Université du Québec
Institut National de la Recherche Scientifique
Institut Armand Frappier

Mise au point d'un système polymérique pour assurer la protection et la libération contrôlée de molécules bioactives

Par

Afia BOUMAIL

Thèse présentée pour l'obtention du grade de
Philosophiae doctor (Ph.D.)
en Biologie

Jury d'évaluation

Président du jury et examinateur interne	Philippe CONSTANT INRS - Institut Armand-Frappier
Examinateur externe	Patrick FUSTIER Agriculture et Agroalimentaire Canada
Examinateur interne	Noureddine BOUSSERRHINE Université Paris-Est Créteil
Directeur de recherche	Monique LACROIX INRS - Institut Armand-Frappier

REMERCIEMENTS

Je remercie ma directrice de recherche, Pre Monique Lacroix, pour m'avoir accueillie au sein de son équipe et pour m'avoir permis de mener ce projet jusqu'à la fin.

Je tiens à remercier particulièrement Stéphane Salmieri. Tes conseils et tes recommandations pendant mon stage m'ont convaincue de continuer pour cette thèse. Malgré ta charge de travail, je te suis reconnaissante pour le temps que tu m'as accordé pendant ces quatre années, ainsi que ton support et ton sens de l'humour.

Ces quatre années n'auraient pas été les mêmes sans la présence des autres étudiants au labo, mais surtout Julie, Kerlynn, Olivier, Paula et Marie-Christine. Nous en avons vu de toutes couleurs, mais avec un peu de persévérance et de courage, on finit bien par passer au travers !

Merci aussi à tous ceux qui ont rempli le rôle d'examinateur interne ou externe au cours des quatre dernières années !

Je souhaite également remercier toutes les personnes qui, sans toujours s'en rendre compte, ont fait en sorte que je revienne chaque jour et m'ont permis d'aller jusqu'au bout de ce long parcours.

Je remercie tous les employés de Nordion pour les nombreuses irradiations qui ont été faites mais aussi pour leur support. Merci à Jacques (pour m'avoir fait une petite place à côté de ton bureau mais aussi pour toutes les conversations qu'on a eu, pour ton écoute et tes bons conseils) ainsi qu'à Sébastien, Guy, Yanick et Carlo pour les petites distractions et conversations du midi. Merci à vous pour avoir bien voulu goûter à mes "créations culinaires". Je n'oublie pas Jeannot, qui nous a quitté trop tôt, mais qui m'a encouragée à continuer jusqu'au bout malgré les obstacles.

Merci à Blanche, Gabrielle, Adrien et Ivan, qui ont été les stagiaires parfaits pour moi et qui ont bien souvent réussi à me faire retrouver le sourire malgré les circonstances particulières et les nombreux contretemps de dernière minute.

Merci aussi à Richard Villemur, pour m'avoir laissée travailler dans un petit coin de son laboratoire, ainsi qu'à toutes les personnes qui m'ont permis de terminer mon projet après notre déménagement à l'édifice 18.

Je n'oublie pas notre équipe de hockey du midi. Merci à Benoit, Fred et Bruno pour avoir permis l'organisation de tant de matchs tout au long de l'année et dans toutes les conditions météorologiques possibles. Malgré les quelques coups, bleus et blessures, j'ai vraiment aimé faire partie de ce groupe. Merci à Alex, pour m'avoir montré comment se débrouiller au poste de gardien de but. J'ai peut-être laissé passer quelques buts, mais certains arrêts "spectaculaires" m'ont valu un trophée bien mérité. Merci aussi à tous les autres joueurs pour votre participation et votre enthousiasme.

Merci au petit groupe (Sonia, Martine, Johanne, Danielle, Louise, Fernando, Chantima, Joannie, Steve et Alex) que j'ai intégré dernièrement après mon arrivée à l'édifice 18. C'était un plaisir de vous rencontrer, je garde en tête votre bonne humeur et c'est certain que l'heure du lunch en votre compagnie va me manquer. Je remercie également Maitté, qui a accepté de "faire tout pour la science" et m'a rendue service de nombreuses fois. Enfin, merci à toutes les personnes qui m'ont encouragées à leur façon, avec un sourire, un *lift* d'un édifice à un autre ou une simple conversation (en particulier Diane, Sylvain, Michel et Marco).

Une petite pensée également à Lina (ces fameux cours sur les polymères et les colloïdes ont fini par m'être bien utiles !!), Michel Troupel (j'essaye toujours d'adopter votre fameux raisonnement de chimie analytique, sans succès...) mais surtout Rachid Barhdadi (maintenant que nous sommes collègues, je peux te tutoyer !).

Enfin, merci à mes parents et à mon frère pour m'avoir encouragée et soutenue pendant ces nombreuses années d'études, depuis ma première journée d'école jusqu'à la dernière !

RÉSUMÉ

Malgré la surveillance accrue et la mise en place d'une réglementation sur l'hygiène et la salubrité alimentaire, il est encore possible de faire face à des cas de contamination bactérienne en industrie. Ces contaminations alimentaires provoquent des effets sur la santé humaine mais également au niveau de l'économie. De nombreux moyens permettent d'empêcher la croissance microbienne dans les aliments mais les agences de santé imposent des limites au niveau de leur utilisation. Les consommateurs exercent aussi une certaine influence, en demandant de plus en plus la présence de produits d'origine naturelle. Les huiles essentielles vont dans ce sens et leurs propriétés antimicrobiennes sont reconnues depuis de nombreuses années. Leur utilisation reste pourtant difficile puisqu'elles peuvent modifier la saveur des aliments et leur caractère volatile peut réduire leur usage à long terme. Le but de cette étude était donc développer un système polymérique permettant de protéger ces composés bioactifs pendant la conservation, tout en assurant une libération contrôlée, dans le but de maintenir un effet antimicrobien contre plusieurs bactéries pathogènes.

Dans un premier temps, un film d'emballage à base de polysaccharides et contenant un mélange de composés antimicrobiens a été développé. Les résultats ont montré la présence d'un effet antimicrobien aussi bien *in vitro* qu'*in situ*. Le suivi des composés antimicrobiens a permis de mettre en évidence la libération contrôlée de ces molécules et une nouvelle méthode de quantification des composés antimicrobiens par spectroscopie infrarouge a été développée. L'étude des propriétés physico-chimiques de ces films a également permis de caractériser la résistance et l'élasticité des films. La présence d'antimicrobiens a permis d'améliorer leur effet barrière face à l'humidité et au dioxyde de carbone.

Par la suite, la recherche s'est portée sur le développement de films d'enrobage, directement appliqués sur des légumes. Un criblage *in vitro* suivi de l'analyse des propriétés organoleptiques ont permis de sélectionner l'enrobage possédant les meilleures effets antimicrobiens tout en gardant des caractéristiques organoleptiques similaires aux légumes non traités. L'effet de cet enrobage sur la respiration, la couleur et la texture des légumes a été évalué. Les changements mineurs observés ne sont toutefois pas visibles pour le

consommateur tout au long de la conservation. Cet enrobage a également montré un bon effet antimicrobien, permettant une inhibition de la croissance de *Listeria* après 7 jours.

Enfin, la dernière étape de ce projet était d'évaluer l'effet de cet enrobage antimicrobien en combinaison avec deux types de traitements physiques : l'irradiation γ ou l'ozonation de l'air. Chacun des traitements utilisé seul a montré une diminution de la charge microbienne. La combinaison de l'enrobage avec l'irradiation γ a montré que la présence de l'enrobage permet de réduire de 4 fois la dose d'irradiation maximale autorisée. Cette combinaison a induit une synergie entre les traitements et les bactéries *Listeria innocua* et *Escherichia coli* n'étaient plus détectées. La combinaison de l'enrobage avec l'ozonation de l'air a montré une réduction totale de *L. innocua* et *E. coli* de 3.3 et 3.8 log UFC/g respectivement. De plus, les résultats obtenus laissent supposer un effet antioxydant de l'enrobage.

Ce projet de doctorat aura permis de développer deux types de films antimicrobiens pouvant être facilement transposés en industrie et intégrés lors de la transformation alimentaire.

TABLE DES MATIERES

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES SIGLES

ACC : Aminocyclopropane-1-carboxylique

ACIA : Agence canadienne d'inspection des aliments

ADF : *Antimicrobial Diffusion Film*

ADN : Acide désoxyribonucléique

ASPC : Agence de santé publique

ATP : Adénosine triphosphate

ATR : *Attenuated total reflectance*

BHT : *Butylated hydroxytoluene*

CFU : *Colony forming unit*

CMI : Concentration minimale inhibitrice

CO_2TR : *Carbon dioxide transmission rate*

DE : Dextrose équivalent

EO : *Essential oil*

FDA : *Federal and drug administration*

FTIR : *Fourier transformed infrared*

FWA : *Films without antimicrobials*

GAE : *Gallic acid equivalent*

HE : Huile essentielle

IR : Infrarouge

MAPAQ : Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec

MC : Méthylcellulose

MD : Maltodextrine

MIC : *Minimal inhibitory concentration*

NCC : Nanocellulose cristalline

PCL : Polycaprolactone

PPO : Polyphénol oxydase

RIV : *Relative inactivation value*

RH : *Relative humidity*

S : *Starch*

SEM : *Scanning electron microscopy*

TAM : *Total aerobic microbiota*

TM : *Tensile modulus*

TP : *Total phenol*

TS : *Tensile strength*

TSA : *Tryptic Soy Agar*

TSB : *Tryptic Soy Broth*

UFC : Unité formatrice de colonies

VO : *Vegetable oil*

WVP : *Water vapor permeability*

LISTE DES SYMBOLES ET UNITES HORS DU SYSTEME INTERNATIONAL

Unités :

cP : centipoise; mesure de la viscosité (1 P = 10^{-1} Pa.s)
kGy : kilogray; mesure de dose de radiation (1 Gy = 1 $J.kg^{-1}$)
mil : mil; mesure de longueur (1 mil = 25.4 µm)
rpm : *revolution per minute*; mesure de vitesse de rotation (1 rpm = 0.1047 $rad.s^{-1}$)

Symboles :

Mg : Magnésium
M_n : Masse molaire moyenne en nombre

PARTIE 1 : SYNTHÈSE

1 REVUE DE LITTÉRATURE

1.1 Consommation des fruits et légumes et normes industrielles

Les fruits et légumes font partie de la base de notre alimentation et apportent à l'organisme des fibres, des vitamines, des minéraux ainsi que de l'énergie (European Food Information Council, 2012). Selon Berger *et al.* (2010), la consommation de fruits et de légumes permettrait de réduire les risques de maladies cardiovasculaires ainsi que certains cancers. Le guide alimentaire canadien recommande aux adultes de consommer 7 à 10 portions de fruits et légumes chaque jour (Santé Canada, 2007). Selon Statistique Canada (2011), entre 1990 et 2010, les disponibilités en fruits et en légumes frais par personne au Canada ont augmenté de 21% et 4% respectivement.

Dû au mode de vie actuel, les consommateurs demandent de plus en plus de produits prêts à manger ou nécessitant peu de préparation. Cette demande a fait en sorte que le marché des fruits transformés au Canada a explosé entre 1990 et 2010, avec une augmentation de 137% (Statistique Canada, 2011). Cependant, la présence de produits déjà prêts pour la consommation implique une ou plusieurs étapes supplémentaires lors de la transformation industrielle. Dans le but d'assurer l'innocuité des aliments, plusieurs lois et règlements sont en vigueur et s'appliquent à "tout lieu où l'on prépare des aliments pour la consommation humaine en vue de leur vente ou de leur service moyennant rémunération" (MAPAQ, 2009a).

1.2 Sénescence des fruits et légumes

1.2.1 Perte de couleur

Les fruits et légumes peuvent être classés pour la plupart selon leur couleur, et il existe ainsi trois principales familles : fruits et légumes à chair blanche (pêches, pommes, artichauts, pommes de terre, etc.), légumes verts (brocolis, haricots verts, etc.) et fruits et légumes orangés (carottes, courges, citrouilles, etc.). Selon le type de fruit ou légume concerné, les changements de couleur observés (principalement le brunissement) sont liés à différents mécanismes.

Les cellules des fruits et légumes à peau blanche sont constituées du cytoplasme et d'une vacuole. Le cytoplasme contient les organites habituels (mitochondries, réticulum endoplasmique, ribosome, etc.) ainsi que des plastes qui renferment un groupe d'enzymes : les polyphénoloxydases (PPO) (Toivonen *et al.*, 2008). La vacuole, quant à elle, contient la majorité des composés phénoliques de la cellule. Certaines étapes de la transformation industrielle, en particulier celles faisant intervenir une coupe, vont engendrer une perturbation des membranes cellulaires. Les composés phénoliques initialement présents dans la vacuole vont alors entrer en contact avec les PPO et l'oxygène présents dans le cytoplasme. L'oxydation de ces phénols va alors entrainer une réaction en chaine, présentée à la **Figure 1.1**, aboutissant à la formation de mélanine, un composé brun (Artés *et al.*, 2007b, Falguera *et al.*, 2012). Terefe *et al.* (2014) citent plusieurs moyens permettant de retarder le brunissement enzymatique des fruits et légumes à peau blanche : on retrouve ainsi l'inactivation thermique/chimique des PPO, la présence d'agents réducteurs/chélateurs ou encore l'absence d'oxygène. Comme pour toute enzyme, la température et le pH jouent un rôle important au niveau de l'activité des PPO. Cependant, des changements de température peuvent modifier la structure des fruits et légumes (cuisson ou encore lésion due au froid). L'acide citrique, utilisé pour réduire le brunissement, est un agent chélateur qui va former un complexe avec le noyau cuivre, et modifier ainsi la structure des PPO (Terefe *et al.*, 2014). D'autres composés tels que l'acide ascorbique ou les thiols vont agir comme agents réducteurs, permettant la réduction des quinones en phénols (Terefe *et al.*, 2014). Enfin, l'ajout de chlorure de sodium ou de calcium peut agir directement sur les PPO et les inhiber (Terefe *et al.*, 2014).

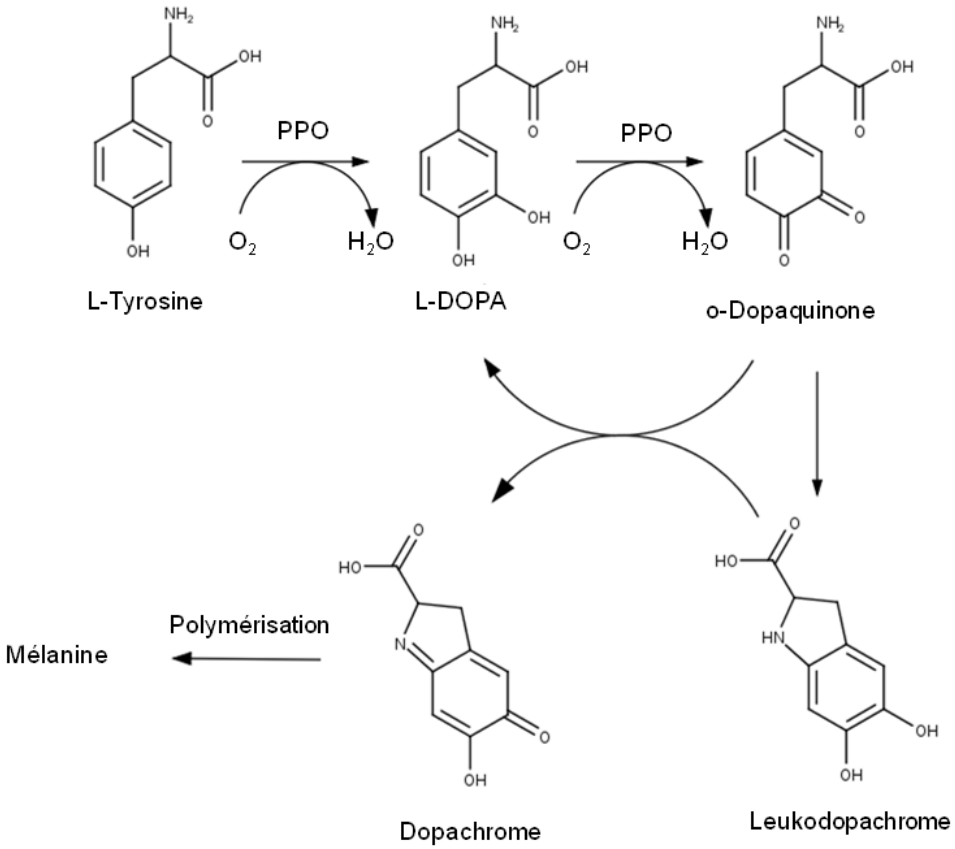


Figure 1.1 Mécanisme de dégradation de phénol aboutissant au brunissement par la formation de mélanine, adapté de Falguera *et al.* (2010).

Après la récolte, les légumes verts (brocoli, haricots verts) peuvent perdre leur couleur, diminuant ainsi leur attrait visuel. Ceci est dû à la dégradation de la chlorophylle, dont différents mécanismes ont été décrits (Costa *et al.*, 2005, Toivonen *et al.*, 2008, Yamauchi *et al.*, 2004). La première étape est la formation de chlorophyllide *a*, suite à la perte de phytol par la chlorophyllase. La deuxième étape est l'élimination du magnésium (Mg) par une Mg-déchélatase pour produire la phéophorbide *a*, qui est décomposée en un catabolite rouge par la phéophorbide oxygénase. La dernière étape est la formation d'un composé incolore par une réductase. Un deuxième mécanisme de transformation serait la séquence alternative : la chlorophylle *a* est dégradée en phéophytine *a* par une Mg-déchélatase. La chlorophyllase agit ensuite sur la phéophytine *a* pour produire la phéophorbide *a*, qui est finalement éliminée en un

produit incolore. Cette perte de couleur, perçue comme un brunissement, correspond en réalité à un jaunissement.

En ce qui concerne les fruits et légumes orangés, la décoloration peut faire suite à une déshydratation ou à une accumulation de phénols et de lignine (Goldberg *et al.*, 1985, Lavelli *et al.*, 2006). C'est le cas des carottes par exemple, qui subissent un desséchement après avoir été pelées et/ou coupées. La lignification, une guérison naturelle des tissus résulte en un changement irréversible de la couleur et provoque la formation de composés phénoliques solubles avant leur conversion en lignine (Artés *et al.*, 2007b, Costa *et al.*, 2005). Cependant, Simões *et al.* (2010) ont montré que la décoloration pouvait être réversible dans les cas où la déshydratation n'était pas extrême.

1.2.2 **Modification de la texture**

Les différences de structure qui existent entre les fruits et les légumes induisent un comportement différent au cours du mûrissement. Les cellules des légumes sont épaisses et rigides alors que celles des fruits sont généralement minces et extensibles. Ces dernières contiennent de la pectine, qui va être dégradée lors du mûrissement. C'est cette dégradation qui va provoquer une diminution de la fermeté ainsi qu'un ramollissement de la chair (Toivonen *et al.*, 2008).

Lors de la transformation des fruits et des légumes, les blessures créées accélèrent leur détérioration. L'endommagement des cellules provoque ainsi une fuite et un mélange des différents composés cellulaires. Suite à ces blessures, le métabolisme des tissus sains environnants est affecté (Hodges *et al.*, 2008). Il semble que de nombreux composés phénoliques soient libérés, précipitant le brunissement (Degl'Innocenti *et al.*, 2005). Le phénomène de respiration augmente, tout comme la production d'éthylène, une hormone végétale. D'ailleurs, la présence de cette hormone en quantité plus importante accélère le phénomène de maturation et de sénescence.

1.2.3 **Respiration**

La photosynthèse et la respiration sont des phénomènes particulièrement importants au niveau des plantes. La **Figure 1.2** décrit, de façon simplifiée, les places que prennent la photosynthèse et la respiration dans la production d'énergie, principalement sous forme d'adénosine triphosphate (ATP), par les cellules végétales. La photosynthèse, ayant lieu dans les chloroplastes situés majoritairement dans les feuilles de la plante, utilise l'énergie solaire et le dioxyde de carbone pour synthétiser des glucides (Grace, 2007, Yoshihara et al., 2000). Ceux-ci sont par la suite dégradés lors de la respiration. Ce mécanisme, se traduisant par l'oxydation des glucides en CO_2 et H_2O, est associé à la libération d'ATP (Grace, 2007, Hill, 2007, Mishra *et al.*, 2007). Cette énergie va par la suite pouvoir être utilisée par la plante, principalement pour sa croissance (Grace, 2007). La récolte des fruits et des légumes ainsi que les transformations industrielles vont déclencher un stress, qui a pour effet d'augmenter la respiration. L'absence de photosynthèse limitant les réserves en glucides, l'augmentation de la respiration aboutit à une dégradation plus rapide des fruits et des légumes (Perera, 2007).

Les réactions biochimiques impliquées lors de la respiration (glycolyse, cycle de Krebs et chaine respiratoire mitochondriale) nécessitent la présence d'enzymes, dont l'activité peut être réduite (Bhande *et al.*, 2008). La température est un des facteurs influençant l'activité enzymatique et donc la respiration. Ainsi, une augmentation de température de 10°C augmenterait de 2 à 3 fois le taux de respiration (Hong *et al.*, 2001), d'où l'importance de réfrigérer les fruits et légumes.

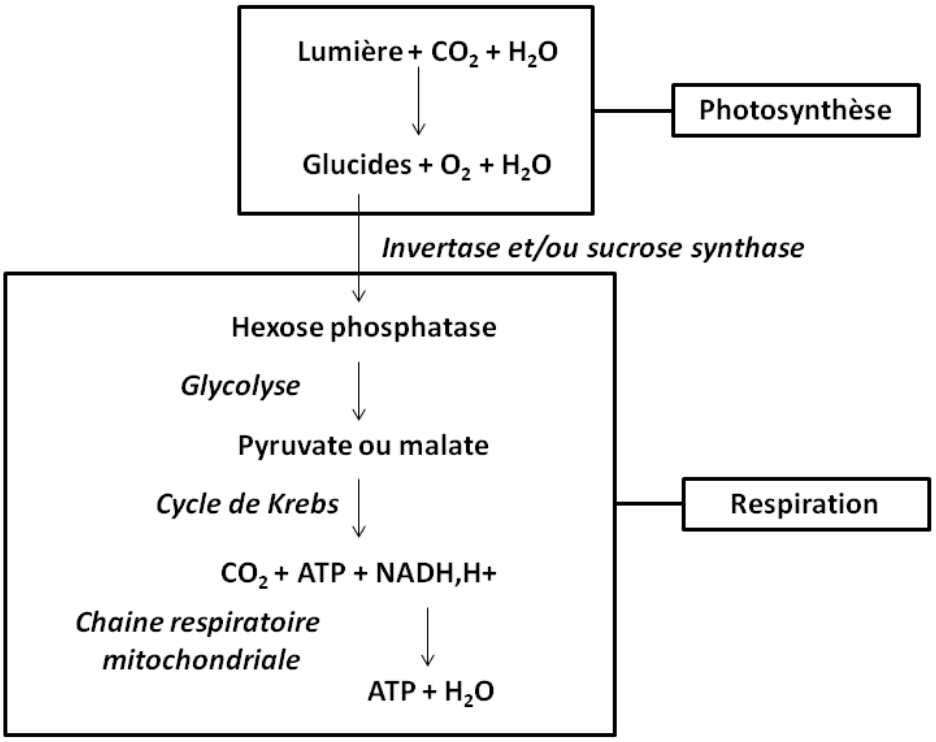


Figure 1.2 Aperçu simplifié de la production d'énergie chez les végétaux

1.3 Développement bactérien

1.3.1 Détérioration des aliments

Les fruits et légumes contiennent une flore microbienne naturelle, pouvant être composée de bactéries, de levures et/ou de moisissures (Rico *et al.*, 2007). La nature de ces micro-organismes dépend du type d'aliment mais aussi de l'environnement (Francis *et al.*, 1999). Dans leur revue, Francis *et al.* (1999) indiquent que la majorité des bactéries présentes dans cette flore sont des bactéries Gram négatif.

