara). *Food Chemistry, 100*, 914–920.

Han, J. H., & Floros, J. D. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film and Sheeting, 13*, 287–298.

Hsu, S. L. C., Chen, H. T., & Tsai. (2004). Novel positive-working and aqueous-base-developable photosensitive poly (imide benzoxazole) precursor. *Journal of Polymer Science, 42*, 5990–5998.

Imran, M., El-Fahmy, S., Revol-Junelles, A.-M., & Desobry, S. (2010). Cellulose derivative based active coatings: effects of nisin and plasticizer on physico-chemical and antimicrobial properties of hydroxypropyl methylcellulose films. *Carbohydrate Polymers, 81*, 219–225.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2010). Effect of lipid self-association on the microstructure and physical properties of hydroxypropyl methylcellulose edible films containing fatty acids. *Carbohydrate Polymers, 82*, 585–593.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006). Effects of plasticizers on the properties of edible films from skin gelatin of big eye snapper and brown stripe red snapper. *European Food Research and Technology, 222*, 229–235.

Kanner, J., Harel, S., & Granit, R. (2001). Betalains a new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry, 49*, 5178–5185.

Khwaldia, K., Banon, S., Perez, C., & Desobry, S. (2004). Properties of sodium caseinate film forming dispersions and films. *Journal of Dairy Science, 87*, 2011–2016.

Kim, K., Weller, C., Hanna, M., & Gennadios, A. (2002). Heat curing of soy protein films at selected temperatures and pressures. *Lebensmittel Wissenschaft und–Technologie, 35*, 140–145.

Kristo, E., Biliaderis, C. G., & Zampraka, A. (2007). Water vapour barrier and tensile properties of composite caseinate-pullulan films: biopolymer composition effects and impact of beeswax lamination. *Food Chemistry, 101*, 753–764.

Kristo, E., Koutsoumanis, K. P., & Biliaderis, C. G. (2008). Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. *Food Hydrocolloids, 22*, 373–386.

Liu, H., Qiu, N., Ding, H., & Yao, R. (2008). Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Research International, 41*, 363–370.

Lourdin, D., Coignard, L., Bizot, H., & Colonna, P. (1997). Influence of equilibrium relative humidity and plasticizer concentration on the water content and glass transition of starch materials. *Polymer, 21*, 5401–5406.

Mali, S., Grossmann, M. V., Garcia, M. A., Martino, M. N., & Zaritzky, N. E. (2006). Effect of controlled storage on thermal, mechanical and barrier properties of plasticized films different starch sources. *Journal of Food Engineering, 75*, 453–460.

Maria, T. M. C., Carvalho, R. A., Sobral, P. J. A., Habitante, A. M. B. Q., & Solorza-Feria, J. (2008). The effect of the degree of hydrolysis of the PVA and the plasticizer concentration on the color, opacity, and thermal and mechanical properties of films based on PVA and gelatin blends. *Journal of Food Engineering, 87*, 191–199.

Maté, J. L., & Krochta, J. M. (1996). Comparison of oxygen and water vapor permeabilities of whey protein isolate and β-lactoglobulin edible films. *Journal of Agricultural and Food Chemistry, 44*, 3001–3004.

Möller, H., Grelier, S., Pardon, P., & Coma, V. (2004). Antimicrobial and physico-chemical properties of chitosan–HPMC based films. *Journal of Agricultural and Food Chemistry, 52*, 6585–6591.

Müller, C. M. O., Laurindo, J. B., & Yamashita, F. (2009). Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films. *Food Hydrocolloids, 23*, 1328–1333.

Olivas Guadalupe, I., & Barbosa-Canovas Gustavo, V. (2008). Alginate-calcium films: water vapor permeability and mechanical properties as affected by plasticizer and relative humidity. *LWT–Food Science and Technology, 41*, 359–366.

Pedreño, M. A., & Escribano, J. (2000). Studying the oxidation and the antiradical activity of betalain from beetroot. *Journal of Biological Education, 35*, 49–51.

Peressini, D., Bravin, B., Lapasin, R., Rizzotti, C., & Sensidoni, A. (2003). Starch-methylcellulose based edible films: rheological properties of film-forming dispersions. *Journal of Food Engineering, 59*, 25–32.

Petersen, J. H., & Breindahl, T. (1998). Specific migration of di-(2-ethylhexyl) adipate (DEHA) from plasticized PVC film: results from an enforcement campaign. *Food Additives and Contaminants, 15*, 600–608.

Quirijns, E. J., Van Boxtel, A. J. B., Van Loon, W. K. P., & Van Straten, G. (2005). An improved experimental and regression methodology for sorption isotherms. *Journal of the Science of Food and Agriculture, 85*, 175–185.

Ray, S. S., & Bousmina, M. (2005). Biodegradable polymers and their layered silicate nanocomposites: in greening the 21st century materials world. *Progress in Materials Science, 50*, 962–1079.

Roos, Y., & Karel, M. (1991). Plasticizing effect of water of thermal the behaviour and crystallization of amorphous food models. *Journal of Food Science, 56*, 38–43.

Sánchez-González, L., Pastor, C., Vargas, M., Chiralt, A., González-Martínez, C., & Cháfer, M. (2011). Effect of hydroxypropyl methylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes. *Postharvest Biology and Technology, 60*, 57–63.

Sebti, I., Chollet, E., Degraeve, P., Noel, C., & Peyrol, E. (2007). Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *Journal of Agricultural and Food Chemistry, 55*, 693–699.

Sobral, P. J. A., Menegalli, F. C., Hubinguer, M. D., & Roques, M. A. (2001). Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids, 15*, 423–432.

Turhan, K. N., & Sahbaz, F. (2004). Water vapor permeability, tensile properties and solubility of methylcellulose based edible films. *Journal of Food Engineering, 61*, 459–466.

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. *Food Research International, 42*, 762–769.

Villalobos, R., Hernandez-Munoz, P., & Chiralt, A. (2006). Effect of surfactants on water sorption and barrier properties of hydroxypropyl methylcellulose films. *Food Hydrocolloids, 20*, 502–509.

Chapitre IV.3 : Activité antioxydante et photo-vieillissement des films d'HPMC fonctionnalisés avec des extraits naturels de plantes

Transition: L'étude de la dégradation et de la stabilité des polymères est extrêmement importante car elle permettra d'améliorer son utilisation et accroitre la durée de vie des produits emballés. Les matériaux polymériques exposés aux conditions extérieures (climat, vieillissement, enfouissement) sont sujets à des transformations (mécaniques, optiques, thermiques et chimiques) plus ou moins importantes. Dans la plus part des cas, les conditions abiotiques déstructurent le polymère et ainsi favorisent son altération (Helbling et al., 2006; Ipekoglu et al., 2007). Dans la littérature scientifique, peu de travaux ont été réalisés sur la stabilité de l'HPMC sous ces conditions alors qu'un nombre important d'étude porte sur l'optimisation de la préparation de films et de ses propriétés mécaniques.

Dans cette partie, nous avons cherché à remplacé l'extrait commercial précédemment utilisé par des extraits concentres de bétanines et d'antocyanines. Plusieurs études portent sur l'activité antiradicalaire et antioxydante de bétanines issues d'extraits de betterave (Escribano et al., 1998; Pedreño & Escribano, 2000; Kanner, Harel, & Granit, 2001). En plus de leur potentiel colorant, les bétanines permettraient une protection contre le stress oxydatif qui chez l'Homme peut conduire à différents problèmes de santé (Kanner, Harel, & Granit, 2001). Notre objectif est donc d'évaluer l'évolution des propriétés physico-chimiques de nos films d'HPMC fonctionnalisés avec de la bétanine et de l'antocyanine ainsi que leurs stabilités lorsqu'ils sont soumis à un photo-vieillissement prolongé.

Chapter IV.3: Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract

Transition: The study of degradation and stabilization of polymers is extremely important from the scientific and industrial point of view and a better understanding of polymer degradation will ensure the long life of the product. Polymeric materials that are exposed to outdoor conditions (i.e. weather, ageing and burying) can undergo transformations (mechanical, light, thermal, and chemical) more or less important. In most cases, abiotic parameters contribute to weaken the polymeric structure, and in this way favor undesirable alterations (Helbling et al., 2006; Ipekoglu et al., 2007). Not enough attention has been given to the study of durability of HPMC films as compared to their preparation techniques and evaluation of mechanical properties. In the scope of natural active agents, recently, fruit and vegetable extracts have gained a considerable market in food industries (Stintzing & Carle, 2004). The natural coloring agents in comparison with artificial colors show less stability against light, oxidation, temperature or pH change and other factors (Fabre et al., 1993; Laleh et al., 2006). In spite of such factors, these natural colorants are gaining importance due to their coloring potential, hygiene, nutrition, pharmaceutical activities, bioactivity and environmental consciousness, which indicates relative dependence on natural products (Hari et al., 1994; Frank et al., 2005).

Several studies on the antioxidant and antiradical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Escribano et al., 1998; Pedreño & Escribano, 2000; Kanner, Harel, & Granit, 2001). In addition to their coloring properties, they are supposed to provide protection against oxidation stress related disorders in humans when being part of the regular diet (Kanner, Harel, & Granit, 2001). Betalains are reported to exhibit anti-inflammatory effects (Gentile et al., 2004) and antiradical activities (Cai, Sun, & Corke, 2003; Stintzing & Carle, 2004). These bioactive color compounds were added into edible films to give them additional properties such as color, antioxidant and gas barrier capacity.

The scientific objectives of this study were to functionalize HPMC films with natural red color compound to give them additional properties and to investigate the impact of aging on color stability, light transmission, antioxidant capacity and HPMC oxidation.

Chapitre IV.3
Activité antioxydante et photo-vieillissement des films d'HPMC fonctionnalisés avec des extraits naturels de plantes

Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract

Muhammad Javeed Akhtar, Muriel Jacquot, Jordane Jasniewski, Charlotte Jacquot, Muhammad Imran, Majid Jamshidian, Cédric Paris, Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France.

(Carbohydrate Polymers, 2012, 89,1150–1158)

Résumé

Le but de ce travail était de fonctionnaliser des films naturels à base d' hydroxypropyle méthylcellulose (HPMC) avec des biomolécules colorantes naturelles ayant une capacité antioxydante afin d'étudier leur stabilité au photo-vieillissement. Des films d'HPMC contenant un composé naturel de couleur rouge (NRC) à une concentration de 1, 2, 3 ou 4% (v / v) ont été préparés par un procédé de casting. Une légère dégradation de la couleur des films a été observée après 20 jours d'exposition continue à la lumière. L'activité antioxydante des films contenant le NRC a été stable au cours des différentes étapes de fabrication du film et après 20 jours de stockage à l'obscurité. Sous lumière, l'activité antioxydante des films a été significativement affectée après 20 jours. La spectroscopie FTIR (Infrarouge à Transformée de Fourier) a été utilisée pour caractériser les nouvelles structures phénoliques produites dans le biopolymère au cours de la photo-dégradation. Les résultats ont montré un phénomène de polymérisation entre le NRC et l'HPMC responsable de l'apparition d'une couleur rouge homogène. Le NRC a montré une capacité à protéger les films d'HPMC contre la photo-dégradation. Ce phénomène est directement proportionnel à la concentration de NRC introduite dans le film.

Carbohydrate Polymers 89 (2012) 1150–1158

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract

Muhammad Javeed Akhtar, Muriel Jacquot*, Jordane Jasniewski, Charlotte Jacquot, Muhammad Imran, Majid Jamshidian, Cédric Paris, Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 22 March 2012
Accepted 29 March 2012
Available online 5 April 2012

Keywords:
Hydroxypropyl methylcellulose
Natural red color (betacyanins)
Biodegradable & edible packaging
Trolox® equivalent antioxidant capacity (TEAC)
FTIR
Photo-aging

ABSTRACT

The aims of this work were to functionalize edible hydroxypropyl methylcellulose (HPMC) films with natural coloring biomolecules having antioxidant capacity and to study their photo-aging stability in the films. HPMC films containing a natural red color compound (NRC) at the level of 1, 2, 3 or 4% (v/v) were prepared by a casting method. A slight degradation of films color was observed after 20 days of continuous light exposure. The antioxidant activity of NRC incorporated films was stable during different steps of film formation and 20 days of dark storage. On the other hand, antioxidant activity of samples stored under light was significantly affected after 20 days. FTIR (Fourier Transformed Infrared) spectroscopy was used to characterize the new phenolic polymeric structures and to study the photo-degradation of films. The results showed a good polymerization phenomenon between NRC and HPMC in polymer matrix giving a natural color to the films. NRC showed an ability to protect pure HPMC films against photo-degradation. This phenomenon was directly proportional to the concentration of NRC.

1. Introduction

Environment and food safety have been at the forefront of research concern in recent years. Currently, there is an increasing trend to employ environmental friendly materials with the intention of substituting non-degradable materials. To deal with environmental issues, extend the food quality and to reduce non-degradable packaging wastes has catalyzed the use of new bio-based packaging materials in edible food packaging (Burke, 2006). In edible coating, use of bio-degradable polymers such as polysaccharides, proteins, lipids and their complexes derived from natural origin (Ray & Bousmina, 2005), depends on their barrier properties against light, water vapor and oxygen (Turhan & Sahbaz, 2004).

Cellulose based materials are widely used due to their biocompatibility, edibility, barrier properties, non-polluting and being more economical (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). The use of hydroxypropyl methylcellulose is attractive because it is a readily available non-ionic edible plant derivative shown to form transparent, odourless, tasteless, oil resistant, and water soluble edible films (Akhtar et al., 2010). HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC 1995); its safety in food use has been affirmed by the JECFA (Burdock, 2007). The tensile strength of HPMC films is high and flexibility neither too high nor too fragile, which make them suitable for edible coating purposes (Brindle & Krochta, 2008).

In the scope of natural active agents, recently, fruit and vegetable extracts have gained a considerable market in food industries (Stintzing & Carle, 2004). To consider the natural bioactive colors as the colorants, stability, yield and price are mostly constrains. The natural coloring agents in comparison with artificial colors show less stability against light, oxidation, temperature or pH change and other factors (Fabre et al., 1993; Laleh, Frydoonfar, Heidary, Jameei, & Zare, 2006). In spite of such factors, these natural colorants are gaining importance due to their coloring potential, hygiene, nutrition, pharmaceutical activities, bioactivity and environmental consciousness, which indicates relative dependence on natural products (Frank et al., 2005; Hari, Patel, & Martin, 1994).

Although anthocyanins are less stable in various environmental conditions, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Markakis, 1982). Moreover, anthocyanins have many health benefits, including reduced risk of cardiovascular diseases (Bell & Gochenaur, 2006) and decreased risk of cancer (Dai, Patel, & Mumper, 2007). These benefits make them essential to provide a healthier food for consumers. Several studies on the antioxidant and antiradical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Escribano, Pedreño, Garcia-Carmona, & Munoz,

* Corresponding author. Tel.: +33 3 83 59 58 49; fax: +33 3 83 59 58 04.
E-mail address: muriel.jacquot@ensaia.inpl-nancy.fr (M. Jacquot).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.03.088

1998; Kanner, Harel, & Granit, 2001; Pedreño & Escribano, 2000). In addition to their coloring properties, they are supposed to provide protection against oxidation stress related disorders in humans when being part of the regular diet (Kanner et al., 2001). Betalains are reported to exhibit anti-inflammatory effects (Gentile, Tesoriere, Allegra, Livrea, & Alessio, 2004) and antiradical activities (Cai, Sun, & Corke, 2003; Stintzing & Carle, 2004). These bioactive color compounds can be added into edible films to give them additional properties such as color, antioxidant and gas barrier capacity. As previously reported, the red color of HPMC films allows very good control against photo-oxidation of polyunsaturated fatty acids (PUFA) in salmon oil (Akhtar et al., 2010). Such edible films would also provide additional benefits to traditional edible film forming materials by providing unique sensory and antioxidant capacity, thus attracting more potential applications as localizing functional effect at the food surface.

The scientific objectives of this study were to functionalize HPMC films with natural red color compound to give them additional properties and to investigate the impact of aging on color stability, light transmission, antioxidant capacity and HPMC oxidation.

2. Materials and methods

2.1. Materials

Hydroxypropyl methylcellulose (Fluka-Biochemika, Japan) is a biochemical product containing 9% hydroxylpropoxyl and 28% methyl radicals. It had a viscosity of 15 mPas and a water solubility of 2% at 25 °C. Ethanol 96.2% (Pharmaceutics Carlo Erba) was used to improve HPMC solublisation, reduce air bubbles in film forming solution (FFS) and accelerate film drying. Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark). Height and diameter of Petri-dishes were 1 cm and 8.5 cm respectively. A red liquid "natural color blend" of beetroot juice (E162) and Purple Carrot Extract (E163) containing about 20% glycerin was obtained from ColorMaker, CA, USA. It was used as an active coloring agent to investigate the improvement of antioxidant and color properties of HPMC films. HPLC grade reagents and solvents (ethanol & acetonitrile) were purchased from Pharmaceutics Carlo Erba (France).

2.2. Methods

2.2.1. HPLC analysis

The HPLC equipment was a Shimadzu (Tokyo, Japan) with auto sampler (SIL-20AC), communication bus module (CBM-20A), pump (LC-20AD), column-oven (CTO-20AC) with ULFC (Shimadzu) cooling module in series with a diode array detector (SPD-M20A). Optimum separation of anthocyanins and betalains was achieved on an analytical scale (250 mm × 4.6 mm i.d.) Agilent C18 (5 μM) reversed phase column with a particle size of 5 μm (Phenomenex, Torrance, CA), fitted with a security guard C18 ODS (4 mm × 3.0 mm i.d.) at a flow rate of 0.5 mL/min and a constant temperature of 25 °C. Eluent A was 5% formic acid and B was MeCN/H_2O (60/40, v/v). Separation was accomplished starting with 3% B, followed by a linear gradient to 20% B for 30 min and then to 50% B for 40 min. Maximum absorption of betalains tended to be higher than those of the anthocyanins. Therefore, an intermediate monitoring wavelength of 530 nm was chosen for both pigment groups. Aliquots mixed samples of 20 μL were injected for analyzes. Duplicate determinations were performed throughout.

2.2.2. Mass spectrometric conditions

The LC–MS equipment includes a binary solvent delivery pump and a linear ion trap mass spectrometer (LTQ-MS, Thermo Finnigan, San Jose, CA, USA). LC analysis parameters were the same as described above except use of a specific LCMS C18 column (150 mm × 2.1 mm and 5 μm – Alltima, Alltech, France) at a smaller flow rate of 0.2 mL/min. LTQ equipped with an atmospheric pressure ionization interface operating in electro spray positive mode (ESI positive). Data were processed using Xcalibur 2.1 software. The operational parameters of mass spectrometer were as follows. Spray voltage was 4.20 kV and the temperature of heated capillary was set at 300 °C. Flow rates of sheath gas, auxiliary gas, and sweep gas were set (in arbitrary units min^{-1}) to 35, 10, and 10, respectively. Capillary voltage was −48 V, tube lens was −13 V, split lens was −38 V and the front lens was −4.25 V. All parameters were optimized by using a standard rutin solution as representative glycosylated flavonoid (0.1 g/L) in mobile phase (A/B: 50/50) at a flow rate of 5 μL/min. The compounds of interest were monitored through specific MS2 scans in addition of MS full scan (50–1000 *m/z*): MS2 (743), MS2 (581), MS2 (949), MS2 (919), and MS2 (889) for the screening of anthocyanins compounds and MS2 (551), MS2 (507), MS2 (389), and MS2 (549) for screening of betanin compounds.

2.2.3. Preparation of film forming solution and films casting

Film forming solutions were prepared according to Akhtar et al. (2010) by dissolving 6 g of HPMC in a 35% ethanol solution for 40 min at 65 °C using a heating magnetic stirrer (Fisher Bio-block Scientific). For better dissolving and avoiding heat oxidation, NRC was dissolved separately in 35% ethanol solution at 20 °C. Both, HPMC and NRC solutions were then mixed and stirred for 30 min at 20 °C to obtain homogeneous solution. NRC solutions pH was adjusted at 3.17 ± 0.1 with HCl (0.1 M). After stirring, the solutions were degassed at room temperature under vacuum "Handy Aspirator WP-15 (Yamato®)" for 30 min. Films were made by pouring 6 g of each film forming solution (FFS) in the lids of the Petri-dishes. Films were then left in a dark room (pre-equilibrated at 20 °C, 50% RH) for drying on a levelled surface for 48 h. Composition of HPMC films, glycerin and NRC concentrations are shown in Table 2.

2.2.4. Film thickness measurement

Film thickness was measured according to the standard NF Q 03-016 with a manual micrometer (Messmer, London, England) equipped with a measuring head of 1 cm in diameter and a sensitivity of 2 μm. Thicknesses were measured in 10 randomly selected points on each film and an average value was calculated.

2.2.5. Film aging

For photo-aging, the films were conditioned under the fluorescent light (OSRAM L36W/640) or darkness for 20 days in an experimental chamber with controlled conditions of temperature (20 °C) and relative humidity (50%). The distance of fluorescent tube from the films was 14 cm.

2.2.6. Color measurements

Color measurements were carried out with a Minolta CM, CR-210 colorimeter (Minolta, Colombes, France) using the Hunter and CIE scale. A black standard color plate (L^* = 24.60, a^* = 0.16, b^* = −0.28) was used as a background for color measurements. Value L^* describes lightness (0 = black to 100 = white). Value a^* describes the amount of redness (positive) or greenness (negative) present in the specimen, while value b^* describes the amount of yellowness (positive) or blueness (negative) present in the specimen. Combined values a^* and b^* define the hue and intensity (saturation) of the color (Moslemi, 1967). The *L*, *a*, and *b* values of each film were taken as the average of at least five points. Color difference (ΔE) is the magnitude of the resultant vector of three component

Table 1
Peak assignments for betalains and anthocyanins of natural red colour (NRC).

	Compound name	Rt (min)	UV–vis$_{max}$ (nm)	*m/z* $[M+H]^+$	*m/z* MS^2 of $[M+H]^+$	(%) Area at 530 nm
1	Betd 5-glc (betanin)	7.69	271.9/293.6/538.9	551.16	389.12	48.78
2	Isobetd 5-glc (iso-betanin)	11.95	271.9/293.6/538.9	551.16	389.13	47.91
3	Cyd 3-xyl-glc-gal	22.53	283.7/514.3	743.1	287.0	0.23
4	Cyd 3-xyl-gal	24.38	283.7/514.3	581.1	287.0	1.11
5	Cyd 3-xyl-glc-gal-sin	27.81	287.8/335.5/523.8	949.2	287.0	0.29
6	Cyd 3-xyl-glc-gal-fer	29.58	287.8/334.3/519.0	919.2	581.1/287.0	1.19
7	Cyd 3-xyl-glc-gal-coum	30.88	287.8/319.0/519.0	889.2	287.0	0.49

Aglycons: Betd, betanidin; Cyd, cyanidin; Xyl, xylose; Glc, glucose; Gal, galactose; Fer, ferulic acid; Sin, sinapic acid; Coum, p-coumaric acid.

differences. Total color difference (ΔEab), was calculated by following equation:

$$\Delta Eab = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

where $\Delta a = a_i - a_0$, $\Delta b = b_i - b_0$ and $\Delta L = L_i - L_0$. The index *i*, indicates the values observed after storage period and index 0, indicates initial values observed before samples storage (Jutaporn, Suphitchaya, & Thawien, 2011).

2.2.7. Light transmission

The barrier properties of HPMC films against ultraviolet (UV) and visible light were measured at selected wavelengths between 200 and 900 nm, using UV–visible recording spectrophotometer (Ultrospec 4000 UV/visible, Pharmacia Biotech, Orsay, France) according to Fang, Tung, Britt, Yada, and Dalgleish (2002).

2.2.8. ABTS radicals scavenging activity

The evaluation of 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical scavenging activity was based on the ability of antioxidants to inhibit the long-life ABTS radical cation (Sigma, Germany), a blue/green chromophore with characteristic absorption at 734 nm, in comparison with that of Trolox. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in darkness, at room temperature, for 12–16 h before use. To study antiradical activity of NRC, $ABTS^{\bullet+}$ solution was diluted with ethanol at 30 °C, to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 1.0 mL of diluted $ABTS^{\bullet+}$ solution to 10 μL of sample or standard Trolox in ethanol (concentration between 0 and 16 μM), the absorbance was measured at 30 °C exactly 6 min after initial mixing. Appropriate solvent blanks were run in each assay. All experiments were performed in triplicate. A standard curve was obtained by using Trolox standard solution at various concentrations. The absorbance of reaction samples was compared to that of Trolox standard and results were expressed in terms of Trolox equivalents (Re et al., 1999). TEAC value is defined as the concentration of standard Trolox with the same antioxidant capacity as a 1 mM concentration or 1 mg/mL of the antioxidant compound under investigation (Maisuthisakul, Pongsawatmanit, & Gordon, 2007).

2.2.9. FTIR analysis of HPMC films

Changes in structure of HPMC composite films after 20 days of continuous light exposure were followed by Fourier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25 °C with a Tensor 27 mid-FTIR Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with a Platinum ATR optical cell and an RT-DlaTGS detector (Bruker, Karlsruhe, Germany). The diaphragm was set at 4 mm. The scanning rate was 10 kHz, and 80 scans were performed both for the reference and the sample from 4000 to 800 cm^{-1} with 4 cm^{-1} of resolution. All data treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-point Savitsky-Golay smoothing functions. Elastic baseline correction was applied to spectra, which were further cut between 1800 and 800 cm^{-1}, centered and normalized.

The stability of NRC compounds was calculated by spectral deconvolution using second derivative resolution enhancement and the curve-fitting procedure of 1800–1500 cm^{-1} region. Second derivative spectra were calculated on centered and normalized data with an additional nine-points Savitsky-Golay smoothing function. The second derivative spectra were used only for identifying individual peak positions. The spectra were then deconvoluted by a non linear regression curve fitting program of Gaussian peaks to the original spectra (Opus Software). Optimal Fits were supported by favorable RMS (root mean square) values on the order of 10^{-5}, which were less than baseline noise. The resulting curves fitted were analyzed and percentage of each covalent bond was quantified.

2.2.10. Statistical analysis

A factorial design was used to characterize the composite films. Experimental values were given as means ± standard deviation (SD). Analysis of variance (ANOVA) was used to compare mean differences of the samples. If the differences in mean existed, multiple pairwise comparisons were performed using XL STAT software. Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

3.1. HPLC–MS/MS characterization of NRC

Various analytical methods have been reported to differentiate betalains and betacyanins (Charron et al., 2009; Nielson & Harley, 1996). An official HPLC method providing fingerprints of common fruit juices to characterize betacyanins has been published (IFU, 1998). In the present study, newly established HPLC-DAD–MS/MS method (Stintzing et al., 2005) was used allowing simultaneous determination of betacyanins. Betanin and iso-betanin were previously detected in prickly pear (*Opuntia* spp.) by Castellanos-Santiago and Elhadi (2008) and anthocyanin in purple carrots by Kurilich, Clevidence, Britz, Simon, and Novotny (2005). Based on comparison with the standards and bibliographical data (Stintzing et al., 2005), betalains and anthocyanin were readily identified by their retention time order, spectral and mass characteristics including daughter ion and neutral loss scanning (Table 1). As previously reported (IFU, 1998), betacyanins were generally more polar than anthocyanins therefore betanin (betanidin-5-O-β-glucoside) and iso-betanin (isobetanidin-5-O-β-glucoside) eluted considerably earlier than the minor components such as anthocyanins (Fig. 2a).

3.1.1. Identification of major components (betanin & isobetanin)

Major components of NRC, betanin (1) and its C_{15} epimer iso-betanin (2) amounted to 96.69% together were identified on UV–visible chromatogram and single ion chromatogram at

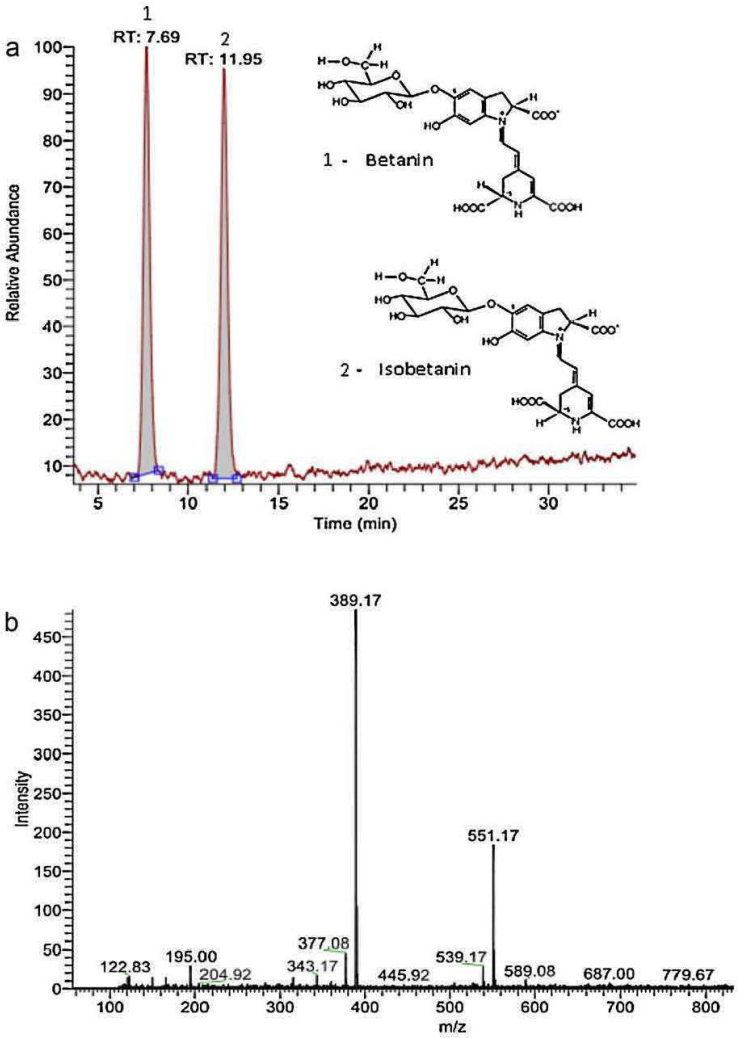


Fig. 1. Identification of major components (betanin & isobetanin) of natural red colour (NRC). Peak assignment is given in Table 1. (a) Single ion chromatogram (SIC) of NRC at $m/z = 551$. Betanin & isobetanin structures were adopted from Herbach et al. (2006a,b). (b) NRC Full MS spectrum at 7.69 min (same at 11.95 min) with parent ion $m/z = 551$ $[M+H]^+$ and source fragmentation ion $m/z = 389$.

retention time of 7.69 min and 11.95 min, respectively (Fig. 1a). They were further confirmed with MS and MS^2 steps.

3.1.2. *Identification of minor components (anthocyanins)*

NRC showed the minor components such as anthocyanins typically cyanidin derivatives (3–6) along with a couma-royl-derivative (7) as also described by Stintzing et al. (2005) and Glässgen, Seitz, and Metzger (1992). Presence of these anthocyanins was confirmed by comparing their specific retention times (Fig. 2a) with those of a standard anthocyanin mixture chromatogram (Fig. 2b).

3.2. *Film thickness measurement*

The thickness of edible films is an important parameter because it directly affects the biological properties and the shelf life of the coated food. Thickness is dependent on the type of dry matter and film preparation methods (Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007). HPMC films incorporated with NRC at the level of 1, 2, 3, or 4% (v/v) were compared for their thickness with 3 types of control HPMC films; composition is shown in Table 2. No significant change in film thickness was found by the addition of glycerin (1% or 4%, w/w of film dry matter). Similar results were reported by Imran, El-Fahmy, Revol-Junelles, and Desobry (2010) by the addition of 10% (w/w) plasticizer into HPMC films. A slight increase in film thickness may be associated with the property of glycerin to retain high moisture content at the end of film drying (Chen & Lai, 2008). However, a gradual but non-significant increase in the thickness of films containing NRC may be combined effect of glycerin and betacyanin molecules containing lots of hydrophilic groups (Díaz, López1, Kerstupp1, Ibarra1, & Scheinvar, 2006). A slight increase in thickness of films containing 4% NRC compared to films containing 1% of glycerin confirmed the plasticizing effect of phenolic compounds on film thickness.

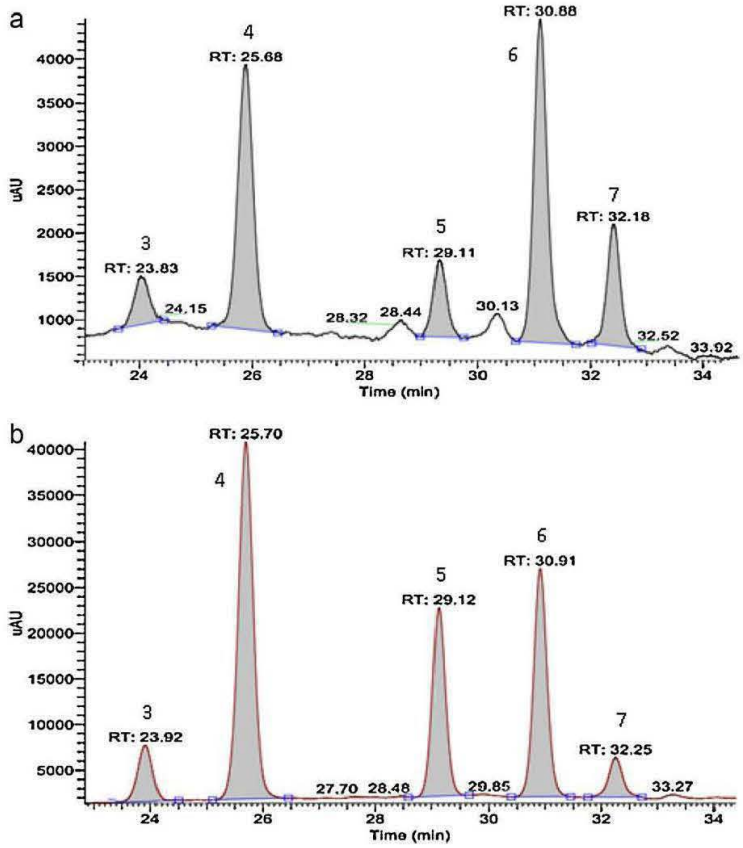


Fig. 2. Identification of NRC minor components (anthocyanins): comparison between NRC UV–visible chromatogram (a) and standard anthocyanins UV–visible chromatogram (b) at 530 nm. Peak assignment is given in Table 1.

3.3. Optical properties of film: color and transparency

Optical properties are the first ones detected by human vision and could affect food quality (Fabra, Talens, & Chiralt, 2009).

3.3.1. Color stability

Color parameters of control and NRC films were analyzed (Table 3). Control HPMC films without NRC and glycerin appeared clear and transparent which showed complete solublisation of HPMC powder in 35% ethanol solution. Incorporation of NRC into HPMC films modified their appearance in both color and transparency. A decrease in L values and an increase in a values were observed in NRC films. Hunter L, a and b values were statistically identical for control HPMC films but they were significantly different for the films containing NRC up to 4% (v/v). Significant increase in redness (a) of NRC films was observed representing its good coloring ability.

Similar results were observed by Park and Zhao (2006) by the addition of Cranberry Pomace Extracts in low methoxyl pectin (LMP) and high methoxyl pectin (HMP) films.

Color stability was studied by conditioning the films under fluorescent light at 20 °C for 20 days. The control samples, HPMC films alone or containing 1 or 4% (w/w) of glycerin exhibited non significant color difference over 20 days of light storage. A significant decrease in a values was observed for all NRC films causing fading of the red surface color (Table 3). The visual color changes observed

Table 2
Thickness and composition of HPMC–NRC–plasticizer composite films and pH values of FFS (mean and standard deviation of triplicate analysis).

Film type	Film composition glycerin, G% (w/w) of dry matter	pH of film forming solutions (FFS)	Film thickness (μm)
HPMC	0.00	7.61 ± 0.50	48.25 ± 3.48[a]
HPMC + G1%	1.00	7.30 ± 0.50	49.03 ± 4.15[a]
HPMC + G4%	4.00	7.35 ± 0.50	49.50 ± 5.03[a]
HPMC + NRC1%	0.20	3.17 ± 0.50	49.38 ± 3.39[a]
HPMC + NRC2%	0.40	3.17 ± 0.50	52.29 ± 3.92[a]
HPMC + NRC3%	0.60	3.17 ± 0.50	53.09 ± 4.85[a]
HPMC + NRC4%	0.80	3.17 ± 0.50	54.29 ± 5.62[a]

Test conditions (temperature 20 ± 2 °C; RH, 50 ± 2%). NRC, natural red colour; HPMC, hydroxypropyl methylcellulose. Same letters within the column (film thickness) indicate non-significant difference at $P < 0.05$.

Table 3
Colour parameters (L^*, a^*, b^*) of edible HPMC films as a function of NRC concentration and 20 days of aging under fluorescent light (mean values of triplicate analysis).