Toutes les étapes intervenant au cours de la transformation industrielle, depuis la récolte dans les champs jusqu'au consommateur, sont susceptibles de fragiliser les fruits et les légumes et peuvent accélérer le processus de décomposition. En effet, les blessures physiques causées pendant la récolte, la manipulation et le transport constituent un point d'entrée pour les micro-

organismes et favorisent leur croissance (Talibi *et al.*, 2014, Uchechukwu-Agua *et al.*, 2015). Prusky (2011) précise ainsi que les pourritures sont plus fréquemment retrouvées lorsque les fruits et les légumes sont endommagés. Ces blessures vont également induire une déshydratation et causer un stress supplémentaire aux fruits et légumes (Iyer *et al.*, 2010).

La présence de micro-organismes pathogènes est moins répandue mais peut avoir diverses origines. Parmi tous les micro-organismes dont la présence sur des aliments peut induire une infection, on retrouve principalement *Escherichia coli* et *Listeria monocytogenes*. Dans leur revue, Kozak *et al.* (2013) mentionnent l'origine de certaines épisodes de contaminations microbiennes. Par exemple, en 2002, la propagation d'*E. coli* dans plusieurs établissements de soins de santé, situés dans la province de l'Île-du-Prince-Édouard au Canada, semble avoir une origine humaine. En effet, un membre du personnel préparant les repas aurait continué à travailler tout en prenant des antibiotiques pour lutter contre les diarrhées (Kozak *et al.*, 2013, Public Health Agency of Canada, 2004). Plus récemment, en 2006 en Californie, plus de 200 cas de contamination par *E. coli* ont été observés (Kozak *et al.*, 2013). La contamination des eaux de rivière et des champs par des matières fécales bovines et porcines pourrait avoir induit la présence de ces bactéries dans des épinards.

1.3.2 **Maladies d'origine alimentaire**

1.3.2.1 *Contaminations par Escherichia coli*

Escherichia coli est une bactérie Gram négatif dont la croissance, optimale à 37°C, peut s'effectuer en présence ou en absence d'oxygène (Croxen *et al.*, 2013). On retrouve naturellement cette bactérie au niveau de l'intestin, mais il existe sept souches entériques pathogènes pour l'être humain, une des plus connues étant *E. coli* O157:H7. Les symptômes observés sont généralement des diarrhées hémorragiques, pouvant être accompagnées de vomissements, de crampes abdominales et/ou de fièvre (Croxen *et al.*, 2013). Les aliments contaminés par cette bactérie sont souvent des produits d'origine bovine, mais la consommation de fruits et légumes crus pose également un problème (Berger *et al.*, 2010, Kozak *et al.*, 2013). Selon l'Agence de santé publique du Canada (ASPC), plus de 400 cas de contaminations par *E. coli* sont recensés en moyenne chaque année au Canada (Agence de Santé Publique du Canada, 2015).

1.3.2.2 *Contaminations par Listeria monocytogenes*

Listeria monocytogenes est une bactérie Gram positive, anaérobie facultative, pouvant croitre à des températures allant de 0.5°C à 45°C et à des pH compris entre 4.3 et 9.8 (Warriner *et al.*, 2009). De par sa capacité à adhérer à de nombreuses surfaces, *L. monocytogenes* peut être distribuée dans l'environnement, que ce soit sur des surfaces d'acier inoxydable, de polystyrène ou encore dans les aliments (Välimaa *et al.*, 2015). La consommation d'un aliment contaminé va provoquer des symptômes gastroentériques tels que des crampes abdominales et/ou des diarrhées. Cependant, certaines souches plus virulentes peuvent entraîner des complications telles qu'une infection du système nerveux, une septicémie, une fausse couche, voire même la mort. Les personnes les plus à risque (femmes enceintes, personnes âgées, enfants, personnes immunodéprimées) doivent donc être particulièrement vigilantes. L'ASPC estime qu'environ 132 cas de listérioses sont recensés chaque année au Canada (Agence de Santé Publique du Canada, 2012).

1.3.2.3 *Agences de santé et de salubrité alimentaire*

Au Canada, les politiques et les normes reliées à la salubrité alimentaire sont mises en place par Santé Canada (2012). L'Agence Canadienne d'Inspection des Aliments procède aux inspections et s'assure que les industries répondent aux exigences imposées par Santé Canada. Les aliments prêts à manger ont été classés par Santé Canada selon deux catégories (Santé Canada, 2011). La première catégorie concerne les produits au sein desquels le développement de *L. monocytogenes* peut être présent pendant toute la durée de conservation (principalement les fromages et les produits à base de viande). La deuxième catégorie d'aliments se subdivise en deux sous-classes : 2A et 2B. La catégorie 2A comprend les aliments prêts à manger dans lesquels le développement de *L. monocytogenes* est limité, c'est-à-dire ne dépassant pas 100 unités formatrices de colonies (UFC)/g. Il s'agit habituellement des aliments dont la durée de conservation est inférieure à 5 jours. Les produits faisant partie de la catégorie 2B sont les aliments dans lesquels *L. monocytogenes* ne peut pas croitre (crème glacée, par exemple). Pour ces deux types de catégories, le seuil de tolérance pour *L. monocytogenes* est de 100 UFC/g, une valeur au-delà de cette limite représentant un risque pour la santé. Au Québec, le MAPAQ (Ministère de l'Agriculture, des Pêcheries et de

l'Alimentation du Québec) applique les lois et règlements permettant l'essor du secteur agroalimentaire. Les lignes directrices du MAPAQ recommandent que, pour des légumes prêts à manger, *L. monocytogenes* ne devrait pas être retrouvé dans 25 g d'aliment, alors que la limite pour *E. coli* ne devrait pas dépasser 10 UFC/g (MAPAQ, 2009b).

Aux États-Unis, l'agence fédérale chargée d'assurer la salubrité alimentaire est la *Federal and Drug Administration* (FDA). En 2013, il a été estimé qu'aux États-Unis, 63 153 personnes ont été infectées par *E. coli*. Cela a mené à l'hospitalisation de 2138 personnes, dont 30 sont décédées. Ces contaminations ont généré des coûts de plus de 270 millions de dollars. Quant à *L. monocytogenes*, la contamination de 1591 personnes a provoqué le décès de 306 patients, dont 59 nouveau-nés contaminés pendant la grossesse. Les frais médicaux reliés ont ainsi atteint près de 3 milliards de dollars (United States Department of Agriculture - Economic Research Service, 2014).

Même si le nombre de cas liés à la présence de *L. monocytogenes* et *E. coli* paraît faible, il semble que de nombreux cas ne soient pas déclarés aux autorités et agences de santé (Agence de Santé Publique du Canada, 2015). En effet, d'après Scavia *et al.* (2012) seuls 36% des personnes présentant des symptômes gastro-intestinaux sévères iraient vers une aide médicale. Parmi ceux-ci, moins de 3% feraient l'objet d'une investigation plus poussée.

De plus, les rappels de produits alimentaires contaminés par des bactéries pathogènes peut provoquer de lourdes pertes économiques aux industries et briser la confiance des consommateurs (Ivanek *et al.*, 2005). Suite à une étude menée par Thomsen *et al.* (2001) entre 1982 et 1998, Ivanek *et al.* (2005) ont estimé que les coûts liés à un rappel pouvaient atteindre jusqu'à 2.4 milliards de dollars par année. La présence des micro-organismes dans les aliments doit donc être prise très au sérieux. Un moyen répandu au niveau industriel consisterait à prévenir leur apparition (Ivanek *et al.*, 2005), permettant ainsi de réduire les coûts associés aux rappels de produits, de réduire les dépenses de santé, en plus d'augmenter la durée et la qualité de vie des consommateurs.

1.4 Lutte contre les contaminations bactériennes

Chaque étape de la transformation alimentaire peut être à l'origine d'une contamination bactérienne. Cependant, il existe plusieurs procédés de désinfection permettant d'assurer la salubrité alimentaire. Les désinfectants utilisés en industrie peuvent être chimiques (hypochlorite de sodium, dioxyde de chlore, peroxyde d'hydrogène, acide peracétique, ozone), physiques (UV-C, irradiation γ) ou biologiques (huiles essentielles, acides organiques). Les principaux traitements utilisés pour la désinfection des aliments sont présentés dans le **Tableau 1.1**.

Tableau 1.1. Principaux désinfectants utilisés en industrie alimentaire

Désinfectant	Mode d'action	Limites réglementaires	Autre	Référence
Hypochlorite de sodium	Inactivation enzymatique, altération du métabolisme	Interdit en Europe sur les produits frais	Puissant oxydant Dégradation en chloroforme, chloramine et/ou trihalométhanes	Gil *et al.* (2009) Artés *et al.* (2009)
Dioxyde de chlore	Inhibition du métabolisme	Rinçage à l'eau	Agent oxydant, élimination des mauvaises odeurs, peut entrainer un brunissement	Ölmez *et al.* (2009) Joshi *et al.* (2013)
Peroxyde d'hydrogène	Inhibition de la croissance	Non autorisé par la FDA pour usage sur des produits frais	Peut entrainer un brunissement Dégradation en eau et oxygène	Parish *et al.* (2003) Joshi *et al.* (2013) Artés *et al.* (2009) Artés *et al.* (2007a)
Acide peracétique	Oxydation des membranes	80 ppm	Efficacité influencée par le pH et la température Dégradation en acide acétique, eau et oxygène	Code of Federal Regulations (2012) Joshi *et al.* (2013)

Suite du Tableau 1.1

Ozone	Oxydation des parois, modification de l'ADN	100 ppm pour 8h d'exposition	Puissant oxydant, dégradation rapide, action en surface Dégradation en oxygène	Alencar *et al.* (2013) Shah *et al.* (2013) Kim *et al.* (1999)
Irradiation γ	Cassures de l'ADN	1 kGy (fruits et légumes)	Pénétration des rayons γ	Hussain *et al.* (2014) Jeong *et al.* (2010)
Irradiation UV-C	Inhibition de la croissance	1 W pour une surface de 5-10 pi^2	Action en surface	USFDA (2000) Hollósy (2002) Labas *et al.* (2005)
Acides organiques	Altération du pH cytoplasmique	Reconnu sans danger ("Generally Recognized As Safe" - GRAS)	Nécessite un long temps d'exposition, peut affecter les qualités organoleptiques	Ricke (2003) Davidson *et al.* (2007)
Huiles essentielles	Modification de la perméabilité membranaire	Reconnu sans danger (GRAS)	Très volatile, peut affecter les qualités organoleptiques	Dima *et al.* (2015) Burt (2004) Nazzaro *et al.* (2013)

1.4.1 **Désinfection par traitement chimique**

L'hypochlorite de sodium, NaClO, est un puissant oxydant. Son effet antimicrobien provient de la formation de l'acide hypochlorite (HClO) après ajout dans de l'eau. Selon Estrela *et al.* (2002), l'hypochlorite de sodium induit une inactivation enzymatique irréversible et altère le métabolisme cellulaire des bactéries. Il a été décrit que l'hypochlorite de sodium réagit avec la matière organique présente dans les aliments, menant à la formation de produits de dégradation potentiellement carcinogènes et/ou toxiques tels que le chloroforme, la chloramine ou les trihalométhanes. Suite à cela, plusieurs pays européens en ont interdit l'usage sur les produits frais (Artés *et al.*, 2009). Pourtant, Gil *et al.* (2009) citent une étude menée sur des salades, qui aurait conclu que la teneur en produits chlorés après traitement avec NaClO était inférieure à la limite autorisée dans l'eau de consommation. Selon Russell (2005), il faudrait consommer plusieurs kilos de salade pour que la teneur en trihalométhanes soit proche des limites toxicologiques.

Le dioxyde de chlore (ClO_2), un agent oxydant puissant, est également utilisé pour la désinfection des aliments. Ce composé pénètre à travers les parois cellulaires et induit également une inhibition du métabolisme (Joshi *et al.*, 2013). Il a été montré que le lavage de salades avec une solution de dioxyde de chlore à 2 mg/L induisait une réduction de la population d'*E. coli* de 1.4 log UFC/g (Petri *et al.*, 2015). Un avantage supplémentaire du dioxyde de chlore est sa capacité à éliminer les mauvaises odeurs alimentaires. Cependant, la réglementation américaine exige que tout produit désinfecté avec du dioxyde de chlore soit rincé à l'eau potable par la suite.

Le peroxyde d'hydrogène (H_2O_2) possède de bonnes propriétés bactéricides et bactériostatiques. En effet, les radicaux hydroxyles produits vont cibler les différents composés cellulaires, induisant une inhibition de la croissance microbienne (Santé Canada, 2013). Les produits de dégradation du peroxyde d'hydrogène sont l'eau et l'oxygène, ce qui le rend particulièrement intéressant (Joshi *et al.*, 2013). Des études ont été menées sur la survie de micro-organismes sur la cantaloup et elles ont montré que des traitements au peroxyde d'hydrogène étaient efficaces contre *L. monocytogenes* et réduisaient la flore totale (Sapers *et al.*, 2001b, Ukuku *et al.*, 2002). Cependant, il semblerait qu'une utilisation à faible concentration ne soit pas suffisante pour éliminer efficacement les micro-organismes (Artés *et al.*, 2009, Joshi *et al.*, 2013). De plus, plusieurs études signalent l'apparition d'un brunissement de certains fruits

et légumes, indiquant que les traitements au peroxyde d'hydrogène nécessitent l'ajout d'un agent anti-brunissement (Parish *et al.*, 2003, Sapers *et al.*, 2001a).

L'acide peracétique peut être utilisé comme alternative face aux désinfectants chlorés. Son effet antimicrobien provient de l'oxydation de la membrane externe des bactéries et ses produits de dégradation (acide acétique, eau et oxygène) sont peu dangereux (Joshi *et al.*, 2013). Cependant, la réglementation américaine impose une concentration maximale de 80 ppm (Code of Federal Regulations, 2012), ce qui ne semble pas être suffisant pour éliminer totalement les micro-organismes. En effet, Rodgers *et al.* (2004) ont montré que l'acide peracétique permettait une réduction de *L. monocytogenes* et d'*E. coli*, mais qu'il était moins efficace que les autres traitements testés (chlore, dioxyde de chlore, ozone). De même, Gonzalez *et al.* (2004) ont démontré que l'acide peracétique était moins efficace que le chlore et le chlorite de sodium.

L'ozone, un allotrope de l'oxygène, peut être produit par l'effet corona. L'air est exposé à un courant à haute tension, provoquant une séparation des électrons après leur excitation. Les atomes d'oxygène se retrouvant seuls vont par la suite se combiner aux molécules d'oxygène, formant ainsi une nouvelle molécule d'ozone possédant une demi-vie plus courte (Alencar *et al.*, 2013, Kim *et al.*, 1999, Shah *et al.*, 2013). L'effet antimicrobien de l'ozone a été étudié et plusieurs mécanismes ont été mis en évidence (Kim *et al.*, 1999). En effet, il a été proposé que l'ozone induit des changements au niveau de l'ADN des micro-organismes, menant à leur inactivation. Selon d'autres auteurs (Beuchat, 1992), l'ozone réagirait sur les parois cellulaires des bactéries en oxydant les composés majoritaires. Il est à noté que l'utilisation d'ozone est déjà autorisée par la FDA pour le traitement, l'entreposage et la transformation des aliments (Khadre *et al.*, 2001). L'usage de l'ozone se fait principalement dans l'eau et plusieurs études ont observé un effet antimicrobien. En effet, César *et al.* (2012) ont contaminé des instruments dentaires avec *E. coli* et ont montré qu'un traitement à l'eau ozonée pendant 10 min permettait de réduire la population bactérienne d'environ 2.7 log UFC/g. Il est aussi possible d'utiliser l'ozone sous forme gazeuse. Ainsi, Akbas *et al.* (2008) ont traité des figues avec de l'air ozoné à 1 ppm pendant 10 min et ont obtenu une réduction de la population d'*E. coli* de 3.5 log UFC/g.

Au cours des dernières années, un nouveau procédé a été combiné à l'ozonation de l'air : l'ionisation négative. Cette nouvelle méthode, utilisée pour purifier de l'air, apporte des charges négatives aux particules, provoquant leur accumulation sur les murs et les sols (Holt *et al.*, 1999). Selon Digel *et al.* (2005), la formation de radicaux libres induirait des dommages au niveau des membranes bactériennes, à l'origine de la mort cellulaire. Dans leur revue, Tyagi *et*

al. (2012) indiquent que les conditions environnementales peuvent influencer l'effet de l'ionisation négative de l'air sur les bactéries. Ainsi, Shargawi *et al.* (1999) ont démontré que plus la distance entre l'échantillon et le générateur d'ions était petite, plus l'inhibition de la croissance de *Candida albicans* augmentait. Le type de micro-organisme ciblé peut également influencer l'effet antimicrobien (Tyagi *et al.*, 2008). Les auteurs ont ainsi montré qu'une exposition aux ions négatifs induisait une réduction de la viabilité d'*E. coli* de 33% alors que celle de *Pseudomonas fluorescens* était de 42%. Challenger *et al.* (1996) ont aussi montré que l'utilisation d'ions négatifs peut aussi mener à la formation de peroxyde d'hydrogène, comme présenté dans l'équation suivante :

$$e^- + H_2O \rightarrow e^-_{aq}$$

$$O_2 + e^-_{aq} \rightarrow O_2^{\cdot}$$

$$2O_2^{\cdot} + 2H^+ \rightleftharpoons 2OH^- + 2O^{\cdot} \rightleftharpoons H_2O_2 + O_2$$

1.4.2 **Désinfection par traitement physique**

L'irradiation γ est un procédé à froid pouvant être utilisé pour la protection des aliments contre les micro-organismes. En effet, les rayons γ pénètrent à l'intérieur des aliments sans induire de radioactivité. Les doses d'irradiation sont exprimées en kilo Gray (kGy), unité pour laquelle 1 Gy équivaut à 1 J/kg de produit irradié. L'irradiation γ produit des effets directs et indirects sur les cellules. En effet, la cible principale du rayonnement γ est l'ADN, induisant des cassures (Hussain *et al.*, 2014, Jeong *et al.*, 2010). Ces modifications peuvent empêcher la réplication de la cellule et même conduire à sa mort en cas de dommages trop importants. Un des effets indirects de l'irradiation γ provient des produits issus de la radiolyse de l'eau. Des espèces réactives de l'oxygène ($OH^{\cdot}$, H^+, $O_2^{\cdot-}$) sont formées et vont à leur tour cibler certaines composantes de l'ADN (Beauchamp *et al.*, 2012). En 2005, il a été estimé que 185 686 tonnes d'épices et légumes secs, 81 593 tonnes de céréales et fruits, 32 471 tonnes de viandes et produits de la mer, 88 196 tonnes de racines alimentaires et 16 858 tonnes d'aliments divers (parmi lesquels les champignons ou le miel) ont été irradiés dans le monde (Kume *et al.*, 2009). Des limites ont été imposées par les autorités : la dose maximale pouvant être appliquée sur des fruits et légumes frais ne devrait pas dépasser 1 kGy (Komolprasert *et al.*, December

2007/January 2008). Cependant, certaines bactéries pathogènes telles que *L. monocytogenes* peuvent survivre et nécessitent des doses supérieures pour être éliminées (Bari *et al.*, 2006). Dans leur étude, Turgis *et al.* (2012) ont montré qu'une dose de 0.50 kGy permettait d'obtenir une réduction de *L. monocytogenes* de 1.3 log UFC/g sur des carottes. Dépendamment du niveau initial de contamination, il est possible que ce traitement ne permette pas une élimination totale des bactéries. Une étude récente de Ndoti-Nembe *et al.* (2015) a montré qu'une dose d'irradiation de 1 kGy sur des carottes inoculés par *Salmonella* Typhimurium à une concentration de 7 log UCF/g induisait une réduction de 1.7 log UFC/g après 24 h et 3.9 log UFC/g après 9 jours.

Les ultraviolets (UV) font partie de la région non ionisante (200-400 nm) et sont divisés en 3 régions : UV-C (longueur d'onde comprise entre 200 et 280 nm), UV-B (longueur d'onde comprise entre 280 et 320 nm) et UV-A (longueur d'onde comprise entre 320 et 400 nm). Le soleil est la principale source de radiation naturelle et émet des longueurs d'ondes entre 250 et 1200 nm (Falguera *et al.*, 2011). Cependant, les radiations correspondantes n'atteignent pas entièrement la surface de la Terre grâce à la couche d'ozone qui agit en tant que filtre et bloque les UV dont la longueur d'onde est inférieure à 280 nm (Falguera *et al.*, 2011, Hollósy, 2002). Hollósy (2002) a décrit différents types de cibles affectées par les radiations UV, la plus importante étant l'ADN. Une fois irradié par les UV-C, l'ADN peut subir des lésions, entrainant une inhibition de la division cellulaire et donc une mort des cellules. D'après Skowron *et al.* (2014) et Labas *et al.* (2005), une longueur d'onde de 254 nm permettrait d'obtenir un effet bactéricide maximal. En effet, les photons sont absorbés par l'ADN microbien à cette longueur d'onde, provoquant des modifications structurelles de l'ADN menant à la mort cellulaire (Bae *et al.*, 2012, Labas *et al.*, 2005). La facilité d'utilisation ainsi que l'absence de produits toxiques figurent parmi les avantages du rayonnement UV-C. Cependant, dû à la faible pénétration des rayons UV, son usage peut être limité à la désinfection en surface (Skowron *et al.*, 2014). Une étude de Chun *et al.* (2009) a montré qu'une dose de 8 kJ/m^2 appliquée sur des tranches de jambon pouvait réduire de 2 et 2.7 log UFC/g les populations de *S.* Typhimurium et *L. monocytogenes* respectivement. Ge *et al.* (2013) ont étudié différentes doses et différents temps de traitement sur la survie de *S.* Typhimurium dans de la salade. Une dose de 0.25 $mW.cm^{-2}$ appliquée pendant 5 ou 10 min a permis de réduire la charge microbienne de 1.4 ou 2.2 log UFC/g respectivement. En augmentant la dose d'irradiation UV-C à 1.5 $mW.cm^{-2}$, les auteurs ont observé une réduction de 2.3 log UFC/g, indépendamment du temps de traitement.

1.4.3 **Désinfection par traitement biologique**

De nombreux composés d'origine naturelle sont reconnus pour leurs propriétés antimicrobiennes. On retrouve ainsi les huiles essentielles (HE) et les acides organiques, qui ont déjà montré leur efficacité sur plusieurs bactéries pathogènes telles que *L. monocytogenes* et *E. coli* O157:H7 (Dussault *et al.*, 2014).

L'utilisation d'acides organiques comme antimicrobiens permet d'agir directement au niveau du cytoplasme des bactéries. Dans sa revue, Ricke (2003) explique qu'après leur passage à travers la membrane, les acides organiques vont se dissocier en anions et protons à l'intérieur du cytoplasme. La neutralité du pH est alors affectée, tout comme les composés cellulaires. Il a également été indiqué que l'énergie des cellules est utilisée pour transférer les protons en dehors de la cellule, résultant en un manque d'énergie pour assurer les fonctions cellulaires (Davidson *et al.*, 2007).

Les HE sont des molécules odorantes issues du métabolisme des plantes. Leur rôle est de protéger les plantes contre les différents stress qu'elle peut subir, tels que virus, bactéries, moisissures, rayonnement UV ou encore changements de températures (Nabavi *et al.*, 2015). Dans leur revue, Dima *et al.* (2015) ont décrit les différentes classes de composés retrouvés dans les HE : les terpènes/terpénoïdes, les phénylpropanoïdes, les composés soufrés et les composés azotés. Les terpènes sont constitués d'unités d'isoprène (présenté en **Figure 1.3**) linéaires ou cycliques alors que les terpénoïdes correspondent à des terpènes possédant un ou plusieurs atomes d'oxygène.

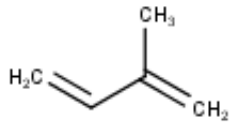


Figure 1.3 Molécule d'isoprène (unité de base des terpènes et terpénoïdes).

Parmi les terpènes les plus répandus, on retrouve le p-cimène, le γ-terpinène ou encore l'α-pinène. La famille des terpénoïdes contient, entre autres, le néral, le géranial, le thymol ainsi que le carvacrol. Enfin, les phénylpropanoïdes comprennent le trans-cinnamaldéhyde, l'eugénol et l'acétate d'eugényle. Les structures chimiques de tous ces composés sont présentées à la **Figure 1.4**. Les composés phénoliques, ayant comme base un cycle aromatique et une fonction

alcool, sont largement décrits dans la littérature (Burt, 2004, Llana-Ruiz-Cabello *et al.*, 2015, Sivakumar *et al.*, 2014, Wen *et al.*, 2003) et contiennent des fragments de terpénoïdes. C'est le cas du thymol et du carvacrol.

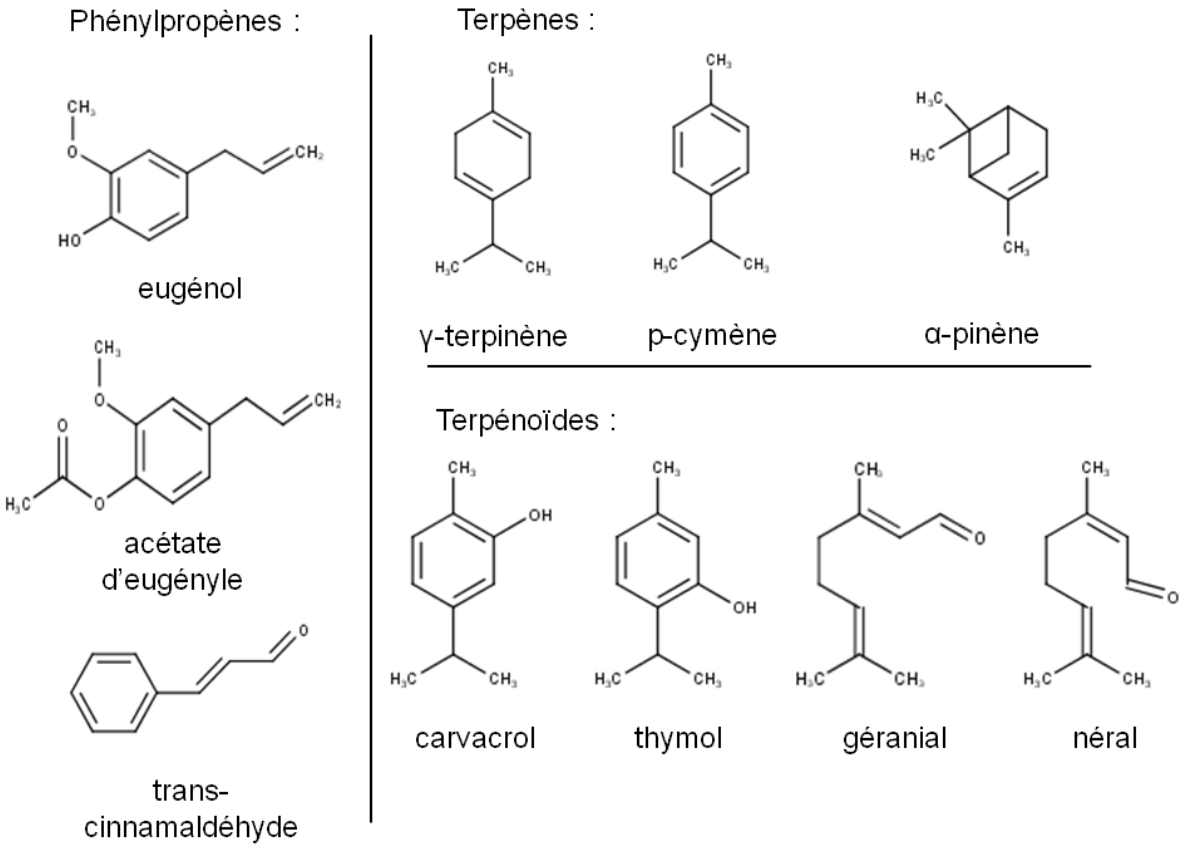


Figure 1.4 Structure chimique des principaux composés retrouvés dans les huiles essentielles.

Dans leur étude, Cosentino *et al.* (1999) ont identifié les composés présents dans trois HE de thym. Ils ont ainsi déterminé que l'effet antimicrobien contre différentes bactéries et levures était dû principalement aux composés phénoliques (thymol et carvacrol) présents dans ces HE.

L'effet des HE diffère selon que la bactérie est un Gram positif ou un Gram négatif (Nazzaro *et al.*, 2013). Les bactéries à Gram négatif possèdent une mince couche de peptidoglycanes reliée à une membrane externe, composée d'une bicouche phospholipidique. Les lipopolysaccharides sont formés de lipides A et de chaines latérales osidiques procurant une certaine résistance aux HE. En effet, le caractère hydrophile de cette membrane limite le passage des composés antimicrobiens, principalement hydrophobes (Calo *et al.*, 2015). En revanche, les bactéries à

Gram positif possèdent une structure membranaire composée de 90 à 95% de peptidoglycanes. La structure de cette membrane permet aux composés hydrophobes de la pénétrer et de se retrouver dans le cytoplasme. Ainsi, l'entrée des HE va perturber les membranes cellulaires des bactéries Gram positif et augmenter leur perméabilité (Burt, 2004, Calo *et al.*, 2015, Nazzaro *et al.*, 2013). Oussalah *et al.* (2006a) ont étudié l'effet des HE sur les composés cellulaires. Les auteurs ont rapporté que la concentration intracellulaire en ATP est réduite en présence d'HE, ce qui mènerait à des modifications au niveau de la membrane. Ces changements provoqueraient par la suite une perte des composés cellulaires et entraineraient la mort cellulaire. Caillet *et al.* (2009) mentionnent également le fait que certaines huiles essentielles pourraient réduire l'activité d'une enzyme à l'origine des liaisons entre les peptides qui forment la paroi cellulaire.

Ainsi, le **Tableau 1.2** montre que la concentration minimale inhibitrice (CMI) des HE peut varier selon la bactérie ciblée. En effet, selon Oussalah *et al.* (2007), l'utilisation de citronnelle à 0.4% permet une inhibition totale de *L. monocytogenes* alors qu'il faut une concentration supérieure à 0.8% pour obtenir le même résultat sur *E. coli*. L'utilisation de mélanges d'HE permettrait aussi d'atteindre une gamme plus large de micro-organismes tout en permettant une inhibition de ceux-ci. Fratini *et al.* (2014) ont ainsi étudié l'effet de la combinaison de deux HE (*Satureja montana* L. et *Thymus vulgaris* L. ct. thymol) sur six souches bactériennes. Des géloses ont été inoculées avec les bactéries ciblées puis un disque imprégné d'une des deux HE ou du mélange des deux a été déposé sur ces géloses. Les auteurs ont ensuite mesuré les zones d'inhibition présentes autour du disque. Les résultats ont montré que les HE utilisées seules provoquaient une inhibition des six souches bactériennes, à l'exception de *Thymus vulgaris* L. ct. thymol qui ne présente pas d'effet sur *E. coli*. En combinant ces deux HE, les auteurs ont observé un effet antimicrobien sur toutes les souches bactériennes, ainsi qu'une augmentation des zones d'inhibition.

Cependant, le caractère très volatile des HE réduit leur durée de vie et leur utilisation industrielle. L'oxydation par l'oxygène présent dans l'air peut également réduire l'efficacité des HE. Ainsi, la protection de ces composés devient donc nécessaire pour garantir des effets antimicrobiens optimaux à plus long terme.

Tableau 1.2 Composition d'huiles essentielles fréquemment utilisées et concentration minimale inhibant la croissance de *L. monocytogenes* et *E. coli*, adapté de Oussalah *et al.* (2007)

Huile essentielle	Composés majoritaires (%)	CMI sur *L. monocytogenes* (% v/v)	CMI sur *E. coli* (% v/v)
Cannelle	Trans-cinnamaldéhyde (87)	0.05	0.025
Citronnelle	Géranial (46) Néral (31)	0.4	> 0.8
Clou de girofle	Eugénol (78) Eugenyl acetate (14)	0.2	0.1
Coriandre	Linalool (70) α-pinène(6)	> 0.8	0.2
Origan	Carvacrol (54) p-cymène (14) γ-terpinène (14)	0.05	0.025
Thym	Carvacrol (33) p-cymène (24) Thymol (12)	0.1	0.05

1.5 Immobilisation des composés bioactifs

Depuis 1997, la recherche s'oriente de plus en plus sur le développement de films de polymère dans lesquels des agents antimicrobiens ont été incorporés (Appendini *et al.*, 2002). Ces films peuvent être directement intégrés à l'emballage des fruits et des légumes, et sont généralement faits à base de polysaccharides. Cependant, ils peuvent contenir d'autres composés tels que du glycérol, utilisé en tant que plastifiant, ou des huiles végétales, augmentant la solubilité des composés bioactifs hydrophobes. Il existe deux types de films : les films d'enrobage et les films d'emballage bioactifs. Les films d'enrobage bioactifs sont directement appliqués sur les aliments et sont ingérés par les consommateurs. Tous les composés présents doivent donc être comestibles et dans les limites autorisées par les agences de santé. Les films d'emballage bioactifs biodégradables peuvent être intégrés dans l'emballage alimentaire et vont bien souvent contenir des composés volatiles qui diffuseront dans l'environnement alimentaire. Ils doivent par contre avoir une certaine résistance et/ou flexibilité afin de faire face aux chocs qui peuvent survenir.

1.5.1 Matrices polymériques

Parmi les polysaccharides régulièrement utilisées pour l'immobilisation de composés bioactifs, on retrouve principalement la cellulose et ses dérivés, l'amidon et ses dérivés, le chitosane, l'alginate, la gomme xanthane ou encore la gomme gellane. La cellulose est le principal constituant des plantes, ce qui en fait le polymère le plus abondant dans la nature. Insoluble dans la majorité des solvants, la cellulose doit être fonctionnalisée avant utilisation. La méthylcellulose (MC), un des éthers cellulosiques les plus hydrophobes, est utilisée dans l'industrie depuis plusieurs années pour la production de gels en pharmaceutique, alimentaire, peinture, adhésifs ou encore cosmétiques (Khan *et al.*, 2010b). La nanocellulose cristalline (NCC) est un dérivé cellulosique composé d'un réseau de nanofibres. Les fibres de NCC ont un diamètre de 2 à 20 nm et une longueur allant de quelques centaines de nanomètres à quelques micromètres (Klemm *et al.*, 2009). Des études ont rapporté que la NCC améliore les propriétés mécaniques, diminue la perméabilité des films à base de MC et augmente la stabilité des agents bioactifs dans les films (Khan *et al.*, 2010b). Azeredo *et al.* (2010) ont également montré que les nanofibres de cellulose améliorent les propriétés mécaniques et réduisent la

perméabilité à la vapeur d'eau de films à base de chitosane. Des études menées sur des cellules endothéliales de cerveau ont montré que l'utilisation de NCC n'avait pas d'effet toxique sur les cellules. Ces résultats encouragent l'usage de la NCC comme transporteur de médicaments (Roman *et al.*, 2009).

L'amidon est un polymère ramifié fréquemment utilisé dans l'industrie alimentaire, dû à son abondance et son coût peu élevé. Il est constitué d'amylose, une chaine linéaire de D-glucose, et d'amylopectine, une chaine ramifiée de D-glucose. L'amylose est responsable des propriétés filmogènes des films à base d'amidon, ceux ayant une forte teneur en amylopectine étant cassants. À cause des groupements hydroxyles, l'amidon natif est très perméable à la vapeur d'eau. Il est cependant possible de le modifier de manière physique, chimique ou enzymatique pour améliorer ses propriétés (Pareta *et al.*, 2006). La maltodextrine (MD) est produite par hydrolyse partielle de l'amidon et se caractérise par son "dextrose équivalent" (DE). Plus le DE est élevé, plus l'amidon a été hydrolysé. Les MD ont un DE inférieur à 20; au-delà, on parle de sirop de glucose (Wang *et al.*, 2015). Il a été décrit que les MD possédant un DE élevé présentaient une meilleure efficacité pour l'encapsulation des composés bioactifs tout en offrant une meilleure protection contre l'oxydation (Jafari *et al.*, 2008, Wang *et al.*, 2015).

Le chitosane est obtenu par désacétylation partielle de la chitine, un composant de l'exosquelette des crustacés. Il s'agit d'un polysaccharide cationique, soluble en solution acide grâce à ses groupements amines et composé de moins de 20 % de β-(1,4)-2-acétaminido-D-glucopyranose et de plus de 80 % de β-(1,4)-2-amino-D-gucopyranose (Zou *et al.*, 2016). Le chitosane est également connu pour ses propriétés antimicrobiennes (Coma *et al.*, 2002, Jayakumar *et al.*, 2007, Kraśniewska *et al.*, 2012, Sánchez-González, Laura *et al.*, 2011a). Pour expliquer cet effet antimicrobien, Goy *et al.* (2009) décrivent en détail trois mécanismes, dont le plus probable serait dû aux interactions entre les charges positives du chitosane et les charges négatives des bactéries. Cependant, le chitosane est principalement utilisé pour des films d'emballage car sa consommation n'est pas autorisée aux États-Unis (Baldwin, 2007).

L'alginate est un polysaccharide extrait des algues marines et est constitué des acides β-D-mannuronique et α-L-guluronique alternés pour former une chaine linéaire. L'alginate est souvent utilisé comme matrice pour colorants et arômes. Une particularité de l'alginate est sa capacité à réagir avec les cations divalents ou trivalents tels que le calcium ou le fer. Les interactions avec le calcium et les groupements carboxyliques permettent la formation d'un réseau tridimensionnel et donc la formation de gels résistants.

La gomme xanthane est un polysaccharide produit par la bactérie *Xanthomonas campestri*. Ce polymère est constitué de deux unités de glucose, deux unités de mannose et une unité d'acide glucuronique (Garcia-Ochoa *et al.*, 2000). La gomme xanthane est reconnue comme additif alimentaire par la FDA et comme agent émulsifiant/stabilisant par la Communauté Européenne (Garcia-Ochoa *et al.*, 2000). La viscosité élevée de ce polysaccharide lui permet d'agir en tant qu'agent épaississant.

La gomme gellane est produite par la bactérie *Sphingomonas elodea* et est constituée de glucose, d'acide glucuronique et de rhamnose (Pérez *et al.*, 2009). Ce polysaccharide est souvent utilisé en tant qu'agent gélifiant et ses interactions avec le calcium lui permettent de former des films résistants (Moreira *et al.*, 2015).

Il est à noter que l'alginate, la gomme xanthane, la gomme gellane et la méthylcellulose sont reconnus par Santé Canada comme étant des additifs alimentaires, et classés dans la catégorie "agent émulsifiant, stabilisant ou épaississant" (Santé Canada, 2006). Leur consommation par le biais des films d'enrobage n'est donc pas problématique.

1.5.2 **Effet des films sur les fruits et légumes**

Les films d'enrobages alimentaires sont principalement développés dans le but d'augmenter la durée de vie du produit, que ce soit par réduction du développement bactérien ou par réduction de la perte de qualité au cours du temps. Le **Tableau 1.3** présente des exemples de films à base de polysaccharides ayant été développés ces dernières années.

Les enrobages à base de gellane développés par Rojas-Graü *et al.* (2007) ont induit un maintien de la couleur des pommes pendant 48h, grâce à la présence des composés bioactifs (N-acétylcystéine ou glutathion). Ce polysaccharide a permis de retarder le brunissement des pommes et constitue donc une bonne matrice pour l'immobilisation de la N-acétylcystéine et du glutathion. Le gellane étant une faible barrière à l'eau, les auteurs ont ajouté de l'huile végétale avant de mesurer la résistance de l'enrobage à l'eau. La présence de lipides dans l'enrobage a permis d'obtenir un enrobage présentant un meilleur effet barrière face à l'eau.

Guerreiro *et al.* (2015) ont développé un enrobage à base d'alginate 1 ou 2% et l'ont appliqué sur des fraises. Les fruits ainsi traités ont gardé leur couleur et leur texture pendant 14 jours. Les auteurs ont noté que l'ajout d'eugénol dans l'enrobage d'alginate permettait d'augmenter la

fermeté des fraises enrobées. Le traitement a également permis de réduire le développement des levures/moisissures tout au long de la conservation. Les meilleurs enrobages se sont révélés être ceux ayant une concentration en alginate de 2%. L'utilisation de concentrations plus faibles en alginate pourrait réduire la rétention des composés bioactifs et favoriser leur évaporation.

Cháfer *et al.* (2012) ont préparé un enrobage à base de chitosane et d'HE. La présence de thymol ou d'huile d'arbre à thé a permis de réduire le développement de moisissures sur des oranges. Les auteurs ont comparé l'effet de ces enrobages selon leur utilisation à des fins préventives ou curatives. Les résultats ont montré que la présence de l'enrobage a provoqué un ralentissement de la croissance des moisissures au cours du temps. L'HE de thym a présenté le meilleur effet préventif alors que l'huile d'arbre à thé est plus efficace pour un traitement curatif.

Randazzo *et al.* (2016) ont préparé des films d'emballage à base de chitosane ou de MC, et contenant de l'HE de citron. Les résultats obtenus ont démontré un effet antimicrobien *in vitro* contre *L. monocytogenes*. Les films de chitosane contenant l'HE de citron ont induit un meilleur effet antimicrobien, ce qui serait dû aux propriétés antimicrobiennes du chitosane. Les auteurs suggèrent également que l'entreposage à basse température favoriserait la diffusion des HE à travers les membranes bactériennes, menant ainsi à un effet antimicrobien plus important. Cette étude indique également qu'une bonne incorporation des composés bioactifs dans la matrice polymérique réduirait le taux de diffusion des antimicrobiens, permettant donc la présence d'un effet à plus long terme.

Das *et al.* (2013) ont utilisé l'amidon comme matrice de base pour la préparation de leur enrobage. L'étude a montré que ces enrobages agissaient en tant que barrière physique face aux micro-organismes mais qu'ils ne possédaient pas de propriétés antimicrobiennes. En revanche, leur utilisation a permis de réduire la maturation des tomates et les changements de couleur ont été retardés. Les auteurs attribuent cet effet à la présence d'extrait de thé vert qui, grâce à ses effets antioxydants, limiterait l'accumulation de caroténoïdes.

Mei *et al.* (2002) ont introduit de la vitamine E ou du calcium dans une matrice de gomme xanthane pour un usage sur des carottes. Ils ont observé une réduction de la décoloration en présence de l'enrobage. Les auteurs proposent deux effets pour expliquer ces observations. La décoloration des carottes étant due à une déshydratation, l'enrobage agirait comme une couche permettant d'hydrater les carottes. L'enrobage pourrait également combler les abrasions causées lors de la transformation alimentaire, permettre une certaine rétention de l'eau, présente sous forme d'humidité, et ainsi hydrater les fruits et légumes.

L'enrobage de MC développé par Maftoonazad *et al.* (2005) a permis d'augmenter la durée de vie d'avocats. En effet, les auteurs ont observé que l'enrobage avec de la MC permettait de ralentir le mûrissement en agissant comme une barrière physique face aux échanges gazeux, allongeant ainsi la conservation des avocats à 10 jours au lieu de 6. Ces études montrent que l'ajout de composés antimicrobiens permet de limiter le développement des micro-organismes présents, réduisant les risques de contaminations (bactéries telles que *L. monocytogenes*) et augmentant la durée de vie en réduisant les moisissures et/ou les bactéries naturellement présentes dans les légumes. Également, la matrice polymérique agit comme une barrière et réduit les échanges avec l'oxygène environnant. Cela a pour effet de diminuer les réactions d'oxydation et donc de retarder les mécanismes aboutissant à des pertes de couleur et de fermeté. L'enrobage joue aussi un rôle quant à la déshydratation des fruits et légumes.