Films types	L^* (lightness)		a^* (redness/greenness)		b^* (yellowness/blueness)		ΔEab^* (0–20 d)
	0 d (P.A.)	20 d (P.A.)	0 d (P.A.)	20 d (P.A.)	0 d (P.A.)	20 d (P.A.)	
HPMC	32.18 ± 0.05^{Aa}	32.16 ± 0.09^{Aa}	-0.067 ± 0.006^{Ae}	-0.10 ± 0.01^{Be}	-0.48 ± 0.04^{Ad}	-0.46 ± 0.03^{Ae}	0.06 ± 0.02^{b}
HPMC + G1%	32.17 ± 0.02^{Ba}	32.25 ± 0.02^{Aa}	-0.097 ± 0.021^{Ae}	-0.11 ± 0.01^{Ae}	-0.44 ± 0.01^{Ad}	-0.50 ± 0.01^{Be}	0.10 ± 0.03^{b}
HPMC + G4%	32.16 ± 0.03^{Aa}	32.17 ± 0.22^{Aa}	-0.093 ± 0.006^{Ae}	-0.10 ± 0.02^{Ae}	-0.44 ± 0.02^{Ad}	-0.52 ± 0.04^{Be}	0.08 ± 0.03^{b}
HPMC + NRC1%	31.78 ± 0.16^{Ab}	31.91 ± 0.04^{Aa}	0.997 ± 0.085^{Ad}	0.66 ± 0.04^{Bd}	2.34 ± 0.19^{Ac}	1.99 ± 0.20^{Ad}	0.56 ± 0.32^{b}
HPMC + NRC2%	29.39 ± 0.19^{Bc}	30.40 ± 0.08^{Ab}	5.013 ± 0.267^{Ac}	3.27 ± 0.11^{Bc}	5.99 ± 0.18^{Ab}	5.77 ± 0.15^{Ac}	2.03 ± 0.27^{a}
HPMC + NRC3%	28.87 ± 0.16^{Bd}	30.03 ± 0.15^{Ac}	5.930 ± 0.267^{Ab}	4.18 ± 0.29^{Bb}	6.35 ± 0.05^{Aa}	6.52 ± 0.08^{Ab}	2.11 ± 0.27^{a}
HPMC + NRC4%	27.96 ± 0.02^{Be}	28.88 ± 0.17^{Ad}	6.943 ± 0.051^{Aa}	5.86 ± 0.24^{Ba}	6.31 ± 0.03^{Aa}	6.95 ± 0.04^{Ba}	1.56 ± 0.22^{a}

0 d (P.A.), 0 day photo-aging; 20 d (P.A.), 20 days photo-aging; ΔEab^* (0–20 d), total colour change during 20 days of photo-aging. Different small letters within each column and different capital letters within each row indicate significant differences among the values of the same colour property at $P < 0.05$.

for light stored films appeared in L values, significantly increased by photo-aging. The lightening of initial red surface color of films was due to photo-degradation of betacyanins (Herbach, Stintzing, & Carle, 2006).

The changes in L, a and b values were summarized by calculating total color difference (ΔEab). There was an increase in ΔE values of NRC added films under fluorescent light conditions (Table 3). The increase in ΔE values was higher for the films incorporated with NRC 2, 3 or 4% over 20 days of photo-aging. This increase in ΔE resulted from a decrease in a values and increase in L values. The ΔE value of 1.0 is the smallest color difference a normal human eye can detect so any ΔE less than 1.0 is imperceptible (Jonathan Sachs, 2001–2002). Some color differences even greater than 1 are perfectly acceptable, may be even unnoticeable, depending on the color, shade and density. For example, the ΔE color difference between two reds may be the same but may not look like same difference to the human eye (Jonathan Sachs, 2001–2002). Keeping in view this statement, the films other than those containing 2, 3 and 4% NRC were stable for their color properties after 20 days of photo-aging.

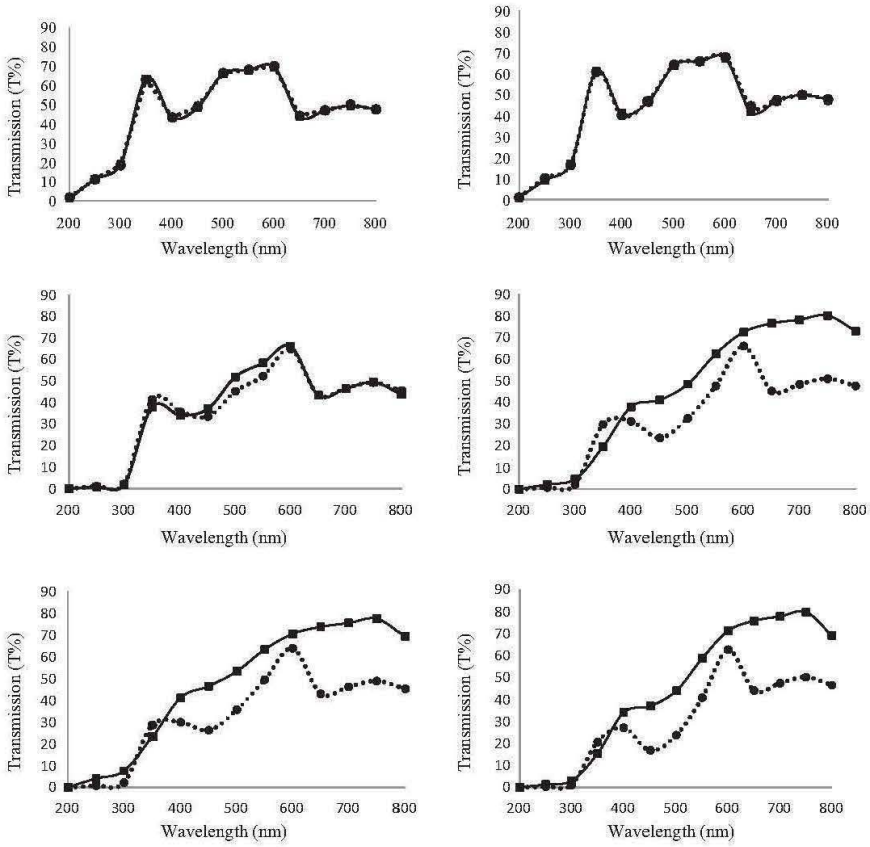


Fig. 3. Light transmission (T%) of UV–visible for HPMC–NRC–plasticizer composite films before and after 20 days of photo-aging. (a) HPMC 0 d (●), HPMC 20 d (■). (b) HPMC + G1% 0 d (●), HPMC + G1% 20 d (■). (c) HPMC + NRC1% 0 d (●), HPMC + NRC1% 20 d (■). (d) HPMC + NRC2% 0 d (●), HPMC + NRC2% 20 d (■). (e) HPMC + NRC3% 0 d (●), HPMC + NRC3% 20 d (■). (f) HPMC + NRC4% 0 d (●), HPMC + NRC4% 20 d (■).

3.3.2. Light transmission

For use as packaging materials, transparency of HPMC films is required to fulfill consumer eagerness to see food through packaging. Comparing the effect of each mixture component on % transmission, NRC concentration was the main factor reducing film transparency. The lowest transmission through films was noticed for the greatest concentration of NRC. Fig. 3 shows transmission of UV and visible light, at selected wavelength between 200 and 700 nm, through films before and after 20 days of light exposure.

Increase in NRC contents of HPMC films showed a decrease in light transmission of films for both UV and visible regions. This result was in accordance with Jutaporn, Suphitchaya, and Thawien (2011) who observed that HPMC films became less transparent with the increase of phayom wood extract contents. No change in transmission of HPMC films alone and with glycerin 1% was noticed after photo-aging as shown in Fig. 3a and b. It is clear that NRC films became more transparent after light exposure due to color degradation. Increase in light transmission after photo-aging was more pronounced in films containing high concentration of NRC and was confirmed by total change in color (ΔE). Indeed, the reflected and transmitted spectrum of a colored layer was based on a material dependent scattering and absorption of light in visible spectra (Hutchings, 1999).

3.4. ABTS radical scavenging activity

The ABTS radical scavenging activity method is based on the ability of molecules to scavenge the ABTS radical cation, in comparison with that of Trolox. The ABTS assay was calibrated with the water soluble alpha-tocopherol analog, Trolox. Antioxidant stability of NRC was investigated for different stages of film formation and light-aging (Fig. 4). All the samples containing NRC displayed antioxidant activities as they were able to scavenge $ABTS^{\bullet+}$ radical cation. They were shown to be antiradical agents compared to the Trolox. FFS, fresh films, films stored under darkness or under light, displayed less free radical scavenging activities than pure NRC solution (TEAC = 0.0133 ± 0.0005). No significant change in TEAC value was observed for FFS (0.0123 ± 0.0004), fresh film (0.0121 ± 0.0005) and those stored under darkness (0.012 ± 0.0004) as compared to pure NRC (0.0133 ± 0.0005), showing their antioxidant stability. The film samples stored under light were significantly different from pure NRC samples for their antioxidant activity (Fig. 4). These results suggested that Trolox equivalent antioxidant activity of NRC was slightly decreased during FFS preparation but no significant change was observed during film casting and film aging after darkness or light exposure. This phenomenon indicated that NRC compound was slightly modified or degraded during aging process. However, the degradation products have conserved some antioxidant capacity. Moreover, NRC was more stable in FFS, fresh films and films stored in darkness. These results were in accordance with those of Díaz et al. (2006) who studied the effect of light and darkness on betalains stability.

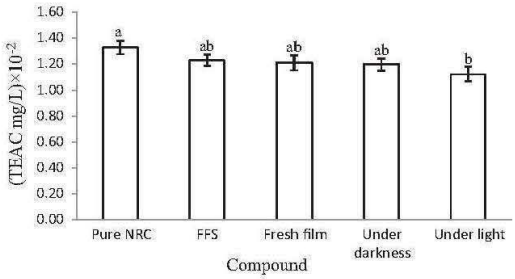


Fig. 4. Antioxidant activities of pure NRC solution, film forming solution (FFS) and NRC incorporated films against $ABTS^{\bullet+}$ radical, expressed as TEAC values. Means within the same column with different letters are significantly different at $P < 0.05$.

3.5. FTIR analysis of HPMC films

FTIR spectroscopy is a rapid technique with minimum samples preparation requirements. It allows qualitative and quantitative determination of organic compounds in samples because intensities of spectrum bands are proportional to concentration (Vlachos et al., 2006). Control and NRC composite films were analyzed by FTIR spectroscopy to characterize new phenolic polymeric structures and light storage effect. FTIR spectra for control and HPMC films colored with NRC (betacyanins), ranging between 1800 and 800 cm^{-1} are shown in Fig. 5. All samples had very strong absorption bands at 1060 cm^{-1} related with a pronounced shoulder at 1115 cm^{-1} attributed to a combination band of C–O stretches and secondary hydroxyl group (O–H).

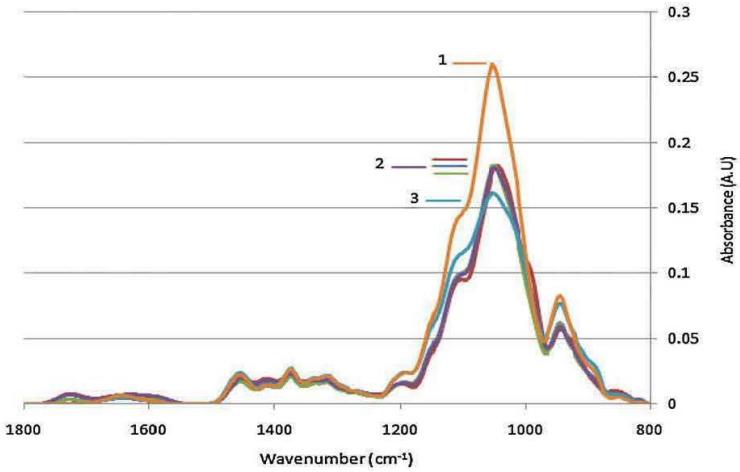


Fig. 5. FTIR spectra of control HPMC films; without light exposure (3), after light exposure (1) and films containing NRC 1% or 4% before and after 20 days of photo-aging (2).

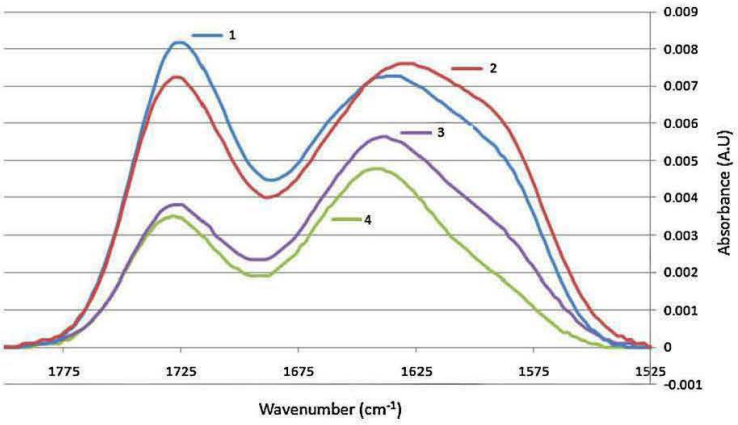


Fig. 6. FTIR spectral region (1525–1775 cm^{-1}) of HPMC films containing 1% NRC; without light exposure (4), after light-ageing (3) and with 4% NRC; without light exposure (1) and after light-ageing (2).

Table 4
Peak assignment of deconvolution FTIR spectra of NRC incorporated HPMC films (HPMC + NRC1% & HPMC + NRC4%) before and after 20 days of photo-aging.

Resonant frequency(cm^{-1})	HPMC + NRC1%			HPMC + NRC4%			Bond types
	% age of covalent bond			% age of covalent bond			
	0 day aging	20 days aging	Variations	0 day aging	20 days aging	Variations	
1581.140	5.38 ± 0.22^{b}	9.64 ± 0.39^{a}	4.260	9.465 ± 1.51^{a}	13.07 ± 0.18^{b}	3.603	NH
1609.688	16.51 ± 0.18^{b}	20.34 ± 0.15^{a}	3.829	16.84 ± 2.75^{a}	19.80 ± 0.17^{a}	2.967	COO^{-}
1636.439	9.48 ± 0.110^{a}	7.39 ± 0.390^{b}	−2.086	2.213 ± 0.59^{b}	3.344 ± 0.11^{a}	1.130	COO^{-}
1654.688	36.50 ± 0.19^{a}	35.60 ± 0.45^{b}	−0.903	37.33 ± 0.34^{a}	33.11 ± 0.05^{b}	−4.220	NH_2
1699.986	0.000 ± 0.00^{b}	0.08 ± 0.027^{a}	0.081	1.115 ± 0.76^{a}	0.000 ± 0.00^{a}	−1.115	C=O
1726.441	32.13 ± 0.41^{a}	26.95 ± 0.48^{b}	−5.181	33.036 ± 4.6^{a}	30.67 ± 0.43^{a}	−2.365	C=O

Different letters within each row indicate significant differences among the values of the same NRC percentage at $P < 0.05$.

3.5.1. *Changes in absorption band at 1060 cm^{-1}*

Absorption band at 1060 cm^{-1} is associated with the hydroxyl group indicating formation of intermolecular hydrogen bonds. In case of pure HPMC films after 20 days of photo-aging, the absorption of this band was increased due to OH groups formation. Increase in peak area in this region was due to the availability of more OH groups of glycerin interacting with cellulosic OH groups. The stretching vibration at 1060 cm^{-1} causing an increase in peak surface indicated light degradation of pure HPMC films after photo-aging (Fig. 5). The increasing slopes of the curves were due to availability of OH groups of phenolic compounds present in NRC for cross-linking with cellulosic OH groups. Same results were observed by Kim, López, Güebitz, and Cavaco-Paulo (2008) from coloration of flax fabrics with flavonoids.

NRC Films were compared with control HPMC films for their FTIR spectra. Absorption band at 1060 cm^{-1} for films incorporated with NRC was stable over 20 days of fluorescent light exposure, which indicated that NRC had an ability to protect pure HPMC films against photo-degradation.

3.5.2. *Changes in the region between 1525 cm^{-1} and 1775 cm^{-1}*

The comparison between the spectra showed an additional peak around 1726 cm^{-1} for NRC films (Fig. 6). This band was attributed to C=O stretching vibrations and indicated the presence of phenolic compounds (betacyanins) on treated HPMC films. The 1726 cm^{-1} band was attributed to C=O ester absorption and could be generated by flavonoids oxidation after 20 days of light exposure. NRC compounds stability was calculated by spectral deconvolution using second derivative resolution enhancement and curve-fitting procedure of 1800–1500 cm^{-1} region. The resulting curves were then analyzed and percentage of each covalent bond was quantified.

Fitting the bands to a variable number of individual contributing vibration modes was most successful using six peaks (Table 4). Stretching vibration of NH bond was reported at 1581 cm^{-1} and increased after light exposure due to NH_2 oxidative breakdown. The increase % in NH groups was greater in films containing 1% NRC (4.26%) as compared to those containing 4% NRC (3.60%) after light exposure showing NRC oxidation. Similarly, stretching vibration of COO^{-} was reported at 1609 cm^{-1} and increased after light exposure due to C=O oxidative breakdown. The increase in COO^{-} groups was greater in films containing 1% NRC (3.82) as compared to those containing 4% NRC (2.96%) after light exposure which indicated NRC oxidation. It could be concluded from Table 4 that NRC was slightly oxidized after 20 days of light exposure.

4. Conclusion

This study demonstrated that natural plant extracts could be used to functionalize edible films with additional benefits. Such films provide unique fruit flavor, color and antioxidant capacity, which would significantly enhance its potential applications in both food and nonfood industries. Miscibility of HPMC and NRC in composite films was confirmed by infrared spectroscopy analysis. Absorption bands in FTIR spectra suggested interactions through hydrogen bonding between components. The additional peak observed for NRC films was due to compounds OH groups interaction with cellulosic OH groups. Increased peak area in this region was directly proportional to NRC concentration making films more hydrophilic. NRC antioxidant capacity during the steps of film

preparation was stable. Color of edible films became darker and redder as NRC increased, while an increase effect of light exposure was noticed on color stability. Results pointed that NRC films has good potential for food applications due to their color, plasticizing property, good antioxidant stability and ability to protect HPMC from photo-degradation. Nevertheless, films stability in respect to color, transparency, microbial growth, and flavor retention needs further studies.

Acknowledgements

The authors would like to thank Carole Jeandel, Céline Charbonnel and Carole Perroud for their technical assistance.

References

Akhtar, M. J., Jacquot, M., Tehrany, E. A., Gaiani, C., Linder, M., & Desobry, S. (2010). Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film color. *Food Chemistry, 120*, 395–401.

Bell, D. R., & Gochenaur, K. (2006). Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. *Journal of Applied Physiology, 100*, 1164–1170.

Brindle, L. P., & Krochta, J. M. (2008). Physical properties of whey protein hydroxypropyl methylcellulose blend edible films. *Journal of Food Science, 73*, 446–454.

Burdock, G. A. (2007). Safety assessment of hydroxypropyl methylcellulose as a food ingredient. *Food and Chemical Toxicology, 45*, 2341–2351.

Burke, J. R. (2006). Biodegradeable-compostable packaging: The promise and the problems. *Paper and Packaging, 47*, 5.

Cai, Y., Sun, M., & Corke, H. (2003). Antioxidant activity of betalains from plants of the amaranthaceae. *Journal of Agricultural and Food Chemistry, 51*, 2288–2294.

Castellanos-Santiago, E., & Elhadi, M. Y. (2008). Identification and quantification of betalains from the fruits of 10 Mexican prickly pear cultivars by high-performance liquid chromatography and electrospray ionization mass spectrometry. *Journal of Agricultural and Food Chemistry, 56*, 5758–5764.

Charron, C. S., Kurilich, A. C., Clevidence, B. A., Simon, P. W., Harrison, D. J., Britz, S. J., et al. (2009). Bioavailability of anthocyanins from purple carrot juice: Effects of acylation and plant matrix. *Journal of Agricultural and Food Chemistry, 57*, 1226–1230.

Chen, C. H., & Lai, L. S. (2008). Mechanical and water vapor barrier properties of tapioca starch/decolorized hsian-tsao leaf gum films in the presence of plasticizer. *Food Hydrocolloids, 22*, 1584–1595.

Dai, J., Patel, J. D., & Mumper, R. J. (2007). Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *Journal of Medicinal Food, 10*, 258–265.

Díaz, F. S., López1, E. M. S., Kerstupp1, S. F., Ibarra1, R. V., & Scheinvar, L. (2006). Colorant extraction from red prickly pear (*Opuntia lasiacantha*) for food application. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 5*, 1330–1337.

Escribano, J., Pedreño, M. A., Garcia-Carmona, F., & Munoz, R. (1998). Characterization of the antiradical activity of betalains from *Beta vulgaris* L. roots. *Phytochemical analysis, 9*, 124–127.

Fabre, C. E., Santeri, A. L., Lorete, M. O., Baberian, R., Pareilleux, A., Goma, G., et al. (1993). Production and food applications of the red pigments of Monascus rubber. *Journal of Food Science, 58*, 1099–1111.

Fabra, M. J., Talens, P., & Chiralt, A. (2009). Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocolloid, 23*, 676–683.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science, 67*, 188–193.

Frank, T., Conrad, F., Carle, S. R., Bitsch, I., Daniela, Q., Gabriele, B., et al. (2005). Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. *Pharmacological Research, 52*, 290–297.

Gentile, C., Tesoriere, L., Allegra, M., Livrea, M. A., & Alessio, P. (2004). Antioxidant betalains from cactus pear (*Opuntia ficus-indica*) inhibits endothelial ICAM-expression. *Annals of the New York Academy of Sciences, 1028*, 481–486.

Glässgen, W. E., Seitz, H. U., & Metzger, J. W. (1992). High performance liquid chromatography/electrospray mass spectrometry and tandem mass spectrometry of anthocyanins from plant tissues and cell cultures of *Daucus carota* L. *Biological Mass Spectrometry, 21*, 271–277.

Hari, R. K., Patel, T. R., & Martin, A. M. (1994). An overview of pigment production in biological systems: Functions, biosynthesis and applications in food industry. *Food Reviews International, 10*, 49–70.

Herbach, K. M., Stintzing, F. C., & Carle, R. (2006). Betalain stability and degradation: Structural and chromatic aspects. *Journal of Food Science, 71*, 41–50.

Herbach, K. M., Stintzing, F. C., Elss, S., Preston, C., Schreier, P., & Carle, R. (2006). Isotope ratio mass spectrometrical analysis of betanin and isobetanin isolates for authenticity evaluation of purple pitaya-based products. *Food Chemistry, 99*, 204–209.

Hutchings, J.-B. (1999). *Food color and appearance*. Maryland: Aspen Publisher, USA.

Imran, M., El-Fahmy, S., Revol-Junelles, A.-M., & Desobry, S. (2010). Cellulose derivative based active coatings: Effects of nisin and plasticizer on physico-chemical and antimicrobial properties of hydroxypropyl methylcellulose films. *Carbohydrate Polymers, 81*, 219–225.

International Federation of Fruit Juice Producers. (1998). *IFU method 71: Anthocyanins by HPLC*. Zug, Switzerland: IFU.

Jonathan Sachs Color Management Terms Copyright© 2001–2002 Digital Light & Color.

Jutaporn, C. T., Suphitchaya, C., & Thawien, W. (2011). Antimicrobial activity and characteristics of edible films incorporated with Phayom wood (*Shorea tolura*) extract. *International Food Research Journal, 18*, 39–54.

Kanner, J., Harel, S., & Granit, R. (2001). Betalains a new class of dietary cationized antioxidants. *Journal of Agricultural and Food Chemistry, 49*, 5178–5185.

Kim, S., López, C., Güebitz, G., & Cavaco-Paulo, A. (2008). Biological coloration of flax fabrics with flavonoids using laccase from trametes hirsute. *Engineering in Life Sciences, 8*, 324–330.

Kurilich, A. C., Clevidence, B. A., Britz, S. J., Simon, P. W., & Novotny, J. A. (2005). Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. *Journal of Agricultural and Food Chemistry, 53*, 6537–6542.

Laleh, G. H., Frydoonfar, H., Heidary, R., Jameei, R., & Zare, S. (2006). The effect of light, temperature, pH and species on stability of anthocyanin pigments in four berberis species. *Pakistan Journal of Nutrition, 5*, 90–92.

Maisuthisakul, P., Pongsawatmanit, R., & Gordon, M. H. (2007). Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (*Cratoxylum formosum* Dyer). *Food Chemistry, 100*, 1620–1629.

Markakis, P. (1982). *Introduction in anthocyanin in fruits, vegetables and grain*. Boca Roton, FL: CRC Press. (Chapter 1).

Moslemi, A. A. (1967). Quantitative color measurement for black walnut wood. US Forest Service, Research Paper NC-17. St. Paul, Minnesota: USDA, Forest Service, North Central Forest Experiment Station. 15 p.

Nielson, L. R., & Harley, S. M. (1996). Chemotaxonomy: Simple test for distinguishing between anthocyanins and betacyanins. *Journal of Biological Education, 30*, 88–90.

Park, S., & Zhao, Y. (2006). Development and characterization of edible films from cranberry pomace extracts. *Journal of food Science, 71*, 95–101.

Pedreño, M. A., & Escribano, J. (2000). Studying the oxidation and the antiradical activity of betalain from beetroot. *Journal of Biological Education, 35*, 49–51.

Ray, S. S., & Bousmina, M. (2005). Biodegradable polymers and their layered silicate nanocomposites: In greening the 21st century materials world. *Progress in Materials Science, 50*, 962–1079.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*, 1231–1237.

Sebti, I., Chollet, E., Degraeve, P., Noel, C., & Peyrol, E. (2007). Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *Journal of Agricultural and Food Chemistry, 55*, 693–699.

Stintzing, F. C., & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food and in human nutrition. *Trends in Food Science & Technology, 15*, 19–38.

Stintzing, F. C., Herbach, K. M., Mosshammer, M. R., Carle, R., Yi, W., Sellappan, S., et al. (2005). Color, betalains pattern and antioxidant properties of cactus pear (*Opuntia* sp.) clone. *Journal of Agricultural and Food Chemistry, 53*, 442–451.

Turhan, K. N., & Sahbaz, F. (2004). Water vapor permeability, tensile properties and solubility of methylcellulose based edible films. *Journal of Food Engineering, 61*, 459–466.

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. *Food Research International, 42*, 762–769.

Vlachos, N., Skopelitis, Y., Psaroudaki, M., Konstantinidou, V., Chatzilazarou, A., & Tegou, E. (2006). Applications of Fourier transform-infrared spectroscopy to edible oils. *Analytica Chimica Acta, 573-574*, 459–465.

Chapitre IV.4 : Cinétiques de libration et stabilité de l'activité antioxydante de composes phénoliques naturels incorporés dans des films de biopolymère

Transition: Les acides gras insaturés sont très sensibles à l'oxydation qui va entrainer la perte de qualité nutritionnelle, l'apparition de faux goût et d'autres produits de dégradation dont certains sont suspectés d'être dangereux pour la santé humaine (Drusch and Berg, 2008; Choe and Min, 2006). Les antioxydants ont la propriété de contrôler l'oxydation par le piégeage des radicaux libres, la chélation des métaux, quenching des oxygènes singulets et l'inactivation des photosensibilisateurs et des lipoxygénases (Choe and Min, 2006). Cependant, certains procédés et le stockage altèrent ces composés naturels ce qui conduit à une réduction de leur capacité à protéger les aliments. C'est pourquoi, souvent d'autres antioxydants sont ajoutés volontairement au produit. Les sources naturelles telles que la betterave, le thé, l'origan, les baies, la moutarde, sont riches en molécules actives qui présentent un fort potentiel à neutraliser les radicaux libres (Murphy et al., 2009; Bhale et al., 2007; Houhoula et al., 2003; Wanasundara and Shahidi, 1998).

Des rapports contradictoires sur les antioxydants synthétiques ont conduit les états à réglementer leur utilisation. Une réglementation a été votée par La Food and Drug Administration (FDA) et le U.S. Department of Agriculture (USDA); au Canada, la Food and Drug Regulations (National Health and Welfare); en Europe, la European Food Safety Authority (EFSA); et au Japon, la Food Sanitation Law.

De nombreux autres pays adoptent une réglementation similaire à celle établie soit aux USA soit en Europe, lesquelles présentent des différences quant à l'approbation des antioxydants, leur application et leurs doses autorisées (Reische et al., 1998; Pratt and Hui, 1996). De ce fait, les antioxydants synthétiques sont progressivement remplacés par des molécules naturelles. Cependant, dans les deux cas, la vigilance est attirée sur les possibles interactions entre les molécules et l'aliment en surface ou dans la masse (Gemili et al., 2010). Pour ces différentes raisons, les emballages actifs semblent présenter un fort potentiel pour éviter des réactions directes avec le produit tout en maintenant un niveau de protection performant. Dans la littérature, nous retrouvons plusieurs formulations de films bioactifs qui permettent de contrôler l'oxydation (Jamshidian et al., 2012; Mayachiew and Devahastin, 2010; Gemili et al., 2010; Gómez-Estaca et al., 2009; Han and Krochta, 2007). Mayachiew & Devahastin (2010) ont étudié le relargage d'un extrait de groseilles indiennes sous différentes conditions de séchage et de température. Les méthodes de séchage et les conditions thermiques testées ont un impact significatif sur le pourcentage de composés phénoliques

résiduel. Tovar, Salafranca, Sanchez & Nerin (2005) ont montré que l'extrait de romarin avait un fort potentiel pour fonctionnaliser des emballages car sa libération est inférieure à 20 fois la limite autorisée. De la même façon, des extraits de betterave et de carottes pourpres présentent une bonne activité antioxydante (Akhtar et al., 2012). L'objectif de cette étude est de déterminer les cinétiques de libération (coefficients de diffusion et de partage) de ces composés (betanine, anthocyanine et leur mélange à 50:50) lorsqu'ils sont incorporés dans les films d'HPMC. L'étude est réalisée avec un simulant alimentaire (éthanol 95%) à 20 et 4°C. Leur stabilité durant la préparation des films et lors de leur stockage est étudiée. Les interactions entre les groupements fonctionnels de l'HPMC et les molécules actives est étudiée par spectroscopie infrarouge à transformée de Fourier (FTIR).

Chapter IV.4: Release kinetics and antioxidant stability of natural phenolic compounds integrated in Bio-films

Transition: Unsaturated fatty acids are very sensitive to oxidation which is a destructive process, causing loss of nutritional value and changes in chemical composition. Oxidation of fats and oils leads to rancidity, discoloration, off-flavor and other oxidation product formation; some of which are supposed to be against human health (Drusch and Berg, 2008; Choe and Min, 2006). Antioxidants control oxidation rate by a combination of free radicals scavenging, pro-oxidative metals chelating, quenching of singlet oxygen and photosensitizers and lipoxygenase inactivating (Choe and Min, 2006). However, processing and storage alter these natural compounds, resulting in decreased protection against food oxidation. Thus exogenous antioxidants are intentionally added to delay oxidation onset.

Natural and synthetic compounds have been evaluated for their effectiveness as radical scavengers or for their other inhibitory effects. Among them, natural antioxidants from different sources (beetroot, tea, rosemary, oregano, spices, herbs, clove, blueberries, mustard etc.) have shown a great capacity to neutralize free radicals and subsequently delay foods deterioration (Murphy et al., 2009; Bhale et al., 2007; Houhoula et al., 2003; Wanasundara and Shahidi, 1998). Due to positive and negative reports of synthetic antioxidants on human health, their use is subject to regulation, in the United States, under the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA); in Canada, the Food and Drug Regulations (National Health and Welfare); in Europe, the European Food Safety Authority (EFSA); and in Japan, the Food Sanitation Law. Many other countries adopted regulations similar to those used in the United States or Europe, with significant differences

existing both in the antioxidants approved and in their application and usage level (Reische et al., 1998; Pratt and Hui, 1996). Concerns about synthetic antioxidants safety increased use of natural antioxidants in foodstuffs. Use limitations are nevertheless the reduced protective effect once active compounds are engaged in complex reactions of food system or due to a lack of selectivity to target food surface where most oxidative reactions occur intensively (Gemili et al., 2010).

Active food packaging is an alternative to overcome these limitations by providing gradual release of antioxidants on food surfaces, thus, maintaining only their critical concentration necessary for controlling oxidative change. Recently, many antioxidant edible films have been successfully developed to reduce oxidation in packed foods (Jamshidian et al., 2012; Mayachiew and Devahastin, 2010; Gemili et al., 2010; Gómez-Estaca et al., 2009; Han and Krochta, 2007). Mayachiew and Devahastin (2010) studied the release of Indian gooseberry extract rich in natural antioxidants under various drying methods and temperature conditions. Drying methods and temperature conditions were found to have significant effects on the percentage of residual total phenolic content. Tovar, Salafranca, Sanchez and Nerin (2005) showed that rosemary extract can act as an effective active agent for antioxidant active packaging because its release values were 20 times lower than the established limits in migration worst cases. Similarly, other plant extracts including beetroot extract (*Beta vulgaris* L.) and purple carrot extract presenting antioxidant capacity (Akhtar et al., 2012) can protect packed food through their gradual release from packaging to food surface. The objective of this study was to determine the release kinetic and calculate release parameters (diffusion and partition coefficients) of phenolic compounds (betalains, anthocyanins and their 50:50 mixtures) from HPMC films. Antioxidants release was studied into food simulant (95 % ethanol in water) at 20°C and 4°C. Antioxidant stability during film processing and light-dark storage was evaluated. Fourier-transform infrared (FTIR) spectroscopy was also performed to investigate functional group interaction between HPMC and added active agents.

Chapitre IV.4
Cinétiques de libration et stabilité de l'activité antioxydante de composes phénoliques naturels incorporés dans des films de biopolymère

Release kinetics and antioxidant stability of natural phenolic compounds integrated in Bio-films

Muhammad Javeed Akhtar, Muriel Jacquot, Ghoul Mohamed & Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France.

(Food Engineering, 2012, Soumise)

Résumé

L'oxydation est un des facteurs critiques de la dégradation des aliments. Elle peut être contrôlée ou limitée par le maintien de fortes concentrations d'antioxydants particulièrement sur les surfaces des aliments. Le premier objectif de cette étude était d'évaluer et de comparer les taux de libération d'antioxydants naturels [(extrait d'anthocyanes (AC), extrait de betterave (B); mélange 50:50 (v / v) des deux (AC + B) et un extrait commerciale rouge naturel (NRC)] à partir de films d'hydroxypropyle méthylcellulose (HPMC). L'étude de la libération des antioxydants naturels à partir des films HPMC dans un simulant alimentaire (éthanol à 95%) à 20 °C et 4 °C a été réalisée. Les betalaïnes présentent la plus forte perte de PTC (contenu phénolique total), 91% de perte contre 80% pour les anthocyanes à 20 °C. Le NRC a montré la plus faible diffusion avec un PTC de 75 et 49% respectivement à 20 °C et 4 °C. La seconde loi de Fick a été appliquée pour déterminer le coefficient de diffusion (D) des antioxydants. La valeur de D pour la betalaïne ($2,58 \times 10^{-9}$ $cm^2.s^{-1}$) était plus grande que ceux de l'anthocyane ($1,32 \times 10^{-9}$ $cm^2.s^{-1}$) et du NRC ($6,79 \times 10^{-10}$ $cm^2.\ s^{-1}$) à 20 °C. La valeur minimum de K pour les bétalaïnes à 20 et 4 °C indiquaient des taux de libération élevés. L'extrait de bétalaïnes présente initialement le plus élevé taux de composés phénoliques totaux et, par conséquence une activité antioxydante plus importante avec des valeurs de TEAC plus élevées (0,256 ± 0,002) que ceux de l'anthocyane (0,155 ± 0,003) ou du NRC (0,053 ± 0,002). Tous les antioxydants ont montré une grande stabilité de leur activité dans la matrice d'HPMC par rapport à celle des solutions pures d'extrait traitées à la lumière directe ou dans l'obscurité. Lors de l'analyse FTIR, la disparition d'une bande d'absorption à $1735cm^{-1}$ a indiqué la perte de la quasi totalité des composés phénoliques de la surface du film après la conservation dans le simulant alimentaire. De plus, la bande à $1060cm^{-1}$ a indiqué la dégradation de l'HPMC dans tous les échantillons de films après le relargage des antioxydants.

Abstract

Oxidation is one of the critical factors concerning food spoilage. It can be controlled or limited by maintaining high concentration of antioxidants especially on food surfaces. The first objective of this study was to evaluate and compare release rates of natural antioxidants [(anthocyanin extract (AC), beetroot extract (B); 50:50 (v/v) mixture of both (AC+B) and commercially available natural red color (NRC)] from hydroxypropyl methylcellulose (HPMC) films. The natural antioxidants release study from HPMC films into food simulant (95% ethanol) at 20°C and 4°C was accomplished. Betalain showed high TPC (Total Phenolic

Content) release (91%) as compared to that of anthocyanins (80%) at 20°C. NRC showed the minimum TPC release of 75 and 49% at 20°C and 4°C respectively. Fick's second law was applied to determine the diffusion coefficient (D) of antioxidants. D value for betalain (2.58 $\times 10^{-9}$ $cm^2.s^{-1}$) was greater than those of anthocyanin (1.32×10^{-9} $cm^2.s^{-1}$) and NRC (6.79×10^{-10} $cm^2.s^{-1}$) at 20°C. The minimum K value of betalains at 20 and 4°C compared to other film samples indicated high rates of betalain release. Betalains showed higher total phenolic content (TPC) and accordingly higher TEAC values (0.256±0.002) than those of anthocyanin (0.155±0.003) and NRC (0.053±0.002). All antioxidants showed high stability in HPMC matrix compared to those in pure extract solutions treated in direct light or darkness. During FTIR analysis, stretching vibration at 1060cm^{-1} of all film samples indicated HPMC degradation after release study. An absorption band at 1735cm^{-1} disappearance indicated removal of almost all phenolic compounds from film surface.