Tableau 1.3 Exemple de matrices utilisées pour améliorer la qualité des fruits et légumes

Matrices	Composés bioactifs	Type de film	Aliments	Effets	Références
Gellane	Glycérol N-acétylcystéine Glutathion	Enrobage (épaisseur de 155 nm)	Pomme	Amélioration de la résistance à l'eau Maintien de la couleur pendant 48h	Rojas-Graü *et al.* (2007)
Alginate	Acide ascorbique Citral Eugénol	Enrobage	Fraise	Maintien de la couleur et de la fermeté Activité antioxydante plus élevée Effet antimicrobien contre les levures/moisissures et les micro-organismes mésophiles	Guerreiro *et al.* (2015)
Chitosane	Huile essentielle de bergamote Thymol Huile d'arbre à thé	Enrobage	Orange	Réduction des moisissures Pas de changement majeur de la qualité des fruits	Cháfer *et al.* (2012)
Chitosane ou méthylcellulose	Huile essentielle de citron	Emballage	(Tests réalisés *in vitro*)	Fort effet antimicrobien contre *L. monocytogenes*	Randazzo *et al.* (2016)

Suite du Tableau 1.3

Amidon	Extrait de thé vert	Enrobage (épaisseur de 0.05 nm)	Tomate	Réduction de la perte de masse Changements de couleur plus tardifs Pas d'effet antimicrobien	Das *et al.* (2013)
Xanthane	Vitamine E Calcium	Enrobage	Carotte	Contrôle de la déshydratation et de la décoloration Pas de modification des propriétés organoleptiques	Mei *et al.* (2002)
Méthylcellulose	(Aucun composé bioactif)	Enrobage	Avocat	Réduction de la respiration, des changements de couleur Maintien de la fermeté Augmentation de la durée de vie	Maftoonazad *et al.* (2005)

1.5.3 **Libération des composés bioactifs**

La formation de films bioactifs implique souvent l'immobilisation d'agents hydrophobes dans une matrice hydrophile. Il est ainsi important de stabiliser cette suspension dans le but d'obtenir par la suite les meilleurs effets. Pour cela, il est nécessaire de former une émulsion dite "huile dans eau", correspondant à la dispersion d'une phase huileuse, sous forme de gouttelettes, dans une phase aqueuse (McClements *et al.*, 2007, Perrier-Cornet *et al.*, 2005). Cela permet par la même occasion d'avoir une suspension uniforme. Cette stabilisation des composés bioactifs permet ainsi d'avoir une libération continue et contrôlée au cours du temps.

Le suivi de l'évolution de la teneur en composés bioactifs permet d'avoir une estimation de la libération de ces composés et d'optimiser, si nécessaire, leurs effets biologiques. Une étape d'extraction est bien souvent nécessaire et aboutit à l'obtention d'une solution contenant les composés bioactifs à l'étude. Ainsi, les composés phénoliques, à l'origine de l'effet antimicrobien des HE, peuvent être dosés grâce à la méthode de Folin-Ciocalteu. Dans sa revue, Gülçin (2012) explique de manière détaillée le dosage des composés phénoliques par le réactif de Folin-Ciocalteu. Ce réactif, un mélange de phosphomolybdate et de phosphotungstate en solution basique, permet d'oxyder les phénols. L'espèce $O_2^{\bullet -}$ nouvellement formée va réagir avec le molybdate, aboutissant à la formation d'oxyde de molybdène. Ce composé a une absorbance très élevée à une longueur d'onde de 750 nm. Cependant, toujours selon Gülçin (2012), le réactif de Folin-Ciocalteu va également réagir avec toute espèce réductrice présente dans l'échantillon. Bien que précise, cette méthode n'est donc spécifique qu'en l'absence d'autres composés réducteurs. De plus, comme toute technique nécessitant une extraction, le dosage des composés phénoliques est une méthode destructrice.

La spectroscopie infrarouge est une technique utilisée pour l'identification et la caractérisation de structures chimiques. Dans leur chapitre de livre, Davis *et al.* (2010) ont expliqué les principes de base de la spectroscopie. Un rayonnement infrarouge, dont les longueurs d'onde sont comprises entre 4000 et 400 cm^{-1}, est envoyé à travers l'échantillon et l'absorption d'une partie de ce rayonnement va provoquer des vibrations au niveau des liaisons chimiques. Ces vibrations peuvent être des élongations (allongement de la liaison entre deux atomes) ou des déformations (modification de l'angle entre deux liaisons). Le faisceau infrarouge est par la suite renvoyé sur un détecteur qui va transformer les informations reçues en signal électrique. Une opération mathématique, appelée "transformée de Fourier", va être appliquée et le signal va devenir un spectre FTIR. Dépendamment de son environnement, une structure chimique peut

subir plusieurs vibrations, ce qui se reflétera sur le spectre par la présence de plusieurs bandes à différentes longueurs d'onde. La zone située entre 1600 et 600 cm^{-1} correspond à "l'empreinte digitale", c'est-à-dire que cette zone est spécifique d'une molécule. Un des avantages de l'utilisation du FTIR est qu'il s'agit d'une méthode non-destructrice et que les échantillons analysés peuvent être récupérés par la suite.

Ainsi, Martins *et al.* (2012) ont utilisé la spectroscopie FTIR pour caractériser des films à base d'un mélange de carraghénine et de gomme de caroube. Ils se sont intéressés aux changements visibles dans la région 1300-750 cm^{-1} du spectre FTIR. Ils ont montré que l'ajout de gomme de caroube induisait une diminution de l'intensité de certaines bandes spécifiques de la carraghénine entre 1295 et 1204 cm^{-1}. Également, le déplacement de certaines bandes caractéristiques permet d'établir les interactions entre les groupements chimiques. Ainsi, les auteurs relient le déplacement d'une bande de 1162 à 1151 cm^{-1} à des ponts hydrogènes entre les groupements OH de la gomme de caroube et la carraghénine. Martínez-Abad *et al.* (2013) ont utilisé le FTIR pour caractériser des films antimicrobiens. Ils ont identifié les bandes spécifiques du cinnamaldéhyde et ont montré qu'il était possible d'en suivre la libération après incorporation dans des films de polycaprolactone (PCL). Cette étude a ainsi montré que le cinnamaldéhyde était en majorité libéré pendant les dix premières heures suivant son introduction et que la teneur en composés bioactifs était stable après 24h.

1.6 Combinaison de traitements

1.6.1 Combinaison avec l'irradiation gamma

L'utilisation de composés bioactifs en combinaison avec l'irradiation γ devrait permettre de fragiliser les bactéries et de les rendre ainsi plus sensibles au traitement d'irradiation. Plusieurs études portant sur ces combinaisons de traitements ont permis de réduire les doses d'irradiation utilisées tout en obtenant de bons effets antimicrobiens. Chiasson *et al.* (2004) ont étudié l'effet de plusieurs composés bioactifs en combinaison avec l'irradiation γ sur de la viande contaminée par *E. coli*. Les auteurs ont montré que la sensibilité relative de *E. coli* à l'irradiation γ était augmentée de 2.2 fois en présence de carvacrol. Ainsi, la D_{10}, dose d'irradiation permettant de réduire la charge microbienne de 1 log UFC/g, est passée de 0.126 kGy pour le groupe non traité à 0.057 kGy en présence de carvacrol. Une autre étude de

Chiasson *et al.* (2005) a montré que la sensibilité de *S.* Typhimurium était également augmentée de 2 fois en présence de carvacrol. Cependant, la conservation sous atmosphère modifiée n'a pas eu d'effet sur la sensibilité d'*E. coli* alors que celle de *S.* Typhimurium a augmenté de 4.2 fois.

Une autre étude, portant sur l'effet du trans-cinnamaldéhyde combiné à l'irradiation γ, a été effectuée sur de la viande (Ayari et al., 2012). L'irradiation à 3 kGy a permis de réduire la population bactérienne d'environ 1 log UFC/g pendant une durée de 14 jours. La présence de trans-cinnamaldéhyde en combinaison avec l'irradiation γ a induit un effet antimicrobien plus important avec une réduction de 2 log UFC/g. Selon les auteurs, l'altération de la perméabilité membranaire ainsi que les dommages créés à l'ADN par l'irradiation γ favoriseraient l'effet antimicrobien des composés bioactifs, provoquant ainsi une inhibition de la croissance bactérienne.

Takala *et al.* (2011) ont préparé un enrobage à base de MC contenant des acides organiques et un extrait de citrus. Ils ont étudié la croissance d'*E. coli* et *L. monocytogenes* sur des brocolis traités avec l'enrobage antimicrobien suivi de l'irradiation γ. Les résultats obtenus ont montré que la sensibilité des bactéries était augmentée lorsque les brocolis étaient traités avec l'enrobage antimicrobien. Les auteurs ont également montré qu'*E. coli* était plus sensible que *L. monocytogenes*, ce qui s'explique par la différence de structure au niveau de la membrane des bactéries (Gram positif *vs* Gram négatif). Plus récemment, Severino *et al.* (2014b) ont étudié l'effet d'un enrobage à base de chitosane et d'HE de mandarine en combinaison avec l'irradiation γ sur *L. monocytogenes*. Les résultats ont montré une diminution de 1 log UFC/g au jour 0 par rapport au groupe non traité. Les auteurs ont également souligné un effet antimicrobien présent tout au long de la conservation puisque la croissance bactérienne était limitée et bien inférieure au groupe non traité (2.7 et 5.3 log UFC/g respectivement).

1.6.2 **Combinaison avec l'ozone**

À ce jour, les recherches sur les combinaisons de traitements avec l'ozone gazeux sont peu nombreuses. Fan *et al.* (2002) ont étudié les interactions entre l'ozone gazeux et les ions négatifs. Ils ont ainsi montré que la combinaison entre les deux traitements était plus efficace que chaque traitement appliqué séparément, et qu'elle permettait d'inhiber totalement trois souches de bactéries en moins de 24h. En revanche, l'utilisation de l'ozone dans l'eau est bien

répandue et beaucoup d'auteurs ont dirigé leurs recherches sur ce traitement. Ainsi, Selma *et al.* (2008) ont évalué l'effet de l'eau ozonée (3 ppm) et des UV-C sur des oignons inoculés avec des coliformes. Le traitement par UV-C a permis des réductions de 1.4 et 2.4 log UFC/g qui ont été observées après 20 min et 1h respectivement. Des diminutions respectives de 2.7 et 4.0 log UFC/g ont également été observées après traitement à l'eau ozonée pendant ces mêmes temps. Par contre, la combinaison de ces deux traitements a induit des réductions de 4.0 et 4.8 log UFC/g après 20 min et 1h respectivement. L'association des deux traitements a ainsi permis de diminuer la croissance bactérienne de manière plus importante qu'en appliquant un seul traitement pour une même durée. Rong *et al.* (2010) ont évalué l'effet de la combinaison de traitements à base d'ozone et de chitosane. Des huitres ont été trempées dans de l'eau ozonée pendant 2 min puis dans un enrobage à base de chitosane pendant 10 min. Les résultats ont montré que le lavage à l'eau ozonée et l'enrobage ont permis d'augmenter la durée de conservation de 2 et 6 jours respectivement. La combinaison de ces deux traitements a plus que doublé le temps de conservation, qui est passé de 8 jours pour un échantillon non traité à 20 jours pour un échantillon ayant subi la combinaison eau ozonée + enrobage.

Plus récemment, Severino *et al.* (2014a) ont également testé l'eau ozonée combinée à un enrobage à base de chitosane contenant des HE de mandarine. Il a été montré que cette combinaison induisait une réduction de la population de *Listeria* et que celle-ci était présente tout au long de la conservation (15 jours). Par contre, les résultats obtenus ont montré que l'enrobage seul était plus efficace que la combinaison des deux traitements. Les auteurs n'ont pu expliqué cette observation mais il est possible que l'enrobage appliqué joue également un rôle protecteur vis-à-vis des effets oxydants de l'ozone.

1.7 But, hypothèses et objectifs

1.7.1 But

Cette étude a comme but de développer deux systèmes polymériques à base de composés naturels permettant d'assurer l'innocuité, de maintenir les qualités physico-chimiques et organoleptiques des légumes prêts à manger et de vérifier l'effet de la combinaison de traitements sur l'élimination des bactéries. Le chou-fleur et le brocoli ont été sélectionnés comme aliments d'étude. Ces légumes se retrouvent parmi les plus vendeurs au Québec, totalisant plus de 38 millions de dollars en 2009 (Observatoire horticole, 2011). Cependant, les micro-organismes peuvent facilement se loger dans les interstices entourant les fleurets et ainsi être hors d'atteinte des produits de désinfection. Ces légumes deviennent donc deux modèles d'étude intéressants car un film efficace sur ceux-ci devrait induire des résultats similaires ou meilleurs sur les légumes présentant des surfaces lisses.

1.7.2 Hypothèses

Le pouvoir antimicrobien des HE est particulièrement intéressant. Cependant, l'utilisation des HE pures peut modifier l'odeur et/ou le goût des aliments. Il est donc habituel de les diluer avant usage. La présence de tensioactifs devient alors nécessaire pour obtenir une stabilité de ce mélange huile/eau et éviter une séparation de phase. La formation d'émulsions permet également de mieux disperser l'huile dans l'eau par la formation de gouttelettes.

Les légumes ayant une durée de vie de plusieurs jours, il est également important de s'assurer que les composés antimicrobiens soient présents et diffusent tout au long de la conservation. De nombreuses matrices comestibles permettent d'emprisonner différents composés bioactifs, ralentissant ainsi leur dégradation ou leur dispersion.

Enfin, il est possible d'utiliser les HE pour fragiliser les micro-organismes. Ceux-ci peuvent par la suite être éliminés par l'ajout d'un autre traitement antimicrobien. Cette combinaison permet également d'utiliser des doses et/ou concentrations inférieures aux limites maximales autorisées par les agences de santé.

Ainsi, les hypothèses suivantes sont posées :

1. La mise au point d'émulsions stabilisera les molécules bioactives et améliorera leur efficacité antimicrobienne.

2. L'immobilisation des émulsions dans des polymères protègera la bioactivité des antimicrobiens et permettra une libération contrôlée au cours du temps.

3. La combinaison de traitements permettra de diminuer les doses nécessaires pour réduire les bactéries hétérotrophes et éliminer les bactéries pathogènes.

1.7.3 **Objectifs spécifiques**

Les trois objectifs spécifiques suivants ont été fixés afin de répondre au but du projet et de valider les hypothèses :

Objectif 1 : Développer un film d'emballage bioactif et caractériser la libération des composés bioactifs.

Objectif 2 : Développer un enrobage antimicrobien et caractériser son effet antimicrobien *in vitro* et *in situ*. Évaluer les propriétés organoleptiques et les qualités physico-chimiques de choux-fleurs pré-coupés enrobés.

Objectif 3 : Évaluer l'effet antimicrobien de l'enrobage en combinaison avec l'irradiation γ ou avec l'ionisation/ozonation de l'air pendant un entreposage de 7 jours.

1.7.4 **Moyens pour atteindre les objectifs**

Objectif 1 : Le suivi de la libération des composés bioactifs immobilisés dans un film d'emballage a été réalisé selon deux méthodes. D'abord, la quantification a été faite par dosage des composés phénoliques afin d'établir un profil de libération (méthode traditionnelle). Les films ont également été analysés par spectroscopie infra-rouge à transformée de Fourier (FTIR) afin d'identifier les bandes spécifiques des composés bioactifs et de suivre leur évolution dans le temps (méthode innovante). Les propriétés physico-chimiques des films ont également été déterminées par des mesures de colorimétrie, d'élasticité et de résistance à la traction. L'effet antimicrobien des films a été vérifié *in vitro* sur gélose et *in situ* sur des brocolis pré-coupés.

Objectif 2 : L'effet antimicrobien *in vitro* de l'enrobage a été caractérisé par une méthode permettant de déterminer la concentration minimale inhibitrice (CMI). Les propriétés organoleptiques des choux-fleurs enrobés ont été évaluées grâce à une analyse sensorielle selon une échelle hédonique en 9 points. Enfin, la qualité des légumes a été déterminée suite à l'évaluation du taux de respiration ainsi qu'aux changements de couleur et de texture pendant l'entreposage.

Objectif 3 : L'enrobage a été combiné à l'irradiation γ ou à l'ionisation/ozonation de l'air. Les propriétés antimicrobiennes contre *L.* innocua, *E. coli* et les bactéries hétérotrophes ont été évaluées par des analyses microbiologiques effectuées tout au long de la conservation des légumes.

PARTIE 2 : ARTICLES

2 PUBLICATION 1: CHARACTERIZATION OF TRILAYER ANTIMICROBIAL DIFFUSION FILMS (ADFS) BASED ON METHYLCELLULOSE-POLYCAPROLACTONE COMPOSITES

Publié dans Journal of Agricultural and Food Chemistry 2013;61:811-21

Afia Boumail[†], Stephane Salmieri[†], Emilie Klimas[†], Pamphile O. Tawema[†], Jean Bouchard[‡], and Monique Lacroix[*†]

[†] INRS-Institute Armand-Frappier, 531 Blvd des Prairies, Laval, Quebec H7V 1B7, Canada

[‡] FPInnovations, 570 Blvd St-Jean, Pointe-Claire, Quebec, H9R 3J9, Canada

* Author to whom correspondence should be addressed, Professor Monique Lacroix Telephone: +1-450-687-5010; Fax: +1-450-686-5501; E-mail: monique.lacroix@iaf.inrs.ca

L'article est présenté tel que publié dans le journal. Le numéro des figures et des tableaux ainsi que la présentation des références ont été modifiés pour suivre un ordre continu au travers de la thèse.

2.1 Contribution des auteurs

J'ai préparé le plan expérimental, mis au point puis réalisé toutes les expériences de cette étude. J'ai également rédigé la publication scientifique. Émilie Klimas et Pamphile O. Tawema m'ont aidé à la préparation du matériel et à la réalisation des expériences. Stéphane Salmieri a participé à l'élaboration des protocoles et aux discussions scientifiques. Jean Bouchard a participé à la révision de la publication. La Pre Monique Lacroix était la responsable scientifique et la coordinatrice du projet de recherche. Elle a supervisé l'élaboration des protocoles, participé aux discussions scientifiques et révisé cet article.

2.2 Résumé en français

Des films de diffusion antimicrobiens en trois couches (ADF) ont été développés pour des applications alimentaires. Les ADFs étaient composées de deux couches externes de polycaprolactone (PCL) et d'une couche interne composée d'une matrice de nanocellulose (NCC) et de méthylcellulose (MC). Deux mélanges antimicrobiens (formulations A et B) ont été incorporés dans la couche interne de MC puis comparés par évaluation des propriétés du film. Les ADFs obtenus ont été insérés comme "patch" de diffusion dans des emballages contenant des légumes et les échantillons ont été conservés à 4 °C pendant 14 jours. Des tests de diffusion microbiologiques en présence des ADFs ont été effectués avec des bactéries pathogènes. L'étude a ensuite porté sur la caractérisation des propriétés physico-chimiques, structurelles et la libération des phénols totaux depuis les ADFs vers l'emballage. La libération des phénols totaux a été déterminée par la méthode de Folin-Ciocalteu et par analyse FTIR. Les résultats ont indiqué une libération contrôlée des agents antimicrobiens dans l'emballage (16.5% pour la formulation A et 13.4% pour la formulation B). Une bonne corrélation (≥ 90%) entre les deux méthodes a permis de mettre au point une méthode FTIR innovante, précise et rapide permettant de quantifier la diffusion des phénols totaux. La microscopie électronique à balayage a montré une structure fibrillaire due à la NCC et un réseau plus compact dû aux antimicrobiens. Les formulations antimicrobiennes encapsulées ont induit des changements de couleur sans pour autant affecter l'attribut visuel des films. Les ADFs contenant la formulation B ont présenté la plus haute résistance à la traction (17.3 MPa) pendant la conservation.

2.3 **Abstract**

Novel trilayer antimicrobial diffusion films (ADFs) were developed for food applications. ADFs were composed of two external layers of polycaprolactone and one internal layer of nanocellulose (NCC)-reinforced methylcellulose (MC) matrix. Two antimicrobial mixtures (formulations A and B) were incorporated in MC layer and compared via the evaluation of film properties. Resulting ADFs were inserted as diffusion devices into vegetables packages and samples were stored at 4°C for 14 days. Microbiological diffusion assays in presence of ADFs were performed on pathogenic bacteria. From this, the study focused on characterizing the structural, physicochemical properties and total phenols (TP) release from ADFs. This TP release was determined by Folin-Ciocalteu's method and by FTIR analysis. Results indicated a controlled release of antimicrobials into headspace (16.5% for formulation A and 13.4% for B). Good correlations (≥ 90%) between both methods allowed validating an innovative, accurate, rapid FTIR procedure to quantify the diffusion of TP**.** SEM micrographs showed fibrillar structure due to NCC and a more compact network due to antimicrobials. Encapsulated antimicrobial formulations induced color changes without affecting visual attributes of films. ADFs containing formulation B exhibited the highest tensile strength (17.3 MPa) over storage.

Keywords: Antimicrobial film, diffusion, methylcellulose, polycaprolactone, nanocrystalline cellulose, composite.

2.4 **Introduction**

Recently, food-borne microbial outbreaks have generated many research programs for the development of innovative methods to inhibit microbial growth in foods while maintaining quality, freshness and safety (Devlieghere *et al.*, 2004, Matthews *et al.*, 2010). In this context, the addition of functional antimicrobial ingredients in food presents many perspectives, particularly by focusing on i) the stability of antimicrobial compounds during processing and storage and ii) the need to prevent undesirable sensorial interactions with food matrix. Therefore, the encapsulation of antimicrobial ingredients in polymer could help to solve these two concerns (Dehkharghanian *et al.*, 2009, Han *et al.*, 2008).

Essential oils (EOs), secondary metabolites of plants, are complex mixtures of volatile substances. They contain large amounts of active compounds such as phenolic acids and flavonoids, which provide strong antimicrobial or antioxidant properties and low toxicity compared with those from synthetic phenolic antioxidants, such as BHT (butylated hydroxytoluene) (Caillet *et al.*, 2006b, Conner, D. E. *et al.*, 1984, Oussalah *et al.*, 2004). These remarkable properties induce these natural agents to be used as alternative food preservatives (Conner, D.E., 1993). However, due to their high relative volatility and thereby the difficulty to control their release into food products, the use of EOs may not be fully effective when directly applied on food. Their encapsulation in a polymeric matrix such as packaging films, edible coatings or diffusion films could provide an alternative issue in order to ensure their stability in such a way that only desired levels of the preservatives diffuse progressively and come into contact with the food (Dehkharghanian *et al.*, 2009, Oussalah *et al.*, 2004, Oussalah *et al.*, 2006b, Oussalah *et al.*, 2007, Takala *et al.*, 2011, Vu *et al.*, 2011). Previous studies showed that the incorporation of essential oils in alginate-based films could provide a reduction of the bacterial load up to five days due to the liberation of antimicrobial compounds.

Polycaprolactone (PCL) is a semicrystalline biodegradable polyester providing good water resistance and good processability (Yoshii *et al.*, 2000). It can be used to produce biodegradable water-resistant films, compatible with various polymer blends (Goupil, 1996, Kohn *et al.*, 1996, Lewis *et al.*, 1997) and therefore provides excellent properties for patch applications in humid environments. Methylcellulose (MC) is a cellulose ether which has been widely used for many years in industry to produce gels and fine chemicals in pharmaceuticals, foods, paints and cosmetics (Khan *et al.*, 2010b). Nanocrystalline cellulose (NCC) is another

cellulose derivative which particles have a diameter of 2-20 nm and a length of a few hundred nanometers. The network created within the matrix provides mechanical reinforcement into polymer bulk (Favier *et al.*, 1995). Some studies have reported that NCC can increase the stability of encapsulated antimicrobial agents into film matrices (Azeredo *et al.*, 2010, Khan *et al.*, 2010b, Klemm *et al.*, 2009, Klemm *et al.*, 2006).

The aim of the present study was to characterize trilayer antimicrobial diffusion films (ADFs) composed of PCL/MC/PCL. Natural antimicrobial agents (formulations A and B) were incorporated into internal MC based-films. A preliminary antimicrobial assay was performed against *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* to evaluate *in vitro* antimicrobial properties of ADFs. Films were also introduced in packages containing pre-cut vegetables and stored for 14 days at 4°C. The molecular structure of ADFs was revealed by Fourier transform infrared (FTIR) spectroscopy and their cross-section morphology was investigated by scanning electron microscopy (SEM). The total phenolic (TP) volatiles release was evaluated by Folin-Ciocalteu's method but also by FTIR analysis, via semi-quantification of encapsulated antimicrobials, for correlations purposes. Colorimetric parameters and mechanical properties of ADFs were measured to determine their variations over storage.

The innovation of this work consists in the development of trilayer diffusion films containing natural antimicrobials for direct application on vegetables. The second aspect is the determination of the percentage of bioactive compounds released from the films over storage by fast FTIR method.

2.5 Experimental section

2.5.1 Materials

2.5.1.1 *Antimicrobial formulations*

EOs mixtures (formulations A and B) were provided from BSA Ingredients s.e.c./l.p. (Montreal, Quebec, Canada). Liquid smoke, composed of a mixture of organic acids, was from Kerry

Ingredients and Flavours (Monterrey, TN, USA). Rosemary extract was from P.L. Thomas and Co., Inc. (Morristown, NJ, USA).

2.5.1.2 *Film ingredients*

MC (Mn ~ 40,000; viscosity 400 cP, 2% in water at 20°C) and PCL (Mn ~ 80,000) were from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada). Nanocrystalline cellulose (NCC) was provided by FPInnovations (Pointe Claire, QC, Canada). Glycerol, used as a plasticizer, and Tween®80, as an emulsifier, were purchased from Laboratoire Mat (Beauport, QC, Canada). Vegetable oil (VO) was used as a hydrophobic agent in film-formation process in order to stabilize hydrophobic compounds via emulsion systems and was bought in a local distributor (Metro, Laval, Quebec, Canada).

2.5.1.3 *Reagents*

Sodium carbonate (monohydrate), Folin-Ciocalteu's phenol reagent and gallic acid were from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada).

2.5.2 **Methods**

2.5.2.1 *Preparation of PCL films (external layers)*

PCL compressed films were prepared by compression molding, according to a procedure developed by Sharmin *et al.* (2012).

2.5.2.2 *Preparation of MC-based films (antimicrobial internal layer)*

(i) Preparation of NCC suspension

A 2% (w/v) NCC aqueous suspension was prepared under stirring before sonication at room temperature for 30 min, using a sonicator bath Branson DHA-1000 (Branson Ultrasonics Corporation, Danbury, CT, USA) at a frequency of 40 kHz. Tween® 80 was then added under stirring at room temperature.

(ii) Dispersion of antimicrobials in the NCC suspension

EOs were added to the NCC suspension along with the liquid smoke. Rosemary extract was solubilized in water, at 40°C under stirring, then added to the former suspension. The solutions containing the antimicrobial agents were then mixed with the NCC suspension under stirring, for 5 min at room temperature. The resulting antimicrobial suspensions were then homogenized at room temperature, using a digital Ultra-Turrax T25 disperser (IKA® Works Inc., Wilmington, NC, USA), at 20,000 rpm for 1min.

(iii) Preparation of antimicrobial MC matrix

MC was solubilized in distilled water, under stirring at 40°C (for pre-gelatinization) and was then cooled in an ice bath to ensure complete solubilization. Then, VO and glycerol were added to the MC suspension and the mixture was stirred strongly for 10 min at room temperature. The NCC suspension (containing antimicrobials) was slowly added to the MC suspension, under strong stirring. Composite films were cast by applying 15 mL of the film-forming suspension onto Petri dishes (95 $\times$ 15 mm; Fisher Scientific, Ottawa, ON, Canada) and allowed to dry i) for 2 h at 40°C and ii) for 24 h at 20°C. Otherwise, for comparison purposes, MC-based films containing formulations A and B were assigned to MC-A and MC-B respectively whereas MC-based films containing no antimicrobials were designated as MC-control.

2.5.2.3 *Preparation of ADFs as trilayer composites PCL/MC/PCL*

Trilayer composite films were fabricated similarly to the compression molding process of PCL films described above. This compression molding step was used to form insoluble films. Each internal and external film weighed around 500 mg. Compression molding was operated at 120°C for 1 min without compression, then with 1 ton pressure for 1 min before cooling for 2 min. PCL/MC/PCL trilayer films (namely ADF; thickness ~ 225-280 µm) were collected and used immediately for testing. For comparison purposes, ADFs containing formulations A and B were assigned to ADF-A and ADF-B respectively while ADFs containing no antimicrobial were designated as ADF-control.

2.5.2.4 *Evaluation of the antimicrobial properties of films*

(i) Preparation of bacterial strains

Escherichia coli O157:H7 EDL 933 (INRS-Institut Armand Frappier, Laval, QC, Canada), *Salmonella* Typhimurium SL1344 (INRS-Institut Armand-Frappier) and *Listeria monocytogenes* 2812 1/2a (Health Canada, St-Hyacinthe, QC, Canada) were maintained at – 80°C in Tryptic Soy Broth (TSB, Difco Laboratories, Detroit, MI, USA) containing 10% glycerol. Prior to the experiment, stock cultures were propagated through two consecutive 24 h growth cycles in TSB at 37°C to obtain working cultures containing approximately 10^9 CFU/mL.

(ii) Antimicrobial assay of pathogenic bacteria

The antimicrobial activity of MC-based films was evaluated according to a modified procedure used by Rojas-Grau *et al.* (2006). Films were cut into 7.5 mm diameter disks and were placed on Mueller Hinton (Oxoid Ltd, Basingstoke, England) previously streaked with a solution containing 10^9 CFU/mL of bacteria.

2.5.2.5 *Treatments of vegetables with ADFs*

ADFs were inserted into high barrier packaging bags (Deli One, 3 mil nylon/EvOH/polyethylene, 8 × 10; Winpak Division Ltd, Montreal, QC, Canada) containing vegetable (100 g) and sealed under air. Sealed packages were then stored immediately at 4°C for physicochemical analysis of films during 14 days of storage.

2.5.2.6 *TP release of antimicrobial compounds*

(i) Determination by spectrophotometry

The availability of total phenols (TP) in ADF-A and ADF-B was determined using Folin-Ciocalteu's procedure, according to a modified method developed by Salmieri and Lacroix (Salmieri *et al.*, 2006).

The concentration of TP was determined according to the following equation:

$$\mathrm{TP\ (\mu g\ GAE/film)} = \frac{\left((\mathrm{A_{ADF-A\ or-B}} - \mathrm{A_{ADF-control}}) + \mathrm{b}\right) \times \mathrm{V}}{\mathrm{a} \times \mathrm{m_{ADF-A\ or-B}}}$$

where:

$\mathrm{A_{ADF-A\ or-B\ or-control}}$ = Absorbance of ADF-A or -B or -control.

V = volume of extract.

$\mathrm{m_{ADF-A\ or-B}}$ = Mass of ADF-A or -B extracted.

a = slope of the standard curve

b = Y-intercept of the standard curve.

TP release was deduced from TP availability in film, according to the equation:

$$\mathrm{TP\ release\ (\%)} = \left(1 - \frac{\mathrm{TP}}{\mathrm{TP_i}}\right) \times 100$$

where $\mathrm{TP_i}$ is the initial concentration of TP in films at Day 0.

A 2 periods-moving average regression was used to represent the TP release of antimicrobials from ADF-A and -B in order to smooth out short-term variations and highlight long-term trends over storage, via periodic extrapolation.

(ii) Determination by ATR-FTIR spectroscopy

FTIR spectra of the films were recorded using a Spectrum One spectrophotometer (Perkin-Elmer, Woodbridge, ON, Canada) equipped with an attenuated total reflectance (ATR) device for solids analysis and a high-linearity lithium tantalate (HLLT) detector. Spectra were analyzed using Spectrum® 6.3.5 software. Film components, PCL/MC/PCL trilayer films and MC-based films (internal layer), were analyzed for structural characterization and for evaluating the available content of TP in ADFs during storage. Samples were placed onto a zinc selenide crystal and the analysis was performed within the spectral region of 650-4000 cm^{-1}, with 64 scans recorded at 4 cm^{-1} resolution. After attenuation of total reflectance and baseline correction, spectra were normalized with a limit ordinate of 1.5 absorbance units. Hence, resulting FTIR spectra were compared i) to identify typical vibration bands related to film components but also ii) to attribute vibration bands assigned to antimicrobial components specifically and their respective peak intensities for estimation of the TP release. Peak absorbance was measured for MC-A and MC-B during storage by determining the height of peaks (with MC-control as a blank) with Spectrum® software. Finally, data obtained by Folin-Ciocalteu's method and by FTIR were compiled and mathematical correlations were performed between absorbance of FTIR peaks related to antimicrobial volatiles and TP measurements in order to validate the feasibility of FTIR analysis to evaluate the TP release of antimicrobial compounds from ADFs.

(iii) Scanning Electron Microscopy Analysis (SEM)

SEM was used to investigate the effect of NCC and antimicrobial incorporation on the cross-section morphology of MC internal layers. MC layers were prepared for SEM using freeze fracture technique by allowing a MC piece (5 $\times$ 5 mm) to equilibrate under liquid nitrogen. Film samples were then fractured in the liquid nitrogen held between tweezers. Samples were then deposited on an aluminum holder and sputtered with gold-palladium alloy (Au/Pd deposition rate of 30 s equivalent to a coating thickness of 50 Å) in a Hummer IV sputter coater. SEM photographs were taken using a Hitachi S-4700 FEG-SEM (Hitachi Canada Ltd., Mississauga,

ON, Canada) at a magnification of 40,000 x and 100,000 x at room temperature and a X-ray detector (model 7200, Oxford Instruments, Abingdon, UK) with a resolution of 1.36 eV at 5.9 keV. The working distance was maintained around 8 mm, and the acceleration voltage used was 2 kV.

2.5.2.7 *Physicochemical properties of films*

(i) Colorimetry of films

Color of the films was measured using a Colormet® with flat window (Instrumar Engineering Ltd, St. Johns, NF, Canada). Measurements of spectral reflectance were performed directly onto the film surface (viewing area: 10 x 20 mm). The L*, a*, b* system (CIELAB) was employed, L* axis represents the lightness from black (L* = 0) to absolute white (L* = 100), a* axis varies from green (-) to red (+) and b* axis from blue (-) to yellow (+). In order to characterize more precisely the color of ADFs, hue angle (hue = Arctan (b*/a*) if a* > 0 and hue = Arctan (b*/a*) + 180° if a* < 0) was determined to indicate color changes between a* (green color) and the intersection of a* and b*, from green (hue = 0°) to yellow color (hue = 90°).

(ii) Mechanical properties of films

Tensile strength (TS) and tensile modulus (TM) were measured according to the method developed by Khan *et al.* (2010b).

2.5.2.8 *Experimental design and statistical analysis*

Samples were separated into 3 groups: (i) ADF-control; (ii) ADF-A; (iii) ADF-B. All measurements were performed in triplicate (n = 3). FTIR, TP measurements and determination of mechanical properties were done using a 2 x 3 x 3 x 7 factorial design: 2 repetitions, 3 replicates, 3 treatments (ADF, ADF-A, ADF-B), 7 days of storage (0, 1, 2, 6, 8, 10 and 13). SEM was performed at Day 0 (day of fabrication) in order to characterize films after compression molding. Color measurements were realized according to a 3 x 3 x 3 factorial design: 3 replicates, 3 treatments, 3 days of storage (0, 6 and 13). Analysis of variance, Duncan's

multiple-range test and Student *t* test were performed for statistical analysis by using PASW Statistics 18.0 software (IBM Corporation, Somers, NY, USA). Differences between means were considered to be significant at a 5% level.

2.6 Results and discussion

2.6.1 Antimicrobial assay

Antibacterial activity of internal films from ADFs is shown in **Table 2.1** and illustrated in **Fig. 2.1**. MC-control did not inhibit any growth of tested pathogenic bacteria. Notable inhibitory zones were detected for MC-A and -B against *E. coli*, *S.* Typhimurium and *L. monocytogenes*. Results showed no significant difference ($p > 0.05$) between MC-A and -B for each bacteria (10.7-12.8 mm for MC-A and 11.5-13.6 mm for MC-B), suggesting that both antimicrobial films had similar inhibitory efficiency. Also, MC-A and -B induced similar inhibitory zones against *L. monocytogenes* and *E. coli* ($p > 0.05$), with diameter values around 10.7-10.8 mm for MC-A and 11.5 mm for MC-B. Besides, both films generated significant larger inhibitory areas ($p \leq 0.05$) against *S.* Typhimurium as compared to other bacteria, with values comprised between 12.8 and 13.6 mm (**Fig. 2.1**). Such observations of high efficiency of antimicrobials against Gram-negative bacteria were reported by Jin *et al.* (2008) and López *et al.* (2007), in relation with pH and external conditions during storage. Although EOs are generally less efficient against Gram-negative bacteria (due to a more resistant structure of their cell wall membrane), the authors explained such unexpected results by the antimicrobial mechanism generated via the diffusion of volatile compounds and their effectiveness from the film to the package headspace, in relation with the concentration of antimicrobials in the closed atmosphere as a function of time *i.e.* the difference of diffusion through the polymeric matrix. Oussalah *et al.* (2007) have determined the minimum inhibitory concentrations (MIC) of several EOs on four pathogenic bacteria, showing that the inhibition of bacteria depends on the concentration of EOs. They indicated that the MIC on *L. monocytogenes* and *E. coli* can vary from two to four times the MIC of *S.* Typhimurium. Those results are consistent with those obtained from the antimicrobial assay.

Table 2.1 Antibacterial activity of ADFs against *E. coli*, *L. monocytogenes* and *S.* Typhimurium.

Type of film	Inhibitory zone (mm) against pathogens[a]		
	E. coli	*L. monocytogenes*	*S.* Typhimurium
ADF-control	ND a,A	ND a,A	ND a,A
ADF-A	10.7 ± 0.5 b,A	10.8 ± 0.3 b,A	12.8 ± 1.2 b,B
ADF-B	11.5 ± 0.4 b,A	11.5 ± 0.3 b,A	13.6 ± 1.1 b,B

[a]Means followed by the same lowercase letter in each column are not significantly different at the 5% level. Means followed by the same uppercase letter in each row are not significantly different at the 5% level. ND : Non-detectable (no inhibitory zone detected).

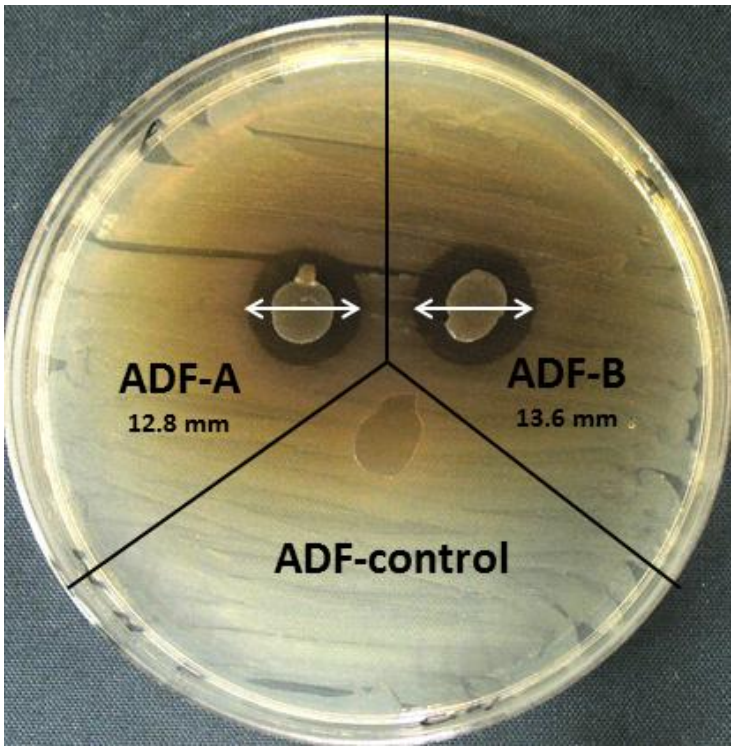


Figure 2.1 Inhibitory zones of *S.* Typhimurium growth on a bacterial plate induced by trilayer ADF-control, ADF-A and ADF-B.

2.6.2 **TP release by spectrophotometry**

The availability of TP content of MC-A and MC-B internal layers during storage is presented in **Table 2.2** and their corresponding release is depicted in **Fig. 2.2**. The profiles of diffusion in **Fig. 2.2** were designed mathematically according to a 2 periods-moving average regression in order to smooth out short-term variations and highlight long-term trends over storage. The moving average model is very useful to estimate the global trend of TP release by periodic extrapolation. TP contents in MC-A and MC-B films at Day 0 were similar (68.3 and 68.2 µg GAE/mg film for MC-A and MC-B respectively), as standardized initial TP concentration in films. Overall, results show that both TP availability in MC-A and MC-B decreased continuously from Day 0 to Day 13 with TP values ranging from 68.3 to 57.0 µg GAE/mg for MC-A and from 68.2 to 59.0 µg GAE/mg for MC-B. These values represent a diffusion of 21.2 and 14.0% respectively for MC-A and MC-B films after 14 days of storage. Between Days 0-2, similar releases of TP were observed in both films ($p > 0.05$), showing a value of 61.5-62.0 µg GAE/mg of film at Day 2. The diffusion curve (**Fig. 2.2**) indicated a TP release at Day 2 of 8.7 and 6.8% for MC-A and MC-B films respectively. These data generated slopes of 4.4 and 3.4% TP/day, hence indicating a higher diffusion over this first sub-period. Between Days 2-8, TP releases of ADF-A and ADF-B were significantly different ($p \leq 0.05$), showing releases of 19.0 and 10.2% for ADF-A and ADF-B respectively at Day 8. These data generated slopes of 1.7 and 0.6% TP/day suggesting a slower release during this second sub-period. Also, it can be observed that the slowdown of ADF-B diffusion was emphasized with a plateau observed from Day 6 whereas a plateau was noted at Day 10 for ADF-A. As a result, ADF-B films showed the lowest TP release during all storage, which may represent an advantage for long term storage of food. From these results, it could be hypothesized that chemical interactions between antimicrobials and MC matrix could influence the TP release of antimicrobial volatiles, as suggested by previous studies (López *et al.*, 2007). Hence, the different rates obtained for ADF-A and –B could be related to the chemical nature and volatility of major phenolic components in formulations A and B during diffusion process. Chemical interactions between bioactive molecules and film components, and the resulting diffusion of volatiles, are described below in FTIR analysis.

Table 2.2 Determination of total phenolics (TP) content in ADF-A and ADF-B during storage.

Films	TP concentration (µg GAE/mg film)[a] Days						
	0	**1**	**2**	**6**	**8**	**10**	**13**
ADF-A	68.3 ± 4.9 a,E	63.3 ± 5.1 a,D	61.5 ± 4.3 a,D	53.4 ± 5.2 a,B	57.2 ± 4.6 a,C	50.6 ± 7.9 a,A	57.0 ± 4.7 a,C
ADF-B	68.2 ± 4.9 a,D	65.1 ± 6.2 a,C	62.0 ± 5.2 a,B	60.2 ± 4.0 b,AB	62.2 ± 4.7 b,B	58.3 ± 5.2 b,A	59.0 ± 4.5 b,A

[a]Means followed by the same lowercase letter in each column are not significantly different at the 5% level. Means followed by the same uppercase letter in each row are not significantly different at the 5% level.

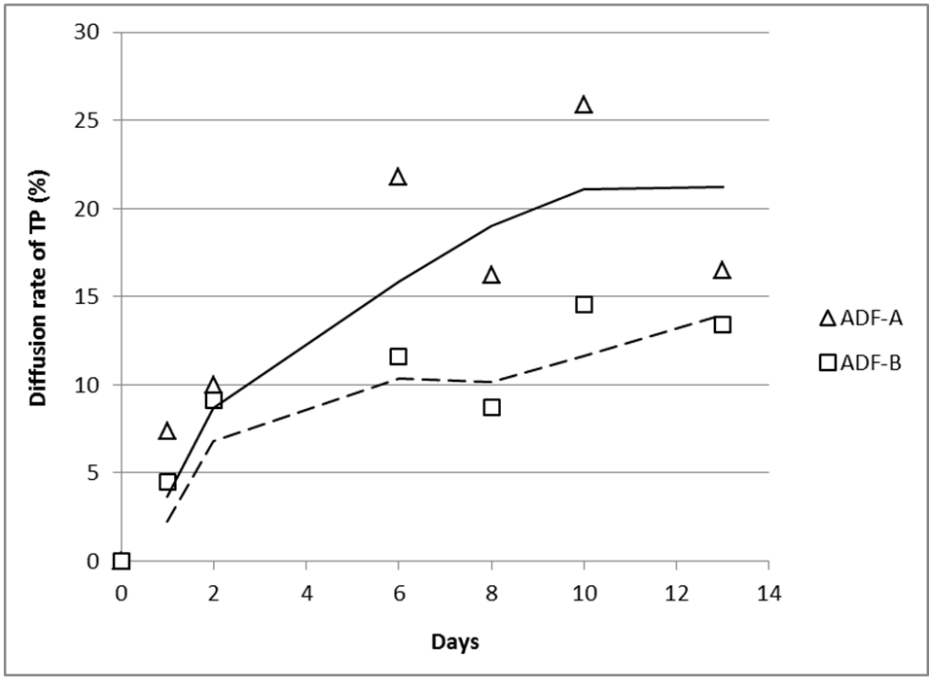


Figure 2.2 Profile of diffusion of TP (%) from ADF-A and ADF-B according to a 2 periods-moving average regression.