Keywords: Active packaging. Natural phenolic antioxidants. Release. Diffusion & partition coefficients. Total phenolic content. Trolox® equivalent antioxidant capacity.

1. Introduction

Unsaturated fatty acids are very sensitive to oxidation which is a destructive process, causing loss of nutritional value and changes in chemical composition. Oxidation of fats and oils leads to rancidity, discoloration, off-flavor and other oxidation product formation; some of which are supposed to be against human health (Drusch and Berg, 2008; Choe and Min, 2006). Antioxidants control oxidation rate by a combination of free radicals scavenging, pro-oxidative metals chelating, quenching of singlet oxygen and photosensitizers and lipoxygenase inactivating (Choe and Min, 2006). However, processing and storage alter these natural compounds, resulting in decreased protection against food oxidation. Thus exogenous antioxidants are intentionally added to delay oxidation onset.

Natural and synthetic compounds have been evaluated for their effectiveness as radical scavengers. Among them, only four synthetic antioxidants are widely used in food industry, namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ) (Wanasundara and Shahidi, 2005). Nevertheless, many natural antioxidants from different sources (beetroot, tea, rosemary, oregano, spices, herbs, clove, blueberries, mustard etc.) have shown a great capacity to neutralize free radicals and subsequently delay foods deterioration (Akhtar et al., 2012; Murphy et al., 2009; Bhale et al., 2007; Houhoula et al., 2003; Wanasundara and Shahidi, 1998). Due to positive and

negative reports of synthetic antioxidants on human health, their use is subject to regulation, in the United States, under the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA); in Canada, the Food and Drug Regulations (National Health and Welfare); in Europe, the European Food Safety Authority (EFSA); and in Japan, the Food Sanitation Law. Many other countries adopted regulations similar to those used in the United States or Europe, with significant differences existing both in the antioxidants approved and in their application and usage level (Reische et al., 1998; Pratt and Hui, 1996). There are many foodstuffs which can not be protected by the addition of synthetic antioxidants, as they are fresh or raw foodstuffs in which the addition of these substances is prohibited (Tovar, Salafranca, Sanchez and Nerin, 2005). Other limitations of antioxidants direct addition to foods include specific limit of activity because of their incorporation in complex reactions in food system and lack of selectivity to target the food surface where most oxidative reactions occur (Gemili et al., 2010).

Active food packaging is an alternative to overcome these limitations by providing gradual release of antioxidants on food surfaces, thus, maintaining only their critical concentration necessary for controlling oxidative change. Recently, many antioxidant edible films have been successfully developed to reduce oxidation in packed foods (Jamshidian et al., 2012; Mayachiew and Devahastin, 2010; Gemili et al., 2010; Gómez-Estaca et al., 2009; Han and Krochta, 2007). Mayachiew and Devahastin (2010) studied the release of Indian gooseberry extract rich in natural antioxidants under various drying methods and temperature conditions. Drying methods and temperature conditions were found to have significant effects on the percentage of residual total phenolic content. Tovar, Salafranca, Sanchez and Nerin (2005) showed that rosemary extract can act as an effective active agent for antioxidant active packaging because its release values were 20 times lower than the established limits in migration worst cases. Similarly, other plant extracts including beetroot extract (*Beta vulgaris* L.) and purple carrot extract presenting antioxidant capacity (Akhtar et al., 2012) can protect packed food through their gradual release from packaging to food surface. Due to consumer health concerns and environmental problems derived from packaging wastes, researches were focused towards bio-polymers as biodegradable alternatives to synthetic plastic packages (Jamshidian et al., 2010). In previous studies, hydroxypropyl methylcellulose has been extensively used as a potential replacer for synthetic plastics due to good film forming properties, flexibility, biodegradability, transparency and gas barrier properties (de Moura et al., 2011; Jiménez et al., 2010; Sánchez-González et al., 2009; Akhtar et al., 2010; Villalobos

et al., 2006). However, very little research studies concerning antioxidant release from HPMC films into foods or food simulants have been reported.

The objective of this study was to determine the release kinetic and calculate mass transfer parameters (diffusion and partition coefficients) of phenolic compounds (Beetroot extract, anthocyanins and their 50:50 mixtures) from HPMC films. Antioxidants release was studied into food simulant (95 % ethanol in water) at 20°C and 4°C. Antioxidant stability during film processing and light-dark storage was evaluated. Fourier-transform infrared (FTIR) spectroscopy was also performed to investigate functional group interaction between HPMC and added active agents.

2. Materials and methods

2.1.Chemicals and Reagents

A viscous red juice of beetroot (*Beta vulgaris*), standardized for color (E 162) with water solubility 100% and pH 4.9 was provided by Naturex, France. A red liquid extract of anthocyanin (amaranthine, E163) with water solubility 100% and pH 3.3 was also provided by Naturex, France. Natural red color (NRC) mixture of beetroot juice (E162) and purple carrot juice (E163) containing about 20% glycerin was obtained from ColorMaker (California, USA). HPMC powder (Fluka-Biochemika, Japan) is a biochemical product containing 9% hydroxylpropoxyl and 28% methyl radicals. It had a viscosity of 15mPa*s and a water solubility of 2% at 25°C. Ethanol (purity > 99%) (Pharmaceutics Carlo, Erba) was used to improve HPMC solublization. The Folin-Ciocalteu reagent (Merck, Darmstadt, France) and sodium carbonate (Sigma-Aldrich, Steinheim, France) were employed for the measurement of the Folin-Ciocalteu total phenolic content. The calibration curve was constructed with gallic acid (Sigma-Aldrich, France). Trolox (6-hydroxy-2, 5, 7, 8-tetramethychroman-2-carboxylic acid; Fluka, 56510) was used as antioxidant standard. For antioxidant capacity measurements ABTS (2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) and potassium persulfate were sourced from Sigma-Aldrich (France). All organic solvents were analytical grade reagents. Phosphorus pentoxide (P_2O_5) was purchased from Sigma–Aldrich (France). Petri-dishes (optilux) were provided by Nunclon^{TM} Fisher (DK-4000 Roskilde, Denmark).

2.2.Methods

2.2.1. HPLC analysis

The natural phenolic extracts were characterized by Shimadzu HPLC equipment (Tokyo, Japan) with auto sampler (SIL-20AC), communication bus module (CBM-20A), pump (LC-20AD), column-oven (CTO-20AC) with ULFC (Shimadzu) cooling module in series with a diode array detector (SPD-M20A). Optimum separation of anthocyanins was achieved on an analytical scale (250 × 4.6mm i.d.) Agilent C18 (5µM) reversed phase column with a particle size of 5µm (Phenomenex, Torrance, CA), fitted with a security guard C18 ODS (4 × 3.0mm i.d.) at a flow rate of 0.5mL/min and a constant temperature of 25°C. Eluent A was 5% formic acid and B was MeCN/H_2O (60/40, v/v). Separation was accomplished starting with 3%B, followed by a linear gradient to 20%B for 30min and then to 50%B for 40min. A monitoring wavelength of 530nm was chosen for different dilutions of natural extracts. Aliquots mixed samples of 20µL were injected for analyses. Duplicate determinations were performed throughout.

2.2.2. Mass spectrometric conditions

The LC–MS equipment includes a binary solvent delivery pump and a linear ion trap mass spectrometer (LTQ-MS, Thermo Finnigan, San Jose, CA, USA). LC analysis parameters were the same as described above except use of a specific LCMS C18 column (150 * 2.1 and 5µm – Alltima, Alltech, France) at a smaller flow rate of 0.2ml/min. LTQ equipped with an atmospheric pressure ionization interface operating in electro spray positive mode (ESI positive). Data were processed using Xcalibur 2.1 software. The operational parameters of mass spectrometer were as follows. Spray voltage was 4.20kV and the temperature of heated capillary was set at 300°C. Flow rates of sheath gas, auxiliary gas, and sweep gas were set (in arbitrary units min^{-1}) to 35, 10, and 10, respectively. Capillary voltage was -48V, tube lens was -13V, split lens was -38V and the front lens was -4.25V. All parameters were optimized by using a standard rutin solution as representative glycosylated flavonoid (0.1g/L) in mobile phase (A/B: 50/50) at a flow rate of 5µL/min. The compounds of interest were monitored through specific MS2 scans in addition of MS full scan (50 to 1000m/z): MS2 (743), MS2 (581), MS2 (949), MS2 (919), MS2 (889) for the screening of anthocyanins compounds.

2.2.3. Preparation of antioxidant-HPMC composite solutions

Initially, HPMC solutions were prepared by dissolving 6g of HPMC powder in ethanol solution (35% v/v) for 40min at 65°C. Secondly, 2% (v/v) of beetroot extract (E-162)

containing betalains, anthocyanin extract, their mixture (B+AC/50:50) and commercial natural red color (NRC) were dissolved separately in ethanol solution (35% v/v) at 20°C because of their higher light and temperature sensitivity. All antioxidant solutions were then mixed separately with HPMC solutions and stirred for 30min at 20°C to obtain homogeneous solutions. The pH was adjusted at 3.17±0.08 for NRC, 3.30±0.08 for anthocyanin and 4.90±0.08 for betanin solutions with HCl (0.1M). After mixing, the solutions were degassed at room temperature under vacuum "Handy Aspirator WP-15 (Yamato®)" for 30min (Akhtar et al., 2010).

2.2.4. Fabrication of antioxidant films

6g of each film forming solution were put in the Petri-dishes and dried in a dark room at ambient temperature for 48h. For complete evaporation of solvent, films were then incubated at 30°C for one week in a hermetic container containing P_2O_5 powder before each analysis. Finally, antioxidant films were obtained with thickness 48±3µm measured by a mechanical micrometer (Messmer, London, UK) according to ASTM D374–99. The thickness was measured in 10 randomly selected points on each film and then an average value was determined. To study light stability of natural antioxidants directly and within HPMC films, samples (2% extract solutions and films containing 2% of extracts) were conditioned under fluorescent light (OSRAM L36W/640) or darkness for 10 days. The distance of fluorescent tube from the samples was 14cm at 20°C and 50% relative humidity.

2.2.5. Determination of total phenolic contents

Pure extracts and film samples total phenolic content (TPC) was determined using slightly modified Folin-Ciocalteu method (Singelton et al., 1999). Briefly, 500µL of sample was mixed with 500µL of 100g/L sodium carbonate (Na_2CO_3, Labosi, Paris, France) and was left for 10min in a water bath at 38°C. 500µL of Folin-Ciocalteu reagent were added and after stirring solution was stored for 15min in darkness at 20°C. Absorbance at 660 nm was measured using a Shimadzu UV-2101 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) which was used to calculate the TPC, using gallic acid as a standard. The mean of three measures was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/L of sample.

2.2.6. Release of antioxidant agents from films

Release test was performed in an environmental incubator (Memert, Germany) at 20°C and 4°C in a dark thermostat room. The total phenolic content (TPC) of samples was evaluated by adapting the elution technique described by Zhang and Kosaraju, (2007). Each film sample (0.541mg) was placed in a beaker containing 100 ml of ethanol (95%) as food simulant and was stirred slowly/continuously during release test. The samples were taken out at 10min intervals until 1h and every 1h afterward. Solutions were than diluted and examined for total phenolic content released from the films. Mean of three readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/L of sample solution.

2.2.7. Estimation of Diffusion and Partition coefficient

To determine diffusion coefficient (D) of antioxidants, Fick's second law was used which describes changes of antioxidant agents concentration in the film with respect to time and position. Fick's second law of diffusion is presented in Crank (1975). The total amount of active compound desorbed from film at any time t (M_t) normalized with respect to amount desorbed at equilibrium ($M\infty$) is given below:

$$\frac{M_t}{M\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left[\frac{(2n+1)^2\pi^2}{L^2}Dt\right] \quad (1)$$

Where $Mt/M\infty$ is the mass of natural antioxidants released from the film at time t, divided by the amount of antioxidants released at equilibrium and L is the film thickness. For short times ($Mt/M\infty < 2/3$), equation 1 can be simplified to equation 2 and then equation 3 for easy calculation of diffusion coefficient (D) as given below:

$$\frac{M_t}{M\infty} = 4(Dt/4L^2\pi)^{0.5} \quad (2)$$

$$D = (S.L/2)^2\pi \quad (3)$$

where S is the slope of a plot representing $Mt/M\infty$ against time ($t^{0.5}$).

Partition coefficient (K) is defined as the ratio of migrant equilibrium concentration in the polymeric material, (C_p) to its equilibrium concentration, in food simulant (C_s) (Tehrany and Desobry, 2004). It can be determined by the following equation.

$$K = C_p/C_s \quad (4)$$

2.2.8. ABTS radicals scavenging activity

Evaluation of 2,-2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{\bullet+}$) radical scavenging activity was based on the ability of antioxidants to inhibit long-life ABTS radical cation, a blue/green chromophore with characteristic absorption at 734nm, in comparison with that of Trolox. The scavenging of $ABTS^{+\bullet}$ is assumed to be an electron transfer process (Xican et al., 2011):

$$ABTS^{+\bullet} + e \rightarrow ABTS \quad (5)$$

$ABTS^{+\bullet}$ was previously produced by the reaction between ABTS diammonium salt and potassium persulfate:

$$\underset{\text{Yellow}}{2(NH_4)_2ABTS} + S_2O_8^{2-} \rightarrow 2SO_4^{2-} + \underset{\text{Green}}{2ABTS^{+\bullet}} + 2NH_4^+ \quad (6)$$

ABTS radical cation was produced by reacting ABTS stock solution (7mM) with 2.45mM potassium persulfate and allowing the mixture to stand in darkness, at room temperature, for 12–16h before use. To study antiradical activity of phenolic compounds, $ABTS^{\bullet+}$ solution was diluted with ethanol at 30°C, to obtain an absorbance of 0.70±0.02 at 734nm. After addition of 1.0mL of diluted $ABTS^{\bullet+}$ solution to 10µL of sample or standard Trolox in ethanol (concentration between 0 and 16µM), the absorbance was measured at 30°C exactly 6min after initial mixing. Appropriate solvent blanks were run with each assay. All experiments were performed in triplicate. A standard curve was obtained by using Trolox standard solution at various concentrations. The absorbance of reaction samples was compared to that of Trolox standard and results were expressed in terms of Trolox equivalents (Re et al., 1999). TEAC value is defined as the concentration of standard Trolox with the same antioxidant capacity as 1mM concentration or 1mg/mL of the antioxidant compound under investigation (Maisuthisakul et al., 2007). The antioxidant films were solubilized in water which was then evaporated under the stream of nitrogen in darkness to avoid the oxidation. After evaporation of water, equal quantity of ethanol was added into the film residue. Different dilutions of these film solutions were used to assess the antioxidant capacity of respective film.

2.2.9. FTIR analysis of antioxidant films

Changes in structure of HPMC composite films before and after antioxidants release were followed by Fourier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25°C with 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 was set at 4mm. The scanning rate was 10 kHz and 80 scans were performed both for the reference and the sample from 4000 to 800cm^{-1} with 4cm^{-1} resolution. All data treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-point Savitsky-Golay smoothing functions. Elastic baseline correction was applied to spectra, which were further cut between 1800 and 800cm^{-1}, centered and normalized

3. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using XLSTAT (Version 2011.5.01) and were presented as mean values with standard deviations. Differences between mean values were established using Duncan's multiple range tests at a confidence level of 95%. All experiments were performed in triplicate.

4. Results and discussion

4.1.HPLC-MS/MS Characterization of beetroot extract and (AC+B) mixture

Natural phenolic compounds have been characterized by various analytical methods previously reported in the literature (Nielson & Harley, 1996; Charron et al. 2009). An official HPLC method providing fingerprints of common fruit juices to characterize these types of compounds has been published (IFU 1998). In the present study, newly established HPLC-DAD-MS/MS method (Stintzing et al., 2005) was used allowing simultaneous determination of betacyanins. Based on comparison with the standards and bibliographical data (Stintzing et al., 2005), anthocyanins were readily identified by their retention time order, spectral and mass characteristics including daughter ion and neutral loss scanning. Components of beetroot extract (B), betanin (1) and its C 15 epimer iso-betanin (2) amounted to 99.78% together were identified on UV-visible chromatogram and single ion chromatogram at retention time 8.31min and 11.98min, respectively (Fig. 1).

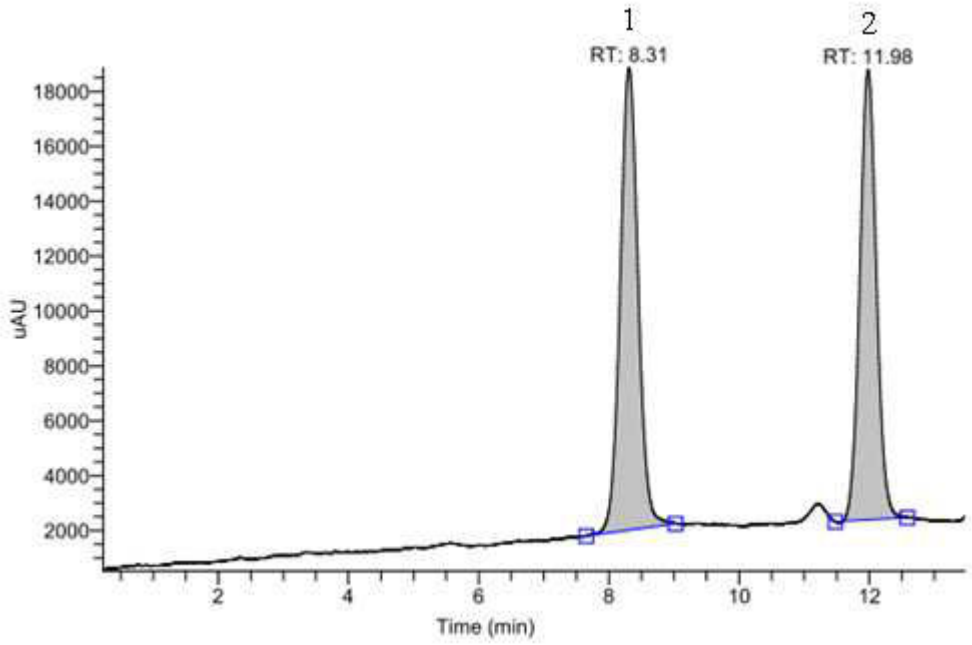


Fig. 1 *UV–visible chromatogram of beetroot extract (B) at 530 nm. Peak assignment is given in Table 1.*

They were further confirmed with MS and MS 2 steps. Peak assignment is given in Table 1. (AC+B) mixture showed betanin (1) and its C 15 epimer iso-betanin (2) amounted to only 17.30% together along with other components such as anthocyanins typically cyanidin derivatives (1-4) along with a couma-royl-derivative (5) as also described by Stintzing et al., (2005) and Glässgen, Seitz & Metzger (1992). These components amounted to 85.65% together were identified on UV-visible chromatogram and single ion chromatogram at retention time 23.20, 24.99, 28.43, 30.20 and 31.53min, respectively (Fig. 2).

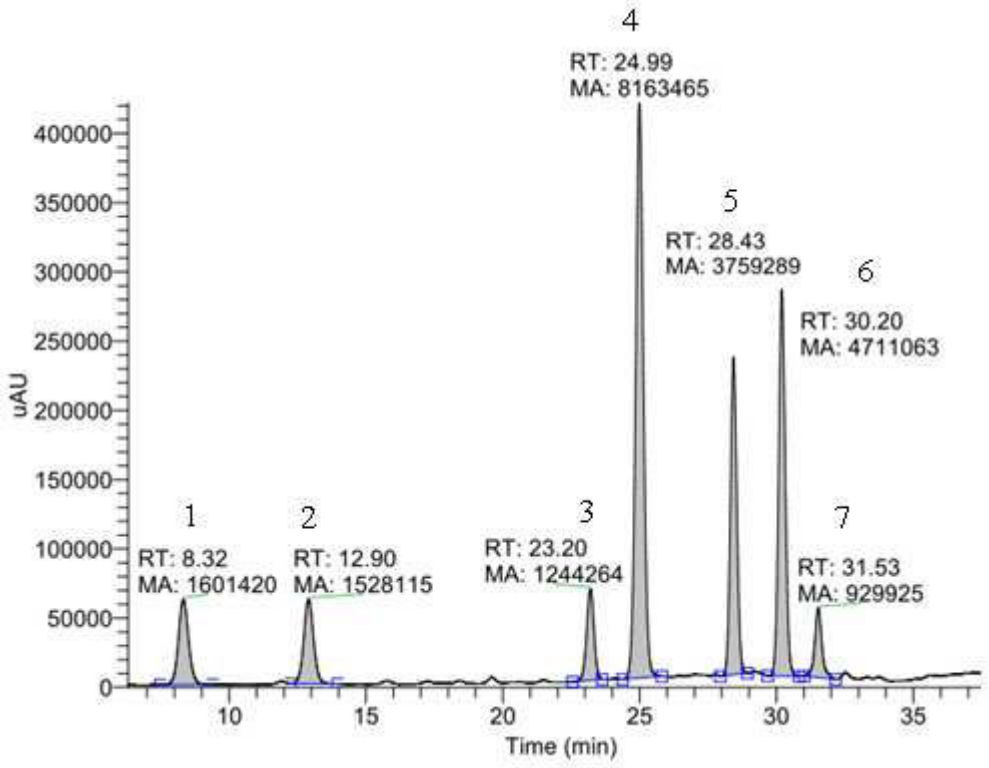


Fig. 2 *UV–visible chromatogram of beetroot extract and anthocyanins compound mixture (B+AC) at 530 nm. Peak assignment is given in Table 2.*

They were further confirmed with MS and MS^2 steps and by comparing their specific retention times with those of previous studies (Stintzing et al., 2005). Peak assignment is given in Table 2. Similarly, Anthocyanin compound (AC) and Natural red color (NRC) were characterized by HPLC-MS/MS as published in our previous work (Akhtar et al., 2013) and (Akhtar et al., 2012) respectively.

Table 1. *Peak assignments for beetroot extract (B).*

Compound name	Rt (min)	UV-Vis_{max} (nm)	m/z $[M+H]^+$	m/z MS^2 of $[M+H]^+$	(%) Area at 530nm
Betd 5-glc (betanin)	8.31	271.9/293.6/538.9	551.16	389.12	49.87
Isobetd 5-glc (iso-betanin)	11.98	271.9/293.6/538.9	551.16	389.13	49.91

Aglycons: Betd. betanidin; Glc. glucose.

Table 2. *Peak assignments for mixture of both beetroot extract and anthocyanins (B+AC).*

	Compound name	Rt (min)	UV-Vis$_{max}$ (nm)	m/z $[M+H]^+$	m/z MS^2 of $[M+H]^+$	(%) Area at 530nm
1	Betd 5-glc (betanin)	8.32	271.9/293.6/538.9	551.16	389.12	7 .3
2	Isobetd 5-glc (iso-betanin)	12.90	271.9/293.6/538.9	551.16	389.13	7.0
3	Cyd 3-xyl-glc-gal	23.20	283.7/514.3	743.1	287.0	5.6
4	Cyd 3-xyl-gal	24.99	283.7/514.3	581.1	287.0	37.21
5	Cyd 3-xyl-glc-gal-sin	28.43	287.8/335.5/523.8	949.2	287.0	17.14
6	Cyd 3-xyl-glc-gal-fer	30.20	287.8/334.3/519.0	919.2	581.1/287.0	21.47
7	Cyd 3-xyl-glc-gal-coum	31.53	287.8/319.0/519.0	889.2	287.0	4.23

Aglycons: Betd. betanidin; Cyd. cyanidin; Xyl. xylose; Glc. glucose; Gal. galactose; Fer. ferulic acid; Sin. sinapic acid; Coum. P-coumaric acid.

Table 3. *Residual total phenolic contents and percentage of TPC release of antioxidant films at 20 and 4°C*

Film type	Film Thickness (µm)	Film weight (mg)	Film area (cm^2)	TPC of film		% TPC release of film	
				Initial content (mg)	Gallic acid equivalent (mg/L)	20°C	4°C
HPMC (C)	48 ± 2	459 ± 4	56.74	------	------	------	------
C+B2%	49 ± 2	468 ± 8	56.74	142.7	132.7 ± 0.5	91.0±3.7	79.2±6.3
C+AC2%	50 ± 2	462 ± 5	56.74	127.0	118.6 ± 1.7	80.5±4.8	71.6±6.7
C+NRC2%	49 ± 2	465 ± 6	56.74	171.2	081.8 ± 0.2	75.6±5.7	49.8±3.8
C + (AC+B)2%	51 ± 3	467 ± 5	56.74	134.8	123.8 ± 2.1	83.5±4.3	60.7±4.7

TPC. total phenolic content, C. control HPMC film, B. beetroot extract, AC. anthocyanin extract, NRC. natural red color. (AC+B) 50:50 (v/v) mixtures of both anthocyanin and betalain extracts.

4.2.Determination of total phenolic contents

Initial total phenolic content (TPC) of films was determined spectrophotometrically according to the Folin-Ciocalteu method and expressed as gallic acid equivalents GAE/L of sample. Table 3 shows the effective initial percentage TPC in HPMC films incorporated with 2% (v/v) of beetroot extract (B), anthocyanins extract (AC), mixture of both (B+AC/50:50) and commercial natural red color (NRC). The beetroot extract showed the highest total phenolic content (132.7 mg/L) and NRC showed minimum phenolic (81.8 mg/L) expressed as mg of gallic acid equivalents per L film solution. Same initial concentration of each phenolic compound (2%) led to different initial content of TPC. The initial antioxidant concentrations were used to calculate diffusion and partition coefficient of released antioxidants.

4.3.Release of antioxidant agents from films

Due to hydrophilic nature of both HPMC polymer and natural phenolic compounds they were easily soluble in water or other high hydrophilic food simulants. Ethanol 95% solution was used as liquid food simulant to avoid films hydrolysis due to hydrophilic nature of these films (Siro et al., 2006). Ethanol 95% is generally used as a fatty simulant for fats, oils and fatty foods due to its resembling hydrophobicity (Commission Directive, 2007/19/EC). Ethanol is fully miscible with water and at the same time it provides a more hydrophobic environment. Usually, release of bioactive compound from biopolymer matrix occurs in two stages. First, water molecules penetrate into film polymeric complex from outer solution. Thus the polymeric network becomes increasingly wider, allowing active agents to diffuse through film into outer solution until a thermodynamic equilibrium between the two phases is reached (Del Nobile et al., 2008).

Fig. 3 and 4 show evolution of percentage TPC release from antioxidant films at 20 and 4°C respectively. Beetroot extract showed the high release at 20°C by having 91.04±3.75% of its initial concentration as compared to anthocyanins having 80.52±4.84% into simulant after about 10h (Table 3). This can be associated with betalains lower molecular weight (m/z 551 as compared to those of anthocyanins m/z 581-949) (Akhtar et al., 2012) leading a high molecular mobility in polymer chains (Jamshidian et al., 2012). The mixture (AC+B) showed the amount of released TPC close to the average of both anthocyanins and beetroot extract. In case of commercial NRC, lowest percent TPC release was observed which may be due to the presence of glycerin used as a stabilizing agent. Temperature and polymer-additive interaction strongly influenced the respective kinetic release of active compounds (Teerakarn et al., 2002;

Rossi-Marquez et al., 2009). Lowering temperature down to 4°C showed a decreasing TPC release from each film type (Fig. 4).

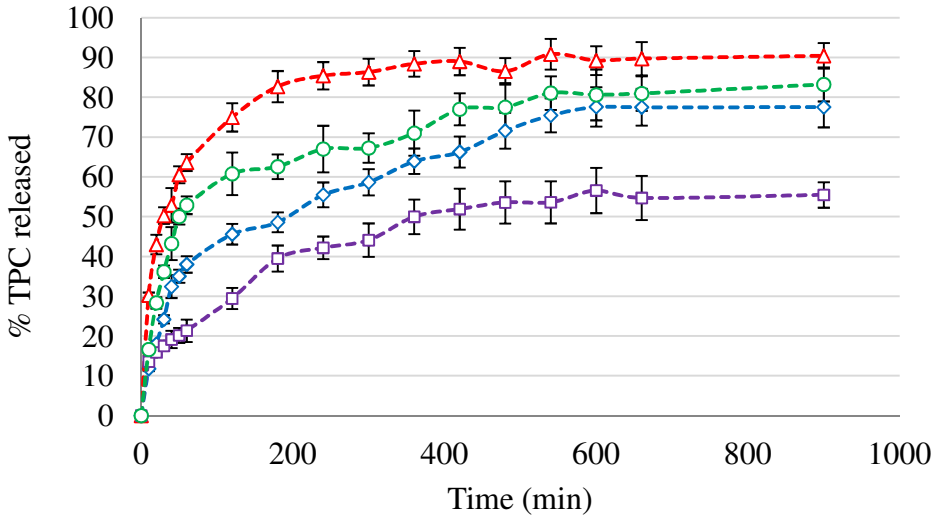


Fig. 3. *TPC release from HPMC films enriched with 2% (v/v) B (Δ), AC (◊), AC+B (○) and NRC (□) compounds at 20°C.*

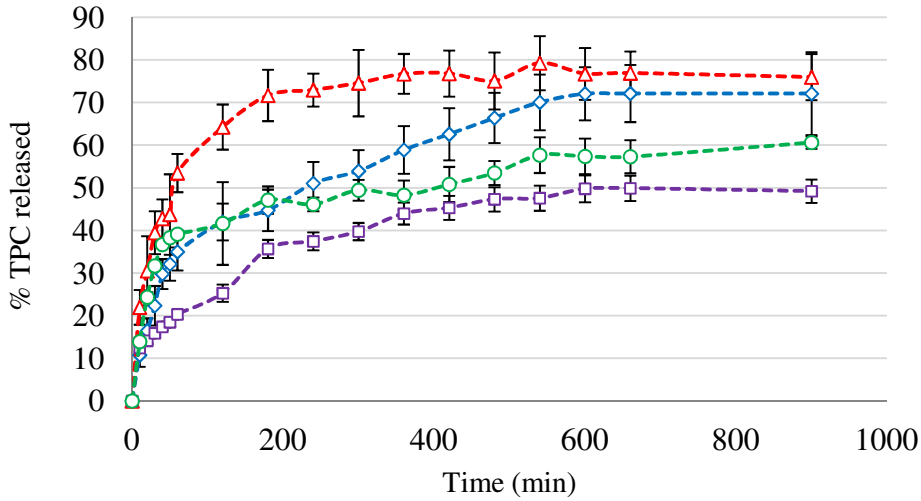


Fig. 4. *TPC release from HPMC films enriched with 2% (v/v) B (Δ), AC (◊), AC+B (○) and NRC (□) compounds at 4°C.*

4.4.Diffusion and Partition coefficients

The diffusion coefficients (D) of natural phenolic compounds into food simulant (ethanol 95%) are summarized in Table 4. D coefficient was observed to be decreased by reducing temperature. HPMC films containing betalains showed maximum value of D coefficient at 20 and 4°C compared to other films. The D coefficient of beetroot extract in simulant at 20°C was greater than at 4°C. As expected, other films showed higher D coefficient values at higher temperature due to higher molecular mobility of antioxidants through polymer matrix

toward simulant (Jamshidian et al., 2012). D coefficient of anthocyanins was also studied by Turker and Erdogdu (2006) directly from carrot slice into extraction medium. Chetan et al. (2006) studied the D coefficient of betanin during its extraction from red beetroot. Both showed direct relation of temperature on D values of anthocyanins and betanin. No work on D coefficient of betanin and anthocyanins in edible food packaging has been reported in literature.

Table 4. *Estimated Diffusion and Partition coefficient of natural phenolic antioxidants from HPMC films into food simulant (ethanol 95%) at 20 and 4°C.*

Films with	D ($cm^2.s^{-1}$)		K	
(2%) extract	20°C	4°C	20°C	4°C
C+B	2.58×10^{-9}	2.32×10^{-9}	0.166±0.037	0.418±0.045
C+AC	1.32×10^{-9}	1.29×10^{-9}	0.330±0.102	0.497±0.108
C+NRC	6.79×10^{-10}	6.13×10^{-10}	1.681±0.420	2.084±0.270
C + (AC+B)	2.52×10^{-9}	2.75×10^{-9}	0.253±0.037	0.907±0.047

D. diffusion coefficient and K. partition coefficient, C. control HPMC film, B. betalain extract, AC. anthocyanin extract, NRC. natural red color. (AC+B) 50:50 (v/v) mixtures of both anthocyanin and beetroot extract.

K coefficients of natural phenolic compounds are presented in Table 4. In the present work, it was possible to calculate the K coefficient because thermodynamic equilibration of antioxidant concentration in both film and simulant was established. Higher K values appeared when the migrant tends more to remains in polymer than to transfer into simulant and vice-versa (Jamshidian et al., 2012). K increased by reducing temperature. Films containing betalains showed minimum K compared to other films indicating its more hydrophilic nature and high betalain release in ethanol 95%. The lower K values of antioxidants indicate the lower retention of these antioxidants into the film. Generally, rapid or zero release of antioxidants from active packaging to foods acts against continuous control of oxidation for which gradual release is required. D and K coefficients could be used to predict antioxidants release rate into different foodstuffs having diverse properties as well as the fat, alcohol and organic acid.

4.5.ABTS radicals scavenging activity

ABTS assay was calibrated with Trolox, water soluble alpha-tocopherol analog. The results of ABTS assays were expressed in comparison to Trolox standard curve. Antioxidant stability of natural active compounds was compared at different stages of film formation and light-ageing.

Table 5 presents antioxidant stability of natural extracts during film functionalization and ageing process while Fig. 5 shows antioxidant stability of natural extracts directly exposed to the light or darkness. All samples showed antioxidant activities as they were able to scavenge $ABTS^{\bullet+}$ radical cation. They were shown to be antiradical agents compared to the Trolox. Crude beetroot extract containing betalains (Yizhong et al., 2001b; Yizhong et al., 1998) showed maximum TEAC values (0.256±0.002) as compared to anthocyanins (0.155±0.003) and other mixtures. These results are in accordance with Shyamala and Jamuna (2010), who demonstrated the anthocyanins and betalains antioxidant activity as 40.8% and 78.6% respectively.

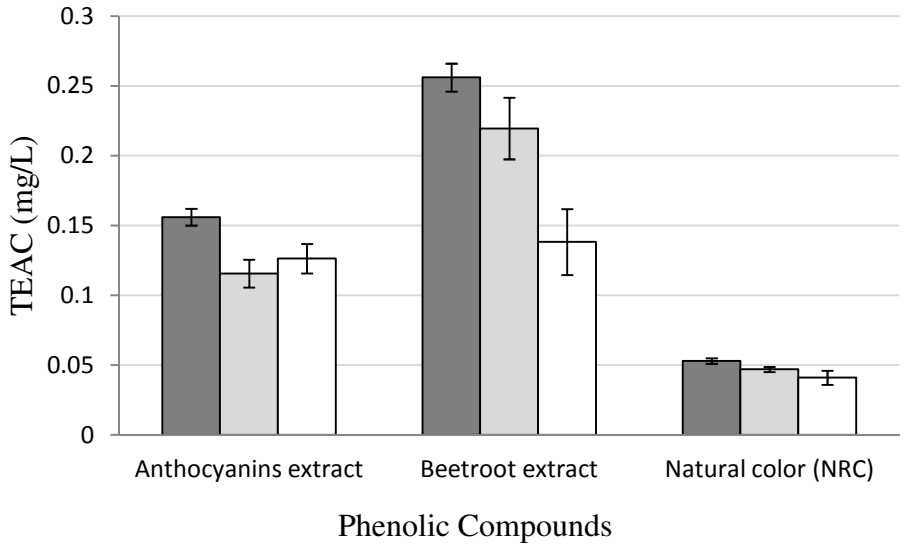


Fig. 5. *Antioxidant stability of natural extracts during light and dark storage. Antioxidant capacity against $ABTS^{\bullet+}$ radical was expressed as TEAC (mg/L) values. Zero treatment (■), 10 days under darkness (□) and 10 days under light storage (□).*

High antioxidant capacity of beetroot extract was due to higher TPC amount (Hanif et al., 2011) compared to those of anthocyanins and other mixtures as discussed in section 4.1. Beetroot extract in film forming solution (FFS), fresh films, films stored in darkness or light, displayed less free radical scavenging activities than pure extract solution. No significant change in TEAC value was observed for FFS (0.253±0.001), fresh film (0.255±0.003) and those stored under darkness (0.254±0.001) as compared to pure solution, showing their antioxidant stability. The films stored under light were significantly different from pure samples for their antioxidant activity (Table 5). These results were in accordance with those of Díaz et al. (2006) who studied the effect of light and darkness on betalains stability. In case of anthocyanins stability, significant difference of TEAC values was observed for films stored

in light and darkness. They were more stable in film forming solution and fresh films. Commercial natural red color (NRC) was more stable than all other samples which may be due to the presence of ascorbic acid.