2.6.3 **FTIR analysis of ADFs**

2.6.3.1 *Analysis of ADFs in function of time*

FTIR spectra of ADFs (composite trilayer films) at different days of storage, with spectrum corresponding to Day 0, 6 and 13, are presented in **Fig. 2.3**. As shown in **Table 2.3**, the absorption sharp peaks of spectrum at Day 0 (2950-2850 cm^{-1}, 1720 cm^{-1} and 1180-1160 cm^{-1}) are mainly assigned to IR vibrations of PCL (external layer of ADFs). The comparison of these 3 spectra allowed characterizing the hydration of ADFs during storage, by progressive increase of peak intensity related to water absorption and by resulting decrease of intensity related to PCL functional groups. Indeed, typical bands from water such as O–H stretching and O–H bending appeared during storage, as indicated by spectra at Day 6 and Day 13. Meanwhile, this hydration resulted in a decrease of intensity of all main peaks attributed to PCL. In view of these results, it is supported that water detection would come from hydration of internal water soluble MC-based film whereas PCL is strongly hydrophobic. Similar observations suggesting a reduction of signal from hydrophobic compounds to the detriment of a hydration process were already reported by Salmieri *et al.* (2006).

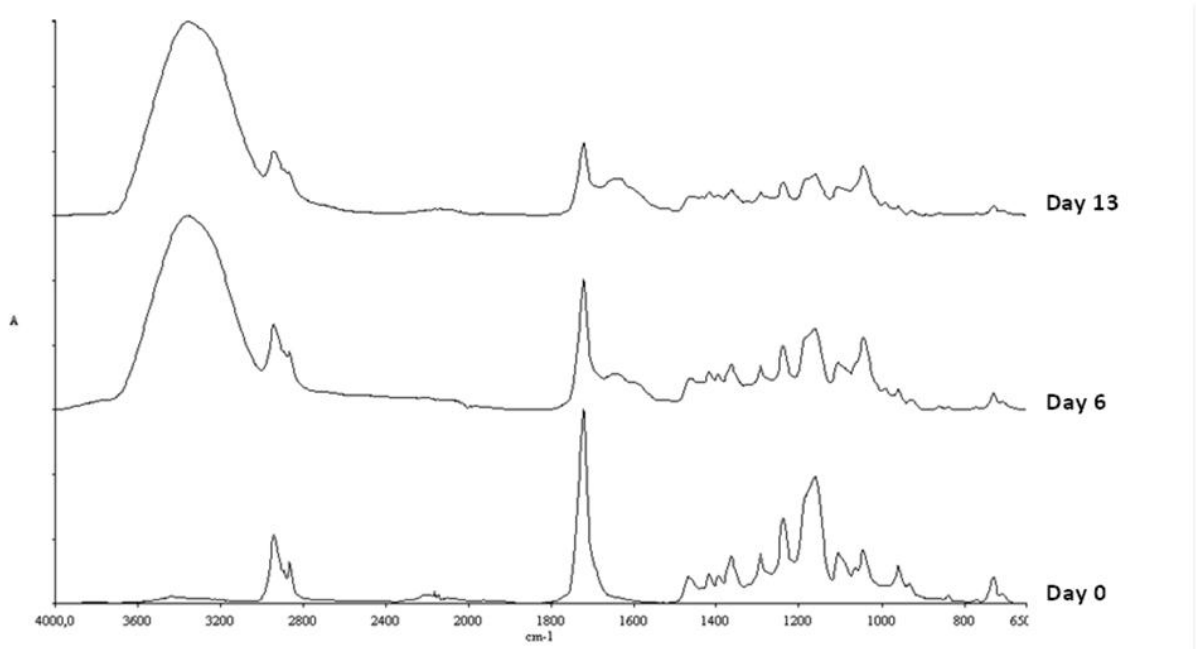


Figure 2.3 FTIR spectra of composite trilayer ADFs as related to time of storage at Day 0, Day 6 and Day 13. Typical vibration bands of PCL based-external layers, related to water content in the films, are shown in function of time.

Table 2.3 Characteristic IR absorption frequencies as related to Figures 3-4 and assignment of typical vibrations to the chemical groups of film components.

Related Spectrum	Wavenumber (cm^{-1})	Vibration	Functional group
Fig. 3 (trilayer ADFs)	3600-3000	OH stretching	Water
	2950-2850	C-H antisymmetric and symmetric stretching	-CH_2 and –CH_3 in aliphatic compounds
	1720	C=O stretching	-C=O in lactones
	1635	-OH bending	-OH in water
	1240	C-O-C antisymmetric stretching	-C-O-C in ethers and esters
	1180-1160	C-O stretching	-C-O in lactones
Fig. 4 – spectrum a (internal layer MC-control)	3600-3200	-OH stretching	-OH in alcohols/phenols (Khan *et al.*, 2010b)
	2960-2870	–C-H symmetric stretching	-CH_2 and –CH_3 in aliphatic compounds (Khan *et al.*, 2010b)
	1800-1600	-OH bending	-OH in water associated with MC (Khan *et al.*, 2010b, Velasquez *et al.*, 2003)
	1500-1270	–C-H symmetric and asymmetric bending	Degree of order in MC (Filho *et al.*, 2007, Velasquez *et al.*, 2003)
	1160-950	-C-O-C antisymmetric stretching and carbon ring	-C-O-C cellulose ethers and -C-CH from carbon rings in cyclic compounds (Khan *et al.*, 2010b, Sharmin *et al.*, 2012)
Fig. 4 – spectrum b (rosemary extract)	3600-3200	-OH stretching	-OH in alcohols/phenols
	3100-2400	-OH stretching	-OH in carboxylic acid groups
	3100-3000	=CH stretching	=CH in aromatic and unsaturated hydrocarbons
	1690	-C=O stretching	-C=O in unsaturated ester and carboxylic acid

Suite du Tableau 2.3.

Fig. 4 – spectra c, d (formulations A and B)	1600 and 1516	Aromatic ring stretching	Aromatic compounds
	1256	C-O-C antisymmetric stretching	C-O-C in ester groups
	3600-3200	OH stretching (predominant in spectrum d)	-OH in alcohols/phenols
	3100-2850	-C-H symmetric stretching	-C-H in aliphatic and unsaturated hydrocarbons
	1670-1660	-C=O stretching	-C=O in unsaturated esters, aldehydes and ketones
	1600 and 1515	Aromatic ring stretching	Aromatic compounds
	1450-1250	-C-H bending and C-O-C stretching	Terpenic compounds and esters from EOs
Fig. 4 – spectrum e (internal layer MC-B)	3600-3100	-OH stretching	-OH in aromatics/phenolics
	3100-2850	-C-H symmetric stretching	-C-H in aliphatic and unsaturated hydrocarbons
	1600 and 1515	Aromatic ring stretching	Aromatic compounds
	1265	C-O-C antisymmetric stretching	C-O-C in ester groups

2.6.3.2 *Analysis of the composition of internal MC layer*

FTIR spectra of **(a)** MC-control, **(b)** rosemary extract powder, **(c)** formulation A, **(d)** formulation B and **(e)** MC-B *i.e.* MC matrix containing formulation B are presented in **Fig. 2.4**. It is to be noted that spectrum of MC-A was not presented since it was identical to that of MC-B (totally overlapped spectra). Also, FTIR spectrum of liquid smoke was not used in FTIR comparison due to its very weak signal as compared to other antimicrobial compounds, implying totally masked absorption bands in resulting spectrum **(e)**.

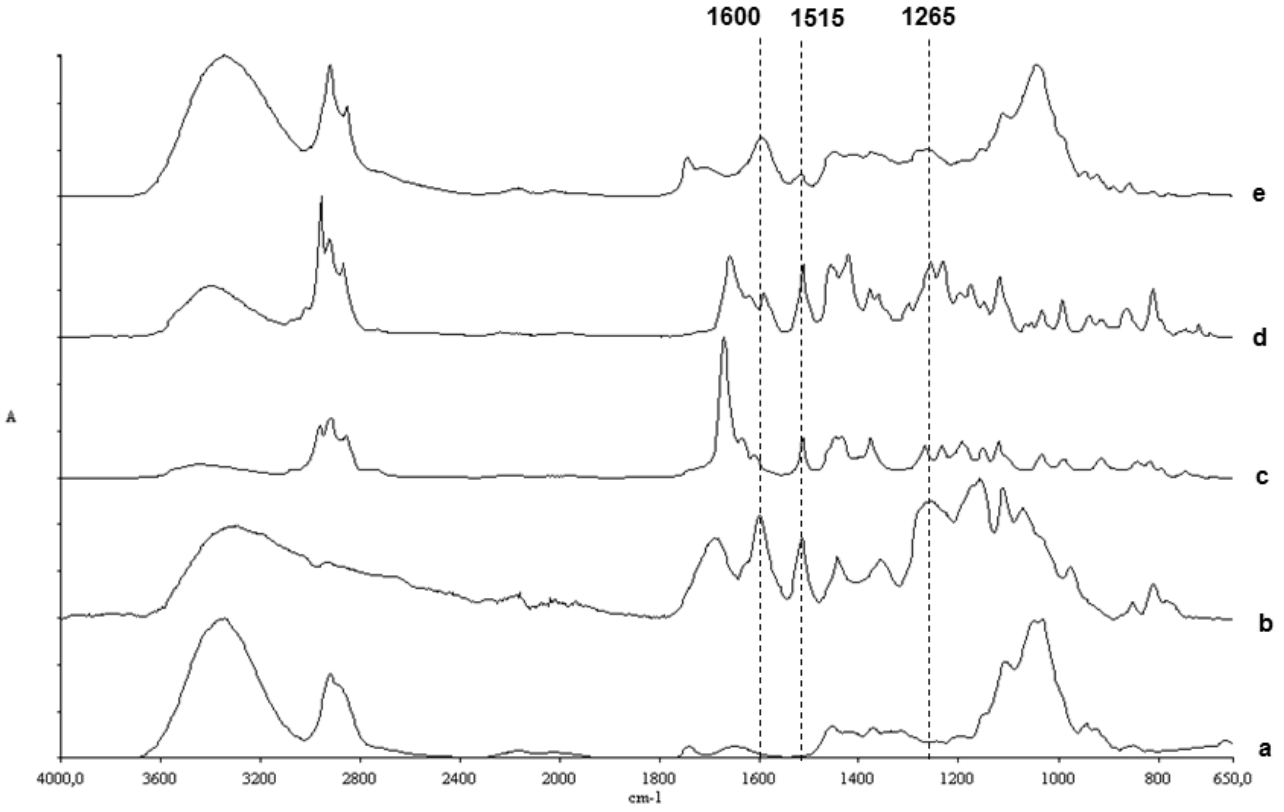


Figure 2.4 FTIR spectra related to the composition of MC-control (a), rosemary extract (b), formulation A (c), formulation B (d) and MC-B (e). Bands at 1600, 1515 and 1265 cm^{-1} are related to aromatic and ester groups from antimicrobial compounds (spectra b, c, d) and are exploitable in ADF-B (spectrum e) for further quantification of the TP release of bioactive ADFs.

Spectra **(c)** and **(d)** are typical FTIR profiles of EOs, with vibration bands mainly attributable to phenolic and terpenic groups. However, some differences can be noted between spectrum **(c)**

(formulation A) and **(d)** (formulation B), due to their respective major components. Indeed, a strong band related to O–H stretching can be observed in spectrum **(d)**, suggesting that formulation B is composed of a major part of phenolic compounds as compared to formulation A that would contain more hydrocarbon terpenic compounds. Spectrum **(e)** represents the FTIR profile of MC-B, hence containing typical absorption bands of MC-matrix and antimicrobial compounds such as rosmarinic acid and formulation B. Some differences can be highlighted in the whole infrared (IR) region after incorporation of formulation B in MC matrix. Indeed, as referred to spectrum **(a)**, a broader band is observed in spectrum **(e)** at 3600-3100 cm^{-1}, related to typical O–H vibrations of aromatic/phenolic in formulation B. In addition, a stronger and asymmetric band occurs at 3100-2850 cm^{-1}, underlying the presence of aliphatic and unsaturated hydrocarbons related to terpenoid components. Clearer changes in spectrum **(e)** can be noted with the appearance of vibrational bands associated with antimicrobials and involving ring stretching modes of aromatic groups at 1600 and 1515 cm^{-1} and C–O–C antisymmetric stretch mode of esters at 1265 cm^{-1}.

Hence, **Fig. 2.4** shows that the evolution of the spectra from **(a)** to **(e)** was consistent as related to the chemical nature of each film component **(a)** to **(d)** and the additive effect of their strong peaks in the whole MC-A or -B film formulation.

2.6.4 **TP release by FTIR analysis**

2.6.4.1 *Identification of bands related to diffused antimicrobials*

The evolution of FTIR spectra of MC-control and MC-B during storage, at Days 0, 2, 6 and 13 is presented in **Fig. 2.5-2.6**. Note that FTIR spectra of MC-A-based films were not shown since they presented similar evolving peaks as those of MC-B-based films. This analysis focused on the fingerprint region (1800-1200 cm^{-1}) of MC internal layer in order i) to take into consideration the evolution of the bands ascribed to the hydration of MC matrix (**Fig. 2.5**) but also ii) to evaluate the diffusion of antimicrobial volatile compounds (**Fig. 2.6**). Spectra were normalized by setting O–H stretching bands up to 1.5 absorbance units (AU) to subtract water absorption in MC matrix. Moreover, the analysis of fingerprint region allowed differentiating the peaks that were associated to antimicrobial compounds. In general, spectra in **Fig. 2.5-2.6** presented the same evolution in higher frequencies (4000-2400 cm^{-1}). For each sample (MC-control and MC-

B), the O–H stretching vibration at 3600-3200 cm^{-1} broadened out during storage (Day 0 to Day 13), suggesting hydration of MC matrix, which is in agreement with results obtained for ADFs (**Fig. 2.3**) and similar observations have already been reported by Le Tien *et al.* (2000) for cellulose-based films. In the meantime, this band broadening due to hydration was also accompanied by a reduced intensity of C–H stretching vibrations at 2950-2850 cm^{-1} (hydrophobic groups), as described above (**Fig. 2.3**). The fingerprint IR region in **Fig. 2.5** shows that the bound water vibration of MC-control at 1650 cm^{-1} increased during storage (relatively to hydration effect) while its vibration modes related to degree of order decreased at 1500-1270 cm^{-1}, possibly due to a reduced resonance of methylated groups in MC, as reported by Khan *et al.* (2010b).

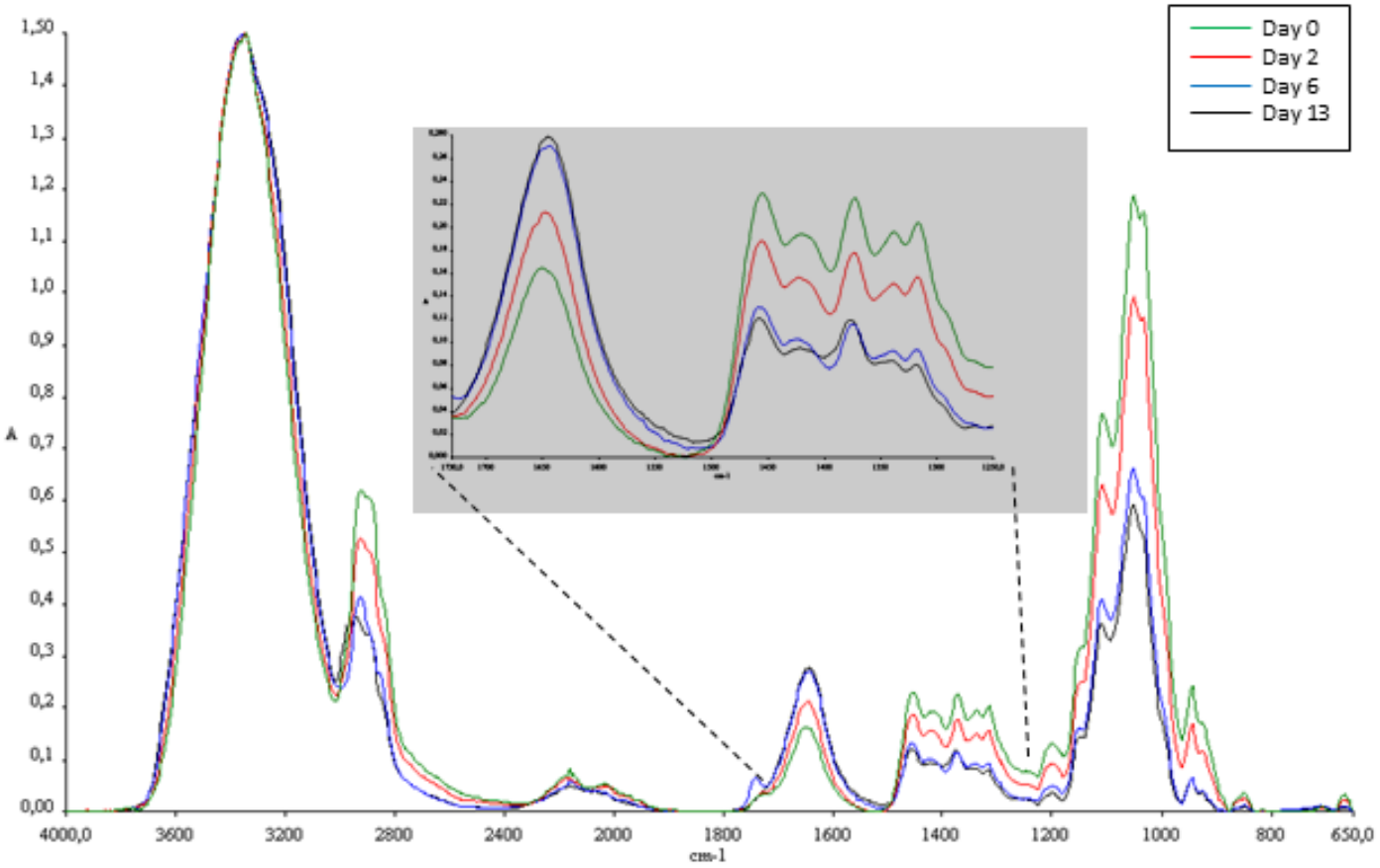


Figure 2.5 FTIR spectra of MC-control internal films as related to storage time, with focus on fingerprint region (1250-1730 cm^{-1}). Color of spectra indicates the day of analysis: green (Day 0), red (Day 2), blue (Day 6) and black (Day 13).

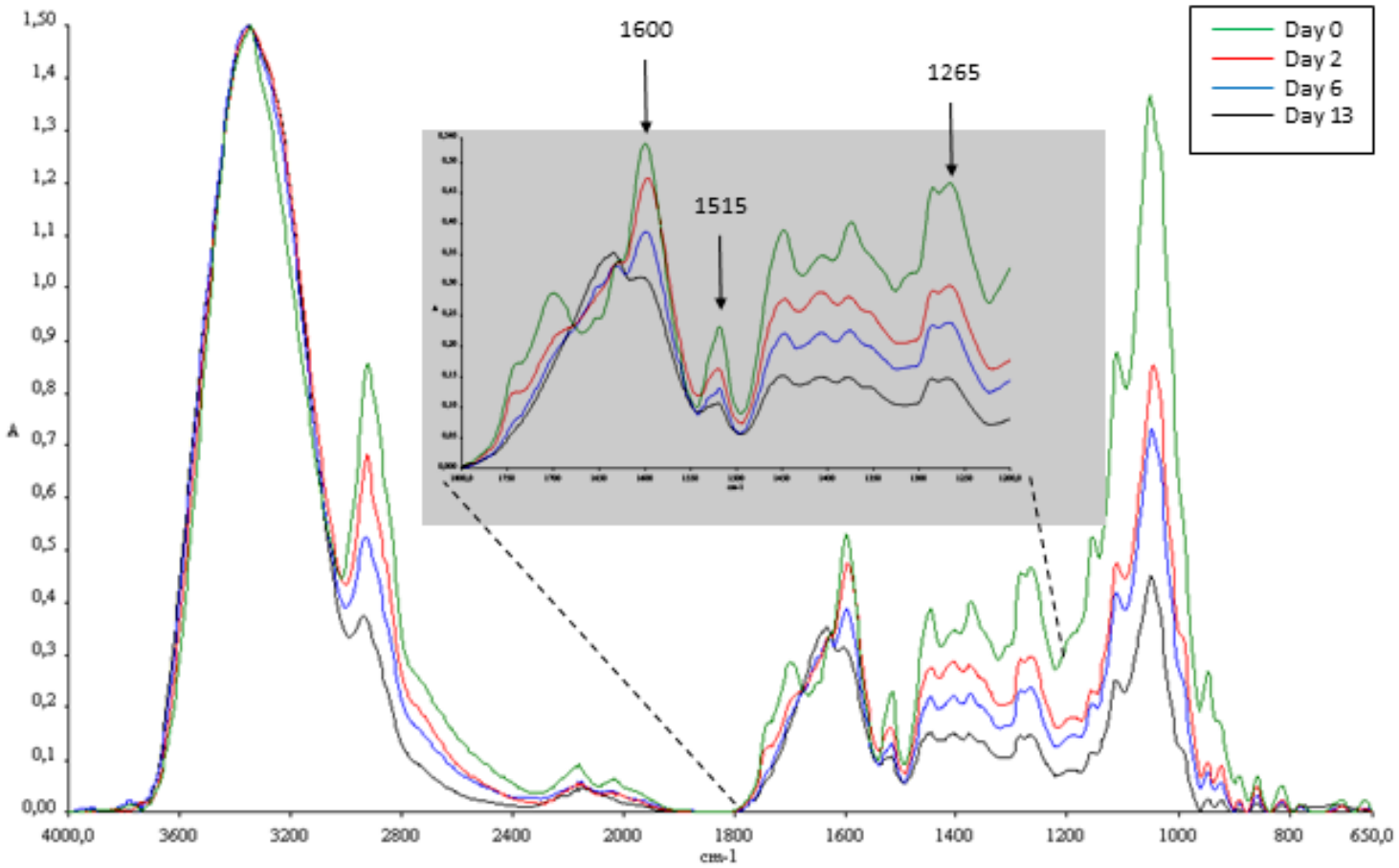


Figure 2.6 FTIR spectra of MC-B internal films as related to storage time, with focus on fingerprint region (1200-1800 cm^{-1}). Color of spectra indicates the day of analysis: green (Day 0), red (Day 2), blue (Day 6) and black (Day 13). Note that FTIR spectra of MC-A films presented exactly the same peaks that evolved in a very similar way

Fig. 2.6 shows the evolution of FTIR spectra of MC-B internal films, during storage. In comparison with **Fig. 2.5** (MC-control), the fingerprint region of **Fig. 2.6** indicates the appearance of 3 typical peaks associated with antimicrobial formulation B such as ring stretching modes of aromatic groups at 1600 and 1515 cm^{-1} and C–O–C antisymmetric stretch mode of esters at 1265 cm^{-1}, which is congruent with observations in **Fig. 2.6-e**. It can also be observed that the intensity of these 3 peaks decreased during storage, thereby indicating a diminution of antimicrobial contents in MC matrix. This profile suggests a diffusion of antimicrobial volatiles towards external environment (headspace), which is in accordance with results obtained for TP quantification (**Table 2.2** and **Fig. 2.2**).

2.6.4.2 *Semi-quantification of IR bands*

In order to correlate FTIR analysis of the bands related to diffused antimicrobials with TP results (**Table 2.2**), the peak heights (absorbance) of IR bands at 1600, 1515 and 1265 cm^{-1} of MC-A and MC-B films, and their relative percentage during storage, were measured and presented in **Table 2.4**. Results show that the percentage of IR peaks related to antimicrobial volatiles decreased progressively over time, suggesting a slow diffusion of volatiles from the films to packaging headspace. If considering proportionality of peak height with availability of compounds in the film, the evolution of percentage of peak height in MC-A at 1600 cm^{-1} (aromatic compounds) is analogous to MC-B, with variations from 100 to 54-58% between Day 0 and 13. In comparison, the progression of peak heights at 1515 and 1265 cm^{-1} seems to be less analogous between the two types of films, with reductions to 45-56% at 1515 cm^{-1} and to 16-23% at 1265 cm^{-1}, over same period. These observations allow demonstrating that the diffusion of antimicrobial compounds during time storage could be monitored by FTIR quantification of related bands.

Table 2.4 Percentage of peak height (%) of typical IR bands at 1600, 1515 and 1265 cm^{-1} for semi-quantitative estimation of the diffusion of antimicrobial compounds from MC-A and MC-B films during storage.

Films	Days	Peak height			Percentage of peak height[1] (%)		
Wavenumbers[2] (cm^{-1})		1600	1515	1265	1600	1515	1265
MC-A	**0**	0.4609	0.1838	0.2832	100.00	100.00	100.00
	1	0.4073	0.1506	0.1901	88.37	81.94	67.13
	2	0.3986	0.1503	0.1932	86.48	81.77	68.22
	6	0.2866	0.1106	0.0860	62.18	60.17	30.37
	13	0.2651	0.1022	0.0652	57.52	55.60	23.02
MC-B	**0**	0.4845	0.2294	0.3825	100.00	100.00	100.00
	1	0.4248	0.1705	0.2430	87.68	74.32	63.53
	2	0.3923	0.1597	0.2154	80.97	69.62	56.31
	6	0.3402	0.1279	0.1549	70.22	55.75	40.50
	13	0.2636	0.1033	0.0629	54.41	45.03	16.44

[1] Percentage of relative peak (%) = (Peak height/peak height at Day 0) × 100.

[2] Peak heights at 1600, 1515 and 1265 cm^{-1} are associated to semi-quantitative estimation of quantities of antimicrobial compounds according to their chemical nature. Therefore, measurements of decreasing values at 1600 and 1515 cm^{-1} were used to evaluate the diffusion of aromatic volatile compounds whereas measurements at 1265 cm^{-1} were used to estimate the diffusion of esters volatile compounds.

2.6.4.3 *Correlation between FTIR analysis and TP measurements*

Correlations between TP concentrations and FTIR peak heights were investigated by a simple type of regression to verify linear correlations between the two methods. Calculations allowed concluding it was possible to establish a satisfying correlation (> 0.90) between TP measurements and the absorbance of FTIR peaks at 1600 cm^{-1} for both antimicrobial films ADF-A and -B. Linear correlation of IR absorbance at 1600 cm^{-1} *vs* TP concentration (µg GAE/mg) is presented in **Fig. 2.7** and shows that i) plotting of ADF-A data for ADF-A resulted in a linear equation y = 0.0063x – 0.0116 (R^2 = 0.92) and ii) plotting of ADF-B data generated an equation y = 0.0063x – 0.0134 (R^2 = 0.90). Consequently, semi-quantitative estimation of peak absorbance at 1600 cm^{-1} (group of aromatic volatiles including phenolic compounds) can offer an accurate, rapid and non-destructive analytical method to evaluate the TP release towards the package headspace over storage time.

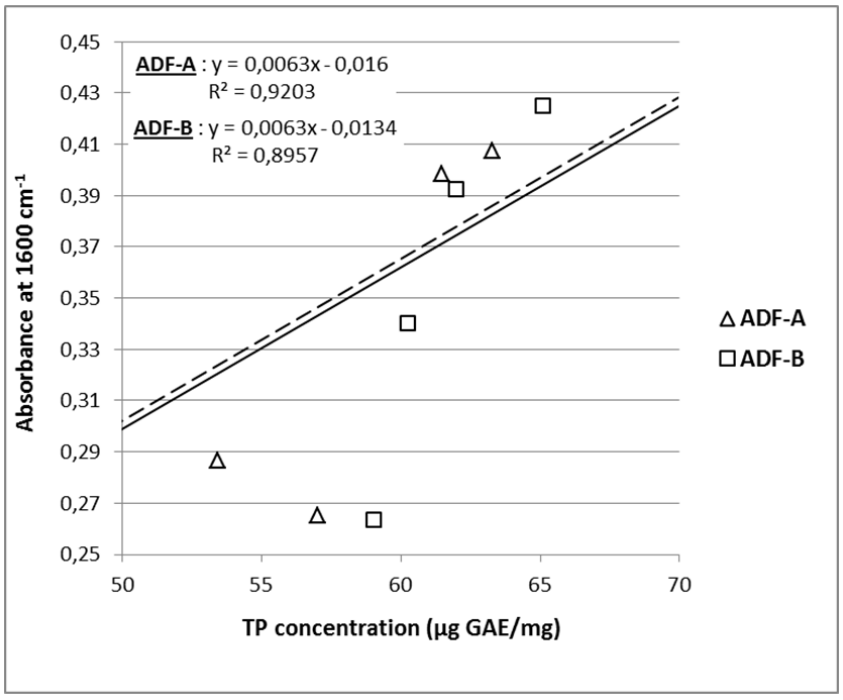


Figure 2.7 Linear correlation between FTIR absorbance at 1600 cm^{-1} of ADF-A and ADF-B and their availability of TP concentration (µg GAE/mg) determined by spectrophotometry, as deduced from data obtained in Table 2 and Table 4.

2.6.5 **SEM analysis**

SEM was carried out for extensive morphological inspection of cross-section in MC internal layer and to evaluate the effect of NCC filling and antimicrobial incorporation on the internal structure of MC matrix. The effect of NCC alone (without antimicrobials) was justified due to the fact that antimicrobials were preliminarily dispersed in NCC suspension before their incorporation in MC matrix for the preparation of ADFs. SEM micrographs of three different internal MC layers from ADFs containing i) no NCC and no antimicrobials (**A-1**; **A-2**), ii) NCC with no antimicrobials (MC-control) (**B-1**; **B-2**) and iii) NCC with formulation B (MC-B) (**C-1**; **C-2**) are presented in **Fig. 2.8**. Two magnifications (600× and 10,000×) were used to evaluate the cross-section of films. Parts **A-1** and **A-2** represent a non-fibrillar structure that tends to adopt a globular organization (smooth structure with globular cavities). The addition of NCC in MC matrix clearly affected the structure of films. Indeed, as shown in parts **B-1** and **B-2**, the cross-section of MC-control appears to be rougher and denser, implying the filling of NCC into the polymer bulk, while maintaining some more dispersed globular heterogeneous areas. Analogous SEM images of NCC into polysaccharide matrices were reported by other studies (Azeredo *et al.*, 2009, Khan *et al.*, 2010b). On the other hand, the addition of bioactive agents (formulation B), as described in **C-1** and **C-2**, results in a very homogenous (denser structure) and more granular cross-section (less smooth with up and down serration shape as compared to other samples), as compared to MC-control. This finer, homogenous and more regular order can be attributed to the presence of bioactive agents interspaced into polymer network. In particular, these observations could suggest that NCC aggregates inserted into MC network have kept much of their original physical properties in presence of bioactive compounds, therefore increasing the tridimensional morphology of MC layer. Similar morphological trends were reported by Khan *et al.* (2010b) and Salmieri *et al.* (2006).

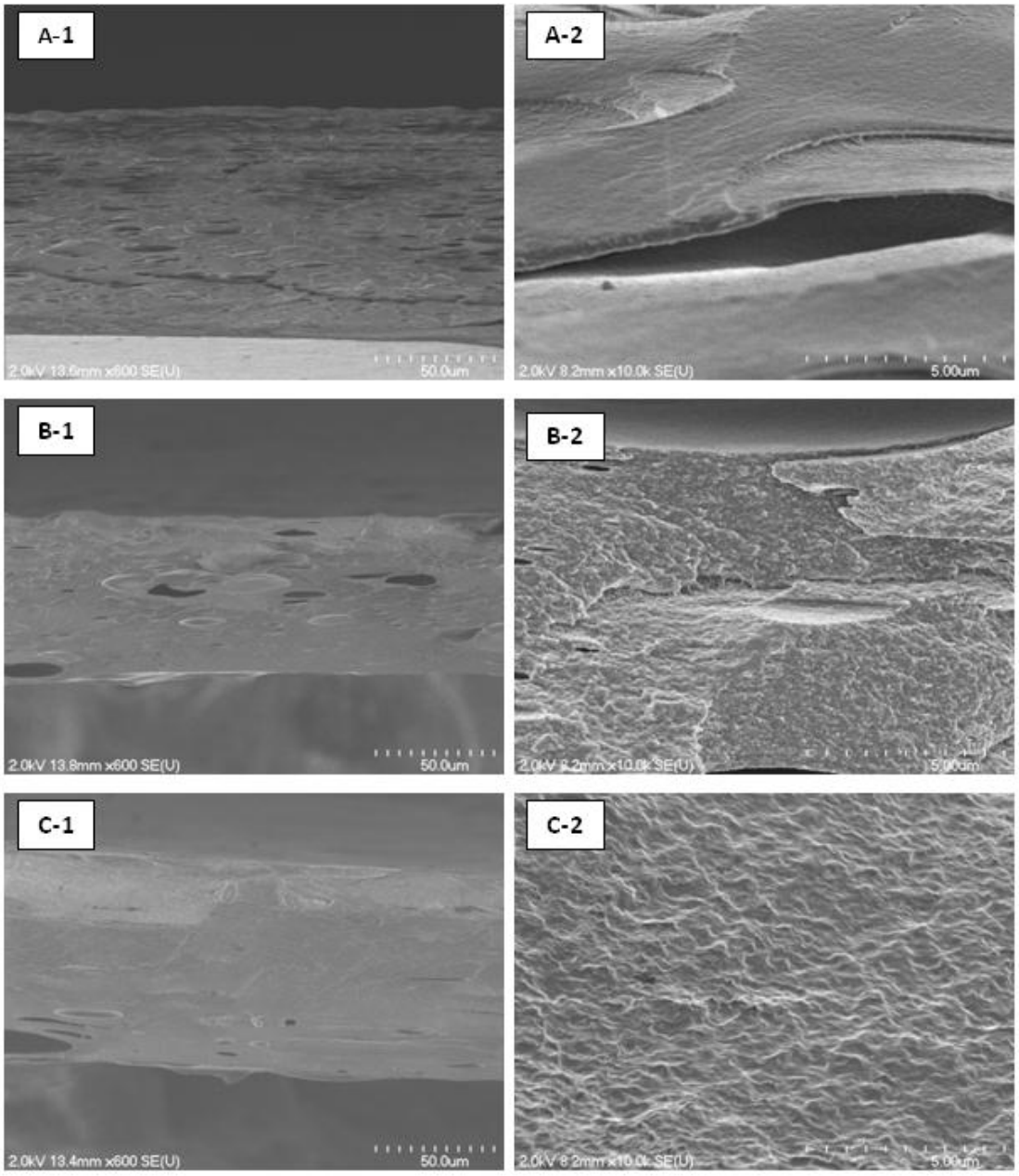


Figure 2.8 Cross-sectional SEM micrographs of internal bioactive MC layers from ADFs. A-1: Control film without NCC at magnification 600×; A-2: Control film without NCC at magnification 10,000×; B-1: Control film at magnification 600×; B-2: Control film at magnification 10,000×; C-1: MC layer containing formulation B at magnification 600×; C-2: MC layer containing formulation B at magnification 10,000×.

2.6.6 **Physicochemical properties of films**

2.6.6.1 *Colorimetry of films*

Colorimetric measurements of ADFs were carried out using the CIELAB system in order to evaluate the effect of the presence of antimicrobial formulations A and B on the lightness (L*) and the hue angle of films during storage.

(i) Evolution of the lightness (L*)

Results presented in **Table 2.5** clearly show that ADF-control exposes a stable lightness over time ($p > 0.05$) that approaches absolute white (L* = 92%) whereas antimicrobial films (ADF-A and ADF-B) display a lower lightness (L* between 60 and 71%) all over storage due to the presence of colored antimicrobial extracts encapsulated in the film matrix (**Table 2.5**). Moreover, as opposed to ADF-control, antimicrobial films present lightness variations over storage. A significant decrease of L* values ($p \leq 0.05$) was observed from 70 to 60 for ADF-A and from 71 to 63 for ADF-B, indicating a significant darkening of these films. Hence, the decrease of the L* values of ADFs during storage could be due to the hydration of ADFs as well as the autoxidation of encapsulated antimicrobial extracts (in particular phenolic compounds), as already observed by numerous studies (Devlieghere *et al.*, 2004, Donhower *et al.*, 1993).

Table 2.5 Colorimetric parameters of ADFs in function of storage time and antimicrobial formulations[a]

	Films	Days		
		0	6	13
$L^{*1,2}$	ADF-control	92.23 ± 0.21 ^{b,A}	92.10 ± 0.26 ^{b,A}	92.43 ± 0.59 ^{c,A}
	ADF-A	69.60 ± 2.48 ^{a,B}	60.80 ± 3.73 ^{a,A}	60.20 ± 1.34 ^{a,A}
	ADF-B	71.00 ± 1.15 ^{a,B}	66.13 ± 4.56 ^{a,AB}	63.10 ± 1.65 ^{b,A}
$h°^{1,2}$	ADF-control	123.42 ± 4.61 ^{b,A}	120.4 ± 3.90 ^{b,A}	122.19 ± 6.59 ^{b,A}
	ADF-A	87.72 ± 1.28 ^{a,B}	83.99 ± 1.88 ^{a,A}	84.37 ± 0.58 ^{a,A}
	ADF-B	88.84 ± 0.80 ^{a,A}	87.06 ± 2.25 ^{a,A}	86.02 ± 0.89 ^{a,A}

[a]Means followed by the same lowercase letter in each column for each property are not significantly different at the 5% level. Means followed by the same uppercase letter in each row are not significantly different at the 5% level. N/A: non-applicable.

(ii) Evolution of the hue angle

A stability of the hue values was observed in ADF-control and ADF-B, showing no significant difference ($p > 0.05$) over time. Indeed, results indicate that the hue of ADF-control was maintained in yellow-green area (hue = 120-123°) and that of ADF-B was maintained in yellow area (hue = 86-89°). In comparison, ADF-A generated a significant decrease ($p \leq 0.05$) of the hue from 88 to 84°, equivalent to a slightly turning-red yellowness of these films, probably due to the chemical nature of antimicrobial compounds that were affected by oxidation in formulation A. Hence, hue values of ADF-A and ADF-B combined with their lower L* values imply a slightly browner color of these films as compared to ADF-control, due to the presence of antimicrobial extracts encapsulated in the film and their oxidation over time, as observed for L* measurements. Furthermore, due to a significant decrease of hue value ($p \leq 0.05$) of ADF-A between Days 0-6, it could be assessed that the composition of formulation A tended to generate more browning reactions by oxidation, as compared to ADF-B. This phenomenon is supported by a significant lower value of L* ($p \leq 0.05$) for ADF-A at Day 13 (60 for ADF-A and 63 for ADF-B). Regarding combined results from L* and hue angle measurements, it can be

noticed that ADF-A seems to be more sensitive than ADF-B in color changes since they demonstrated a more rapid significant variation ($p \leq 0.05$) of lightness and hue from Day 0 to Day 6 (L* from 70 to 60 and hue from 88 to 84°). This propensity for brown-turning color behavior of ADF-A could be explained by a lower antioxidant activity of antimicrobial components in formulation A, which is congruent with results reported by other studies (Bagamboula *et al.*, 2004, Chisari *et al.*, 2010).

2.6.6.2 *Mechanical properties*

(i) Evaluation of the tensile strength (TS)

TS values of ADFs in function of storage time are presented in **Table 2.6**. TS is the ultimate strength (or maximum stress) of a material subjected to tensile loading. Results show that all ADFs possessed similar resistance ($p > 0.05$) at Day 0, with TS values comprised between 20 and 24 MPa. The TS of ADF-control did not vary over time ($p > 0.05$), with values ranging from 20.3 to 18.7 MPa between Days 0-13. On the other hand, the TS of antimicrobial ADFs was reduced significantly ($p \leq 0.05$) during storage. Indeed, the TS values decreased from 23.7 to 7.8 MPa for ADF-A (reduction of 67%) and from 24.0 to 17.3 MPa for ADF-B (reduction of 28%) after 14 days of storage. However, at Day 13, results show no significant difference ($p > 0.05$) between ADF-control and ADF-B with similar TS of 17.3-18.7 MPa whereas the TS of ADF-A was significantly lower ($p \leq 0.05$) with a TS value of 7.8 MPa. Hence, these observations imply that ADF-A was significantly less resistant to tensile stress than ADF-control and ADF-B ($p \leq 0.05$) over storage. These behaviours could be explained by the chemical nature of antimicrobials that interacted differently according to the type of antimicrobial formulation, and in combination with the hydration of films over storage, as described below for TM interpretation. Moreover, it is well-known that the incorporation of EOs into a continuous polymeric matrix tends to decrease its mechanical resistance to fracture because of the structural discontinuities caused by the oil-dispersed phase (Salmieri *et al.*, 2006, Sanchez-Gonzalez *et al.*, 2011).

Table 2.6 Effect of antimicrobial formulation on the tensile strength (TS) of ADFs during storage.

Films	Tensile strength (MPa)[a]		
	Day 0	**Day 6**	**Day 13**
ADF-control	20.3 ± 4.9 ^{a,A}	19.1 ± 2.7 ^{a,A}	18.7 ± 3.7 ^{b,A}
ADF-A	23.7 ± 2.7 ^{a,C}	18.0 ± 2.7 ^{a,B}	7.8 ± 0.7 ^{a,A}
ADF-B	24.0 ± 3.4 ^{a,B}	20.4 ± 1.4 ^{a,A}	17.3 ± 2.0 ^{b,A}

[a]Means followed by the same lowercase letter in each column are not significantly different at the 5% level. Means followed by the same uppercase letter in each row are not significantly different at the 5% level.

(ii) Evaluation of the tensile modulus (TM)

TM values of ADFs in function of storage time are presented in **Table 2.7**. TM (or Young's modulus) is a tangent modulus of elasticity of a material subjected to tensile loading and is expressed by the ratio of stress to elastic strain in tension. A high TM means that the material is rigid. Results show that ADF-control and ADF-B possessed similar viscoelastic properties ($p > 0.05$) at Day 0, with respective TM values of 218 and 210 MPa. Otherwise, TM value of ADF-A was significantly lower ($p \leq 0.05$) than those of ADF-control and ADF-B at Day 0, with a value of 175 MPa, suggesting that the incorporation of formulation A improved the viscoelasticity of ADFs due to chemical nature of its components and their possible plasticizing effect on MC-based internal matrix. Similar observations were reported by Salmieri and Lacroix (Salmieri *et al.*, 2006) for investigation on mechanical properties of films containing different EOs. For each ADF formulation, TM values decreased significantly ($p \leq 0.05$) over storage, from 218 to 111 MPa for ADF-control (reduction of 49%), from 175 to 89 MPa for ADF-A (reduction of 49%) and from 210 to 85 MPa for ADF-B (reduction of 59%). Such TM reductions could be closely related to the hydration of ADFs during time, thereby enhancing their elasticity based on an alteration of hydrogen bonding in MC network (Zsivánovits *et al.*, 2005). These data are in accordance with hydration phenomenon in ADFs characterized by FTIR spectra from **Fig. 2.3** and therefore support these mechanical changes. Additionally, ADF-control tended to be more rigid as compared to antimicrobial films during storage, with higher TM values. It is proposed that the hydration of MC matrix over time could promote the plasticizing action of antimicrobial

compounds. Similar results have already been reported by Salmieri *et al.* (2006) concerning the combined effect of EOs incorporation and dehydrating treatment of polysaccharides-based films.

Table 2.7 Effect of antimicrobial formulation on the tensile modulus (TM) of ADFs during storage.

Films	Tensile modulus (MPa)[a]		
	Day 0	**Day 6**	**Day 13**
ADF-control	218.3 ± 27.2 ^{b,C}	124.1 ± 14.6 ^{b,A}	110.6 ± 3.8^{b,A}
ADF-A	175.2 ± 26.6 ^{a,D}	106.0 ± 10 ^{a,AB}	89.40± 10.5 ^{a,A}
ADF-B	209.6 ± 20.2 ^{b,C}	124.7 ± 22.7 ^{b,B}	84.8 ± 9.6 ^{a,A}

[a]Means followed by the same lowercase letter in each column are not significantly different at the 5% level. Means followed by the same uppercase letter in each row are not significantly different at the 5% level.

As a result, these mechanical measurements allowed characterizing ADF-control as a resistant but less elastic material over time. As opposed, ADF-A was determined as an elastic but less resistant material over time. Meanwhile, ADF-B could be ascribed i) to a more resistant long-term material compared to ADF-A but also ii) to a more elastic material compared to ADF-control, hence gathering higher combined mechanical properties (TM and TS) over storage.