Table 5. *Antioxidant stability of natural antioxidants during film functionalization and ageing process. Antioxidant capacity against* $ABTS^{\bullet+}$ *radical was expressed as TEAC (mg/L) values.*

Sampling Conditions	*TEAC* of each compound at a concentration of 2% (v/v)			
	Betalains (B)	Anthocynins (AC)	(B+AC) 50:50 (v/v)	Natural red color (NRC)
Pure solutions	0.256 ± 0.002^{a}	0.155 ± 0.003^{a}	0.103 ± 0.001^{bc}	0.053 ± 0.002^{a}
FFS	0.253 ± 0.001^{ab}	0.142 ± 0.006^{ab}	0.178 ± 0.013^{a}	0.049 ± 0.002^{ab}
Fresh film	0.255 ± 0.003^{a}	0.148 ± 0.011^{ab}	0.123 ± 0.020^{b}	0.048 ± 0.002^{ab}
Film in darkness	0.254 ± 0.001^{a}	0.118 ± 0.004^{c}	0.095 ± 0.009^{bc}	0.048 ± 0.002^{ab}
Film in light	0.249 ± 0.001^{b}	0.128 ± 0.013^{bc}	0.076 ± 0.016^{c}	0.046 ± 0.003^{b}

TEAC. Trolox equivalent antioxidant capacity. Means within the same column with different letters are significantly different at $P < 0.05$.

Betalain stability was previously reported to be impaired by light exposure (Attoe and von-Elbe, 1981; Cai et al., 1998). Present results indicated that beetroot extract incorporated in HPMC matrix were slightly modified or degraded during light ageing. However, they have conserved more antioxidant capacity as compared to those in pure extract solutions treated with direct light or darkness (Fig. 5). Betalain or anthocyanin light-induced degradation is oxygen dependent, because light exposure effects are negligible under anaerobic conditions (Attoe and von-Elbe, 1981; Huang and von-Elbe, 1986). Thus comparing the results presented in Table 5 and Fig. 5, it can be concluded that HPMC matrix has an ability to preserve antioxidant capacity of natural compounds due to oxygen and light barrier properties.

4.6.FTIR analysis of antioxidant films

FTIR spectroscopy is a rapid technique with minimum sample preparation requirements. It allows qualitative and quantitative determination of organic compounds in samples because intensities of spectrum bands are proportional to concentration (Vlachos et al., 2006). FTIR spectroscopy was performed to determine the characteristics of film matrix as well as the changes of intermolecular interactions both before and after release study. By considering the IR spectra of HPMC films enriched with 2% (v/v) extracts, samples had very strong absorption bands at $1060cm^{-1}$ related with a pronounced shoulder at $1115cm^{-1}$ attributed to a combination band of C-O stretches and secondary hydroxyl group O-H (van der Weerd and Kazarian, 2004; Siepmann and Peppas, 2001; Langkilde and Svantesson, 1995).

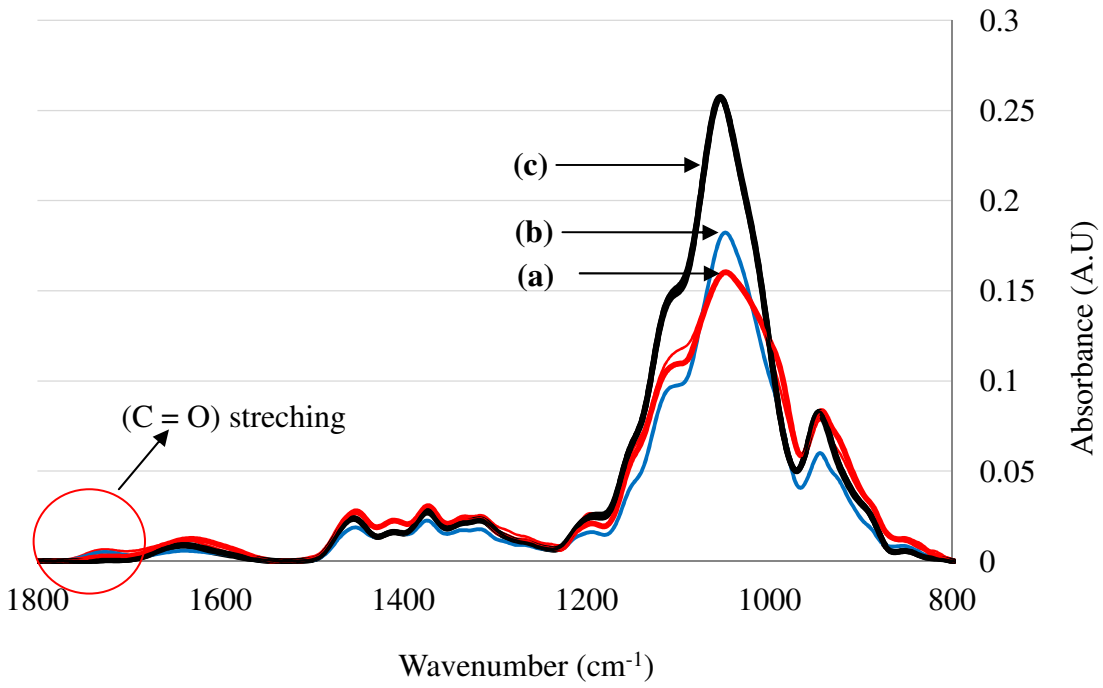


Fig. 6. *FTIR spectra of HPMC films with 2% of each phenolic compound: (a) before release AC, B and (AC+B) films (b) before release NRC films (c) all film samples after release study.*

Note: HPMC films containing 2% anthocyanin extract (AC); beetroot extract (B); 50:50 (v/v) mixture of both (AC+B); commercial natural red color (NRC).

Absorption band at 1060cm^{-1} associated with hydroxyl group indicated intermolecular hydrogen bonds formation (John, 2000). All samples except NRC showed same absorbance at 1060cm^{-1} before TPC release. A slight increase in absorption band of NRC films was due to more OH groups of glycerin interacting with cellulosic OH groups (Fig. 6). Stretching vibration at 1060cm^{-1} causing an increase in peak surface of all film samples indicated HPMC degradation after release study (Fig. 6). Curves increasing slopes were due to availability of OH groups from 95% ethanol simulant cross-linking with cellulosic OH groups. Comparison between spectra showed an additional peak around 1726cm^{-1} for natural extract containing films (Fig. 6). This band was attributed to C=O stretching vibrations and indicated phenolic compounds presence in treated HPMC films. Similar additional band was observed by Abidi et al. (2005) at 1710cm^{-1} by treating cotton fabrics with natural phenolic compounds. Also Kim et al. (2008) observed the same absorption band at 1735cm-1 by coloration of flax fabrics with flavonoids using enzymes. Disappearance of this absorption band after release study indicated the release of phenolic compounds from film surface (Fig. 6).

5. Conclusion

Antioxidants release into food simulants is a complex phenomenon which involves different factors such as film structure, solvent and antioxidant polarities and solubility, antioxidant molecular weight and polymer degradation. The release behavior of natural phenolic antioxidants from HPMC to food simulant at 20 and 4°C was evaluated and compared together. Betalain showed high TPC release as compared to those of anthocyanins at 20°C. It decreased by decreasing temperature. NRC showed minimum TPC release at 20 and 4°C. The antioxidants diffusion from HPMC films indicated that D value for betalain was greater than that of anthocyanin and NRC. Generally, all natural antioxidants showed higher rate of TPC release and reached to equilibrium within 10h which was not in favor of active food packaging. On the other hand, antioxidant capacity of active compounds during film formation was almost stable rather significantly different under light. Betalains showed higher TEAC values than those of mixture (AC+B/50:50), anthocyanin and NRC. All antioxidants showed high stability in HPMC matrix compared to those in pure extract solutions treated directly in light or darkness. Concerning FTIR analysis, stretching vibration at 1060cm^{-1} of all film samples indicated HPMC degradation after release study. A mutual beneficial relation was found between natural phenolic compounds and HPMC matrix i.e. phenolic compounds showed an ability to control oxidation of HPMC, previously discussed in (Akhtar et al., 2012) and HPMC showed an ability to preserve antioxidant capacity of these phenolic compounds. Non polar food simulants such as fats may be more suitable for slow release of phenolic antioxidants. Such bioactive films based on HPMC and natural phenolic antioxidants may possibly apply for non-polar foods preservation.

6. Acknowledgements

The authors would like to thank Céline Charbonnel, Jordane Jasniewski and Carole Jeandel for their technical assistance

Chapitre IV.5 : Effet de la couleur des films d'HPMC–anthocyanines et de leur perméabilité à l'oxygène sur la conservation de l'huile de saumon

Transition: Les acides gras polyinsaturés (AGPI) sont très sensibles à la photo-oxydation entrainant des modifications chimiques indésirables telles que l'apparition de faux gout, la formation de produits d'oxydation et la baisse de la qualité nutritionnelle des produits (Drusch & Berg, 2008; Frankel, 1985). Ces modifications chimiques sont directement liées au type de source lumineuse, aux longueurs d'ondes émises, leurs intensités, le temps d'exposition et la température mais aussi aux caractéristiques de l'emballage utilisé comme les barrières à l'oxygène et à la lumière (Akhtar et al., 2010).

Dans les huiles de poissons, les antioxydants naturels comme l'astaxanthine, le coenzyme Q10, le sélénium, la taurine et les vitamines (A, D et E) peuvent protéger les AGPI (Rita Mattei et al., 2011) Cependant, les procédés de fabrication et la conservation altèrent ces molécules réduisant de fait leur capacité à protéger les AGPI. Il est donc nécessaire d'apporter les antioxydants par des voies extérieures sachant que leur application directe sur les produits frais est interdite dans de nombreux pays. Une solution peut donc être le contrôle et la filtration des rayons lumineux qui atteignent le produit (Moyssiadi et al., 2004). Dans les emballages transparents toutes les longueurs d'onde traversent le matériau. Cependant, les longueurs d'ondes les plus néfastes (400-500 nm) peuvent être bloquées par l'ajout de couleur à l'emballage (Mortensen et al., 2003).

Nos films d'HPMC fonctionnalisés avec les composés phénoliques naturels permettent un contrôle de la lumière qui traverse le film ainsi que de l'oxygène. Nous les avons donc testés sur un produit modèle riche en AGPI, l'huile de saumon.

Chapter IV.5: Effect of HPMC-Anthocyanins Packaging Color and Oxygen Permeability on Salmon oil Preservation

Transition: Lipids containing PUFA are very sensitive to light-oxidation causing a number of unfavorable chemical changes including off-flavors formation, oxidation products formation and nutritional quality decrease, some of which are harmful to human health (Drusch & Berg, 2008; Frankel, 1985). Chemical changes in lipids are directly related to light source, wavelength, intensity, exposure time and temperature as well as packaging material characteristics such as oxygen and light barrier properties (Akhtar et al., 2010). In fish oil natural antioxidants such as astaxanthin, coenzyme-Q10, selenium, taurine and vitamins (A, D and E) can protect lipids against oxidation (Rita Mattei et al., 2011). However, processing and storage alter these natural compounds resulting in decreased protection against lipid oxidation. Antioxidants may be added from external sources but their direct application on fresh foods is prohibited in many countries.

Most appropriate way to protect lipid rich products may be oxygen removal and decrease of light exposure (Moyssiadi et al., 2004); which is not feasible for transparent packaging. The most harmful wavelength (400–500 nm) of light (Mortensen et al., 2003) can be avoided by application of colored packaging with high oxygen barrier properties. Anthocyanins present in these phenolic compounds cover a large variety of colors such as orange, red and blue which make them an attractive alternative to synthetic food dyes (Markakis, 1982). Moreover, anthocyanins have many health benefits including reduced risk of cardiovascular diseases (Bell & Gochenaur, 2006).

HPMC films functionalized with phenolic compounds comprising antioxidant capacity, coloring potential, hygiene, nutrition, pharmaceutical activities and bioactivity (Stintzing & Carle, 2004; Frank et al., 2005) showed good control of light and oxygen permeability. Finally these functionalized HPMC films with additional properties were tested as packaging materials for salmon oil preservation.

Chapitre IV.5
Effet de la couleur des films d'HPMC – anthocyanines et de leur perméabilité à l'oxygène sur la conservation de l'huile de saumon

Effect of HPMC-Anthocyanins Packaging Color and Oxygen Permeability on Salmon oil Preservation

Muhammad Javeed Akhtar. Muriel Jacquot. Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France.

(Food & Bioprocessing Technology, 2013, Accepté)

Résumé

Des films d'emballage alimentaire à base d'hydroxypropyle méthylcellulose (HPMC) à activité antioxydante ont été développé avec succès grâce à l'incorporation d'anthocyanine (AC, extrait liquide de source naturelle). La couleur des films et les propriétés barrière à l'oxygène ont été mesurées. La couleur rouge des films d'AC ont montré une bonne maîtrise de la transmission de la lumière en comparaison avec les témoins transparents. Le suivi des propriétés barrière des films ont montré que l'addition d'AC diminue la perméabilité à l'oxygène, probablement en raison de liaisons hydrogène entre les groupements OH des polymères et des composés d'AC. L'efficacité des films a été étudiée en les utilisant comme emballage pour de l'huile de saumon. Les changements de couleur, la consommation d'oxygène dans l'espace de tête, les dienes conjugués, l'index de polyène et les vibrations C-H d'élongation de la double liaison cis (= CH) ont montré que, en général, les films à base d'AC permettaient d'améliorer la stabilité de l'huile de saumon au cours de sa conservation. Les films avec 2, 3 et 4% d'AC offraient la meilleure protection contre l'oxydation des lipides en raison de propriétés barrières à la lumière et l'oxygène amélior

Abstract

Antioxidant food packaging films were successfully developed by incorporation of anthocyanin compound (liquid extract from natural sources) into hydroxypropyl methylcellulose (HPMC) matrix. Films color and oxygen barrier properties were measured. Red color of AC films showed good control of light transmission in comparison with control (transparent) films. Barrier properties of these films showed that addition of AC compounds decreased oxygen permeability, possibly due to hydrogen bonding between polymer OH groups and those of AC compounds. Bio-active films effectiveness was investigated by packaging applications for salmon oil. Changes in oil color, headspace oxygen consumption, conjugated dienes, polyene index and C–H stretching vibration of cis-double bond (=CH) showed that, in general AC films improved salmon oil stability. Films with 2, 3 and 4% AC offered the best protection against lipid oxidation due to improved barrier properties against light and oxygen.

Keywords Hydroxypropyl methylcellulose. Anthocyanins. Barrier properties. Lipid oxidation. Fourier transform infrared spectroscopy.

1. Introduction

Fish oil contains high levels of polyunsaturated fatty acids (PUFA) particularly eicosapentaenoic acid (EPA, omega-3) and docosahexaenoic acid (DHA, omega-3). Most of beneficial attributes of fish and fish products are due DHA and EPA presence. For instance, they have been shown to have protective effects against coronary heart disease, inflammatory processes, thrombosis, carcinomatosis, and metabolic syndrome (Miller et al., 2008; Tsuzuki et al., 2004; Mori & Beilin, 2001). Such nutritional benefits have promoted significant research into methods of stabilizing unhydrogenated fish oil against oxidative deterioration (Hamilton et al., 1998).

Lipids containing PUFA are very sensitive to light-oxidation causing a number of unfavourable chemical changes including off-flavors formation, nutritional quality decrease, economic losses and oxidation products formation, some of which are supposed to be against human health (Drusch & Berg, 2008; Frankel, 1985). Chemical changes in lipids are directly related to light source, wavelength, intensity, exposure time, and temperature, as well as packaging material characteristics such as color and oxygen barrier properties (Akhtar et al., 2010). In fish oil, natural antioxidants such as astaxanthin, coenzyme-Q10, selenium, taurine, and vitamins (A, D, and E) can protect lipids against oxidation. (Rita Mattei et al., 2011). However, processing and storage alter these natural compounds, resulting in decreased protection against lipid oxidation. Antioxidants may be added from external sources but their direct application on fresh foods is prohibited in many countries.

Most appropriate way to protect lipid rich products may be oxygen removal and decrease of light exposure (Moyssiadi et al., 2004) which is not very easy for transparent packaging, but the most harmful wavelength (400–500 nm) of light (Mortensen et al., 2003) can be avoid by application of food colored packaging (Akhtar et al., 2010) with high oxygen barrier properties.

Phenolic compounds from fruits and vegetable extracts have gained a considerable market in food industries due to their antioxidant capacity, coloring potential, hygiene, nutrition, pharmaceutical activities, and bioactivity (Stintzing & Carle, 2004; Frank et al., 2005). Anthocyanins present in these phenolic compounds cover a large variety of colors such as orange, red, and blue which make them an attractive alternative to synthetic food dyes (Markakis, 1982). Moreover, anthocyanins have many health benefits, including reduced risk of cardiovascular diseases (Bell & Gochenaur, 2006). The scientific aims of this study were to use HPMC films functionalized with anthocyanins as packaging materials for salmon oil and

investigate effects of packaging color and oxygen permeability on light induced lipid oxidative.

2. Materials and Methods

Raw Materials

Salmon oil was purchased from Polaris, France. A red liquid extract of anthocyanin (E163) having water solubility 100% and pH 3.3 was provided by Naturex, France. Hydroxypropyl methylcellulose (Fluka-Biochemika, Japan) is a biochemical product containing 9% hydroxylpropoxyl and 28% methyl radicals. It had a viscosity of 15mPas and a water solubility of 2% at 25°C. Methanol (purity = 99%) and hexane (purity = 97%) used for CPG and conjugated dienes were purchased by Fisher (France). Ethanol (purity > 99%) (Pharmaceutics Carlo, Erba) was used to improve HPMC solublization. All organic solvents were analytical grade reagents. Phosphorus pentoxide (P_2O_5) was purchased from Sigma–Aldrich (France). Petri-dishes (optilux) were provided by Nunclon™ Fisher (DK-4000 Roskilde, Denmark).

2.1. Film Forming Solutions

At first, solutions were prepared by dissolving 6g of HPMC powder in a 35% ethanol solution (v/v) for 40min at 65°C. Secondly, anthocyanin at the level of 1, 2, 3 or 4% (v/v) was dissolved separately in a 35% ethanol solution (v/v) at 20°C in darkness due to high sensitivity of anthocyanin to light and temperature. Both, HPMC and anthocyanin solutions were then mixed and stirred for 30min at 20°C to get a homogeneous solution. A heating magnetic stirrer (Fisher Bio-block Scientific) was used to mix the solutions. Solutions pH was adjusted at 3.3±0.1 with HCl (0.1M). After mixing, solutions were degassed at 20°C under vacuum "Handy Aspirator WP-15 (Yamato®)" for 30min.

2.2. Film Casting

Films were made by pouring 6g of each film forming solution in the lids of Petri-dishes and were dried in a dark room at 20°C for one week to reach complete solvent evaporation. Petri dishes containing films were kept in a hermetic dark container containing P_2O_5 powder 48 hours before each analysis. Finally red color films were obtained having thickness 48±2µm measured by a mechanical micrometer (Messmer, London, UK) according to ASTM D374. Thicknesses were measured in 10 randomly selected points on each film and then an average value was determined.

2.3. Oxygen Permeability (PO_2)

Films were kept under controlled conditions of temperature (20°C) and relative humidity (50%) for one week before analysis. Each film was first sealed into a test cell of 26.42cm^2 exposed area and 0.8 bar oxygen pressure gradient across the film. Film PO_2 was measured by directly injecting samples with a gas sampling syringe at suitable intervals (Dynatec Pressure Lok, Baton Rouge, LA, USA) into a gas chromatograph (Shimadzu GC-4A; Shimadzu Corp., Kyoto, Japan) equipped with a thermal conductivity detector and molecular sieve columns (Desobry & Hardy, 1997). Helium gas at flow rate of 25ml/min was used as carrier gas and column temperature was 50°C. Oxygen transmission rate (OTR) and then oxygen permeability were determined as follows (Khwaldia et al., 2004).

Eq. 1 $$OTR = \frac{\Delta C \times V}{A \Delta t} \quad (cm^3.\ m^{-2}.\ h^{-1})$$

Eq. 2 $$PO_2 = \frac{OTR \times X}{\Delta p} \quad (cm^3\ .m^{-1}\ .s^{-1}\ .Pa^{-1})$$

where $\Delta C/\Delta t$ is the slope of O_2 concentration loss from the PO_2 cell over time (t), V is the volume of cell, A is the area of exposed film sample, X is the film thickness and Δp is the partial pressure difference of oxygen across the film.

2.4. Films Application on Salmon Oil

5ml of salmon oil sample was taken in each Petri-dish. Firstly, Petri dishes containing salmon oil were covered from the top with transparent lids along with films inside to study the effect of film color alone (shown below in Fig. 1a). Secondly, they were covered directly with films (shown below in Fig. 1b) to study color and PO_2 effects on light induced oxidation of salmon

oil. Each side of Petri-dish was covered with black scotch to control oxygen and light permeation. Analyses to study light-induced oxidation of salmon oil were done at 3rd, 6th and 12th days of storage.

2.5. Experimental Conditions

Prepared Petri-dishes with salmon oil and films were placed under fluorescent light (Osram L36w/640) for 12 days in an experimental chamber with controlled conditions of temperature (20°C) and relative humidity (50%). The distance of fluorescent tube from samples was 14cm.

3. Salmon Oil Quality Parameters

Numerous analytical methods are routinely used for measuring lipid oxidation in foods. However, there is no uniform and standard method for detecting all oxidative changes in all food systems. We observed lipids oxidation by analysing change in oil color, headspace oxygen uptake, conjugated dienes, polyene index and FTIR spectra.

3.1. Oil Color Measurement

Color measurements were carried out with a Minolta CM, CR-210 colorimeter (Minolta, Colombes, France) using the Hunter and CIE-Lab scale. The L* value describes lightness (0 = black to 100 = white). The value a* describes the amount of redness (positive) or greenness (negative), while the b* value describes the amount of yellowness (positive) or blueness (negative). Combined a* and b* values define the hue and intensity (saturation) of the color (Moslemi, 1967). The L, a, b values of each oil sample were taken as the average of at least 3 replications. Color difference (ΔEab) is the magnitude of the resultant vector of three component differences which was calculated by following equation:

Eq. 3 $$\Delta Eab = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

where Δa = ai - a0, Δb = bi - b0 and ΔL = Li - L0. The index i, indicates the values observed after a given storage period and index 0, indicates initial values observed before samples storage (Jutaporn et al., 2011).

3.2. Headspace Oxygen Uptake

The analytical method described above to measure O_2 concentration in the PO_2 cell was also used for headspace oxygen uptake measurement. The Petri-dishes containing oil samples to be studied for oxidation were sealed with their lids containing inside edible films and were placed under fluorescent light. At 3rd, 6th and 12th day, gas samples were withdrawn from the lower compartment of Petri-dish via a sampling valve air tightened with glue (UHU® Patafix) and analyzed by gas chromatography. Tests were performed in controlled conditions of temperature (20°C) and relative humidity (50%). Oxygen content is reported as percent of detected peaks (O_2 and N_2). Analyses were made in triplicate.

3.3. Conjugated Dienes Determination of Salmon Oil

For conjugated dienes determination, solutions were prepared according to Pazos et al. (2005) by sampling 0.002g of oil in 4ml of hexane (purity = 97%) in a sterilise test tube. After mixing (1min), solutions were analysed for conjugated dienes by spectrophotometer (Ultrospec 4000 UV/visible, Pharmacia Biotech, Orsay, France) at a wavelength of 233nm. Absorbance values obtained are averages ± standard deviations of at least 3 replicates.

3.4. Fatty Acid Composition of Salmon Oil

The fatty acid composition of salmon oil was determined using a Perichrom™ 2000 gas chromatograph (Perichrom, Saulx-lès-Chartreux, France), equipped with a flame-ionization detector and a fused silica capillary column (50m, 0.25mm i.d. × 0.25µm film thicknesses, CP 7419 Varian, Middelburg, Netherlands). Injector and detector temperatures were set at 260°C. A temperature programme of column initially set at 145°C for 5min, then rising up to 210°C at a rate of 2°C/min and held at 210°C for 10min was used. Fatty acid methyl esters (FAMEs) were prepared by transmethylation of lipid aliquots as described by Ackman (1998). 100mg of salmon oil was dissolved in 5ml hexane in test tubes in which 200µl KOH methanol solutions (2M) were added. Tubes were then strewed for 1min at nitrogen.

After mixing, the solutions were kept at rest for 30min, when both phases were clearly separated. The upper phase was evaporated under a nitrogen stream in order to obtain 100mg/mL FAMEs hexane. The analysis was done by the injection of 1µl FAMEs in gas chromatograph device. Standard mixtures (PUFA 1 from marine source and PUFA 2 from animal source (Supelco, Sigma–Aldrich, Bellfonte, PA, USA) were used to identify fatty acids (internal standard C21:0). The results were presented from triplicate analyses. To

evaluate oxidation extent, polyene index was determined, based on the following formula (Rodriguez et al., 2007):

Eq. 4 $$\text{Polyene index} = \frac{\%\,\text{EPA}+\%\,\text{DHA}}{\%\,\text{C16:0}}$$

3.5. FTIR Analysis of Salmon Oil

Salmon oil oxidative stability after 12 days of continuous light exposure was followed by Fourier transform infrared spectroscopy in total attenuated reflection mode (ATR-FTIR). Measurements were performed at 25°C with 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 was set at 4mm. The scanning rate was 10 kHz, and 80 scans were performed both for reference and sample from 4000 to 800cm^{-1} with 4cm^{-1} of resolution. All data treatments were carried out using OPUS software (Bruker, Karlsruhe, Germany). Raw absorbance spectra were smoothed using a nine-point Savitsky-Golay smoothing functions. Elastic baseline correction was applied to spectra, which were further cut in required regions, centered and normalized.

Statistical Analysis

Experimental values were given as means ± standard deviation. Variance analysis (ANOVA) was used to compare mean differences of the samples. Differences at $P < 0.05$ were considered to be significant.

4. Results and Discussion

The effect of packaging color and oxygen permeability on packed salmon oil is schematically outlined in Fig. 1, showing the factors acting on packed product, light absorption and oxygen content. Fig. 1a represents effect of film color alone and Fig. 1b presents combined effect of film color and PO_2.

Factors influencing light absorbed ($I_{abs,p}$):

- Intensity of light (I_0)
- Spectrum of light source
- Absorption ($I_{abs,f}$) and reflection ($I_{r,m}$) of packaging depending on colour
- Product reflection ($I_{r,p}$)
- Product absorption ($I_{abs,p}$)

Factors influencing oxygen (O_2) present:

- Initial gas composition ($O_{2,p}$+ $O_{2,h}$)
- Product-to-headspace volume ratio
- Product respiration
- Oxygen permeability of packaging material

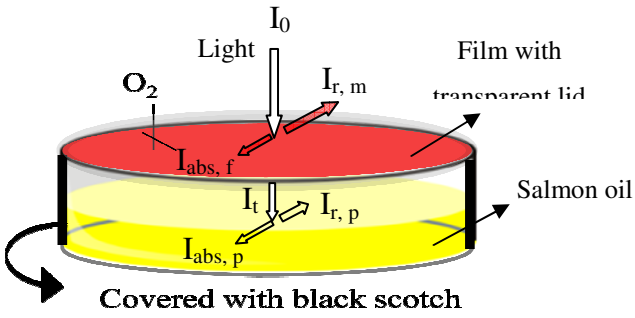


Fig. 1a Film color effect

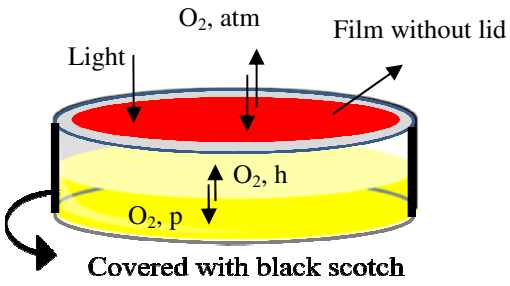


Fig. 1b Film color and PO_2 effect

Fig. 1 *Schematic overview of salmon oil/packaging interactions related to film color and oxygen permeability*

4.1. Film O_2 Permeability and Color

The capability of food packaging materials to modify oxygen transport is very important for suitable applications. For instance, high oxygen availability is reported to be protective for fresh foods (Gill & Gill, 2005) and low oxygen concentration is protective for lipid oxidation (Lee et al., 2008).

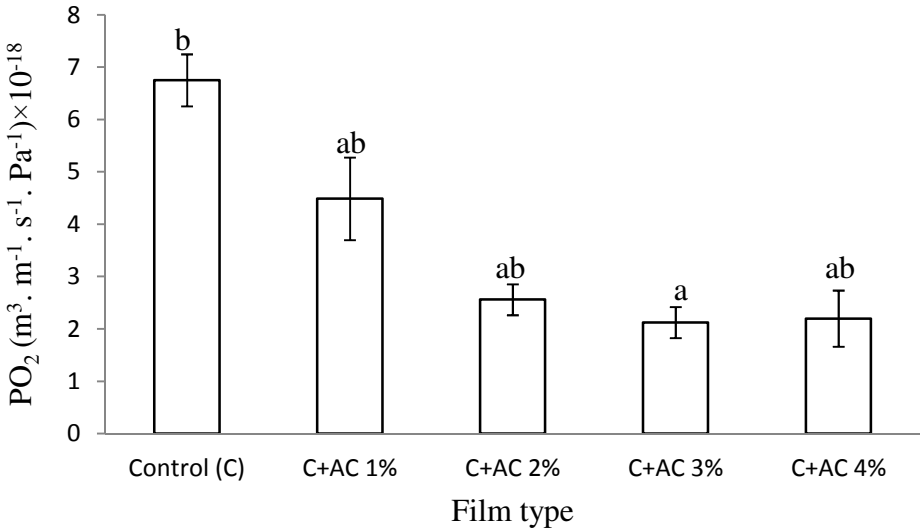


Fig. 2 *Oxygen permeability of films based on HPMC alone and with anthocyanin (AC) 1, 2, 3 and 4% (v/v)*

Fig. 2 shows effect of anthocyanin extract (AC) addition on PO_2 of HPMC films at controlled conditions of temperature (20°C) and relative humidity (50%). There was an increase in oxygen barrier properties by increasing AC up to 4%. This effect can be attributed to the availability of free hydroxyl groups of AC which took part in hydrogen bonding with HPMC matrix giving more compact structure (Aouada et al., 2011). This indicates that HPMC films with added AC are good oxygen barriers at low to intermediate relative humidity. However, PO_2 is expected to be higher at increased relative humidity, due to hydrophilic nature of both, HPMC and AC. leading to absorption of water molecules that plasticize the film structure. Thus, polymer chain mobility increases, resulting in increased PO_2 (Ayranci & Tunc, 2003). Such edible packaging with improved oxygen and light barrier properties can be an application for unsaturated fatty acids conservation. Effect of packaging color on product quality has been published in previous studies (Akhtar et al., 2010; Lennersten & Lingnert, 2000).

4.2. Oil Color

Total change in color (ΔE) of salmon oil before and after 12 days of continuous light exposure is shown in Fig. 3. The oil samples treated with control HPMC films without free PO_2 showed ΔE values of 5.83±0.29 that were further increased up to 7.74±1.91 with free PO_2 after 12 days of light exposure. This ΔE increase was due to films PO_2 accelerating oxidation of

salmon oil. This may be due to oxidation of astaxanthin, a major carotenoid orange pigment present in salmon oil (Mendes-Pinto et al., 2004).

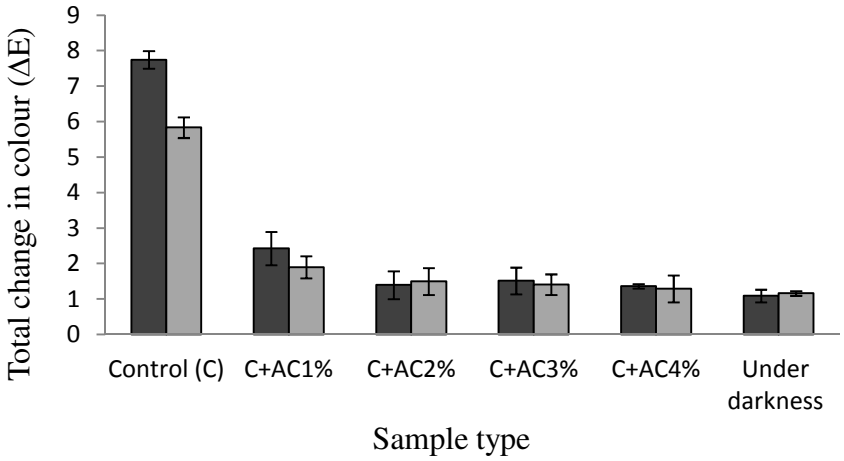


Fig. 3 *Total change in salmon oil colour (ΔE) after 12 days of light exposure with different antioxidant films. (□) Effect of film color alone, (■) combined effect of color and O_2 permeability*

On the other hand, samples covered with AC (1% & 2%) films without free PO_2 showed ΔE values of 1.90±0.31 and 1.50±0.38 respectively. These values were slightly increased up to 2.43±0.47 and 1.39±0.39 respectively with free PO_2. These small differences in ΔE values indicated the effect of PO_2 was lower as compared to those of control HPMC films. Samples stored under light after covering with AC (3 & 4%) films with free PO_2 were stable for oil color properties and similar to those stored under darkness. Results indicate the effects of colored films improved oxygen barrier properties on light induced lipid oxidation.

4.3. Headspace Oxygen Uptake

The headspace gas chromatography method is a simple and reproducible to evaluate oxidative stability of fats and oils (Chung et al., 2004). During light exposure, oxygen concentration changes in Petri-dishes covered with control HPMC and AC films are shown in Fig. 4.

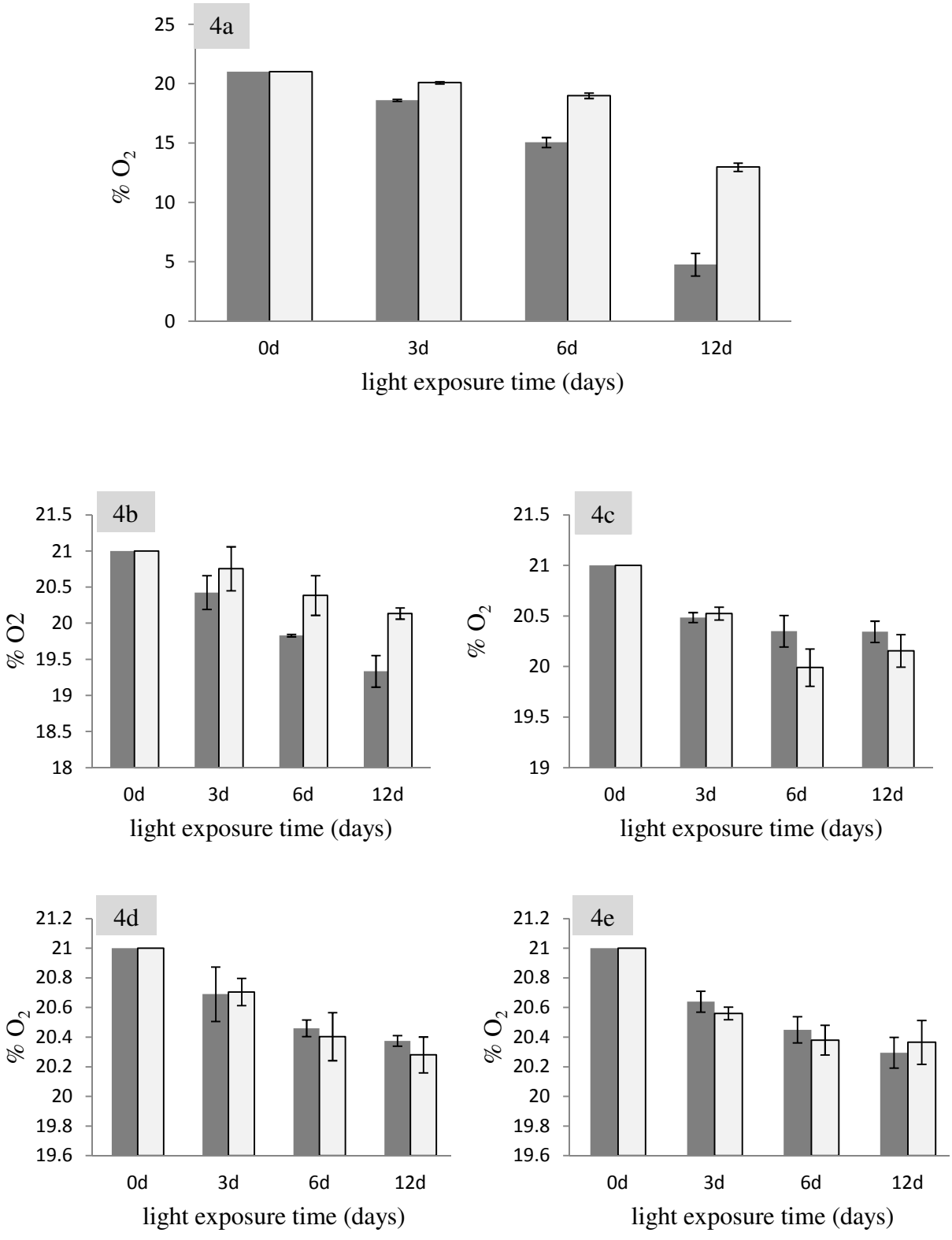


Fig. 4 *Quantity of oxygen in Petri-dishes containing salmon oil exposed to fluorescent light after covering with edible films, Control (Fig. a), C+AC1% (Fig. b), C+AC2% (Fig. c), C+AC3% (Fig. d) and C+AC4% (Fig. e).Effect of film colour (■). Combined effect of colour and* PO_2 *(□)*

Sample covered with control HPMC films without free PO_2 showed a gradual decrease in O_2 concentration (21 to 4.77±0.9 %) indicating lipid oxidation over time. O_2 content of samples treated with control HPMC films with free PO_2 was almost stable during first 6 days and decreased down to 12.98±0.35% at the 12^{th} day of analysis (Fig. 4a). This indicated availability of more O_2 transmitting through edible films, which accelerated lipid oxidation. These results were in accordance with Borle et al. (2001), Mortensen et al. (2002b, 2004) and Rosa et al. (2007). AC1% films demonstrated a gradual decrease of oxygen contents (21 to 19.33±0.22) when considering the effect of film color alone. This phenomenon was less pronounced (21 to 20.13±0.08) by same films with free PO_2 due to poor oxygen barrier properties (Fig. 4b). On the other hand, AC2, 3 & 4% films showed a good control against oxidation by minimizing transmission of both light and oxygen (Fig. 4c, 4d & 4e). Results were in accordance with Akhtar et al. (2010), Mortensen et al. (2002b) & Alves et al. (2002). It can be concluded that films functionalized with AC had an ability to control salmon oil oxidation.

4.4. Conjugated Dienes of Salmon Oil

During hydroperoxides formation from unsaturated fatty acids, conjugated dienes are typically produced due to the rearrangement of double bonds. The resulting conjugated dienes exhibit a strong absorbance at 233 nm (Shahidi & Wanasundara, 2002). The transparent films transmitting more than 90% wavelengths of light (Bosset et al., 1994; Lennersten, 1998) may cause serious oxidative changes in packed products. Conjugated dienes content was gradually increased over storage time in samples covered with control HPMC films without free PO_2 (Fig. 5a). This gradual increase in conjugated dienes content was more pronounced with free PO_2 films (Fig. 5b) indicating more oxidation caused by high oxygen transmission rate through films. Comparing control HPMC films results shown in Fig. 5a & Fig. 5b, an additional films PO_2 effect on salmon oil quality was observed. Red films have the ability to transmit red spectral light (Chandrasekaran, 2001) which has a specific wavelength of 510nm (Lennersten & Lingnert, 2000) that was not deteriorative for salmon oil (Akhtar et al., 2010). Oil samples covered with AC films showed absolute stability against oxidation, unlike those covered with control HPMC films. This indicated films red color positive effect on lipids light oxidation.

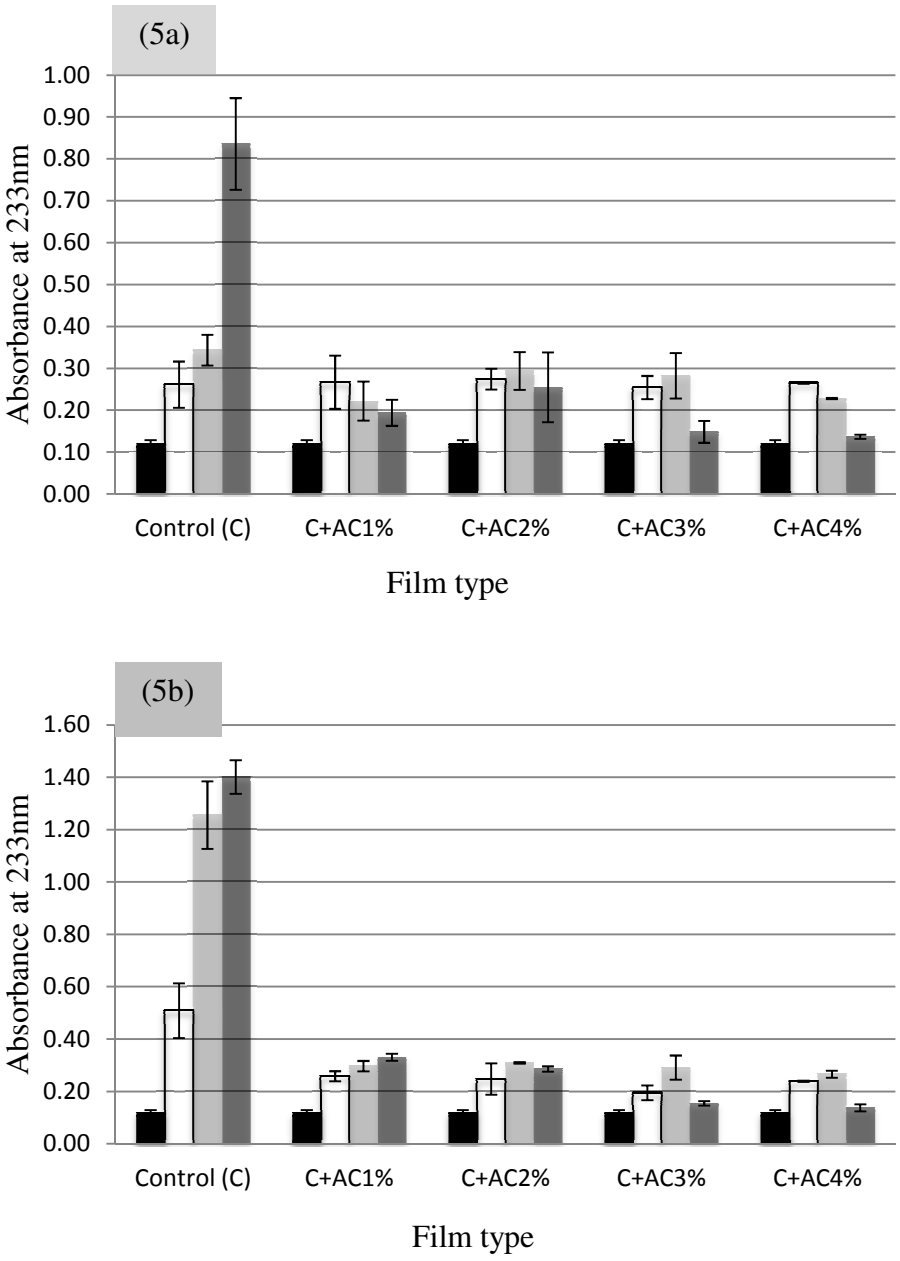


Fig. 5 *Evaluation of the absorbance of conjugated dienes of salmon oil exposed to fluorescent light after covering with edible films, at 0 day (■), 3rd day (□), 6th day (■) and 12th day (■). Effect of film colour (5a), combined effect of colour and O_2 permeability (5b)*

AC1% films without O_2 transmission showed lower conjugated dienes content as compared to those with free PO_2 representing films PO_2 effect on lipid oxidation. On the other hand, conjugated dienes formation in samples covered with AC (2, 3 & 4%) films with and without free PO_2 was almost similar showing good oxygen barrier properties.

4.5. Polyene Index (PI)

Polyunsaturated fatty acids such as palmitic acid (C16), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were determined by gas chromatography (Table 1). According to Rodriguez et al. (2007) polyene index (PI) was calculated from these polyunsaturated fatty acids and was used to identify oxidation of salmon oil (Table 2). EPA and DHA, which represented the major part of unsaturated fatty acids, were most susceptible to oxidation.

Table 1. *Fatty acid composition of salmon oil exposed to direct fluorescent light after covering with edible HPMC films, measured by gas chromatography at 0 day and after 12 day of storage at 20°C*

Edible films	Fatty acids	Light exposure time		
		0 Reading	12^{th} $d_{(C)}$	12^{th} $d_{(C+O)}$
Control (C)	C16	11.44±0.057	12.76±0.096	13.849±0.61
	C20:5n3	5.203±0.007	3.753±0.122	2.253±0.684
	C22:6n3	6.470±0.047	4.252±0.143	2.191±0.727
C+AC1%	C16	11.44±0.057	11.483±0.01	11.71±0.026
	C20:5n3	5.203±0.007	5.074±0.006	5.116±0.108
	C22:6n3	6.470±0.047	6.289±0.037	6.525±0.345
C+AC2%	C16	11.44±0.057	11.53±0.142	11.44±0.057
	C20:5n3	5.203±0.007	5.145±0.013	5.128±0.148
	C22:6n3	6.470±0.047	6.555±0.022	6.394±0.021
C+AC3%	C16	11.44±0.057	11.64±0.001	11.601±0.02
	C20:5n3	5.203±0.007	5.108±0.016	5.115±0.006
	C22:6n3	6.470±0.047	6.388±0.082	6.439±0.727
C+AC4%	C16	11.44±0.057	11.59±0.026	11.564±0.06
	C20:5n3	5.203±0.007	5.179±0.072	5.138±0.084
	C22:6n3	6.470±0.047	6.599±0.266	6.502±0.273

12th d(C) = effect of film color at 12th day of light exposure, 12th d(C+O) = combined effect of film color and O2 permeability at 12th day of light exposure

Table 1 shows the quantity of (C16) increased from 11.44±0.057 to 12.76±0.096 while those of EPA (C20:5n3) and DHA (C22:6n3) decreased from 5.203±0.007 to 3.753±0.122 and 6.470±0.047 to 4.252±0.143 respectively in samples covered by control HPMC films without free PO_2. Similar behavior but more oxidation was resulted in samples covered by same films with free PO_2. These results indicated film PO_2 effect on salmon oil quality. On the other hand decrease in PI (1.020±0.009 to 0.998±0.006) in samples treated with AC1% films was very small as compared to those treated with transparent films indicating film color effect.

Table 2. *Evolution of polyene index calculated from C16, EPA, and DHA values during a storage à 20°C*

Edible films	Light exposure time		
	0 Reading	12^{th} $d_{(C)}$	12^{th} $d_{(C+O)}$
Control (C)	1.020±0.009	0.627±0.025	0.323±0.116
C+AC1%	1.020±0.009	0.998±0.006	0.994±0.041
C+AC2%	1.020±0.009	1.015±0.016	1.007±0.000
C+AC3%	1.020±0.009	0.988±0.009	0.996±0.000
C+AC4%	1.020±0.009	1.016±0.031	1.007±0.036

12^{th} $d_{(C)}$ = effect of film color at 12^{th} day of light exposure, 12^{th} $d_{(C+O)}$ = combined effect of film color and O_2 permeability at 12^{th} day of light exposure

Further increase in PI (0.998±0.006 to 0.994±0.041) with films free PO_2 indicated the effect of oxygen transmission rate. Samples treated by all other AC films (2, 3 & 4%) were stable for their PI values with and without free PO_2 conditions showing combined effect of color and PO_2 on lipid oxidation.

4.6. FTIR Analysis

FTIR spectroscopy is a simple, rapid, and highly precise method with minimum samples preparation necessary. This method provides an automated, efficient and low-cost means of evaluating oils oxidation and has gained considerable interest for quality control in industry (Innawong et al., 2004; Dobarganes & Velasco, 2002; Kiritsakis et al., 2002). Recently, Klaypradit Wanwimol et al. (2011) and Gimenez et al. (2011) have studied oxidation of lipids

by FTIR. Oil samples covered with HPMC films and stored under light were characterized by FTIR for oxidation stability (Fig. 6).

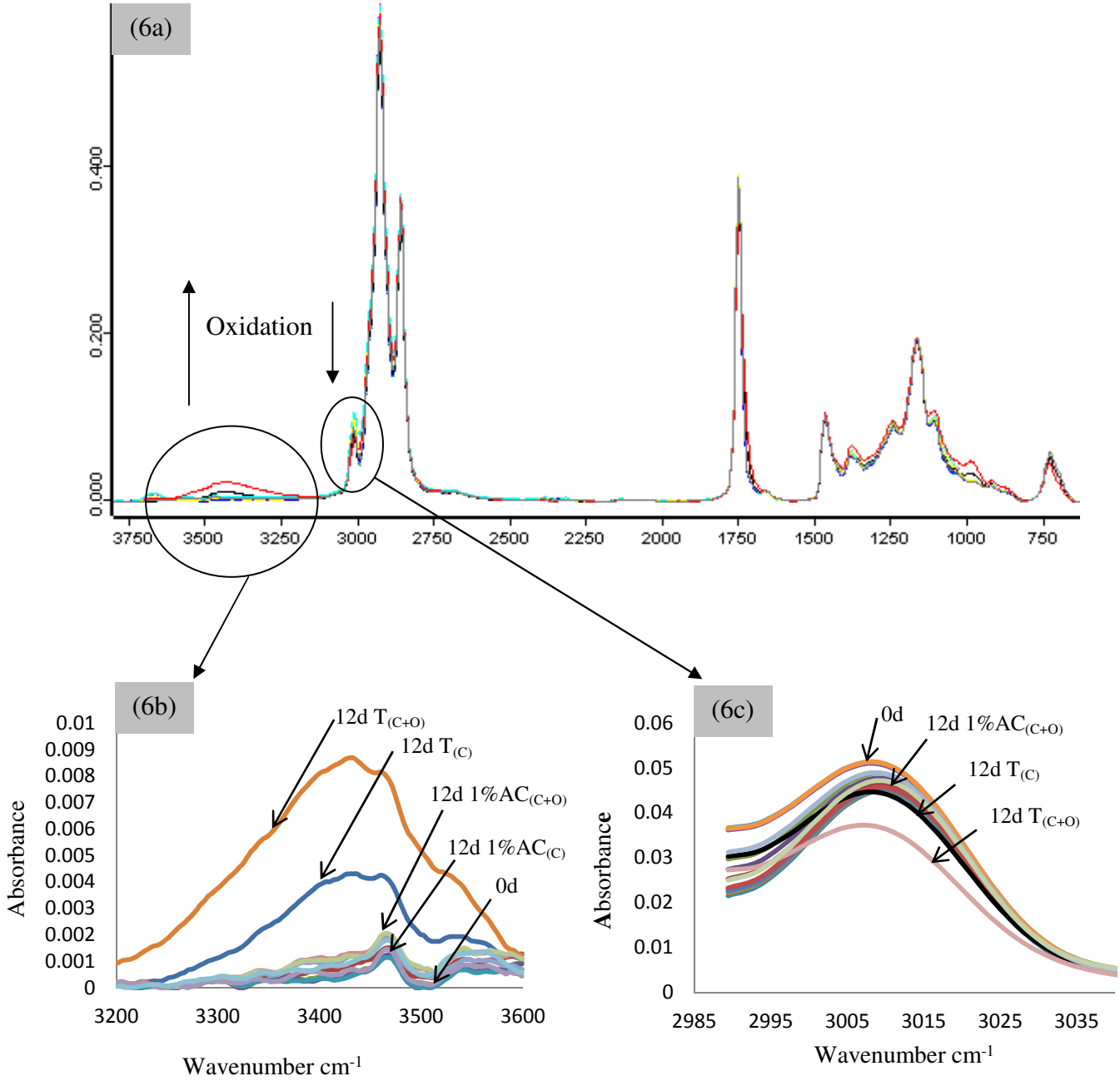


Fig. 6 *FTIR spectra of salmon oil stored over 12 days under light and packaging conditions at 20°C (a). Hydro peroxides band between 3200 and 3600 cm^{-1}(6b). Cis-Double bound band at 3010 cm^{-1} (6c)*

Generally, the FTIR spectrum exhibited similar regions of functional groups vibrations as reported previously for farmed salmon fillets lipids (Guillén et al., 2004), sardine muscle

lipids (Chaijan et al., 2006), some vegetable oils (Vlachos et al., 2006), as well as for salmon oil (Belhaj et al., 2009). Oxidation increase was monitored by growth of hydroxyl region absorption band (H_2O, R-OH and ROOH) observed between 3100 and 3700cm^{-1} (Fig 6b) and the peak at 3012cm^{-1} related to the C–H stretching vibration of *cis*-double bonds (Fig. 6c).

4.6.1. Changes in Spectral Region between 3200 and 3600cm^{-1}

The stretching vibrations were observed between 3100 and 3700cm^{-1}, due to the accumulation of OH from moisture, hydroperoxides (ROOH) and their breakdown products alcohols (ROH). The weak band associated with the overtone of the glyceride ester carbonyl absorption showed a maximum absorbance near 3470cm^{-1} in salmon lipid (Guillén et al., 2004). The frequency of same band in this study appeared approximately at 3455cm^{-1} (Fig 6b). Samples stored in control HPMC films without free PO_2 showed an increase in absorbance of this band indicating light induced oxidation which was further increased with films free PO_2. In case of samples covered with AC films, only AC1% films with combined effect of color and PO_2 showed a slight increase in absorbance. On other hand, samples treated with AC (2, 3, & 4%) films with and without free PO_2 were stable after 12 days of light exposure.

4.6.2. Changes in Spectral Region around 3012cm^{-1}

The absorption band at 3012cm^{-1} is associated with the C–H stretching vibration of the *cis*-double bond. According to Vlachos et al. (2006) the C–H stretching vibration of the *cis*-double bond (=CH) varies from 3009 to 3006cm^{-1}. Whereas, Klaypradit Wanwimol et al. (2011) and Gimenez et al. (2011) found same band at 3012cm^{-1}. The reduced absorbance in this region indicated a reduction in the degree of lipid unsaturation due to oxidation. As can be seen in Fig. 6c, the oil samples covered with transparent HPMC films showed decrease in this region indicating photo-oxidation. On the other hand, samples covered with AC films showed no change of absorbance in this region. It was difficult to make the difference of absorbance for different samples (Fig 6c). Similarly, Guillén et al. (2004) and Vlachos et al. (2006) reported that oxidative process during the storage period could not be evidenced by monitoring the absorbance band at 3012 cm^{-1}.

5. Conclusion

Bio-active films, based on hydroxypropyl methylcellulose and anthocyanins (natural antioxidants), were successfully developed with good oxygen and light barrier properties. Incorporation of AC compound into HPMC films was a suitable replacement of synthetic dyes due to its functional properties including color, oxygen barrier, and antioxidant. The efficiency of theses films was examined by packaging applications of salmon oil under the extended period of light storage. Some conclusions can be made from this application study of bio-active films as follows:

HPMC packaging with high oxygen transmission rate such as control HPMC films without AC are not suitable for salmon oil. Moreover, they showed poor light and a minimum control of photo-oxidation.

Films with AC1% were not suitable for salmon oil packaging due to unsatisfactory lipid oxidation control. On the other hand, films with AC (2, 3, & 4%) showed very good control of lipid oxidation, very close to samples stored under darkness.

In general, large headspace volumes should be avoided to decrease oxygen contents.

This type of biodegradable edible packaging may be a suitable application for products rich in unsaturated fatty acids.

6. Acknowledgements

The authors acknowledge Jordane Jasniewski, Carole Jeandel and Céline Charbonnel for their technical assistance.

V. Conclusion et perspectives

V.1. Conclusions

Pour élargir l'éventail des applications potentielles des emballages alimentaires, les chercheurs s'intéressent de plus en plus à la fonctionnalisation de matrices polymériques, ou d'un coating, en incorporant une variété d'additifs alimentaires, dont des antioxydants, des antimicrobiens ou des colorants naturels. L'objectif principal de ce projet était de développer un emballage biodégradable actif avec l'amélioration des propriétés physico-chimiques fonctionnelles, y compris l'effet barrière à la lumière, à l'oxygène et la libération contrôlée d'antioxydants afin de maîtriser les processus d'oxydation dans les aliments.

Dans la première partie de ce projet, des films d'HPMC de différentes couleurs ont été développés et leur impact sur le contrôle de la photo-oxydation de l'huile de saumon riche en AGPI et stockée sous une lumière fluorescente à 25 ° C est étudiée. L'oxydation des lipides observée était plus élevée dans les échantillons d'huile traités avec des films transparents, bleus ou verts. Les films jaunes fournirent une protection à peu près équivalente à un stockage dans l'obscurité alors que les films blancs et rouges permirent la meilleure protection contre la photo-oxydation. En effet, la perte en O2 de l'espace de tête de l'emballage, mesurée par chromatographie en phase gazeuse (GC), a été maximale dans les échantillons traités avec des films transparents et bleus et tandis minimale dans les échantillons traités avec les films blancs et rouges. Les valeurs mesurées des diènes conjugués étaient élevés dans les échantillons traités avec des films transparents et bleu tandis que faibles dans ceux avec des films rouges et blancs. Grace à l'absorption spécifique de certaines longueurs d'onde, mise en évidence par les courbes de transmission spectrale des films, il est suggéré que l'utilisation du blanc et du rouge pour l'emballage d'huile de saumon fournira la meilleure protection contre sa photo-dégradation.

La deuxième partie de ce projet comprend le développement et la caractérisation de films d'HPMC en incorporant un mélange colorant rouge du commerce à activité antioxydante (NRC). Il a été vérifié que l'addition de composés phénoliques contenus dans le NRC a fortement modifié le comportement de sorption de l'eau, de la vapeur d'eau, de la perméabilité à l'oxygène, des propriétés mécaniques, et de la température de transition vitreuse des films d'HPMC. En effet, ces films ont une perméabilité à l'oxygène bien inférieure au témoin non fonctionnalisé. Ils ont une plus forte humidité et capacité de sorption WVP. Au niveau mécanique, ils présentent une augmentation de l'allongement à la rupture et diminution de la force à rupture et du module de Young. Ces résultats ont donc montré le fort potentiel de l'utilisation de ces composés phénoliques dans le développement de matériaux d'emballage souples bioactifs. Le NRC pur ou incorporé dans la matrice polymérique a aussi

été testé pour son activité antioxydante et sa stabilité à la photo-dégradation. La capacité antioxydante du NRC au cours de la fabrication des films est stable. La couleur rouge des films devient plus foncée lorsque la concentration en NRC augmente dans le film ce qui est corrélé avec l'impact de la photo-dégradation sur la stabilité de la couleur. L'ensemble des résultats a montré que les molécules contenues dans le NRC ont un fort potentiel pour des applications en emballage alimentaire du fait de leur couleur, leur effet plastifiant, leur bonne activité antioxydante et leur capacité à protéger la matrice polymérique de la photo-dégradation. Cependant, la stabilité des films en termes de couleur, transparence, développement microbien et capacité de rétention des arômes doit encore être améliorée.

Le relargage dans un simulant alimentaire (ethanol 95%) des composés phénoliques incorporés dans l'HPMC a été étudié à 20 et 4°C. Des tests ont été réalisés avec le NRC, un mélange de betalaïnes, un mélange d'anthocyanine et un mélange à part égale de ces deux dernières molécules. Les batalaïnes présentent la plus forte libération de contenu phénolique (TPC) par rapport aux antocyanines à 20°C. Ce phénomène diminue avec l'abaissement de la température. Le NRC présente le plus faible relargage de TPC à 20 et à 4°C. La diffusion des antioxydants à partir des films montre une valeur plus importante de D pour les betalaïnes que pour les anthocyanines et le NRC. Généralement, le relargage des composés phénoliques est, de façon globale, très élevé et il atteint un équilibre en environ 10h, ce qui n'est pas en faveur de leurs applications en emballage. D'un autre côté, l'activité antioxydante des films fonctionnalisés est stable. Les betalaïnes présentent la valeur de TEAC plus élevée que celle des autres mélanges. Tous les antioxydants ont une meilleure stabilité dans l'HPMC qu'en solution lorsqu'ils sont photo-dégradés. Au niveau structural, les données de FTIR montrent l'évolution de la vibration à 1060 cm-1 pour tous les échantillons après la libération des antioxydants ce qui indique une dégradation de l'HPMC. Un simulant non polaire tel que de l'huile aurait été plus adapté pour étudier la libération des composés phénoliques. Ces films fonctionnalisés à activité antioxydantes trouveront donc plutôt des applications pour la conservation d'aliments non polaires.

Des films d'HPMC contenant des anthocyanines (AC) ont aussi été développés avec succès. Ils ont de bonnes propriétés barrières à l'oxygène et à la lumière. Ces films ont été étudiés comme emballage expérimental pour de l'huile de saumon afin d'augmenter sa conservation à la lumière. Les films sans AC ou avec une teneur en AC d'1% n'ont pas permis le contrôle de la photo-oxydation de l'huile de saumon. Cependant, les films contenant 2, 3 ou 4% d'AC présentent un très bon contrôle de la dégradation de l'huile, équivalent à un stockage à l'obscurité.

De façon globale, l'ensemble des études a montré que les composés phénoliques naturels pourraient devenir de bons candidats pour remplacer les colorants artificiels d'autant qu'ils apportent de nouvelles fonctionnalités à l'emballage comme l'activité antioxydante ou encore une perméabilité à l'oxygène contrôlée. Le type d'emballage fonctionnalisé obtenu pourrait trouver diverses applications pour la conservation d'aliments riches en matière grasse. De plus, la large variété de couleurs apportées par les composés phénoliques permet de ne pas perdre de vue le rôle attractif que se doit aussi de jouer un emballage.

V.2. Perspectives

L'oxydation des acides gras insaturés est l'une des principales causes de la dégradation de la qualité d'un aliment suite au développement de faux-goûts et la perte des qualités nutritionnelles. L'emballage à base de biopolymères et d'antioxydants naturels est une technologie prometteuse pour contrôler les phénomènes d'oxydation des produits emballés du fait de leurs propriétés physico-chimiques et antioxydantes améliorées. Les différents résultats obtenus lors de cette étude ouvrent plusieurs perspectives :

- L'étude de ces composés phénoliques colorés à fort potentiel dans d'autres matrices polymériques telles que le chitosan, le PLGA ou le PLA ou des mélanges avec l'HPMC serait intéressante.
- L'étude d'autres aliments modèles sensibles à la lumière et différents de l'huile de saumon devront aussi être étudiés afin d'élargir les applications de ces films fonctionnalisés. Des aliments solides ou visqueux seraient probablement de bons modèles présentant des profils de libération complètement différents.
- La sensibilité des composés phénoliques lors de la photo-dégradation et leur fort tôt de relargage dans le simulant alimentaire résultent d'interactions non-covalentes de faibles énergie entre les molécules et le polymère. L'étude d'une fonctionnalisation enzymatique covalente par, par exemple, des laccases pourrait être une piste intéressante pour maîtriser ces phénomènes.

V.3. Conclusions

To broaden the range of potential applications where food packaging could be used, researchers are increasingly investigating additional fonctionnalization of edible films and coatings by incorporating a variety of active food additives including antioxidants, antimicrobials, and natural colorants etc. The overall objective of this project was to develop a biodegradable active packaging with improved physico-chemical functional properties including light barrier, oxygen barrier and antioxidant release etc. to continuously control the oxidation processes in foods. In the first part of this project HPMC films containing different colors provided an attractive and convenient form of packaging, offering protection against photo-oxidation in PUFA rich salmon oil stored under fluorescent light at 25°C. Observed lipid oxidation was higher in oil samples treated with transparent, blue and green films. The yellow films provided almost equivalent protection with regard to photo-degradation as compared to the samples stored under darkness while white and red films provided maximum amount of protection against photo-oxidation. Based on analytical evaluation the loss in O_2 percentage measured by gas chromatography (GC) was maximum in samples treated with transparent and blue films while minimum in that treated with white and red films. The conjugated dienes values measured by spectrophotometer were high in samples treated with transparent and blue films while low in that treated with red and white films. Based on the fact of specific wavelength absorption, as well as spectral transmission curves of edible films tested by spectrophotometer, it is suggested that the use of white and red HPMC films in salmon oil packaging will provide a better protection to its light-sensitivity.

Second part of this project comprises the development and characterization of HPMC films by incorporating commercially available natural red color (NRC). It was verified that addition of NRC containing phenolic compounds greatly affected the water sorption behaviour, water vapor permeability, oxygen permeability, mechanical properties, and glass transition temperature of HPMC films. Films containing NRC presented lower oxygen permeability. On the other hand, NRC films showed high moisture sorption capacity and WVP. NRC films presented an increase of ultimate elongation at break and decrease of both, TS and YM. Results showed the high potential of using NRC phenolic compounds in developing flexible bioactive packaging materials.

Pure NRC and NRC incorporated HPMC films were tested for their antioxidant and photo-ageing stability. NRC antioxidant capacity during film preparation steps was stable. Color of edible films became darker and redder as NRC increased, while an increase effect of light

exposure was noticed on color stability. Results pointed that NRC films has good potential for food applications due to their color, plasticizing property, good antioxidant stability and ability to protect HPMC from photo-degradation. Nevertheless, films stability in respect to color, transparency, microbial growth, and flavor retention needs further studies.

The release behavior of natural phenolic antioxidants from HPMC to food simulant at 20°C and 4°C was evaluated and compared together. Betalain showed high total phenolic contents (TPC) release as compared to those of anthocyanins at 20°C. It decreased by decreasing temperature. NRC showed minimum TPC release at 20 and 4°C. The antioxidants diffusion from HPMC films indicated that D value for betalain was greater than that of anthocyanin and NRC. Generally, all natural antioxidants showed higher rate of TPC release and reached to equilibrium within 10h which was not in favor of active food packaging. On the other hand, antioxidant capacity of active films was almost stable. Betalains showed higher TEAC values than those of mixture (AC+B/50:50), anthocyanin and NRC. All antioxidants showed high stability in HPMC matrix compared to those in pure extract solutions treated directly in light or darkness. Concerning FTIR analysis, stretching vibration at 1060cm^{-1} of all film samples indicated HPMC degradation after release study. HPMC showed an ability to preserve antioxidant capacity of these phenolic compounds. Non polar food simulants such as fats may be more suitable for slow release of phenolic antioxidants. Such bioactive films based on HPMC and natural phenolic antioxidants may possibly apply for non-polar foods preservation.

HPMC films with anthocyanins (AC) were successfully developed with good oxygen and light barrier properties. The efficiency of these films was examined by packaging applications of salmon oil under the extended period of light storage. HPMC packaging with high oxygen transmission rate such as control HPMC films (without AC) and that containing AC1% were not suitable for salmon oil packaging due to unsatisfactory lipid oxidation control. On the other hand, films with AC (2, 3, & 4%) showed very good control of lipid oxidation, very close to samples stored under darkness.

Generally, study demonstrated that the use of natural extracts into HPMC films was a suitable replacement of synthetic dyes due to their functional properties including unique fruit flavor, color, oxygen barrier and antioxidant capacity, which would significantly enhance their potential applications in both food and nonfood industries. This type of biodegradable edible packaging may be a suitable application for products rich in unsaturated fatty acids. In addition, the use of such active films with suitable colors may also provide a unique opportunity to attract the consumers.

V.4. Perspectives

Unsaturated lipids oxidation is a major cause of food quality deterioration by the development of off-flavor compounds and loss of nutritional value of food products. Edible packaging containing natural antioxidant is a promising technology to reduce oxidation in packed foods due to their improved physico-chemical and antioxidant properties. The suitability of HPMC biodegradable polymer as colored antioxidant packaging for unsaturated lipids preservation was studied in this project. The following perspectives appear from the present study:

- Incorporation of these natural color extracts into other biopolymers such as chitosan, PLGA and PLA or their blends may be interesting.
- HPMC films containing suitable edible colors provided an attractive and convenient form of packaging which may be investigated for the preservation of all other light sensitive foods.
- We observed the instability of films color during photo-aging because of non-covalent interaction between HPMC and antioxidant compound. Biopolymers may be covalently colored and functionalized by using enzymes such as laccases.
- The fast release of natural antioxidants into 95% ethanol at 20°C and even at 4°C shows the requirement of further studies to deaccelerate their release for ensuring a continuous control of antioxidants.
- As food stimulant (ethanol 95%) was used for natural antioxidants release, complimentary studies using real foods in contact with HPMC-antioxidant films are needed to fulfill this project in real conditions. Probably solids or viscous models will offer interesting and very different behaviour for antioxidant release.

The blend of HPMC with other biopolymer such as cellulose acetate, chitosan, PLGA, PLA and others, can be developed to provide a controlled release antioxidant active packaging.

VI. PUBLICATIONS ET COMMUNICATIONS SCIENTIFIQUE

VI.1. Scientific publications

1. **Akhtar, M. J.,** Jacquot, M., Tehrany, E. A., Gaïani, C., Linder, M., & Desobry, S. (2010). Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film colour. Food Chemistry, 120, 395–401.
2. **Akhtar, M. J.,** Jacquot, M., Jasniewski, J., Jacquot, C., Imran, M., Jamshidian, M., Paris, C., & Desobry, S. (2012). Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract, Carbohydrate Polymers, 89, 1150–1158.
3. **Muhammad Javeed Akhtar,** Muriel Jacquot, Majid Jamshidian, Muhammad Imran, Elmira-Arab Tehrany & Stéphane Desobry. (2012). Fabrication and physicochemical characterization of HPMC films with commercial plant extract: Influence of light and film composition. Food Hydrocolloids 31, 420–427.
4. **Muhammad Javeed Akhtar,** Muriel Jacquot, Stéphane Desobry. (2013). Effect of HPMC–Anthocyanin Packaging Color and Oxygen Permeability on Salmon Oil Preservation (Food and Bioprocessing Technology).
5. **M. J. Akhtar,** M. Jacquot, M. Ghoul, S. Desobry. (2013). Release kinetics and antioxidant stability of natural phenolic compounds integrated in Bio-films (Food Engineering– Accepted).
6. Imran M., Revol-junelles A.M., René N., Jamshidian M., **Akhtar M. J.,** Tehrany E.A., Jacquot M., Desobry S. (2012). Microstructure and physico-chemical evaluation of nano-emulsion-based antimicrobial peptides embedded in bioactive packaging films. Food Hydrocolloids 29, 407–419.
7. Majid Jamshidian, Elmira Arab Tehrany, Muhammad Imran, **Muhammad Javeed Akhtar,** Franck Cleymand, Stéphane Desobry. (2012). Structural, mechanical and barrier properties of active PLA–antioxidant films. Journal of Food Engineering 110, 380–389.
8. Jamshidian, M., Tehrany, E. A., Cleymand, F., Leconte, S., **Akhtar, M. J.,** Falher, T., & Desobry, S. (2012). Effects of Synthetic Phenolic Antioxidants on Physical, Structural, Mechanical and Barrier Properties of Poly Lactic Acid Film, Carbohydrate polymers 87, 1763–1773.

9. Jacquot, C. Jacquot, M. Marques, P. Jasniewski, J. **Akhtar, M. J.** Didelot, A. S. Desobry, S. (2014). Influence of microwave heating time on structure and properties of Chitosan films, Journal of Applied Polymer Science-Accepted.
10. Imran M., Klouj A., **Akhtar M. J.,** Jamshidian M., Revol-junelles A.M., Desobry S. Controlled release of an antimicrobial bio-preservative nisin from bio-membranes: comparison of HPMC, sodium caseinate, poly-lactic acid and chitosan efficacy for packaging applications (Under process of submission).

VI.2. Scientific communications

11. **Akhtar, M. J.,** Jacquot, M., Tehrany, E. A., Jamshidian, M., Imran M., Desobry S. Control of photo-oxidation of salmon oil by the conservation in HPMC packaging: Influence of film colour. Oral presentation. AIC Interim Meeting "Color and Food" 12-15 October 2010 Hotel Provincial, Mar del Plata, Argentina.
12. **Muhammad Javeed Akhtar**, Muriel Jacquot and Stéphane Desobry. Effect of active-packaging color and oxygen permeability on salmon oil quality. Oral presentation. The 6th International Conference on Environmental Science and Technology, June 25-29, 2012, Hilton Hotel, Houston, Texas, USA.
13. **Akhtar, M. J.,** Jacquot, M., Tehrany, E. A., Desobry S. Physico-chemical characterization of coloured HPMC films with anthocyanins and ediblecolours. Poster presentation. Séminaire de l'école doctorale RP2E, Janvier 2010.
14. Jacquot, M., **Akhtar, M. J.,** Tehrany, E. A., Gaïani, C., Linder, M., Desobry S. Edible packaging based on hydroxypropyl methyl cellulose (HPMC) effect of HPMC film's color on photo-oxidation of salmon oil. Poster presentation. IFT 2008, Annual meeting + Expo, New Orleans, USA.
15. Muriel Jacquot, **Muhammad Javeed Akhtar**, Elmira Arab-Tehrany, Claire Gaïani, Michel Linder, Stéphane Desobry. Role of coloured bio-polymer films against photo-oxidation of salmon oil. Poster presentation. 8th World Congress of Chemical Engineering, August, 23-27, 2009, Montreal, Canada.

VII. REFERENCES BIBLIOGRAPHIQUES

Abidi, N., Hequet, E., Turner, C., & Sari-Sarraf, H. (2005). FTIR analysis of crosslinked cotton fabric using a ZnSe-universal attenuated total reflectance. *Journal of Applied Polymer Science, 96,* 392–399.

Ackman R. G. (1998). Laboratory preparation of conjugated linoleic acids. *Journal of the American Oil Chemists Society, 75,* 541-545.

AFNOR, (2007). Association Française de Normalisation, FD X08-012-2, *(www.afnor.org).*

Aider, M. (2010). Chitosan application for active bio-based films production and potential in the food industry: review. *LWT-Food Science and Technology, 43,* 837–842.

Akhtar, M. J., Jacquot, Desobry, S. (2013). Effect of HPMC–Anthocyanin Packaging Color and Oxygen Permeability on Salmon Oil Preservation. Food and Bioprocess Technology, Article in press.

Akhtar, M. J., Jacquot, M., Jasniewski, J., Jacquot, C., Imran, M., Jamshidian, M., Paris, C., & Desobry, S. (2012). Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract, *Carbohydrate Polymers, 89,* 1150–1158.

Akhtar, M. J., Jacquot, M., Tehrany, E. A., Gaiani, C., Linder, M., & Desobry, S. (2010). Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film colour. *Food Chemistry, 120,* 395–401.

Alexander Saffert, G. P. J. J. (2006). Effect of package light transmittance on the vitamin content of pasteurized whole milk. *Packaging Technology and Science, 19,* 211-218.

Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A., Bora, U. (2008). Indian medicinal herbs as sources of antioxidants. *Food Research International, 41,* 1–15.

Al-Tabakha, M. M., (2010). HPMC capsules: Current status and future prospects. *Journal of Pharmacy and Pharmaceutical Sciences, 13,* 428–442.

Altenhofen, M., Krause, A. C., & Guenter, T. (2009). Alginate and pectin composite films crosslinked with Ca^{+2} ions: effect of the plasticizer concentration. *Carbohydrate Polymers, 77,* 736–742.

Alves, R. M. V., Van Dender, A. G. F., Jaime, S. B. M., & Moreno, I. (2002). Stability of ‘‘Requeijâo cremoso’’ in different packages exposed to light. Poster, Congrilait 2002, 26th International Dairy Conference, Paris, France, 24–27 September, 2002.

AMS/USDA, (2004). Hydroxy methylcellulose processing. Agricultural Marketing Service (AMS)/United States Department of Agriculture (USDA), Washington, DC.

Anker, M., Stading, M., & Hermansson, A. M. (2000). Relationship between the Microstructure and the Mechanical and Barrier Properties of Whey Protein Films. *Journal of Agricultural and Food Chemistry, 48,* 3806–3816.

Anker, M., Stading, M., & Hermansson, A. M. (2001). Aging of Whey Protein Films and the Effect on Mechanical and Barrier Properties. *Journal of Agricultural and Food Chemistry, 49,* 989–995.

Anon, (1994). Packaging and Packaging Waste Directive. 94/62/EC, EU Commision, Brussels, Belgium.

Aouada, F. A., deMoura, M. R., Orts, W. J., & Mattoso, L. H. C. (2011). Preparation and characterization of novel micro- and nanocomposite hydrogels containing cellulosic fibrils. *Journal of Agricultural and Food Chemistry, 59,* 9433–9442.

Arcan, I., Yemenicioğlu, A. (2011). Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. *Food Research International, 44,* 550–556.

Arvanitoyannis, I., Psomiadou, E., Nakayama, A., Aiba, S., & Yamamoto, N. (1997). Edible films made from gelatin, soluble starch and polyols, Part 3. *Food Chemistry, 60,* 593–604.

ASTM, (1999). Standard test methods for thickness of solid electrical insulation. In Annual book of American Standard Testing Methods, D374–99. Philadelphia, PA: ASTM.

ASTM. (2002). Standard method for tensile properties of thin plastic sheeting. In Annual book of American Standard Testing Methods, D 882-02. Philadelphia, PA: ASTM.

Attoe, E. L. & von Elbe, J. H. (1981). Photochemical degradation of betanine and selected anthocyanins. *Journal of Food Science, 46,* 1934–1937.

Avérous, L., & Martin, O. (2001). Poly(lactic acid): plasticization and properties of biodegradable multiphase systems. *Polymer, 42,* 6209–6219.

Averous, L., Moro, L., Dole, P., & Fringant. (2000). Properties of thermoplastic blends: starchepolycaprolactone. *Polymer, 41,* 4157–4167.

Ayranci, E., & Tunc, S. (2003). A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. *Food Chemistry, 80,* 423–431.

Ayranci, E., Tunc, S. (1997). Cellulose-based edible films and their effects on fresh beans and strawberries. *Z Lebensm Unters Forsch A, 205,* 470-473.

Bailey, D. T., Freeberg, D. R., Gertenbach, D., Gourdin, G. T., Richheimer, S. L., Tempesta, M. S., Daugherty, J. (2005). Compositions enriched in anthocyanins. US20050037095.

Baldwin, E. A., Nisperos-Carriedo, M. O., & Baker, R. A. (1995). Use of edible coatings to preserve quality of lightly (and slightly) processed products. *Crit. Rev. Food Sci.* 35, 509–524.

Baldwin, E. A., Wood, B. (2006). Use of edible coating to preserve pecans at room temperature. *Hort Science, 41,* 188–192.

Barnes, D. K. A., Galgani, F., Thompson, R. C. & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. *Phil. Trans. R. Soc. B 364,* 1985– 1998.

Bekbölet, M. (1990). Light effects on food. Journal of Food Protection, 53, 430–440.

Bekhit, A. E. D., Faustman, C. (2005). Metmyoglobin reducing activity. *Meat Science, 71,* 407–439.

Belhaj N, Arab-Tehrany E, Linder M. (2010). Oxidative kinetics of salmon oil in bulk and in nanoemulsion stabilized by marine lecithin. *Process Biochemistry, 45,*187–95.

Bell, D. R., Gochenaur, K. (2006). Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. *Journal of Applied Physiology 100,* 1164–1170.

BeMiller, J. N., & Whistler, R. L. (1996). Carbohydrates. In: Fennema, O.R. (Ed.), *Food Chemistry*, third ed. Marcel Dekker, Inc., New York, NY, pp. 205-207.

Bertuzzi, M. A., Armada, M., & Gottifredi, J. C. (2007). Physicochemical characterization of starch based films. *J. Food Eng. 82,* 17–25.

Bhale, S. D., Xu, Z., Prinyawiwatkul, W., King, J. M., & Godber, J. S. (2007). Oregano and rosemary extracts inhibit oxidation of long-chain n-3 fatty acids in menhaden oil. *Food Science, 72,* 504–508.

Bico, S. L. S., Raposo, R. M. S., Morais, C., & Morais, A. M. M. B. (2009). Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut banana. *Food Control, 20,* 508–514.

Bifani, V., Ramirez, C., Ihl, M., Rubilar, M., Garcia, A., & Zaritzky, N. (2007). Effects of murta (Ugni molinae Turcz) extract on gas and water vapor permeability of carboxymethylcellulose-based edible films. *LWT–Food Science and Technology, 40,* 1473–1481.

Bleach, C. N., Nazhat, N. S., Tanner, E. K, Kellomäki, M., & Tormälä P. (2002). Effect of filler content on mechanical and dynamic mechanical properties of particular biphasic calcium phosphate-polylactide composites. *Biomaterials 23,* 1579–1585.

Bonilla, F., Mayen, M., Merida, J., & Medina, M. (1999). Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chemistry, 66,* 209–215.

Borle, F., Sieber, R., & Bosset, J. O. (2001). Photo-oxidation and photoprotection of foods, with particular reference to dairy products. An update of a review article (1993–2000). *Sciences des Aliments, 21,* 571–590.

Borzi, A. (1887). Le communicazioni intracellulari delle Nostochinae. *Malpighia 1,* 28–74.

Bosquez-Molina, E., Tomás, S. A., & Rodríguez-Huezo, M. E. (2010). Influence of $CaCl_2$ on the water vapor permeability and the surface morphology of mesquite gum based edible films. *LWT-Food Science and Technology, 43,* 1419–1425.

Bosset, J. O., & Flückiger, E. (1989). Packaging and maintenance of food quality: Effect on photosensitivity of different types of yoghurt. *Lebensmittel Wissenschaft und-Technologie, 22,* 292–300.

Bosset, J. O., Gallmann, P. U., & Sieber, R. (1994). Influence of light transmittance of packaging materials on the shelf life of milk and dairy products–a review. In M. Mathlouthi (Ed.), *Food Packaging and Preservation* (pp. 222–268). Glasgow: Blackie Academic & Professional.

Braunegg, G., Bona, R., Schellauf, F., & Wallner, E. (2002). Polyhydroxyalkanoates (PHAs): sustainable biopolyester production. *Polymer, 47,* 479–484.

Breivik, H., Jacobsen, C., Moen, V., Vojnovic, T. (2010). Antioxidant composition for marine oils comprising tocopherol, rosemary extract, ascorbic acid and green tea extract. WO2010033034

Brindle, L. P., & Krochta, J. M. (2008). Physical properties of whey protein hydroxypropyl methylcellulose blend edible films. *Journal of Food Science, 73,* 446-454.

Brown, A. J. (1886). On an acetic ferment which forms cellulose. *J. Chem. Soc. 49,* 432–439.

Brown, R. M. (1996). The Biosynthesis of Cellulose. *Pureand Applied Chemistry, 33,* 1345–1373.

Burdock, G. A. (2007). Safety assessment of hydroxypropyl methylcellulose as a food ingredient. *Food and Chemical Toxicology, 45,* 2341–2351.

Burke, J. R. (2006). Biodegradeable compostable packaging: The promise and the problems. *Paper and Packaging, 47,* 5.

Burt, S. A. (2004). Essential oils: their antibacterial properties and potential applications in food–a review. *Int. J. Food Microbiol. 94,* 223–53.

Cai, Y., & Corke, H. (1990). Amaranthus β -cyanin pigments applied in model food systems. *J. Food Sci. 64,* 869–873.

Cai, Y., Sun, M. & Corke, H. (1998). Colourant properties and stability of Amaranthus betacyanin pigments. *Journal of Agricultural and Food Chemistry, 46,* 4491–4495.

Cai, Y., Sun, M., & Corke, H. (2003). Antioxidant activity of betalains from plants of the amaranthaceae. *Journal of Agricultural and Food Chemistry, 51,* 2288–94.

Cai, Y., Sun, M., Schliemann, W., and Corke, H. (2001). Chemical stability and colorant properties of β-xanthin pigments from Celosia argentea. *J. Agric. Food Chem. 49,* 4429–4435.

Caraballo, I. (2010). Factors affecting drug release from hydroxypropyl methylcellulose matrix systems in the light of classical and percolation theories. *Expert Opinion on Drug Delivery, 7,* 1291–1301.

Carneiro-da-Cunha, M. G., Cerqueira, M. A., Souza, W. M. B., Souza, M. P., Teixeira, J. A., & Vicente, A. A. (2009). Physical properties of edible coatings and films made with a polysaccharide from Anacardium occidentale L. *Journal of Food Engineering, 95,* 379–385.

Castellanos-Santiago, E., & Elhadi M. Y. (2008). Identification and quantification of betalains from the fruits of 10 Mexican prickly pear cultivars by high-performance liquid chromatography and electrospray ionization mas spectrometry. *Journal of Agricultural and Food Chemistry, 56,* 5758–5764.

Cemeli E, Baumgartner A, Anderson D. (2009). Antioxidants and the Comet assay. *Mutation, 681*: 51-67.

Cerqueira, M. A., Pinheiro, A. C., Souza, B. W. S., Lima, A. M. P., Ribeiro, C., Miranda, C. (2009a). Extraction, purification and characterization of galactomannans from non-traditional sources. *Carbohydrate Polymers, 75,* 408–414.

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2006). Changes of lipids in sardine (Sardinella gibbosa) muscle during iced storage. *Food Chemistry, 99,* 81–83.

Chambi, H., & Grosso, C. (2006). Edible films produced with gelatin and casein cross-linked with transglutaminase. *Food Research International, 39,* 456–458.

Chandra, R. and Rustgi, R. (1996). The Biosynthesis of Cellulose. *Pureand Applied Chemistry, 33,* 1345–1373.

Chandrasekaran, J. (2001). Chemistry of colours. *Resonance, 6,* 66–75.

Chapman, K. W., Whited, L. J., & Boor, K. J. (2002). Sensory threshold of light-oxidized flavor defects in milk. *Journal of Food Science, 67,* 2770–2773.

Charron, C. S., Kurilich, A. C., Clevidence, B. A., Simon, P. W., Harrison, D. J., Britz, S. J., Baer, D. J., & Novotny, J. A. (2009). Bioavailability of anthocyanins from purple carrot juice: Effects of acylation and plant matrix. *Journal of Agricultural and Food Chemistry, 57,* 1226–1230.

Chaudiere J, Ferrari-Iliou R. (1999). Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem Toxicol; 37,* 949–62.

Chen H, Tappel A. (1996). Protection by multiple antioxidants against lipid peroxidation in rat liver homogenates. *Lipids, 31,* 47–50.

Chen, C. H., & Lai, L. S. (2008). Mechanical and water vapor barrier properties of tapioca starch/decolorized hsian-tsao leaf gum films in the presence of plasticizer. *Food Hydrocolloids, 22,* 1584–1595.

Chen, H. H. (2007). Rheological properties of HPMC enhanced Surimi analyzed by small- and large-strain tests: I. The effect of concentration and temperature on HPMC flow properties. *Food Hydrocolloids*, *21*, 1201-1208.

Chen, M-C., Yeh, G. H-C., & Chiang, B-H. (1996). Antimicrobial and physico-chemical properties of methylcellulose and chitosan films containing a preservative. *J. Food Process. Preserv., 20,* 379–390.

Chetan, A., Nayak, S., Chethana, N. K., Rastogi, K.S.M.S. Raghavarao, (2006). Enhanced mass transfer during solid-liquid extraction of gamma-irradiated red beetroot. *Radiation Physics and Chemistry, 75,* 173–178.

Chidanandaiah, R. C. K., Sanyal, M. K. (2009). Effect of sodium alginate coating with preservatives on the quality of meat patties during refrigerated (4± 1ºC) storage. *Journal of Muscle Foods, 20,* 275–292.

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety, 5,* 169–186.

Cholli, A. L., Kumar, V., Kumar, J., Parmar, V. S., Samuelson, L. A., Bruno. F. F., Dhawan, A. (2004). New substituted benzene antioxidant polymer useful for inhibiting oxidation of substance, e.g. edible products or oils. WO2003087260, WO2003087260, US2003230743, US2004214935 & EP1492856.

Christen S, Woodall A. A, Shigenaga M. K, Southwell-Keely P. T, Duncan M. W, Ames B. N. (1997). Gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. Proc Natl Acad Sci USA: 3217–22.

Chung, H. J., Colakoglu, A. S., & Min, D. B. J. (2004). Relationships among headspace oxygen, peroxide value and conjugated diene content of soybean oil oxidation. *Journal of Food Science, 69,* 83–88.

CIR, (1986). Cosmetic ingredient review (CIR): final report on the safety assessment of hydroxyethylcellulose, hydroxypropyl cellulose, methylcellulose, hydroxypropyl methylcellulose, and cellulose gum. *Journal of the American College of Toxicology 5,* 1–59.

Cladman, W., Scheffer, S. Goodrich, N., & Griffiths, M. W. (1998). Shelf-life of milk packaged in plastic containers with and without treatment to reduce light transmission. *International Dairy Journal, 8,* 629–636.

Clydesdale, F. M. (1997). Hydroxypropyl methylcellulose. In: Food Additives: Toxicology, Regulation and Properties. CRC Press, Boca Raton, FL, CD-ROM.

Colliver, S. P., Hughes, S. G., Muir, S. R., van Tunen, A. J., Verhoeyen, M. E. (2007). Process for increasing the flavonoid content of a plant and plants obtainable thereby. US7208659.

Coma V., Sebti I., Pardon P., Pichavant F. H. Deschamps A. (2003). Film properties from crosslinking of cellulosic derivatives with a polyfunctional carboxylic acid *Carbohydrate Polymers, 51,* 265–271.

Coma, V. (2008). *Meat sciences, 78,* 90–103.

Corrales, M., Han, J. H., & Tauscher, B. (2009). Antimicrobial properties of grape seed extracts and their effectiveness after incorporation into pea starch films. *Int. J. Food Sci. Technol. 44,* 425–433.

Crompton, T. R. (1979). Additive migration from plastics into food, Pergamon press, Oxford.

Cuomo, J., Rabowskiy, A. B. (2002). Antioxidant compositions extracted from olives and olive by-products. US6358542.

Cuq, B., Aymard, C., Cuq, J., & Guilbert, S. (1995). Edible packaging films based on fish myofibrillar proteins: Formulation and functional properties. *J. Food Sci., 60,* 1369−1374.

Cuq, B., Gontard N., & Guilbert, S. (1998). Proteins as agricultural polymers for packaging production. *Cereal Chemistry, 75,* 1-9.

Dai, J., Patel, J. D., & Mumper, R. J. (2007). Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *Journal of Medicinal Food, 10,* 258–265.

Davis, G., & Song, J. H. (2006). Biodegradable packaging based on raw materials from crops and their impact on waste management. *Industrial Crops and Products, 23,* 147-161.

DEFRA, (2004). A strategy for non-food crops and uses. London: Department for Environment, Food and Rural Affairs, PB10188.

DEFRA, (2007). Waste strategy for England 2007. PB12596. London, UK: Department for Environment, Food and Rural Affairs.

Deger, D., & Ashoor, S. H. (1987). Light-induced changes in taste, appearance, odor, and riboflavin content of cheese. *Journal of Dairy Science, 70,* 1371–1376.

Del Nobile, M. A., Conte, A., Incoronato, A. L., & Panza, O. (2008). Antimicrobial efficacy and release kinetics of thymol from zein films. *Journal of Food Engineering, 89,* 157–63.

DeMan, J., M. (1978). Possibilities of prevention of light-induced quality loss of milk. *Canadian Institute of Food Science and Technology Journal, 11,* 152–154.

deMoura, M. R., Avena-Bustillos, R., McHugh, T. H., Wood, D. F., Otoni, C. G., & Mattoso, L. H. C. (2011). Miniaturization of cellulose fibers and effect of addition on the mechanical and barrier properties of hydroxypropyl methylcellulose films. *Journal of Food Engineering, 104,* 154–160.

Desobry, S., & Hardy, J. (1997). The increase of CO_2 permeability of paper packaging with increasing hydration. *International Journal of Food Science and Technology, 32,* 407–410.

Devlieghere, A., Vermeulen, F., & Debevere, J. (2004). Chitosan: antimicrobial activity, interaction with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21,* 703–714.

Díaz, F. S., López1, E. M. S., Kerstupp1, S. F., Ibarra1, R. V., & Scheinvar, L. (2006). Colorant extraction from red prickly pear (opuntia lasiacantha) for food application. *Electronic Journal of Environmental, Agricultural and Food Chemistry, 5,* 1330–1337.

Dobarganes, M. C. & Velasco, J. (2002). Analysis of lipid hydroperoxides. *European Journal of Lipid Science and Technology, 104,* 420–428.

Drusch, S., & Berg, S. (2008). Extractable oil in microcapsules prepared by spray- drying: Localization, determination and impact on oxidative stability. *Food Chemistry, 109,* 17–24.

Du, W. X., Olsen, C. W., Avena-Bustillos, R. J., McHugh, T. H., Levin, C. E., & Friedman, M. (2008). Antibacterial activity against E. coli O157:H7, physical properties, and storage stability of novel carvacrol-containing edible tomato films. *J. Food Sci. 73,* 378–383.

Duan, J., Cherian, G., Zhao, Y. (2010). Quality enhancement in fresh and frozen lingcod (Ophiodon elongates) fillets by employment of fish oil incorporated chitosan coatings. *Food Chemistry, 119,* 524–532.

Eberhardt M. V, Lee C. Y, Liu R. H. (2000). Nutrition- Antioxidant activity of fresh apples. *Nature, 405,* 903-4.

EC, (1995). European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners. *Official Journal of the European Communities No. L6*1, 18–3.

Einbond L. S, Reynertson K. A, Luo X. D, Basile M. J, Kennelly E. J. (2003). Anthocyanin antioxidants from edible fruits. *Food Chem. 84,* 23–8.

Enrione, J. I., Hill, S. E., & Mitchell, J. R. (2007). Sorption behavior of mixtures of glycerol and starch. *Journal of Agricultural and Food Chemistry, 55,* 2956–2963.

Erkan, N., Ayranci, G., Ayranci, E. (2008). Antioxidant activities of rosemary (Rosmarinus officialis L.) Extract, blackseed (Nigella sativa L.) Essential oil, carnosic acid, rosmarinic acid and sesamol. *Food Chemistry 110,* 76–82.

Escribano, J., Pedreño, M. A., Garcia-Carmona, F., & Munoz, R. (1998). Characterization of the antiradical activity of betalains from Beta vulgaris L. roots. *Phytochemical analysis, 9,* 124–127.

Eswaranandam, S., Hettiarachchy, N. S., & Meullenet, J. F. (2006). Effect of malic and lactic acid incorporation to soy protein coatings on the sensory attributes of whole apple and fresh-cut cantaluope. *J. Food Sci. 71,* 307–313.

EU. (2004). Regulation (EC) No. 1935/2004 European Parliament and the Council of 27 October 2004 on materials and articles intended to come into contact with food repealing.

Fabra, M. J., Jimenez, A., Atares, L., Talens, P., & Chiralt, A. (2009). Effect of fatty acids and beeswax addition on properties of sodium caseinate dispersions and films. *Biomacromolecules, 10,* 1500–1507.

Fabra, M. J., Talens, P., & Chiralt, A. (2009). Microstructure and optical properties of sodium caseinate films containing oleic acid-beeswax mixtures. *Food Hydrocolloid, 23,* 676–683.

Fabre, C. E., Santeri, A. L., Lorete, M. O., Baberian, R., Pareilleux, A., Goma, G., & Blanc, P. J. (1993). Production and food applications of the red pigments of Monascus rubber. *Journal of Food Science, 58,* 1099–1111.

Falguera, V., Pagan, J., & Ibarz, A. (2011). Effect of UV irradiation on enzymatic activities and physicochemical properties of apple juices from different varieties. *LWT-Food Science and Technology, 44,* 115–119.

Falguera, V., Quintero, J. P., Jiménez, A., Muñoz, J. A., Ibarz, A. (2011). Edible films and coatings: Structures, active functions and trends in their use. *Trends in Food Science & Technology 22,* 292-303.

Fang, J., & Fowler, P. (2003). The use of starch and its derivatives as biopolymer sources of packaging materials. *Food, Agriculture & Environment, 1,* 82–84.

Fang, Y., Tung, M. A., Britt, I. J., Yada, S., & Dalgleish, D. G. (2002). Tensile and barrier properties of edible films made from whey proteins. *Journal of Food Science, 67,* 188-193.

Fasseas M. K, Mountzouris K. C, Tarantilis P. A, Polissiou M, Zervas G. (2007). Antioxidant activity in meat treated with oregano and sage essential oils. *Food Chem, 106,* 1188–94.

Fellenberg, P. M. A., Alarcon, C. J. G., Sainz, L. J. I. (2008). Favoring composition for favoring the preservation of meat food, preferably through antioxidant effect, includes barksoap tree extract from barksoap tree polyphenols and polysaccharides, where small part of saponins may be included. EP1849367 & US2008038431.

Feng T, Yumin D, Li J, Hu Y, Kennedy J. F. Enhancement of antioxidant activity of chitosan by irradiation. *Carbohydr Polym 2007, 73,* 126–32.

Flores, S., Haedo, A., Campos, C., & Gerschenson, L. (2007b). Antimicrobial performance of potassium sorbate supported in tapioca starch edible films. *Eur. Food Res. Technol. 225,* 375–384.

Fossen, M. & Mulder. W. (1998). Die Anwendung vonPflanzenproteinen im Non-Food Bereich, LVT, nr. 43, p. 108.

Frank, T., Conrad, F., Carle, S. R. Bitsch, I., Daniela, Q., Gabriele, B., Roland, B., & Michael, N. (2005). Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. *Pharmacological Research, 52,* 290–297.

Frankel E.N., (1985). Chemistry of autoxidation: mechanism, products and flavor significance. *Flavor chemistry of fats and oils*. Eds D.B. Min, T.H. Smouse. AOCS Champaign: 1-37.

Frankel, E. N., Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture, 80,* 1925–1941.

French, D. (1984). Organization of starch granules. In: Whistler LR, Bemiller N. J, Paschall FE, editors. Starch: Chemistry and Technology, 2nd ed. New York: Academic Press. p. 184–248.

Fukumoto, S., Tsuruhami, K., Mori, S. (2007). Antioxidant material, antideterioration agent and food or beverage. US20070244189.

Fullmer, L., Emmick T. (2005). Carotenoid nanodispersions for use in water-based systems and a process for their preparation. US20050037115.

Galati, E. M., Mondello, M. R., Giuffrida, D., Dugo, G., Miceli, N., and Pergolizzi, S. (2003). Chemical characterization and biological effects of Sicilian Opuntia ficus indica (L.) Mill. Fruit juice: Antioxidant and antiulcerogenic activity. *J. Agric. Food Chem. 51*, 4903–4908.

Garcia, M. A., Martino, M. N., & Zaritzky, N. E. (2000). Lipid addition to improve barrier properties of edible starch-based films and coating. *Journal of Food Science, 65,* 941–946.

Garde, A., Schmidt, A. S., Jonsson, G., Andersen, M., Thomsen,A. B.,Ahring, B. K. and Kiel, P. (2000). Agricultural Cropsand Residuals ASA Basis for Polylactate Production in Denmark. Proceedings of the Food Biopack Conference, Copenhagen, 27–29 August 2000, pp. 45–51.

Garlotta, D. (2001). A literature review of poly(lactic acid). *J Polym Environ, 9,* 63–84.

Gaynor, D. A., Nicholson, M. D., Ducharme, P. E. (2004). Casing for processing foodstuff, e.g. meat product, has cellulose or polymeric film with meat emulsion contacting surface coated with antibacterial shirring solution of antibacterial nisin and ascorbate or erythrobate antioxidant. EP1369045.

Gemili, S., Yemenicioglu, A., & Altinkaya, S. A. (2010). Development of antioxidant food packaging materials with controlled release properties. *Journal of Food Engineering, 96,* 325–332.

Gentile, C., Tesoriere, L., Allegra, M., Livrea, M. A., & Alessio, P. (2004). Antioxidant betalains from cactus pear (Opuntia ficus-indica) inhibits endothelial ICAM-expression. *Annals of the New York Academy of Sciences, 1028,* 481–486.

Gertz, C. (2003). Improving the useful life and cooking characteristics of fats by addition of a water-in-oil emulsion containing antioxidants in combination with water-soluble carboxylic acids. EP1277408 & US2003026887.

Gill, A. O., & Gill, C. O. (2005). Preservative packaging for fresh meats, poultry, and fin fish. In: Han, J.H. (Ed.), Innovation in Food Packaging. Elsevier Academic Press, pp. 205–226.

Gimenez, B., Gomez-Guillen, M. C., Perez-Mateos, M. (2011). Evaluation of lipid oxidation in horse mackerel patties covered with borage-containing film during frozen storage. *Food Chemistry, 124,* 1393–1403.

Glässgen, W. E., Seitz, H. U., & Metzger, J. W. (1992). High performance liquid chromatography electrospray mass spectrometry and tandem mass spectrometry of anthocyanins from plant tissues and cell cultures of Daucus carota L. *Biological Mass Spectrometry, 21,* 271–277.

Gómez-Estaca, J., Bravo, L., Gómez-Guillén, M. C., Alemán, A., & Montero, P. (2009a). Antioxidant properties of tuna-skin and bovine hide gelatin films induced by the addition of oregano and rosemary extracts. *Food Chemistry, 112,* 18–25.

Gómez-Estaca, J., Giménez, B., Montero, P., Gómez-Guillén, M. C. (2009b). Incorporation of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. *Journal of Food Engineering, 92,* 78–85.

Gómez-Estaca, J., Montero, P., Giménez, B., Gómez-Guillén, M. C. (2007). Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (Sardina pilchardus). *Food Chemistry, 105,* 511–520.

Gontard, N., Guilbert, S., & Cuq, J. L. (1993). Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *Journal of Food Science. 58,* 206–211.

Goursaud, J. (1996). Packaging of milk products. In Food Packaging Technology, Vol. 2. (pp. 251–276). Weinheim: VCH.

Graaf, de L. A. and Kolster, P. (1998). Industrial Proteins as Green Alternative for "petro" polymers: Potentials and Limitations. Macro-Molecular Symposia, 127, p. 51.

Greaves, J. A., Brinkhaus, F., Haworth, J. E. (2005). Method for simultaneous extraction of essential oils and antioxidants from Labiatae species and the products thereof. US6855349.

Greenfield, A. B. (2005). A Perfect Red: Empire, Espionage, and the Quest for the Color of Desire, pp. 338. Harper Collins, New York.

Gregory, M. R. (2009). Environmental implications of plastic debris in marine settings entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Phil. Trans. R. Soc. B 364,* 2013–2025.

Gross, R. A., & Scholz, C. editors. (2001). Biopolymers from polysaccharides and agroproteins. Washington, DC: American Chemical Society.

Guiga, W., Galland, S., Peyrol, E., Degraeve, P., Carnet-Pantiez, A., & Sebti, I. (2009). Antimicrobial plastic films: Physico-chemical characterization and nisin desorption modeling. *Innovative Food Science and Emerging Technologies, 10,* 203–207.

Guilbert, S., Ed. (1986). Technology and Application of Edible Protective Films; Elsevier Applied Science Publishers Ltd.: New York.

Guilbert, S., Gontard, N., & Gorris, L. G. M. (1996). Prolongation of the shelf-life of perishable food products using biodegradable films and coatings. *Lebensmittel Wissenschaft und–Technologie, 29,* 10–17.

Guillén, M. D., Ruiz, A., & Cabo, N. (2004). Study of the oxidative degradation of farmed salmon lipids by jeans of Fourier transform infrared spectroscopy. Influence of salting. *Journal of the Science of Food and Agriculture, 84,* 1528–1534.

Gülçin I. (2010). Antioxidant properties of resveratrol: A structure activity insight. Innov Food Sci Emerg Technol; 11: 210-8.

Haisman, D. R., Groenendijk, C. B. M., & O'Sullivan, G. J. (1992). The effect of light on flavour and nutritional content of milk. Paperboard vs. plastic containers. *Food Technology New Zealand, 27,* 16–20.

Hamaguchi, P. Y., Weng, W. Y., & Tanaka, M. (2007). Effect of pH on the formation of edible films made from the muscle proteins of Blue marin (Makaira mazara). *Food Chemistry, 100,* 914–920.

Hamid, S. H., Amin, M. B., & Maadhah, A. G. (1992). Handbook of polymer degradation. Marcel Dekker, New York, Hong Kong, p 55.

Hamilton, R. J., Kula, C., McNeill, G. P., Padley, F. B., & Pierce, J. H. (1998). Effects of tocopherols, ascorbyl palmitate and lecithin on autoxidation of fish oil. Journal of the American Oil Chemists' Society, *75,* 813-823.

Han, J. H., & Krochta, J. M. (2007). Physical properties of whey protein coating solutions and films containing antioxidants. *Journal of Food Science, 72,* 308–314.

Han, J. H., & Floros, J. D. (1997). Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. *Journal of Plastic Film and Sheet*, *13,* 287-298.

Han, J.H. and Gennadios, A. (2005). Edible films and coatings: a review. *In Innovations in Food Packaging.* Elsevier Ltd.

Hanif, M. A., Al-Maskari, M. Y., Al-Maskari, A., Al-Shukaili, A., Al-Maskari, A. Y., & Al-Sabahi, J. N. (2011). Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil. *Journal of Medicinal Plants Research, 5,* 751–757.

Hansen, E. (1996). Light-induced oxidative changes in dairy spreads. Project report. Copenhagen: Department of Dairy and Food Science. The Royal Veterinary and Agricultural University.

Hara, N., Kiyama, K., Akamatsu, T., Yasue, R. Microcapsules for foods and drugs-have multi-core structure containing natural carotenoid, tablets and additives. JP9302379, US5780056 & JP3555640 (1997).

Hari, R. K., Patel, T. R., & Martin, A. M. (1994). An overview of pigment production in biological systems: functions, biosynthesis and applications in food industry. *Food reviews International, 10,* 49–70.

Herbach, K. M., Stintzing, F. C., & Carle, R. (2006b). Betalain stability and degradation: structural and chromatic aspects. *Journal of Food Science, 71,* 41–50.

Herbach, K. M., Stintzing, F. C., Elss, S., Preston, C., Schreier, P., Carle, R. (2006). Isotope ratio mass spectrometrical analysis of betanin and isobetanin isolates for authenticity evaluation of purple pitaya-based products. *Food Chemistry, 99,* 204-209.

Hocking, P. J., Marchessault, R. H., Timmins, M. R., Scherer, T. M. Lenz, R. W., & Fuller, R. C. (1994). Enzymatic degradability of isotactic versus syndiotactic poly(Beta-Hydroxybutyrate). *Macromolecular Rapid Communications, 15,* 447–452.

Hong, C. M., Wendorff, W. L., & Bradley Jr., R. L. (1995b). Effects of packaging and lighting on pink discoloration and lipid oxidation of annatto-colored cheeses. *Journal of Dairy Science, 78,* 1896–1902.

Hong, C. M., Wendorff, W. L., & Bradley Jr., R. L. (1995b). Effects of packaging and lighting on pink discoloration and lipid oxidation of annatto-colored cheeses. *Journal of Dairy Science, 78,* 1896–1902.

Honkanen, O., Steppe, H., Eerikainen, S., Tuominen, R., Marvola, M. (2001). Bioavailability of ibuprofen from orally and rectally administered hydroxypropyl methyl cellulose capsules compared to corresponding gelatine capsules. STP (Sciences Techniques et Pratiques Pharmaceutiques) *Pharma Sciences, 11,* 181–185.

Houhoula, D. P., Oreopoulou, V., & Tzia, C. (2003). Antioxidant efficiency of oregano during frying and storage of potato chips. *Journal of the Science of Food and Agriculture, 83,* 1499–1503.

Hsu, S. L. C., Chen, H. T., & Tsai, (2004). Novel positive-working and aqueous-base-developable photosensitive poly (imide benzoxazole) precursor. *Journal of polymer science, 42,* 5990–5998.

Huang, A. S., & von Elbe, J. H. (1986). Stability comparison of two betacyanine pigments amaranthine and betanine. *Journal of Food Science, 51,* 670–674.

Hutchings, J-B (1999) Food color and appearance, Maryland: Aspen Publisher, USA.

Iguchi, M. and Yamanaka, S. and Budhini, A. (2000). BacterialCellulose-A Masterpiece of Natures Arts. *Journal of Materials Science. 35,* 1-10.

Imran, M., El-Fahmy, S., Revol-Junelles, A-M., Desobry, S. (2010). Cellulose derivative based active coatings: Effects of nisin and plasticizer on physico-chemical and antimicrobial properties of hydroxypropyl methylcellulose films. *Carbohydrate Polymers. 81,* 219–225.

Innawong, B., Mallikarjunan, P., Irudayaraj, J., & Marcy, J. E. (2004).The determination of frying oil quality using Fourier transform infrared attenuated total reflectance *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 37,* 23–28.

International Federation of Fruit Juice Producers (1998). IFU method 71: Anthocyanins by HPLC. IFU, Zug, Switzerland.

Jamshidian, M., Tehrany, E. A., & Desobry, S. (2012). Antioxidants Release from Solvent-Cast PLA Film: Investigation of PLA Antioxidant-Active Packaging. *Food Bioprocess Technol, 10,* 1–14.

Jamshidian, M., Tehrany, E. A., Imran, M., Jacquot, M., & Desobry, S. (2010). Poly-lactic acid: production, applications, nanocomposites, and release studies. *Comprehensive Reviews in Food Science and Food Safety, 9,* 552–571.

JECFA, (2004). Summary of evaluations performed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Hydroxypropylmethyl cellulose. Joint FAO/WHO Expert Committee on Food Additives.

Jeon, Y.-I., Kamil, J. Y. V. A., Shahidi, F. (2002). Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *Journal of Agricultural and Food Chemistry, 20,* 5167–5178.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012b). Influence of hydroxypropyl methylcellulose addition and homogenization conditions on properties and ageing of corn starch based films. *Carbohydrate Polymers, 89,* 676–686.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2010). Effect of lipid self-association on the microstructure and physical properties of hydroxypropyl-methylcellulose edible films containing fatty acids. *Carbohydrate Polymers, 82,* 585–593.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012a). Effect of re-crystallization on tensile, optical and water vapour barrier properties of corn starch films containing fatty acids. *Food Hydrocolloids, 26,* 302–310.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012c). Effect of sodium caseinate on properties and ageing behaviour of corn starch based films. *Food Hydrocolloids, 29,* 265–271.

John Coates, (2000). Interpretation of Infrared Spectra, A Practical Approach in Encyclopedia of Analytical Chemistry R.A. Meyers (Ed.) John Wiley & Sons Ltd, Chichester, pp. 10815–10837.

Jonathan Sachs Color Management Terms Copyright© 2001–2002 Digital Light & Color.

Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. (2006). Effects of plasticizers on the properties of edible films from skin gelatin of big eye snapper and brown stripe red snapper. *European Food Research and Technology, 222,* 229–235.

Jutaporn, C. T., Suphitchaya, C., & Thawien, W. (2011). Antimicrobial activity and characteristics of edible films incorporated with Phayom wood (Shorea tolura) extract. *International Food Research Journal, 18,* 39–54.

Kaiser S, Di Mascio P, Murphy ME, Sies H. (1990). Physical and chemical scavenging of singlet molecular oxygen by tocopherols. *Arch Biochem Biophys, 277,* 101–8.

Kajimoto, G., Yamaguchi, M., Kasutani, S., Yoshida H., & Shibahara, A. (1994). Influence of synthetic food colorants on oxidative deterioration of oil. *Nippon Shokuhin Kogyo Gakkaishi, 41*, 793–796.

Kalia, V. C., Raizada, N., & Sonakya, V. (2000). *Bioplastics. J Sci Ind Res 59,* 433–445.

Kamil, J. Y. V. A., Jeon, Y.-J., Shahidi, F. (2002). Antioxidative activity of chitosans of different viscosity in cooked comminuted flesh of herring (Clupea harengus). *Food Chemistry, 79,* 69–77.

Kechichian, V., Ditchfield, C., Veiga-Santos, P., & Tadini, C. (2010). Natural antimicrobial ingredients incorporated in biodegradable films based on cassava starch. *LWT-Food Science and Technology, 43,* 1088–1094.

Khwaldia, K., Banon, S., Desobry, S., & Hardy, J. (2004). Mechanical and barrier properties of sodium caseinate-anhydrous milk fat edible films. *International Journal Food Science and Technology, 39,* 403-411.

Khwaldia, K., Banon, S., Perez, C., & Desobry, S. (2004). Properties of sodium caseinate film forming dispersions and films. *Journal of Dairy Science, 87,* 2011–2016.

Khwaldia, K., Banon, S., Perez, C., & Desobry, S. (2004). Properties of sodium caseinate film forming dispersions and films. *Journal of Dairy Science, 87,* 2011–2016.

Kibbe, A. H. (2000). Hydroxypropyl methylcellulose. Handbook of Pharmaceutical Excipients, third ed. American Pharmaceutical Association, Washington, DC, pp. 252–255.

Kim, K., Weller, C., Hanna, M., & Gennadios, A. (2002). Heat curing of soy protein films at selected temperatures and pressures. *Lebens. Wiss. Technol, 35,* 140–145.

Kim, S., López, C., Güebitz, G., & Cavaco-Paulo, A. (2008). Biological coloration of flax fabrics with flavonoids using laccase from trametes hirsute. *Engineering in Life Sciences, 8,* 324–330.

Kim, S., López, C., Güebitz, G., Cavaco-Paulo, A., 2008. Biological coloration of flax fabrics with flavonoids using laccase from trametes hirsute. *Engineering in Life Sciences 8,* 324–330.

King, J. W., Grabiel, R. D. (2007). Isolation of polyphenolic compounds from fruits of vegetables utilizing sub-critical water extraction. US7208181.

Kiritsakis, A., Kanavouras, A., & Kiritsakis, K. (2002). Chemical analysis, quality control and packaging issues of olive oil. *European Journal of Lipid Science and Technology, 104,* 628–638.

Kiritsakis, A., Kanavouras, A., & Kiritsakis, K. (2002). Chemical analysis, quality control and packaging issues of olive oil. *European Journal of Lipid Science and Technology, 104,* 628–638.

Kirk Othmer. (1993) b Encyclopedia of Chemical Technology, 4th Ed. vol 5. J. Kroschwitz, ed. Cellulose, p. 476-496. New York, John Wiley.

Kittur, F. S., Kumar, K. R. & Thraranathan, R. N. (1998).Functional Packaging Properties of Chitosan Films. Zeitschrift für Lebensmittel Untersuchung und Forschung, 206: 44 –47.

Klaypradit Wanwimol, Kerdpiboon Soraya, & Singh Rakesh, K. (2011). Application of Artificial Neural Networks to Predict the Oxidation of Menhaden Fish Oil Obtained from Fourier Transform Infrared Spectroscopy Method. *Food and Bioprocess Technology, 4,* 475–480.

Klaypradit Wanwimol, Kerdpiboon Soraya, & Singh Rakesh, K. (2011). Application of Artificial Neural Networks to Predict the Oxidation of Menhaden Fish Oil Obtained from Fourier Transform Infrared Spectroscopy Method. *Food and Bioprocess Technology, 4,* 475–480.

Klemm, D., Philipp, B., Heinze, T., Heinze, U., & Wagenknecht, W. (1998). "Comprehensive Cellulose Chemistry", in: Fundamentals and analytical Methods, vol.1,

Kolar, H. M., Urbancic, S., Hadolin, K. M. (2009). Mixture for the antioxidative and microbiological protection of fats and foodstuffs containing fats e.g. Meat product comprises the extract of at least one plant selected from Labiatae family, and green tea extract having synergistic effect. WO2009044240, EP2207430 & US2010203213.

Kong J. M, Chia L. S, Goh N. K, Chia TF, Brouillard R. (2008). Analysis and biological activities of anthocyanins. *Phytochem, 69,* 1939–40.

Kristo, E., Biliaderis, C. G., & Zampraka, A. (2007). Water vapour barrier and tensile properties of composite caseinate-pullulan films: Biopolymer composition effects and impact of beeswax lamination. *Food Chemistry, 101,* 753–764.

Kristo, E., Koutsoumanis, K. P., & Biliaderis, C. G. (2008). Thermal, mechanical and water vapor barrier properties of sodium caseinate films containing antimicrobials and their inhibitory action on Listeria monocytogenes. *Food Hydrocolloids, 22,* 373–386.

Krochta, J. M., Baldwin, E. A., & Nisperos-Carriedo, M. (1994). Edible coatings and films to improve food quality. Florida, United States of America. CRC Press.

Krochta, J. M., Ed. (1997). Edible Protein Films and Coatings. In Food Proteins and Their Applications; Marcel Dekker Press: New York.

Kurilich, A. C., Clevidence, B. A., Britz, S. J., Simon, P. W., Novotny, J. A. (2005). Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. *Journal of Agricultural and Food Chemistry, 53,* 6537–6542.

L. H. (2001). Influence of light and temperature on the colour and oxidative stability of processed cheese. *International Dairy Journal, 11,* 837–843.

Labuza, T. P, & Breene, W. M. (1989). Applications of «active packaging» for improvement of shelf-life and nutritional quality of fresh and extended shelf-life foods. *J. Food Proc. Preserv., 13,* 1–69.

Laguerre M, Lecomte J, Villeneuve P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Prog Lipid, 46,* 244–82.

Laleh, G. H., Frydoonfar, H., Heidary, R., Jameei, R., & Zare, S. (2006). The Effect of Light, temperature, pH and species on stability of anthocyanin pigments in four berberis species. *Pakistan Journal of Nutrition*, 5, 90–92.

Langkilde, F. W., & Svantesson, A. (1995). Identification of celluloses with Fourier-Transform (FT) mid-infrared, FT-Raman and near-infrared spectrometry. *Journal of Pharmaceutical and Biomedical Analysis, 13,* 409–414.

Lee, D. S., Yam, K. L., & Piergiovanni, L. (2008). Vacuum/modified atmosphere packaging. In: Lee, D.S., Yam, K.L., Piergiovanni, L. (Eds.), *Food Packaging Science and Technology*. CRC Press Taylor & Francis Group, pp. 397–424.

Lee, G. O., Ryu, J. C. (2004). Tocopherol containing milk rice and a method for preparing thereof. KR2004080132 & KR513926.

Lee, J. Y., Park, H. J., Lee, C. Y., & Choi, W. Y. (2003). Extending shelf-life of minimally processed apples with edible coatings and antibrowning agents. *LWT-Food Sci. Technol. 36,* 323–329.

Lee, K. T. (2010). Quality and safety aspects of meat products as affected by various physical manipulations of packaging materials. *Meat Science 86,* 138–150.

Lee, S. H., & Min, D. B. (1990). Effects, quenching mechanisms and kinetics of carotenoids in chlorophyll-sensitised photooxidation of soybean oil. *Journal of Agricultural and Food Chemistry, 38,* 1630–1634.

Lee, S. Y., Krochta, J. M. (2002). Accelerated shelf life testing of whey-protein-coated peanuts analyzed by static headspace gas chromatography. *Journal of Agricultural and Food Chemistry, 50,* 2022–2028.

Lee, S. Y., Trezza, T. A., Guinard, J.X., Krochta, J. M. (2002). Whey-protein-coated peanuts assessed by sensory evaluation and static headspace gas chromatography. *Journal Food Science, 67,* 1212–1218.

Lemoigne, M. (1926). Produits de deshydration et de polymerisation de lácide β-oxybutyrique. *Bull. Soc. Chim. Bio. 8,* 770–782.

Lennersten, M. S. (1998). Light-induced lipid oxidation and colour changes in foods. Influence of wavelength and packaging material. Ph. D. Thesis. Gothenberg: Chalmers University of Technology/ the Swedish Institute for Food and Biotechnology (SIK).

Lennersten, M., & Lingnert, H. (2000). Influence of wavelength and packaging material on lipid oxidation and colour changes in low-fat mayonnaise. *LWT – Food Science and Technology, 33,* 253–260.

Leopoldini M, Russo N, Toscano M. (2010). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry, 10,* 8–12.

Li, C. L., Martini, L. G., Ford, J. L., & Roberts, M. (2005). The use of hypromellose in oral drug delivery. *Journal of Pharmacy and Pharmacology, 57,* 533–546.

Li, Y. D., Zeng, J. B., Wang, X. L., Yang, K. K., & Wang, Y. Z. (2008). Structure and Properties of Soy Protein/Poly(butylenes succinate) Blends with Improved Compatibility. *Biomacromolecules, 9,* 3157–3164.

Liesegang, T. J., (1993). Viscoelastics. *International Ophthalmology Clinics 33,* 127–147.

Lima, A. M., Cerqueira, M. A., Souza, B. W. S., Santos, E. C. M., Teixeira, J. A., Moreira, R. A., et al. (2010). New edible coatings composed of galactomannans and collagen blends to improve the postharvest quality of fruits e Influence on fruits gas transfer rate. *Journal of Food Engineering, 97,* 101–109.

Lin, C. C., Lin, S. Y., & Hwang, L. S. (1995). Microencapsulation of Squid Oil with Hydrophilic Macromolecules for Oxidative and Thermal Stabilization, *Journal of Food Science, 60*, 36-39.

Linder, M., Matouba, E., Fanni J., & Parmentier, M. (2002). Enrichment of salmon oil with n 3 PUFA by lipolysis, filtration and enzymatic re-esterification. *European Journal of Lipid Science and Technology*, *104,* 455–462.

Liu, F., Qin, B., He, L., & Song, R. (2009). Novel starch/chitosan blending membrane: antibacterial, permeable and mechanical properties. *Carbohydrate Polymers, 78,* 146–150.

Liu, H., Qiu, N., Ding, H., & Yao, R. (2008). Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Research International, 41,* 363–370.

López, O. V., García, M. A., & Zaritzky, N. E. (2008). Film forming capacity of chemically modified corn starches. *Carbohydrate Polymers, 73,* 573–581.

López, O., & García, M. A. (2012). Starch films from a novel (Pachyrhizus ahipa) and conventional sources: development and characterization. *Materials Science and Engineering, 32,* 1931–1940.

Lopez-Caballero, M. E., Gómez-Guillén, M. C., Pérez Mateos, M., Montero, P. (2005). A chitosan-gelatin blend as a coating for fish patties. *Food Hydrocolloids, 19,* 303–311.

Lourdin, D., Coignard, L., Bizot, H., & Colonna, P. (1997). Influence of equilibrium relative humidity and plasticizer concentration on the water content and glass transition of starch materials. *Polymer, 21,* 5401–5406.

Maftoonazad, N., Ramaswamy, H. S., Moalemiyan, M., & Kushalappa, A. C. (2007). Effect of pectin-based edible emulsion coating on changes in quality of avocado exposed to Lasiodiplodia theobromae infection. *Carbohydrate Polymers, 68,* 341–349.

Maisuthisakul, P., Pongsawatmanit, R., Gordon, M. H., 2007. Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (Cratoxylum formosum Dyer). *Food Chemistry 100,* 1620–1629.

Maisuthisakul, P., Pongsawatmanit, R., Gordon, M.H. (2007). Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (Cratoxylum formosum Dyer). *Food Chemistry, 100,* 1620–1629.

Maizura, M., Fazilah, A., Norziah, M. H., & Karim, A. A. (2007). Antibacterial activity and mechanical properties of partially hydrolyzed sago starch-alginate edible film containing lemongrass oil. *J. Food Sci. 72,* 324–330.

Mali, S., Grossmann, M. V., Garcia, M. A., Martino, M. N., & Zaritzky, N. E. (2006). Effect of controlled storage on thermal, mechanical and barrier properties of plasticized films different starch sources. *Journal of Food Engineering, 75,* 453–460.

Mallikarjunan, P., Chinnan, M.S., Balasubramaniam, V.M., & Phillips, R.D. (1997). Edible coatings for deep-fat frying of starchy products. *Lebensmittel-Wissenschaft und- Technologie. 30,* 709-714.

Mandal, A. S., Biswas, N., Karim, K. M., Guha, A., Chatterjee, S., Behera, M., et al. (2010). Drug delivery system based on chronobiology–A review. *Journal of Controlled Release, 147,* 314–325.

Maria, T. M. C., Carvalho, R. A., Sobral, P. J. A., Habitante, A. M. B. Q., & Solorza-Feria, J. (2008). The effect of the degree of hydrolysis of the PVA and the plasticizer concentration on the color,

opacity, and thermal and mechanical properties of films based on PVA and gelatin blends. *Journal of Food Engineering 87,* 191–199.

Markakis, P. (1982). Introduction in Anthocyanin in fruits, vegetables and grain. *Boca Roton*, FL: CRC Press (Chapter 1).

Marron, V., Saari, L., Floridi, G, Boelck, C, & Innocenti, F., (2000). The Market of Biobased Packaging Materials.Biobased Packaging Materials for the Food Industry-Status and Perspectives, edited by C.J. Weber (Copenhagen: Department of Dairy and Food Science,The Royal Veterinary and Agricultural University), pp105-112.

Marsh, R., Kajda, P., & Ryley, J. (1994). The effect of light on the vitamin B2 and the vitamin A content in cheese. *Nahrung, 5,* 527–532.

Martínez-Camacho, A. P., Cortez-Rocha, M. O., EzquerraBrauer, J. M., Graciano-Verdugo, A. Z., Rodríguez-Félix, F., Castillo-Ortega, M. M., et al. (2010). Chitosan composite films: thermal, structural, mechanical an antifungal properties. *Carbohydrate Polymers, 82,* 305–315.

Maté, J. I., Frankel, E. N., Krochta, J. M. (1996). Whey protein isolates edible coatings: effect on the rancidity process of dry roasted peanuts. *Journal of Agriculture and Food Chemistry, 44,* 1736-1740.

Mattei Rita, Polotow Tatiana, G., & Vardaris Cristina, V. (2011). Astaxanthin limits fish oil-related oxidative insult in the anterior forebrain of Wistar rats: Putative anxiolytic effects. *Pharmacology Biochemistry and Behavior, 99,* 349–355.

Mayachiew, P., & Devahastin, S. (2010). Effects of drying methods and conditions on release characteristics of edible chitosan films enriched with Indian gooseberry extract. *Food Research International, 43,* 125–132.

McHugh, T. H. (2000). Protein-lipid interactions in edible films and coatings. *Nahrung, 44,* 148–151.

McHugh, T. H., Aujard, J. F., & Krochta, J. M. (1994). Plasticized whey protein edible " lms: water vapor permeability properties. *Journal of Food Science, 59,* 416–420.

Mei, Y., Zhao, Y., Yang, J., & Furr, H. R. (2002). Using edible coating to enhance nutritional and sensory qualities of baby carrots. *J. Food Sci. 67,* 1964-1968.

Mendes-Pinto, M. M., Choubert, G., & Morais, R. (2004). Effect of dietary bile extracts on serum response of astaxanthin in rainbow trout (Oncorhynchus mykiss): a preliminary study. *Aquaculture Nutrition, 10,* 353–357.

Mendes-Pinto, M. M., Choubert, G., Morais, R. (2004). Effect of dietary bile extracts on serum response of astaxanthin in rainbow trout (Oncorhynchus mykiss): a preliminary study. *Aquaculture Nutrition, 10,* 353–357.

Möller, H., Grelier, S., Pardon, P., & Coma, V. (2004). Antimicrobial and physicochemical properties of chitosan-HPMC-based films. *J. Agric. Food Chem., 52,* 6585–6591.

Montenegro, G. (2009). New natural antioxidant composition prepared from phenolic extracts of monofloral honey independently acting as antioxidants, useful as an antioxidant in meat products and in poultry, preferably chicken. WO2009125036.

Moon, J.-K.; Shibamoto, T. (2009). Antioxidant assays for plant and food components. *J. Agric. Food Chem. 57,* 1655−1666.

Mori, T. A., & Beilin, L. J. (2001). Long-chain omega 3 fatty acids, blood lipids and cardiovascular risk reduction. *Current Opinion in Lipidology, 12,* 11–7.

Morillon, V., Debeaufort, F., Bond, G., Capelle, M., & Volley, A. (2002). Factors affecting the moisture permeability of lipid e based edible films: a Review. *Critical Reviews in Food Science and Nutrition, 42,* 67–89.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2002a). Light-induced oxidation in semi-hard cheeses. Evaluation of methods used to determine levels of oxidation. *Journal of Agricultural and Food Chemistry, 50,* 4364–4370.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2002b). Effect of light and oxygen transmission characteristics of packaging materials on photooxidative quality changes in Havarti cheeses. *Packaging Technology and Science, 15,* 121–127.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2003a). Response surface models used for prediction of photooxidative quality changes in havarti cheese. *European Food Research and Technology*, *216*, 93–98.

Mortensen, G., Sørensen, J., & Stapelfeldt, H. (2003b). Response surface models for prediction of photooxidative quality changes in Havarti cheese. *European Food Research and Technology, 216,* 93–98.

Mortensen, G., Sørensen, J., Danielsen, B., & Stapelfeldt, H. (2003c). Effect of specific wavelengths on light-induced quality changes in Havarti cheese. *Journal of Dairy Research, 70,* 413–21.

Moslemi, A. A. (1967) Quantitative color measurement for black walnut wood. US Forest Service, Research Paper NC-17. St. Paul, Minnesota: USDA, Forest Service, North Central Forest Experiment Station. 15 p.

Mottar, J. (1984). The effect of light on the quality of milk and milk products. *Deutsche Milchwirtschaft, 591,* 587–588.

Moyssiadi, T., Badeka, A., Kondyli, E., Vakirtzi, T., Savvaidis, I., & Kontominas, M. G. (2004). Effect of light transmittance and oxygen permeability of various packaging materials on keeping quality of low fat pasteurized milk: Chemical and sensorial aspects. *International Dairy Journal, 14,* 429–436.

Müller, C. M. O., Laurindo, J. B., & Yamashita, F. (2009). Effect of cellulose fibers addition on the mechanical properties and water vapor barrier of starch-based films. *Food Hydrocolloids, 23,* 1328–1333.

Murphy, R. R., Renfroe, M. H., Brevard, P. B., Lee, R. E., & Gloeckner, J. (2009). Cooking does not decrease hydrophilic antioxidant capacity of wild blueberries. *International Journal of Food Sciences and Nutrition, 60,* 88–98.

Murray, L. (2004a). Alcon: Bion® Tears lubricant eye drops. Physicians' Desk Reference, fifty-eighth ed. Thomson PDR, Montvale, NJ, p. 537.

Murray, L. (2004b). Pfizer Consumer Healthcare: Visine Tear® lubricant eye drops. Physicians' Desk Reference for Nonprescription Drugs and Dietary Supplements, twenty-fifth ed. Thomson PDR, Montvale, NJ, pp. 737–738.

Nahas, R., Chadwick, L., Berdahk, D., Mckeague, J., Valalstyne, P. C., Uhlir, A. E., Reynhout, G. S., Jones, T. S., Barren, J. (2010). Compositions and methods for enhancing the stability of foods, beverages, nutritional supplement and cosmetics. US0197812.

Nair, M. G. (2004). Method and compositions for producing berry derived products. US6676978.

Navarro-Tarazaga, M. L., Sothornvit, R., & Peírez-Gago, M. B. (2008). Effect of plasticizer type and amount on hydroxypropyl methylcellulose- beeswax edible film properties and postharvest quality of coated plums (Cv. Angeleno). *Journal of Agricultural and Food Chemistry*, *56*, 9502–9509.

Nazan Turhan, K., & Sahbaz, F. (2004). Water vapor permeability, tensile properties and solubility of methylcellulose-based edible films. *Journal of Food Engineering*, *61*, 459–466

Nelson, K. H., & Cathcart, W. M. (1983). Analytical technique for measuring transmission of light through milk carton materials. *Journal of Food Protection, 46,* 309–314.

Netravali, A. N., Huang, X., Mizuta, K. (2007). Advanced green composites. *Adv. Compos. Mater, 16,* 269–282.

Nielson, L. R., & Harley, S. M. (1996). Chemotaxonomy: simple test for distinguishing between anthocyanins and betacyanins. *Journal of Biological Education, 30,* 88–90.

NLM, (2004). MedlinePlus Drug Information: Hydroxypropyl Methylcellulose (Ophthalmic) National Library of Medicine (NLM).

No, H. K., Park, N. Y., Lee, S. H., & Meyers, S. P. (2002). Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *International Journal of Food Microbiology,* 74, 65–72.

Norajit, K., Kim, K. M., Ryu, G. H. (2010). Comparative studies on the characterization and antioxidant properties of biodegradable alginate films containing ginseng extract. *Journal of Food Engineering, 98,* 377–384.

Ojagh, S. M., Rezaei, M., Razavi, S. H., Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry, 122,* 161–166.

Olin, B. R., (1995). Hydroxypropyl methycellulose. Drug Facts and Comparisons. Facts and Comparisons, Inc., St. Louis, MO, pp. 516–516.

Olivas Guadalupe, I., Barbosa-Canovas Gustavo, V. (2008). Alginate-calcium films: Water vapor permeability and mechanical properties as affected by plasticizer and relative humidity *LWT–Food Science and Technology, 41,* 359–366.

Otles, S. and Otles, S. (2004). Manufacturing of Biobased Packaging Materials for the Food Industry. *Acta Sci. Pol. Technologia Alimentaria, 3,* 13−17.

Ou, S., Wang, Y., Tang, S., Huang, C., Jackson, M. (2005). Role of ferulic acid in preparing edible films from soy protein isolate. *Journal of Food Engineering, 70,* 205–210.

Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., Lacroix, M. (2004). Antimicrobial and antioxidant effects of milk protein-based film containing essential oils from the preservation of whole beef muscle. *Journal of Agriculture and Food Chemistry, 52,* 5598–5605

Paes, S. S., Yakimets, I., & Mitchell, J. R. (2008). Influence of gelatinization process on functional properties of cassava starch films. *Food Hydrocolloids, 22,* 788−797.

Pagella, C., Spigno, G., & De Faveri, D. M. (2002). Characterization of starch based edible coatings. *Trans I Chem E, 80,* 193–198.

Pagella, C., Spigno, G., & De Faveri, D. M. (2002). Characterization of starch based edible coatings. *Food and Bioproducts Processing, 80,* 193−198.

Paine, F. A., & Paine, H. Y. (1992). Using barrier materials efficiently. In A handbook of food packaging (2nd ed.) (pp. 390–425). Glasgow: Blackie Academic & Professional.

Pan, X., Ushio, H., & Ohshima, T. (2005). Effects of molecular configurations of food colorants on their efficacies as photosensitizers in lipid oxidation. *Food Chemistry, 92,* 37-44.

Papachristou, C., Badeka, A., Chouliara, I., Kondyli, E., Kourtis, L., & Kontominas, M. G. (2006). Evaluation of polyethylene terephthalate as a packaging material for premium quality whole pasteurized milk in Greece: Part II. Storage under fluorescent light. *European Food Research and Technology*, *224*, 237−247.

Park, S., & Zhao, Y. (2006). Development and characterization of edible films from cranberry pomace extracts. *Journal of food Science, 71,* 95–101.

Pasteur, L. (1861). On the viscous fermentation and the butyrous fermentation. Bull. *Soc. Chim. Paris 11,* 30–31.

Pazos, M., Gallardo, J., Torres, J., & Medina, I. (2005). Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. *Food chemistry*, 92, 547-557.

Pedreño, M. A., & Escribano, J. (2000). Studying the oxidation and the antiradical activity of betalain from beetroot. *Journal of Biological Education, 35,* 49−51.

Peressini, D., Bravin, B., Lapasin, R., Rizzotti, C., & Sensidoni, A. (2003). Starch-methylcellulose based edible films: Rheological properties of film-forming dispersions. *Journal of Food Engineering, 59,* 25–32.

Pesek, C. A., & Warthesen, J. J. (1987). Photodegradation of carotenoids in a vegetable juice system. *Journal of Food Science, 53,* 744–746.

Petersen, J. H., & Breindahl, T. (1998). Specific migration of di-(2-ethylhexyl) adipate (DEHA) from plasticized PVC film: Results from an enforcement campaign. *Food Additives and Contaminants, 15,* 600–608.

Peterson, K., Nielsen, P.V., Bertelsen, G., Lawther, M., Olsen,M.B., Nilsson, N.H. and Mortensen, G. (1999). Potentialof Biobased Materials for Food Packaging. Trends in *Food Science and Technology, 10,* 52–68.

Petterson, M. K., Løkkeberg, Ø., Solgaard, R. T., & Nilsson, A. (2002). Effect of different packaging materials, drawing depths and light on oxidative stability of cream cheese. Poster, Worldpak 2002, East Lansing, Michigan, 23–28 June, 2002.

Piorkowska, E., Kulinski, Z., Galeski, A., & Masirek, R. (2006). Plasticization of semicrystalline poly(L -lactide) with poly(propylene glycol). *Polymer 47,* 7178–7188.

Podsedek A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT-Food Sci Technol, 40,* 1–11.

Politeo O, Jukic M, Milos M. (2007). Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essential oil. Food Chem, 101, 379–85.

Ponce, A. G., Roura, S. I., del Valle, C. E., & Moreira, M. R. (2008). Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in vivo studies. *Postharvest Biology and Technology, 49,* 294–300.

Popović, S., Peričin, D., Vaštag, Z., Popović, L., Lazić, V. (2011). Evaluation of edible film-forming ability of pumpkin oil cake. Effect of pH and temperature. *Food Hydrocolloids, 25,* 470–476.

Pranoto, Y., Rakshit, S. K., & Salokhe, V. M. (2005a). Enhancing antimicrobial activity of chitosan films by incorporating garlic oil, potassium sorbate and nisin. *LWT-Food Sci. Technol. 38,* 859–865.

Pranoto, Y., Salokhe, V. M., & Rakshit, S. K. (2005b). Physical and antibacterial properties of alginate-based edible films incorporated with garlic oil. *Food Res. Int. 38,* 267–272.

Pratt, D. E., in Y. H. Hui, ed., (1996). Bailey's Industrial Oil & Fat Products, vol. 3, John Wiley & Sons, Inc., New York, pp. 524–545.

Quirijns, E. J., Van Boxtel, A. J. B., Van Loon, W. K. P., & Van Straten, G. (2005). An improved experimental and regression methodology for sorption isotherms. *Journal of the Science of Food and Agriculture, 85,* 175–185.

Rao, A.V., Rao, L.G. (2007). Carotenoids and human health. *Pharmacol Res, 55,* 207–16.

Ray, S. S., & Bousmina, M. (2005). Biodegradable polymers and their layered silicate nanocomposites: In greening the 21st century materials world. *Progress in Materials Science, 50,* 962–1079.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26,* 1231–1237.

Reilly, W. J. (2000). Pharmaceutical necessities. In: Gennaro, A. R. (Ed.), Remington: The Science and Practice of Pharmacy, 20th ed. Lippincott Williams & Wilkins, Philadelphia, PA, p. 10-32.

Reische, D. W., Lillard, D. A., & Eitenmiller, R. R. in C. C. Akoh and D. B. Min, eds., Food Lipids: Chemistry, Nutrition and Biotechnology, Marcel Dekker, Inc., New York, 1998, pp. 423–448.

Rhim, J. W., Hong, S. I., Park, H. M., Ng, P. K. W. (2006). Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *J. Agric. Food Chem, 54,* 5814-5822.

Rice-Evans C, Sampson J, Bramley PM, Holloway D. E. (1997). Why do we expect carotenoids to be antioxidants in vivo. *Free Radic Res, 26,* 381–98.

Rodriguez, A., Losada, V., Larrain, M. A., Quitral, V., Vinagre, J., & Aubourg, P. (2007). Development of lipid changes related to quality loss during the frozen storage of farmed coho salmon (Oncorhynchus kisutch). *Journal of the American Oil Chemists Society, 84,* 727–34.

Rojas-Graü, M. A., Avena-Bustillos, R. J., Olsen, C. O., Friedman, M. F., Martín Belloso, O., Pan, Z., & McHugh, T. H. (2007a). Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate-apple puree edible films. *J. Food Eng. 81,* 634–641.

Rojas-Graü, M. A., Soliva-Fortuny, R., & Martın-Belloso, O. (2009a). Edible coatings to incorporate active ingredients to freshcut fruits: a review. *Trends in Food Science and Technology, 20,* 438–447.

Rojas-Graü, M., Avena-Bustillos, R., Friedman, M., Henika, P., Martín-Belloso, O., & McHugh, T. H. (2006). Mechanical, barrier, and antimicrobial properties of apple puree edible film containing essential oils. *J. Agric. Food Chem. 54,* 9262–9267.

Romanazzi, G., Nigro, F., Ippolito, A., Di Venere, D., & Salerno, M. (2002). Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. *Journal of Food Science, 67,* 1862–1866.

Romanczyk, L. J., Hammerstone, J. F., Buck, M. M. (2004). Foods containing a cocoa polyphenol additive. US6777005.

Roos, Y., & Karel, M. (1991). Plasticizing effect of water of thermal the behaviour and crystallization of amorphous food models. *Journal of Food Science, 56,* 38–43.

Rosa, M., Vercelino-Alves, Ariene, G. F., Van Dender, Sandra, B. M., Jaime, Izildinha Moreno, & Beatriz, C. (2007). Pereira Effect of light and packages on stability of spreadable processed cheese. *International Dairy Journal, 17,* 365–373.

Rossi-Marquez, Giovanna, Han, Jung, H., Garcia-Almendarez Blanca (2009). Effect of temperature, pH and film thickness on nisin release from antimicrobial whey protein isolate edible films. *Journal of Science of Food and Agriculture, 89,* 2492–2497.

Ryan, P. G., Moore, C. J., van Franeker, J. A. & Moloney, C. L. (2009). Monitoring the abundance of plastic debris in the marine environment. Phil. *Trans. R. Soc. B 364,* 1999–2012.

Sánchez-González, L., González-Martínez, C., Chiralt, A., & Cháfer, M. (2010). Physical and antimicrobial properties of chitosan-tea tree essential oil composite films. *Journal of Food Engineering, 98,* 443–452.

Sánchez-González, L., Vargas, M., González-Martínez, C., Cháfer, M., Chiralt, A. (2011). Use of Essentials oils in bioactive edible coatings–A review. *Food Engineering Reviews, 3,* 1–16.

Sánchez-González, L., Vargas, M., González-Martínez, C., Chiralt, A., & Cháfer, M. (2009). Characterization of edible films based on hydroxypropyl methylcellulose and tea essential oil. *Food Hydrocolloids, 23,* 2102–2109.

Sandoval, A. E, Engelbrecht, D. A, McMindes, M. K., Sandoval, A. (2006). Protein composition for preventing discoloration of fresh beef cuts, e.g. beef steaks or roasts, comprises antioxidant comprising alkali metal salt of ascorbic acid or alkali metal salt of isoascorbic acid. US2006177547 & WO2006086461.

Sardo, A., Bompeix, G. (2002). Process for treating fruit and vegetables using tocopherols as antioxidants. US6403139.

Sattar, A., deMan J. M., & Alexander, J. C. (1977). Wavelength effect on light-induced decomposition of vitamin A and b-carotene in solutions and milk fat. *Canadian Institute of Food Science and Technology Journal, 10,* 56–60.

Sattar, A., deMan, J. M., & Alexander, J. C. (1976). Stability of edible oils and fats to fluorescent light irradiation. *Journal of the American Oil Chemists' Society, 53,* 473–477.

Sebti, I. (2002). "Bio-emballage actif incorporant la nisine, diffusion de cette bactériocine en gel d'agarose", Doctorat thesis, Université Bordeaux1, France.

Sebti, I., Chollet, E., Degraeve, P., Noel, C., & Peyrol, E. (2007). Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *Journal of Agricultural and Food Chemistry, 55,* 693–699.

Selleck, R. (2004). Fruit and vegetable preservative. US6749875.

Seydim, A.C., & Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. *Food Res. Int., 39,* 639–644.

Shellhammer, T. H., & Krochta, J. M. (1997). Whey protein emulsion film performance as affected by lipid type and amount. *Journal of Food Science, 62*, 390–394.

Shiku, Y., Hamaguchi, P. Y., & Tanaka, M. (2003). Effect of pH on the preparation of edible films based on fish myofibrillar proteins. *Fisheries Science, 69,* 1026–1032.

Shrestha, A. K., Arcot, J., & Paterson, J. L. (2003). Edible coating materials their properties and use in the fortification of rice with folic acid. *Food Research International, 36,* 921–928.

Shukla, P. T. (1992). Trends in Zein Research and Utilization. *Cereal Foods World, 37*: 225.

Shyamala, B.N., & Jamuna, P. (2010). Nutritional Content and Antioxidant Properties of Pulp Waste from Daucus carota and Beta vulgaris. *Malaysian Journal of Nutrition, 16,* 397–408.

Siepmann, J., Peppas, N. A. (2001). Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Advanced Drug Delivery Reviews, 48,* 139-157.

Sinclair, R. G. (1996). The Case for Polylactic Acid as a Commodity Packaging Plastic. Polymeric Materials: Science and Engineering, 72: 133–135.

Singelton, V., R., Orthifer, R., Lamuela-Raventos, R., M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent *Methods in Enzymology. 299*, 152–178.

Siripatrawan, U. Harte, B. (2010). Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids, 24,* 770–775.

Siro, I., Fenyvesi, E., Szente, L., De Meulenaer, B., Devlieghere, F., Orgovànyi, J., Sényi, J., & Barta, J. (2006). Release of alpha-tocopherol from antioxidative low-density polyethylene film into fatty food simulant: influence of complexation in beta-cyclodextrin. *Food Additives and Contaminants, 23,* 845–853.

Sobral, P. J. A., Menegalli, F. C., Hubinguer, M. D., & Roques, M. A. (2001) Mechanical, water vapor barrier and thermal properties of gelatin based edible films. *Food Hydrocolloids, 15,* 423–432.

Souza, M. P., Cerqueira, M. A., Souza, B. W. S., Teixeira, J. A., Porto, A. L. F., Vicente, A. A., et al. (2010). Polysaccharide from Anacardium occidentale L. tree gum (Policaju) as a coating for Tommy Atkins mangoes. *Chemical Papers, 64,* 475–481.

Stampfer, M. J, Rimm, E. B. (1995). Epidemiologic evidence for vitamin E in prevention of cardiovascular disease. *Am J Clin Nutr, 62,* 1365–9.

Stintzing, F. C., & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food and in human nutrition. *Trends in Food Science & Technology, 15,* 19–38.

Stintzing, F. C., Herbach, K. M., Mosshammer, M. R., Carle, R., Yi, W., Sellappan, S., Akoh, C. C., & Felker, P. (2005). Color, betalains pattern and antioxidant properties of cactus pear (Opuntia sp.) clone. *Journal of Agricultural and Food Chemistry, 53,* 442–451.

Stintzing, F. C., Schieber, A., and Carle, R. (2003). Evaluation of color properties anchemical quality parameters of cactus juices. *Eur. Food Res. Technol. 216,* 303–311.

Suman, S. P., Mancini, R. A., Joseph, P., Ramanathan, R., Konda, M. K. R, Dady, G., Yin, S. (2010). Packaging-specific influence of chitosan on color stability and lipid oxidation in refrigerated ground beef. *Meat Science, 86,* 994–998.

Takeda Chem. Ind. LTD. (2000). Water-dispersible carotenoid composition comprises a mixture of a carotenoid and a dissolving agent or a suspending agent for the carotenoid, and an emulsifier, useful as additive for food pharmaceuticals, cosmetics and feeds. JP2000106844.

Talja, R. A., Helén, H., Roos, Y. H., & Jouppila, K. (2007). Effect of various polyols and polyol contents on physical and mechanical properties of potato starch-based films. *Carbohyd. Polym. 67,* 288–295.

Tan, Y. A., Sambanthmurthi, R., Manickam, K. S., Wahid, B. B. (2010). Palm phenolics and flavonoids as potent biological and chemical antioxidants for applications in foods and edible oils. US20100183786.

Tanada-Palmu, P. S., & Grosso, C. R. (2005). Effect of edible wheat gluten-based films and coatings on refrigerated strawberry (Fragaria ananassa) quality. *Postharvest Biology and Technology, 36,* 199–208.

Tanaka, Y. (1991). Rubber and related polyprenols. In: Charlwood BV, Banthorpe DV, editors. Methods in Plant Biochemistry: Terpenoids. London: Academic Press. p. 519.

Tarvainen, M., Sutinen, R., Peltonen, S., Mikkonen, H., Maunus, J., Vähä-Heikkilä, K., Lehto, V.P., & Paronen, P. (2003). Enhanced film-forming properties for ethyl cellulose and starch acetate using n-alkenyl succinic anhydrides as novel plasticizers. *European Journal of Pharmaceutical Sciences*, *19*, 363–371.

Teerakarn, A., Hirt, D. E., Acton, J. C. (2002). Nisin diffusion in protein films: Effects of film type and temperature. *Journal of Food Science, 67,* 3019–3025.

Tehrany E. A., & Desobry, S. (2004). Partition coefficients in food/packaging systems: a review. *Food Additives and Contaminants, 21,* 1186–1202.

Tharanathan R, Kittur F. (2003). Chitin-The undisputed biomolecule of great potential. *Crit Rev Food Sci Nutr, 43,* 61–87.

Tharanathan, R. N. (2003). Biodegradable films and composite coatings: past, present and future. *Trends in Food Science & Technology*, *14*, 71–78.

Thompson, R. C., Moore, C. J., vom Saal, F. S. & Swan, S. H. (2009b) Plastics, the environment and human health: current consensus and future trends. *Phil. Trans. R. Soc. B 364,* 2153–2166.

Thompson, R. C., Swan, S. H., Moore, C. J. & vom Saal, F. S. (2009a). Our plastic age. *Phil. Trans. R. Soc. B 364,* 1973–1976.

Tovar, L., Salafranca, J., Sanchez, C., & Nerin, C. (2005). Migration studies to assess the safety in use of a new antioxidant active packaging. *Journal of Agricultural and Food Chemistry, 53,* 5270–5275.

Tovar, L., Salafranca, J., Sanchez, C., Nerin, C. (2005). Migration studies to assess the safety in use of a new antioxidant active packaging. *Journal of Agricultural and Food Chemistry 53,* 5270–5275.

Tsuzuki, T, Igarashi, M, Miyazawa T. (2004). Conjugated eicosapentaenoic acid inhibits transplanted tumor growth via membrane lipid peroxidation in nude mice. *Journal of Nutrition, 134,* 1162–1166.

Turhan, K.N., & Sahbaz, F. (2004). Water vapor permeability, tensile properties and solubility of methylcellulose based edible films. *Journal of Food Engineering, 61,* 459–466.

Turker, N., & Erdogdu, F. (2006). Effects of pH and temperature of extraction medium on effective diffusion coefficient of anthocynanin pigments of black carrot (Daucus carota var. L.). *Journal of Food Engineering, 76,* 579–583.

Turro, N. J. (1991). Modern molecular photochemistry. Mill Valley: University Science Books. Kristensen, D., Hansen, E., Arndal, A., Trinderup, R. A., & Skibsted,

Valencia Rodrıguez, M. T. (2001). Efecto del tratamiento de preservacion por depresion e la actividad acuosa en la calidad del alga. Tesis de Magıster en Bromatologıa y Tecnologıa de la Industrializacion de Alimentos. FCEN-UBA. Argentina.

Valencia-Chamorro, S. A., Palou, L., del Río, M. A., & Pérez-Gago, M. B. (2008). Inhibition of Penicillium digitatum and Penicillium italicum by hydroxypropyl methylcellulose-lipid edible composite films containing food additives with antifungal properties. *J. Agric. Food Chem., 56,* 11270 –11278.

van Aardt, M., Duncan, S. E., Long, T. E., O'Keefe, S. F., Marcy, J. E., & Sims, S. R. (2004). Effect of Antioxidants on Oxidative Stability of Edible Fats and Oils: Thermogravimetric Analysis. *J. Agric. Food Chem., 52,* 587–591.

van Beilen Jan, B., & Poirier Yves. (2007). Establishment of new crops for the production of natural rubber, *Trends in Biotechnology, 25,* 522-529.

Van der Weerd, J., & Kazarian, S. G. (2004). Combined approach of FTIR imaging and conventional dissolution tests applied to drug release. *Journal of Controlled Release, 98,* 295–305.

Van Tieghem, P. (1878). On sugar mill gum. *Ann. Sci. Nature Bot. Biol. Veg. 7,* 180–203.

Vasconez, M. B., Flores, S. K., Campos, C. A., Alvarado, J., & Gerschenson, L. N. (2009). Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. *Food Research International, 42,* 762–769.

Vassila, E., Badeka, A., Kondyli, E., Savvaidis, I., & Kontominas, M.G. (2002). Chemical and microbiological changes in fluid milk as affected by packaging conditions. *International Dairy Journal, 12,* 715–722.

Villalobos, R., Hernandez-Munoz, P., & Chiralt, A. (2006). Effect of surfactants on water sorption and barrier properties of hydroxypropyl methylcellulose films. *Food Hydrocolloids, 20,* 502–509.

Vlachos, N., Skopelitis, Y., Psaroudaki, M., Konstantinidou, V., Chatzilazarou, A., & Tegou, E. (2006). Applications of Fourier transform-infrared spectroscopy to edible oils. *Analytica Chimica Acta, 573,* 459–465.

von Elbe J. H, Klement J. T, Amundson C. H, Cassens R. G, Lindsay R. C. (1974). Evaluation of betalain pigments as sausage colorants. *J Food Sci, 39,* 128–32.

von Elbe J. H, Schwartz S. J. (1981). Absence of mutagenic activity and a short term toxicity study of beet pigments as food colorants. *Arch Toxicol 49,* 93–8.

Walle, vander G. A. M, Koning, de G. J. M., Weusthuis, R. A.and Eggink, G (2001). Properties, Modifications and Applications of Biopolyesters In: Advances in Biochemical Engineering/Biotechnology Volume 71, Steinbüchel, A. and Babel, W. (Eds.), Springer Verlag.

Wanasundara, P. K. J. P. D., & Shahidi, F. (2005). Antioxidants: science, technology, and applications. New York: Wiley.

Wanasundara, U. N., & Shahidi, F. (1994). Canola extract as an alternative natural antioxidant for canola oil. *Journal of the American Oil Chemists' Society*, *71*, 817-822.

Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chemistry, 63,* 335–342.

Wang, X. L., Yang, K. K., & Wang, Y. Z. (2003). Properties of Starch Blends with Biodegradable Polymers. *J Macromol Sci Polym Rev 43,* 385–409.

Ward, L. (1998). Isolation of casein fractions by manipulating inherent solubility and association properties of casein. Ph.D. thesis, University of Minnesota, USA.

Wasteline, January (2002). Information Sheet on Packaging. Wastew-atch, UK. Klingbeil, M. 2000 Working document of biodegradable waste management. Brussels: European Commission.

Weber, C.J.; Haugaard, V.; Festersen, R. & Bertelsen, G. (2002). Production and Applications of Biobased Packaging Materials for the Food Industry. Food Additives and Contaminants, 19 Supplement: 172–177.

Westhius, R.A., Van der Walle, G.A.M. and Eggink, G. (2000). Potential of PHA Based Packaging Materials for the FoodIndustry. Proceedings of the Food Biopack Conference,27-29 August, Copenhagen, pp. 24-27.

Wold, J. P., Jørgensen, K., & Lundby, F. (2002). Non-destructive measurement of light-induced oxidation in dairy products by fluorescence spectroscopy and imaging. *Journal of Dairy Science, 85,* 1693–1704.

Xican, Li., Wang, X., Chen, D., & Chen, S. (2011). Antioxidant Activity and Mechanism of Protocatechuic Acid in vitro. *Functional Foods in Health and Disease, 7,* 232–244.

Xing, R., Liu, S., Guo, Z., Yu H., Wang P, Li C, et al. (2007). Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities in vitro. *Bioorg Med Chem, 13,* 1573–7.

Yen, M. T, Yang, J. H, Mau, J. L. (2008). Antioxidant properties of chitosan from crab shells. *Carbohydr Polym, 74,* 840–4.

Yener, F., Korel, F., & Yemenicioglu, A. (2009). Antimicrobial activity of lactoperoxidase system incorporated into cross-linked alginate films. *J. Food Sci. 74,* 73–79.

Yizhong, C., M., Sun, S., Willibald, & Corke, H. (2001b). Chemical Stability and Colorant Properties of Betaxanthin Pigments from Celosia argentea. *Journal of Agricultural and Food Chemistry, 49,* 4429–4435.

Yizhong, C., Sun, M., & Corke. H. (1998). Colorant Properties and Stability of Amaranthus Betacyanin Pigments. *Journal of Agricultural and Food Chemistry, 46,* 4491–4495.

Yizhong, C., Sun, M., Willibald, S., Corke, H. (2001b). Chemical Stability and Colorant Properties of Betaxanthin Pigments from Celosia argentea. *Journal of Agricultural and Food Chemistry 49,* 4429–4435.

Zhang, H. K., Mittal, G. (2010). Biodegradable Protein-based Films from Plant Resources: A Review. *Prog. Sustain. Energy, 29,* 203–220.

Zhang, L., & Kosaraju, S. L. (2007). Biopolymeric delivery system for controlled release of polyphenolic antioxidants. *European Polymer Journal, 43,* 2956–2966.

Zhou, J., Ren, L., Tong, J., Xie, L., & Liu, Z. (2009). Surface esterification of corn starch films: reaction with dodecenyl succinic anhydride. Carbohydrate Polymers, 78, 888–893.

Zhou, J., Zhang, J., Ma, Y., & Tong, J. (2008). Surface photo-crosslinking of corn starch sheets. *Carbohydrate Polymers, 74,* 405–410.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Sci., 82,* 338–345.

VIII. Annexes

Food Chemistry 120 (2010) 395–401

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Control of salmon oil photo-oxidation during storage in HPMC packaging film: Influence of film colour

Muhammad Javeed Akhtar, Muriel Jacquot, Elmira Arab-Tehrany *, Claire Gaïani, Michel Linder, Stéphane Desobry

Institut National Polytechnique de Lorraine (INPL), Laboratoire d'Ingénierie des biomolécules (LIBio), ENSAIA-2,av.de la Forêt de Haye, Boîte postale 172-54505, Vandoeuvre les Nancy Cedex, France

ARTICLE INFO

Article history:
Received 24 March 2009
Received in revised form 1 September 2009
Accepted 6 October 2009

Keywords:
Edible packaging
Edible colours
PUFA
Light-oxidation
Fish oil

ABSTRACT

The efforts are being made to design protective hydroxypropyl methylcellulose (HPMC) colour packages to avoid photo-oxidation of edible fats and oils. In the present study, edible films of HPMC containing different edible colours like blue, green, yellow, red and white were tested for their ability to avoid photo-oxidation in salmon oil. The samples taken in petri-dishes and covered with coloured HPMC films of thickness 40 μm were placed under fluorescent light at 20 °C. During storage, chemical parameter of oil quality such as fat oxidation was monitored during 8 days of storage. Oxygen consumption by gas chromatography, conjugated diene values by spectrophotometery and fatty acid composition by gas chromatography (GC) was measured. The results of our study show that HPMC films with suitable edible colours act as adequate light barrier to avoid photo-oxidation of salmon oil during extended storage. HPMC films containing white, red and yellow edible colours show good control of oil photo-oxidation almost similar to the control samples stored in darkness. Oil samples treated with blue and green edible films show gradual increase in oil oxidation with increasing time of light exposure. Oxidation behaviour of samples treated with blue and green films was almost similar to the samples stored in transparent films.

1. Introduction

Non-bio-degradable materials used in packaging are responsible to cause serious environmental problems. As a consequence, in recent years research attention has turned to develop bio-degradable packaging of natural polymers which may be protein, lipid, or polysaccharide-based. Selection of bio-polymer packaging depends on study of its main permeants like oxygen, water vapours and light because they can transfer through the polymer package wall to cause adverse affect on product quality and its shelf life (Nazan Turhan & Sahbaz, 2004). Polysaccharide-based polymer such as HPMC is used in food Industries as an emulsifier, protective colloid, suspending agent, film former and as a barrier to oxygen and water vapour (Tharanathan, 2003). It gives little or no flavour to food (BeMiller & Whistler, 1996). In addition, HPMC also has a unique property of thermal gelation at high temperature (Chen, 2007). Edible films of bio-degradable polymers, in general, have good barrier properties to oxygen especially in the food components susceptible to oxidation, such as unsaturated lipids (Cuq, Gontard, & Guilbert, 1998). Light barrier properties of these films may be enhanced by the addition of suitable edible colours. The absorption of light by naturally occurring or synthetic pigments is principally related in food products, directly exposed to the light. Certain food colourants have been studied in relation with oxidation of lipids (Kajimoto, Yamaguchi, Kasutani, Yoshida, & Shibahara, 1994; Pan, Ushio, & Ohshima, 2005). The most appropriate way to protect a fat rich product is to remove all kinds of light exposure (Moyssiadi et al., 2004) which is very difficult, but one can try to avoid the most harmful wavelength of light. Several studies show that the 400–500 nm regions are the most harmful part of the visible spectral region with regard to photo-oxidation (Bosset, Gallmann, & Siebar, 1993; Mortensen, Sørensen, & Stapelfeldt, 2003). Lipids containing polyunsaturated fatty acids (PUFA) are more sensitive to oxidation than saturated ones which help to predict lipid susceptibility to oxidation processes (Frankel, 1985). PUFA oxidation causes a number of unfavourable changes like decrease in nutritious quality, health risk and economic losses (Drusch & Berg, 2008). So, scientific objective of the present study was to select the suitable bio-degradable edible film to increase the time of conservation of salmon oil by limiting the happening of photo-oxidation. For this purpose, HPMC films with different edible colours were tested.

* Corresponding author.
E-mail address: elmira.arab-tehrany@ensaia.inpl-nancy.fr (E. Arab-Tehrany).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.10.010

Carbohydrate Polymers 89 (2012) 1150–1158

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antioxidant capacity and light-aging study of HPMC films functionalized with natural plant extract

Muhammad Javeed Akhtar, Muriel Jacquot*, Jordane Jasniewski, Charlotte Jacquot, Muhammad Imran, Majid Jamshidian, Cédric Paris, Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France

ARTICLE INFO

Article history:
Received 12 January 2012
Received in revised form 22 March 2012
Accepted 29 March 2012
Available online 5 April 2012

Keywords:
Hydroxypropyl methylcellulose
Natural red color (betacyanins)
Biodegradable & edible packaging
Trolox® equivalent antioxidant capacity (TEAC)
FTIR
Photo-aging

ABSTRACT

The aims of this work were to functionalize edible hydroxypropyl methylcellulose (HPMC) films with natural coloring biomolecules having antioxidant capacity and to study their photo-aging stability in the films. HPMC films containing a natural red color compound (NRC) at the level of 1, 2, 3 or 4% (v/v) were prepared by a casting method. A slight degradation of films color was observed after 20 days of continuous light exposure. The antioxidant activity of NRC incorporated films was stable during different steps of film formation and 20 days of dark storage. On the other hand, antioxidant activity of samples stored under light was significantly affected after 20 days. FTIR (Fourier Transformed Infrared) spectroscopy was used to characterize the new phenolic polymeric structures and to study the photo-degradation of films. The results showed a good polymerization phenomenon between NRC and HPMC in polymer matrix giving a natural color to the films. NRC showed an ability to protect pure HPMC films against photo-degradation. This phenomenon was directly proportional to the concentration of NRC.

1. Introduction

Environment and food safety have been at the forefront of research concern in recent years. Currently, there is an increasing trend to employ environmental friendly materials with the intention of substituting non-degradable materials. To deal with environmental issues, extend the food quality and to reduce non-degradable packaging wastes has catalyzed the use of new bio-based packaging materials in edible food packaging (Burke, 2006). In edible coating, use of bio-degradable polymers such as polysaccharides, proteins, lipids and their complexes derived from natural origin (Ray & Bousmina, 2005), depends on their barrier properties against light, water vapor and oxygen (Turhan & Sahbaz, 2004).

Cellulose based materials are widely used due to their biocompatibility, edibility, barrier properties, non-polluting and being more economical (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). The use of hydroxypropyl methylcellulose is attractive because it is a readily available non-ionic edible plant derivative shown to form transparent, odourless, tasteless, oil resistant, and water soluble edible films (Akhtar et al., 2010). HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC 1995); its safety in food use has been affirmed by the JECFA (Burdock, 2007). The tensile strength of HPMC films is high and flexibility neither too high nor too fragile, which make them suitable for edible coating purposes (Brindle & Krochta, 2008).

In the scope of natural active agents, recently, fruit and vegetable extracts have gained a considerable market in food industries (Stintzing & Carle, 2004). To consider the natural bioactive colors as the colorants, stability, yield and price are mostly constrains. The natural coloring agents in comparison with artificial colors show less stability against light, oxidation, temperature or pH change and other factors (Fabre et al., 1993; Laleh, Frydoonfar, Heidary, Jameei, & Zare, 2006). In spite of such factors, these natural colorants are gaining importance due to their coloring potential, hygiene, nutrition, pharmaceutical activities, bioactivity and environmental consciousness, which indicates relative dependence on natural products (Frank et al., 2005; Hari, Patel, & Martin, 1994).

Although anthocyanins are less stable in various environmental conditions, they include varieties of colors such as orange, red, maroon and blue which make them an attractive alternative as coloring agents in food industries (Markakis, 1982). Moreover, anthocyanins have many health benefits, including reduced risk of cardiovascular diseases (Bell & Gochenaur, 2006) and decreased risk of cancer (Dai, Patel, & Mumper, 2007). These benefits make them essential to provide a healthier food for consumers. Several studies on the antioxidant and antiradical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Escribano, Pedreño, Garcia-Carmona, & Munoz,

* Corresponding author. Tel.: +33 3 83 59 58 49; fax: +33 3 83 59 58 04.
E-mail address: muriel.jacquot@ensaia.inpl-nancy.fr (M. Jacquot).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.03.088

Food Hydrocolloids 31 (2013) 420–427

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Fabrication and physicochemical characterization of HPMC films with commercial plant extract: Influence of light and film composition

Muhammad-Javeed Akhtar, Muriel Jacquot*, Majid Jamshidian, Muhammad Imran, Elmira Arab-Tehrany, Stéphane Desobry

Université de Lorraine, ENSAIA–INPL, Laboratoire d'Ingénierie des Biomolécules (LIBio), 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France

ARTICLE INFO

Article history:
Received 15 May 2012
Accepted 9 October 2012

Keywords:
Hydroxypropyl methylcellulose
Bioactive films
Natural red color
Photo-aging
Physicochemical properties

ABSTRACT

Betacyanins used as natural red color (NRC) are known as antioxidants. The present paper was focused on their effect on physicochemical properties of hydroxypropyl methylcellulose (HPMC) films. All the films were evaluated for their photo-aging stability on optical, mechanical, barrier, thermal and structural properties. Both, tensile strength and Young's modules of NRC composite films decreased, while elongation significantly increased compared to control films. Dynamic vapor sorption data fitted by Guggenheim–Anderson–de Boer (GAB) model showed lower values of sorption energy for NRC composite films. NRC films showed an initial decrease in oxygen permeability that was more decreased after 20 days of photo-aging. Inversely, a significant increase in water vapor permeability of films by increasing NRC was observed. The films composed of 4% NRC (v/v) showed the highest WVP and lowest oxygen permeability. HPMC films transparency decreased with NRC contents.

1. Introduction

Face to increased health and environmental concerns replacement of synthetic chemical additives with natural edible compounds in packaging is a modern concept in the food industry. In response to this consumer requirement, bioactive films based on natural biodegradable polymers, such as structural polysaccharides, gums, proteins, lipids and their complexes (Ray & Bousmina, 2005), combined with natural antioxidants are one of the most promising technologies. Development of such bioactive films can protect food against chemical and physical damages and reduce food preservatives.

Selection of bio-polymers depends on their barrier properties like oxygen, water vapor and light because they cause adverse affect on product quality and limit shelf life (Turhan & Sahbaz, 2004). Polysaccharides are important biopolymers used to prepare edible films and coatings (Gontard, Guilbert, & Cuq, 1993; Mali, Grossmann, Garcia, Martino, & Zaritzky, 2006; Peressini, Bravin, Lapasin, Rizzotti, & Sensidoni, 2003). Cellulose-based materials are widely used because of their biocompatibility, edibility and barrier properties. Moreover, they are non-polluting and economical materials (Vasconez, Flores, Campos, Alvarado, & Gerschenson, 2009). Use of hydroxypropyl methylcellulose is attractive because, it is a readily available non-ionic edible plant derivative forming transparent, odorless, tasteless, oil resistant, and water soluble edible films. It has also the ability to absorb and retain the color pigments (Akhtar et al., 2010). HPMC is approved for food uses by the FDA (21 CFR 172.874) and the EU (EC, 1995); its safety in food use has been confirmed by the (ECFA) "Joint expert committee on food additives" (Burdock, 2007). Food grade HPMC is listed as suitable for use in applications falling under the provisions of the regulation as additive or polymer production aid with no specific migration limit (Annex I UE N. 10/2011). The tensile strength of HPMC films is high with medium flexibility, which makes them suitable for edible coating purposes (Brindle & Krochta, 2008).

Recently, the use of plant natural products such as fruit or vegetable extracts has gained a considerable market, not only because of their coloring potential but also the positive physiological attributes of their pigments (Frank et al., 2005). These phenolic compounds are among the most effective and abundant bioactive compounds from different fruits and vegetables, agro-industrial wastes, and by-products (Ali et al., 2008; Bonilla, Mayen, Merida, & Medina, 1999; Liu, Qiu, Ding, & Yao, 2008). Several studies on the anti-inflammatory, antioxidant and anti-radical activity of betalains (mainly betanin) from red beetroot extract (*Beta vulgaris* L.) have been published (Gentile, Tesoriere, Allegra, Livrea, & Alessio, 2004; Kanner, Harel, & Granit, 2001; Pedreño & Escribano, 2000). Anthocyanins play a role in industry as synthetic colorant replacer and have health benefits, including

* Corresponding author.
E-mail address: muriel.jacquot@univ-lorraine.fr (M. Jacquot).

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2012.10.008

Food Bioprocess Technol
DOI 10.1007/s11947-013-1075-y

ORIGINAL PAPER

Effect of HPMC–Anthocyanin Packaging Color and Oxygen Permeability on Salmon Oil Preservation

M. J. Akhtar · M. Jacquot · S. Desobry

Received: 5 June 2012 / Accepted: 14 February 2013

Abstract Antioxidant food packaging films were successfully developed by incorporation of anthocyanin compound (liquid extract from natural sources) into hydroxypropyl methylcellulose matrix. Film color and oxygen barrier properties were measured. Red color of films containing anthocyanin compound (AC) showed good control of light transmission in comparison with control (transparent) films. Barrier properties of these films showed that addition of AC decreased oxygen permeability, possibly due to hydrogen bonding between polymer OH groups and those of anthocyanin compound. The effectiveness of bioactive films was investigated by packaging salmon oil. Changes in oil color, headspace oxygen consumption, conjugated dienes, polyene index, and C–H stretching vibration of cis-double bond (=CH) showed that, in general, AC films improved salmon oil stability. Films with 2, 3, and 4 % (v/v) AC offered the best protection against lipid oxidation due to improved barrier properties against light and oxygen.

Keywords Hydroxypropyl methylcellulose · Anthocyanins · Barrier properties · Lipid oxidation · Fourier transform infrared spectroscopy

Introduction

Fish oil contains high levels of polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Most of the beneficial attributes of fish and fish products are due to the presence of DHA and EPA; for instance, they have shown protective effects against coronary heart diseases, inflammatory processes, thrombosis, carcinomatosis, and metabolic syndrome (Miller et al., 2008; Tsuzuki et al. 2004; Mori and Beilin 2001). Such nutritional benefits have promoted significant research into methods of stabilizing unhydrogenated fish oil against oxidative deterioration (Hamilton et al. 1998).

Lipids containing PUFA are very sensitive to light oxidation, causing a number of unfavorable chemical changes, including off-flavor formation, oxidation product formation, and nutritional quality decrease, some of which are harmful to human health (Drusch and Berg 2008; Frankel 1985). Chemical changes in lipids are directly related to light source, wavelength, intensity, exposure time, and temperature, as well as packaging material characteristics such as oxygen and light barrier properties (Akhtar et al. 2012). In fish oil, natural antioxidants such as astaxanthin, coenzyme-Q10, selenium, taurine, and vitamins (A, D, and E) can protect lipids against oxidation (Mattei et al. 2011). However, processing and storage alter these natural compounds, resulting in decreased protection against lipid oxidation. Antioxidants may be added from external sources, but their direct application on fresh foods is prohibited in many countries.

The most appropriate way to protect lipid-rich products may be oxygen removal and decrease of light exposure (Moyssiadi et al. 2004), which is not feasible for transparent packaging. The most harmful wavelength (400–500 nm) of light (Mortensen et al. 2004) can be avoided by application of colored packaging (Akhtar et al. 2010) with high oxygen barrier properties.

M. J. Akhtar · M. Jacquot (✉) · S. Desobry
Laboratoire d'Ingénierie des Biomolécules (LIBio), ENSAIA, Université de Lorraine, 2 avenue de la Forêt de Haye, 54505 Vandœuvre-lès-Nancy Cedex, France
e-mail: muriel.jacquot@univ-lorraine.fr

Published online: 10 March 2013

Journal of Food Engineering 110 (2012) 380–389

Contents lists available at SciVerse ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Structural, mechanical and barrier properties of active PLA–antioxidant films

Majid Jamshidian [a,*], Elmira Arab Tehrany [a], Muhammad Imran [a], Muhammad Javeed Akhtar [a], Franck Cleymand [b], Stéphane Desobry [a]

[a] École nationale supérieure d'agronomie et des industries alimentaires, Institut National Polytechnique de Lorraine, 2 avenue de la Forêt de Haye, 54501 Vandoeuvre les Nancy, France
[b] Institut Jean Lamour, UMR 7198 CNRS, École des Mines de Nancy, INPL, Parc Saurupt, CS14234, 54042 Nancy CEDEX, France

ARTICLE INFO

Article history:
Received 24 June 2011
Received in revised form 14 December 2011
Accepted 24 December 2011
Available online 13 January 2012

Keywords:
Poly-(lactic acid)
Ascorbyl palmitate
α-Tocopherol
X-ray photoelectron spectroscopy
Atomic force microscopy
Microstructural and mechanical characterization
Antioxidants
Mechanical properties

ABSTRACT

Ascorbyl palmitate (AP) and α-tocopherol (AT) were added to poly-(lactic acid) (PLA) film to investigate their effects on PLA's physical, structural, mechanical and barrier properties. AP crystals appeared on the PLA surface after solvent evaporation and changed PLA transparency. AP decreased the PLA contact angle on the recto side and increased film polarity and wettability. X-ray photoelectron spectroscopy showed that AT increased the concentration of C–(C,H), O=C and O–H bonds on the verso side, but decreased the concentration of C–O and O–C=O bonds. Atomic force microscopy confirmed that the PLA surface microstructure was drastically influenced by the addition of antioxidants in terms of roughness parameters (R_a and R_q). AT significantly decreased the film crystallization temperature down to 74.1 °C due to its plasticizing effect. Both AT and AP reduced PLA Young's modulus and tensile strength. PLA water vapor permeability was not significantly influenced by AP and AT. AP is not recommended to be used in PLA active packaging.

1. Introduction

Free radicals have been found to be responsible for lipid oxidation, and hundreds of natural and synthetic compounds have been evaluated for their efficiency as radical scavengers or for their other inhibitory effects. Among synthetic antioxidants, only four are widely used in foods, namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) (Wanasundara and Shahidi, 2005). Recently, however, consumer demand for more natural and functional foods has increased because of their positive health benefits and the potential negative health effects of synthetic antioxidants.

The tocopherols are mainly present in oilseeds, oils, meats and the green parts of higher plants, whereas the tocotrienols are mostly found in the germ and bran fraction of certain seeds and cereals. The most abundant natural antioxidants in vegetable oils are the α- and γ-tocopherols, and the most important commercial compounds are α-, γ- and mixed tocopherols. The first two are commonly synthesized, while the latter is a by-product of vegetable oil processing. The antioxidant effects of tocopherols and tocotrienols are due to their ability to donate their phenolic hydrogen to lipid free radicals and retard the autocatalytic lipid peroxidation processes. Alpha-tocopherol (AT) has the highest in vivo antioxidant activity for higher animals and humans, but the efficiency of tocopherols differs in different oxidation conditions (Seppanen et al., 2010).

Ascorbyl palmitate (AP) is a fat-soluble ester of palmitic acid and ascorbic acid, and could be used in oils or fatty foods. AP is a substance that is generally recognized as safe with no specific limitations or restrictions. Ingestion of this antioxidant would pose no health hazards because metabolic breakdown yields ascorbic acid and palmitic acid, both normal metabolites; furthermore it has been shown to be able to regenerate other antioxidants such as tocopherols (Lee et al., 1999; Karabulut, 2010).

Recently, the modification effects of natural antioxidants on polymers, especially biopolymers, have been investigated. Han and Krochta (2007) studied oxygen permeability parameters including the oxygen solubility and diffusivity of two differently-made whey protein isolate (WPI) coatings (powder blended and ethanol solvent mixing) containing 10% of AT and AP. They showed that AP and AT addition increased the oxygen diffusivity approximately 10-fold, and oxygen solubility decreased 10-fold. As a result, the permeability of antioxidant-incorporated films was not enhanced compared to control WPI films. Lopez-Rubio and Lagaron (2010) described the UV stability and mechanical properties of biopolyesters such as poly-(lactic acid) (PLA), polycaprolac-

* Corresponding author. Tel.: +33 (0)383 595878; fax: +33 (0)383 595804.
E-mail addresses: majid_jamshidian@yahoo.com, majid.jamshidian@ensaia.inpl-nancy.fr (M. Jamshidian).

0260-8774/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jfoodeng.2011.12.034

Food Hydrocolloids 29 (2012) 407–419

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Microstructure and physico-chemical evaluation of nano-emulsion-based antimicrobial peptides embedded in bioactive packaging films

Muhammad Imran, Anne-Marie Revol-Junelles, Noémie René, Majid Jamshidian, Muhammad Javeed Akhtar, Elmira Arab-Tehrany, Muriel Jacquot, Stéphane Desobry*

Laboratoire d'Ingénierie des Biomolécules (LIBio), université de Lorraine, École nationale supérieure d'agronomie et des industries alimentaires (ENSAIA), 2 avenue de la Forêt de Haye, 54505 Vandoeuvre-lès-Nancy Cedex, France

ARTICLE INFO

Article history:
Received 2 March 2011
Accepted 11 April 2012

Keywords:
Biodegradable polymer
Bioactive peptide
Emulsion
Controlled release
Nanoencapsulation

ABSTRACT

Customized application of antimicrobial peptide (AMP) 'nisin' directly into food (neither in active packaging nor encapsulated form) is expensive and associated with loss of activity due to deactivation in complex food systems. The purpose of the present study was to fusion the two concepts for improved bioavailability i.e. AMP nanoencapsulation and biopolymer immobilizing to formulate the next generation biodegradable films embedded with either active agent, nano-encapsulated active agent or both of them. Nanoliposomes were prepared using soy-lecithin by microfluidizer at 2000 bar with 5 cycles to generate an average size of 151 ± 4 nm with 50 ± 3% encapsulation efficiency. For active films, nisin had demonstrated no negative impact on transparency, thickness and water sorption behavior obtained by GAB model (25 °C, 0–0.95 a_w). For nano-active films, the results clearly illustrated that different physico-chemical properties including barrier (oxygen and water vapor permeability), color and transparency (200–900 nm) remained comparable to native hydroxypropyl methylcellulose (HPMC) films and were significantly improved than using lecithin directly without nano-scale restructuring. The microstructure studies (topography and morphology) by scanning and transmission electron microscopes (SEM/TEM) revealed different (pore, lamellar, fusion) modes of nisin release from nanoliposomes embedded in HPMC matrix. As microbiological worth, nisin nano-emulsion (encapsulated and free nisin) films were effective against potential foodborne pathogen *Listeria monocytogenes*. This innovative concept of biodegradable nano-active films may thus be a preventive system toward improved food safety.

1. Introduction

'Green consumerism', a trend since the beginning of the previous decade, had emerged due to increasing consumer demand for natural antimicrobial compounds. Such biomolecules are of natural origin, non-toxic for humans, environmentally safe and effective in preserving foods by controlling microorganism's activity (Mastromatteo, Conte, & Del Nobile, 2010). Antimicrobial peptides (AMPs) are widely recognized as promising alternatives to the current use of antibiotics (Marcos & Gandia, 2009). Nisin is probably the most utilized AMP in the food industry as food biopreservative world-wide (Ercolini et al., 2010). Nisin is a 3.5 kDa cationic polypeptide produced from *Lactococcus lactis* strains approved for specific uses in foods in more than 40 countries (O'Sullivan, Ross, & Hill, 2002). It is the only antimicrobial bacteriocin with the status of generally recognized as safe (GRAS) approved by the Food and Drug Administration (FDA) (Sanjurjo, Flores, Gerschenson, & Jagus, 2006). Nisin offers effective control against broad spectrum of Gram positive bacteria especially against the foodborne pathogens *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus* (Joerger, 2007). This AMP kills susceptible bacteria through a multi-step process that destabilize the phospholipids bilayer of the cell and creates transient pores (Breukink & de Kruijff, 2006).

Stability issues like proteolytic degradation and the potential interaction of the AMP with food components might result in decreased antimicrobial activity. Use of nisin in its free form (unpackaged or unencapsulated) is expensive and is associated with loss of activity due to degradation or deactivation and emergence of nisin-resistant bacteria strains (Benech, Kheadr, Lacroix, & Fliss, 2002; Laridi et al., 2003). Significant loss of nisin activity in different foods due to its interactions with complex food components such as divalent cations, enzymes, fat and other

* Corresponding author. Tel.: +33 (0) 3 83 59 58 80; fax: +33 (0) 3 83 59 57 72.
E-mail addresses: m.imran@comsats.edu.pk (M. Imran), stephane.desobry@univ-lorraine.fr (S. Desobry).

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2012.04.010

Résumé

La fonctionnalisation d'emballages biodégradables avec des antioxydants naturels est l'une des techniques prometteuses pour améliorer la conservation des aliments, diminuer la quantité de conservateurs chimiques utilises, protéger la dégradation aromatique des produits et ainsi conserver une meilleure qualité globale. Le contrôle du relargage de ces composés actifs de l'emballage vers l'aliment permet d'étendre l'efficacité de la fonctionnalisation en libérant progressivement les antioxydants à la surface de l'aliment. L'objectif global de ce travail était de fonctionnaliser le polymère HPMC afin de produire un film d'emballage coloré à activité antioxydante et d'évaluer son aptitude à servir d'emballage actif. Tout d'abord, des films d'HPMC contenant différents colorants synthétiques comme le bleu, le vert, le jaune, le rouge et le blanc ont été testés afin de déterminer la couleur la plus adaptée pour le contrôle de la photo-oxydation de produits gras. Ensuite, la couleur rouge synthétique, montrant un maximum de contrôle contre la photo-oxydation, a été remplacée par des composés actifs naturels de même couleur. Ces composés provenaient soit d'un mélange d'extraits de betterave et de carottes pourpres, d'un mélange de bétalaïnes soit d'un mélange d'anthocyanes. Le mode d'incorporation de ces composés actifs dans la matrice d'HPMC, leurs effets sur les propriétés thermiques, mécaniques, barrière et structurales des films ont été étudiés. Les résultats ont montré que l'intégration de ces différents composés actifs naturels a permis d'améliorer les propriétés des films. Les composés actifs utilisés ont la capacité de contrôler le photo-vieillissement de la matrice polymérique et que l'HPMC est un bon candidat pour incorporer ces molécules et permettre le contrôle de la dégradation de produits alimentaires riches en lipides.

Mots clés: Hydroxypropyl methylcellulose, emballages bioactifs, extraits naturels, antioxydants, photo-vieillissement, l'huile de saumon.

Abstract

Biodegradable packaging functionalized with natural antioxidants is one of the promising techniques to enhance foods shelf-life, lower use of preservatives in food formations, higher protection of flavours and higher food qualities. Controlled release of these bioactive compounds from packaging to food surface provides longer food stability by continuously librating antioxidants at food surface. The overall objective of the present work was to functionalize the HPMC polymer as colored antioxidant packaging and investigate its suitability as active packaging for unsaturated lipids. Firstly, HPMC films containing different synthetic colours like blue, green, yellow, red and white were tested to chose a suitable color having control against photo-oxidation. Secondly, red synthetic color (showing maximum control against pho-oxidation) was replaced by natural active red compounds including "natural red color" (beetroot extract + purple carrot extract), betalains and anthocyanins to produce bioactive food packaging. Mode of incorporation of these active compounds in HPMC matrix and also their potential effects on thermal, mechanical, barrier and structural properties of films were investigated. Controlled release kinetics, antioxidant capacity and light stability of bioactive compounds in HPMC films were also investigated. The overall results showed that successful incorporation of different natural active compounds have capability to improve film properties. The active compounds under discussion have ability to control photo ageing of polymer matrix and HPMC has the capability for being a suitable carrier for antioxidant active packaging for some food products.

Keywords: Hydroxypropyl methylcellulose, bioactive packaging, natural extracts, antioxidants, photo-ageing, salmon oil.