In summary, trilayer ADFs developed in this study showed very satisfactory physicochemical and *in vitro* antimicrobial properties and could further be explored in food applications to prevent pathogenic contamination during storage of ready-to-eat foods. FTIR analysis allowed characterizing the molecular interactions occurring after incorporation of antimicrobials and SEM observations provided a typical morphological structure of the films due to the filling of the NCC-antimicrobials emulsion into polymer bulk. Moreover, ADFs allowed a slow controlled release of the antimicrobial compounds during storage. This study has also demonstrated that it is possible to follow the TP release of the antimicrobial compounds by FTIR with satisfying correlations ($>$ 90%) compared to standard method. Addition of antimicrobials increased the

elasticity of the films, and ADF-B were determined as more resistant than ADF-A. Moreover, the incorporation of antimicrobials in ADFs promoted only slight variations of their color. In order to complete these investigations, further tests are to be carried out on the sensorial quality of the packaged vegetables treated with ADFs during storage but also on *in situ* microbiological analyses during storage using vegetables inoculated by pathogenic foodborne bacteria.

2.6.7 **Acknowledgement**

The Québec Ministry of Agriculture, Fisheries and Food are particularly acknowledged for their financial support through the PSIA program (Programme de Soutien à l'Innovation en Agroalimentaire). Authors are grateful to FPInnovations (Pointe-Claire, Quebec, Canada) for providing NCC generously. The authors are also thankful to P.L. Thomas & Co. Inc., Kerry Ingredients and Flavours and BSA Food Ingredients s.e.c./l.p. to have graciously provided natural antimicrobials. Authors highly appreciated the assistance of Line Mongeon, Technician of Biomedical Engineering Department and Facility Electron Microscopy Research (FEMR) at McGill University, for SEM support. Finally, the authors thank Winpak Division Ltd for providing packaging material.

3 PUBLICATION 2: EFFECT OF ANTIMICROBIAL COATINGS ON MICROBIOLOGICAL, SENSORIAL AND PHYSICO-CHEMICAL PROPERTIES OF PRE-CUT CAULIFLOWERS

Publié dans Posthavest Biology and Technology 2016; 116:1-7

Afia BOUMAIL[a], Stéphane SALMIERI[a], France ST-YVES[b], Martine LAUZON[b], Monique LACROIX[a]*

[a] INRS-Institute Armand-Frappier, Research Laboratories in Sciences Applied to Food, Canadian Irradiation Center, 531 Blvd des Prairies, Laval, Quebec H7V 1B7, Canada

[b] Department of Dietetics, Collège Montmorency, 475 Blvd de l'Avenir, Laval, Quebec H7N 5H9, Canada

*Corresponding author. Tel.: +1 450 687 5010 #4489; fax: +1 450 686 5501. E-mail address: monique.lacroix@iaf.inrs.ca

L'article est présenté tel que publié dans le journal. Le numéro des figures et des tableaux ainsi que la présentation des références ont été modifiés pour suivre un ordre continu au travers de la thèse.

3.1 Contribution des auteurs

J'ai préparé le plan expérimental, mis au point puis réalisé toutes les expériences de cette étude. J'ai également rédigé la publication scientifique. Stéphane Salmieri a participé à l'élaboration des protocoles et aux discussions scientifiques. France St-Yves et Martine Lauzon ont aidé à la préparation et ont supervisé les évaluations organoleptiques. La Pre Monique Lacroix était la responsable scientifique et la coordinatrice du projet de recherche. Elle a supervisé l'élaboration des protocoles, participé aux discussions scientifiques et révisé cet article.

3.2 Résumé en français

Six enrobages contenant différents ratios de polysaccharides et d'antimicrobiens ont été préparés et leur efficacité contre *Listeria monocytogenes* a été testée *in vitro*. Les meilleurs enrobages ont ensuite été appliqués sur des légumes et soumis à une évaluation sensorielle. Finalement, leur effet sur la qualité, la couleur et la consistance des légumes a été déterminé pendant une conservation d'une semaine. Tous les enrobages sélectionnés ont permis une inhibition totale des bactéries *in vitro* à des concentrations de 8-10 mL L^{-1}. Un des enrobages antimicrobiens a induit des caractéristiques (odeur, goût, texture) similaires à celles des légumes non-traités. Les traitements avec cet enrobage ont généré des changements négligeables au niveau du taux de respiration et aucune différence n'était visible sur les choux-fleurs. Enfin, les analyses *in situ* ont montré un bon effet antimicrobien et ont permis une inhibition des bactéries après une conservation pendant 7 jours à 4°C.

3.3 **Abstract**

Six coatings containing different ratios of polysaccharides and antimicrobials were prepared and tested *in vitro* against *Listeria monocytogenes*. Best coating mixtures were then applied on vegetables and submitted to a sensorial analysis. Finally, their effect on the quality, the color and the consistency of vegetables during a one-week storage was determined. All selected coatings showed a total *in vitro* inhibition of bacteria at concentrations of 8-10 mL L^{-1}. One formulation containing the antimicrobials induced vegetables to have similar characteristics (smell, taste and texture) as compared to the non-treated vegetables. Treatments with this coating generated minor changes concerning the respiration rate and no differences were visually observed on cauliflowers. Finally, *in situ* analyses showed a good antimicrobial effect and allowed a complete inhibition of *Listeria innocua* after seven days of storage at 4 °C.

Keywords : antimicrobial coating, ready-to-eat vegetable, quality, shelf-life

3.4 **Introduction**

Nowadays, ready-to-eat (RTE) products are increasingly in demand, mainly because of our lifestyle and their convenience. However, industrial processing can be a source of microbial contamination (Castro-Ibáñez *et al.*, 2016, Zilelidou *et al.*, 2015). During the last past years, food safety has become more important due to resulting illnesses, but also to related economic burdens. Indeed, it has been estimated that, in 2010, the total cost for *Listeria monocytogenes* illness was more than $2 billion in the United States (Scharff, 2012). Since foodborne diseases can induce serious health threats, new methods controlling bacterial growth are being developed. Natural compounds such as essential oils (EOs) and organic acids have already proved their efficiency on a large scale of pathogenic bacteria such as *L. monocytogenes, Escherichia coli* O157:H7 or *Salmonella* Typhimurium (Boumail, A *et al.*, 2013, Dussault *et al.*, 2014). After internalization into cells, organic acids seem to dissociate into protons and anions (Ricke, 2003), leading to pH changes. As for EOs, their action on the membrane permeability results in a loss of metabolites and the denaturation of enzymes and proteins (Nazzaro *et al.*, 2013). However, since the high volatility of those compounds may limit their use during time, their immobilization in a polymer matrix can be used to increase their availability (Oussalah *et al.*, 2004) but also to protect them from possible degradation due to humidity or oxygen (Ribeiro *et al.*, 2015).

Methylcellulose (MC) is a water-soluble cellulose ether that is widely used for industry purposes. Indeed, its physico-chemical properties allow the formation of gels and coatings (Nasatto *et al.*, 2015). It has been demonstrated that coatings based on MC can increase avocado shelf-life by reducing respiration rates and color changes (Maftoonazad *et al.*, 2005). Starch (S), a polymer made of amylose and amylopectin, is also commonly used for the formation of edible coatings, since it increases firmness and enhances gel strength (Galus *et al.*, 2012). Maltodextrins (MD) are produced after hydrolysis of S. Their properties depend on their dextrose equivalent (DE) value. Indeed, MD with a high DE will be more likely to produce an efficient encapsulation of bioactive compounds and will offer a better protection against oxidation (Jafari *et al.*, 2008, Wang *et al.*, 2015). MD has also been described as a polymer which possesses oxygen barrier and water retention properties amongst others (Chronakis, 1998).

The aim of this study was to evaluate the effects of the bioactive coating on the quality of fresh cauliflowers. Coatings containing different ratios of MC, MD and S were prepared and their

antimicrobial properties were evaluated *in vitro*. Best coatings were then used to coat cauliflower florets which were submitted to sensorial evaluations in order to determine the level of appreciation of the smell, the texture and the taste. Physico-chemical parameters such as O_2/CO_2 content, colorimetry and consistency of coated cauliflowers were also measured. Finally, the antimicrobial effect of the coating was evaluated *in situ* on cauliflowers against *Listeria innocua*.

3.5 Materials and Methods

3.5.1 Bacterial suspension

A mixture of five *L. monocytogenes* strains (Health Canada, Health Product and Food Branch, Ottawa, ON, Canada) were used (HPB 1043 1/2a, HPB 2371 1/2b, HPB 2558 1/2b, HPB 2569 1/2a, HPB 2812 1/2a), as well as a *L. innocua* ATCC 51742 strain. Bacteria were stored in tryptic soy broth (TSB, Difco Laboratories, Detroit, MI, USA) containing glycerol (150 g L^{-1}) at -80 °C. Before utilization, bacteria were propagated twice in TSB for 24 h at 37 °C. *L. monocytogenes* strains were mixed together and this bacterial suspension was diluted to reach 10^6 CFU (colony forming unit) mL^{-1} for *in vitro* tests.

3.5.2 Antimicrobial formulation

The antimicrobial formulation was prepared using lactic acid (Sigma-Aldrich Ltd, Oakville, ON, Canada), citrus extract (Biosecur Lab Inc., Otterburn, QC, Canada), lemongrass EO (BSA Ingredients s.e.c./l.p., Montreal, QC, Canada) and Tween 80 (Sigma-Aldrich Ltd) with the following ratio 100:10:1:2. This mixture was then homogenized at room temperature under sterile conditions, using a digital Ultra-Turrax T25 disperser (IKA Works Inc., Wilmington, NC, USA), at a speed of 1,047 rad s^{-1} for 60 s.

3.5.3 **Preparation of coatings**

Stock solutions of MD (dextrose equivalent 16.5-19.5), MC (powder form, viscosity of 1.5 Pa s for a 10 g L^{-1}solution at 20 °C - Sigma-Aldrich, Oakville, ON, Canada) and modified S (National Starch & Chemical Company, Bridgewater, NJ, USA) were prepared at a concentration of 20 g L^{-1}distilled water. Suspensions were autoclaved at 80 °C for 15 min for pre-gelatinization and sterilization, then cooled in an ice bath to ensure complete solubilization. The high viscosity of MC was used to provide a coating matrix, allowing the coating to adhere to vegetables but also limiting the dripping. Glycerol was also used as a plasticizer at a set concentration of 7.5 g L^{-1}. Coatings were homogenized at a speed of 1,047 rad s^{-1} for 60 s with a digital Ultra-Turrax T25 disperser.

3.5.4 ***In vitro* test - Minimum inhibitory concentration (MIC)**

The effect of relative concentrations of MD and S with/without MC (2.5 g L^{-1}) was evaluated on the MIC by insuring a total polymer content of 10 g L^{-1} and the different composition are presented in **Table 3.1**. The antimicrobial formulation was added to reach final concentrations of 0; 0.8; 8; 10; 12; 14; 16 and 34 mL L^{-1} in the coating. The *in vitro* screening of the MIC was evaluated using 96-wells (flat-bottom wells) microtiter plates. One plate was used for the control coating while the others were used for measuring the effect of the coating on *L. monocytogenes* at 10^6 CFU mL^{-1}. The control plate was prepared as follows according to Dussault *et al.* (2014). The first column was filled with 250 µL of TSB and column 2 to 9 were filled with 125 µL of TSB and 125 µL of coating containing the antimicrobial formulation (from 0 to 34 mL L^{-1} respectively). A quantity of 15 µL of peptone water was then added to each well of the 9 columns. The other plates were prepared with a similar design but peptone water was replaced with 15 µL of the bacterial suspension. Plates were then incubated at 37 °C for 24 h. Bacterial growth was detected by absorbance at 595 nm using a microtiter plate reader (ELISA reader, CLX800-Biotek Instruments) and compared to the negative turbidity control. The lowest concentration that inhibited the growth of the bacteria was determined as the minimal inhibitory concentration (MIC).

3.5.5 **Sensorial evaluation**

The sensorial evaluation was performed with 65 panelists who were asked to evaluate the odor, the texture and the taste of 8 samples. Cauliflowers (*Brassica oleracea* L. var. *botrytis* L.) were purchased from a local supermarket, cut into ready-to-eat size florets and dipped for 30 s in the selected coatings. Each side of the cauliflowers was allowed to dry on foil for 10 min. Cauliflowers were deposited in cups, randomly identified with a 3-digit number and refrigerated overnight before organoleptic evaluation. Panelists were asked to eat unsalted crackers and drink water between samples. The answers were based on a 9 point hedonic scale, from 9 ("like extremely") to 1 ("dislike extremely). Samples with scores lower than 5 were considered as not appreciated.

3.5.6 **Physico-chemical properties of coated cauliflowers**

The oxygen/carbon dioxide amount released as well as the color, the resistance of penetration and the consistency of cauliflowers were measured during storage in plastic desiccators (VWR, Mississauga, ON, Canada) at 4 °C.

3.5.6.1 *Respiration rates*

A headspace oxygen and carbon dioxide analyzer (Illinois Instruments Inc, IL, USA) was used to measure the gas content surrounding vegetables during storage. The headspace analyzer was calibrated with concentrations of 209 mL L^{-1} of oxygen and 0 mL L^{-1} of carbon dioxide and a filter was used to prevent bringing humidity in the analyzer. Packaged air was taken during 30 s and the O_2 and CO_2 percentages were displayed. Respiration rates were calculated according to modified equations used by Bhande *et al.* (2008):

$$R_{O_2} = \frac{\left(G_{O_2}\right)_t - \left(G_{O_2}\right)_{t+1}}{\Delta t}$$

$$R_{CO_2} = \frac{\left(G_{CO_2}\right)_{t+1} - \left(G_{CO_2}\right)_t}{\Delta t}$$

where R_{O_2} is the consumption of O_2 (mL L^{-1} h^{-1}), R_{CO_2} is the production of CO_2 (mL L^{-1} h^{-1}), G_{O_2} and G_{CO_2} are the O_2 and CO_2 gas concentration (mL L^{-1}) respectively and Δt is the time storage difference between samples (h).

3.5.6.2 *Color changes*

The color of the coated cauliflowers was measured using a Color reader CR-10 (Konica Minolta, Ramsey, NJ, USA). Measurements of spectral reflectance were performed directly onto the vegetables surface (viewing area = 10 x 10 mm). The L*, a*, b* system (CIELAB) was employed; the L* axis represents the lightness from black (L* = 0) to absolute white (L* = 100), the a* axis varies from green (−) to red (+), and the b* axis varies from blue (−) to yellow (+). To characterize more precisely the color of cauliflowers, the hue angle (hue = arctan(b*/a*) if a* > 0 and hue = arctan(b*/a*) + 180° if a* < 0) was determined to indicate color changes between a* (green color) and the intersection of a* and b*, from green (hue = 0 °) to yellow color (hue = 90 °).

3.5.6.3 *Resistance of penetration and consistency*

The resistance of penetration and the consistency of cauliflowers were measured using a texturometer Stevens-LFRA (modèle TA-1000, Texture Technologies Corp., Scarsdale, NY, USA) connected to a printer (Linseis®, L 6512 model, NJ, USA). The texturometer was calibrated to 700 g with a sensibility of 1 V. Penetration tests were conducted using a needle and the following conditions: penetration distance: 10 mm, test speed: 2.0 mm s^{-1}, printer speed: 3.3 mm s^{-1}. Cauliflowers stems were cut following a longitudinal section. The first peak obtained was considered as the resistance of penetration (strength necessary for a needle to penetrate the external side of stems) (Raybaudi-Massilia *et al.*, 2008a) while the peak area at half-distance of penetration (7.5 mm) was measured to express the consistency of the cauliflowers, according to a procedure by Brookfield Engineering labs Inc. (2015). The resistance of cauliflowers was expressed in N and the consistency was expressed in N m.

3.5.7 *In situ* antimicrobial effect of coating

Cauliflower florets (20 to 25 g) were packaged in 12.7 µm metalized polyester-50.8 µm ethylene vinyl acetate copolymer bags (Winpak Division Ltd., Montreal, QC, Canada). Sterilization was done at the Canadian Irradiation Center by γ-radiation, using a UC-15 A (SS canister) underwater calibrator (Nordion Inc., Kanata, ON, Canada) equipped with a ^{60}Co source. A radiation dose of 10 kJ kg^{-1} was delivered at a dose rate of 16.95 kJ kg^{-1} h^{-1}. Under sterile conditions, cauliflowers were coated and inoculated with a diluted *L. innocua* suspension (500 µL of suspension for 20 g of sample) to reach final concentrations of 3.2 log CFU g^{-1}. Samples were then stored at 4°C and microbiological analysis were performed at days 0, 1, 2, 4 and 7. For microbiological analysis, samples were 3-fold diluted in peptone water (1 g L^{-1}) and homogenized using a stomacher. Serial 10-fold dilutions were made and 1 mL of each dilution was spread on Petri dishes before medium was poured (Palcam supplemented with antibiotics for *L. innocua*). Petri dishes were incubated at 37 °C for 48 h before bacterial enumeration. Detection level was calculated as 0.48 log CFU g^{-1} (3 CFU g^{-1}).

3.5.8 Experimental design and statistical analyses

MIC determination was done according to a 3 x 6 x 8 factorial design: 3 repetitions, 6 treatments, 8 antimicrobial concentrations. For physico-chemical analysis, samples were separated into 2 groups: (i) control; (ii) coating. Oxygen/carbon dioxide, color and firmness measurements were done on the same samples in this respective order. Analyses were done using a 3 x 3 x 2 x 5 factorial design: 3 repetitions, 3 replicates, 2 treatments, 5 d of storage. Finally, antimicrobial analyses were also done according to a 3 x 3 x 2 x 5 factorial design : 3 repetitions, 3 replicates, 2 treatments, 5 d of storage. Analysis of variance and Duncan's multiple-range test were performed for statistical analysis by using SPSS 16.0 software (IBM Corp., Somers, NY, USA). Differences between means were considered to be significant at a 5% level.

3.6 Results and Discussion

3.6.1 *In vitro* test - MIC

The different coating solutions and their *in vitro* antimicrobial effect on *L. monocytogenes* are presented in **Table 3.1**. Results showed that all coating formulations allowed a total inhibition of *L. monocytogenes*. Coatings A and B required an antimicrobial concentration of 10 mL L^{-1} while only 8 mL L^{-1} were needed for coating C to F. The presence of MD in coatings C to F seemed to reduce the MIC from 10 to 8 mL L^{-1}. However, the increase in the MD concentration did not change *in vitro* antimicrobial properties. These results could be due to the good encapsulation properties of MD. Indeed, this polymer has been used for its ability to protect bioactive compounds from oxidation and heat (Fernandes *et al.*, 2014, Wang *et al.*, 2015). However, because of its high water-solubility, its emulsifying properties are very low, thus MC and/or S were added as matrices and co-emulsifiers. This addition ensured the stability of coatings and did not affect the MIC.

Table 3.1 Ratio (g/L) of MD, S and MC in studied bioactive coating formulations[1] and their *in vitro* antimicrobial effect against *L. monocytogenes*.

Coating[1]	MD	S	MC	MIC (mL L^{-1})[2]
A	0	10	0	10
B	0	7.5	2.5	10
C	2.5	5.0	2.5	8
D	5.0	2.5	2.5	8
E	7.5	0	2.5	8
F	10	0	0	8

[1]Coating formulations were also composed of water, glycerol (7.5 g L^{-1}) and the antimicrobial formulation (concentration between 0 and 34 mL L^{-1}).

[2]MIC = minimal inhibitory concentration.

This test also suggest that the mixture of antimicrobial compounds have a strong antimicrobial effect against *L. monocytogenes*. This behavior would most likely be related to the presence of terpenic compounds on the EO. Indeed, Lis-Balchin *et al.* (1998) have demonstrated that EOs containing terpenes (such as citral, eugenol or cinnamaldehyde) as a main component exhibited higher antimicrobial activities. Silva-Angulo *et al.* (2015) also confirmed the antimicrobial properties of citral and suggested to combine it with other bioactive compounds when used with food. Organic acids, such as lactic acid, are also known to possess antimicrobial properties (Davidson *et al.*, 2007, Ricke, 2003). Indeed, their internalization in bacteria cells would induce pH changes, forcing cells in using energy to restore a neutral pH (Davidson *et al.*, 2007).

This *in vitro* test showed that the coatings C to F required only 8 mL L^{-1} of antimicrobials to inhibit *L. monocytogenes*. Since they showed the best in vitro antimicrobial properties, coatings C to F were selected for the following tests. However, for industry feasibility purposes, coatings were developed to be efficient against bacteria over a long period of time. Previous studies focusing on films containing similar antimicrobials showed a controlled release of bioactive compounds of approximately 20% after a one-week storage (Boumail, A *et al.*, 2013, Salmieri *et*

al., 2014). To guarantee a minimal antimicrobial concentration of 8 mL L^{-1} over storage, the initial concentration in the coating solution was increased to 16 mL L^{-1}, representing a 34 mL L^{-1} antimicrobial solution.

3.6.2 **Sensorial evaluation**

Results of the sensorial evaluation are presented in **Table 3.2**. The effect of the addition of antimicrobials in different MD/S/MC ratios was tested on the odor, the texture and the taste. The coating F was not tested because of a lack of adherence of the coating to the vegetables. Results showed that the addition of antimicrobial coatings did not induce any detrimental effects on the odor. However, the coating E + AM was evaluated with a significantly better odor ($p \leq 0.05$) as compared to the control, with scores of 5.9 and 5.6 respectively. Concerning the texture, no differences were observed between the control and the coated samples, with scores ranging around 7. Those results mean that the odor of cauliflowers was neither liked nor disliked while the texture was liked moderately. Concerning the taste of cauliflowers, results showed no significant differences ($p > 0.05$) between the control and the coating E + AM, both evaluated as "liked moderately". However, cauliflowers treated with coatings C + AM or D + AM significantly decreased the appreciation ($p \leq 0.05$) of the taste as compared to the control. This could be due to the interactions between the antimicrobials and the polymers contained in the coatings. Indeed, coatings containing S were significantly less appreciated ($p \leq 0.05$) than the coating E + AM and the control, with scores of 5.6-6.4 for coatings C + AM and D+ AM, and scores of 7.0-7.5 for the coating E + AM and the control. Sosa *et al.* (2014) have reported that the use of modified starches would be appropriate for encapsulation because of their ability to retain volatile compounds. They have shown that matrices composed of modified starch, maltodextrin and either trehalose or sucrose could be used as aroma carriers and the nature of sugars could affect the retention of bioactive compounds. Indeed, their results indicated that limonene concentration was higher when used with coatings containing trehalose rather than sucrose. A concentrated antimicrobial coating could induce a longer efficiency during storage, but might also lead to unacceptable sensorial qualities. Results obtained in our study seem to reflect this behavior. Indeed, coatings C + AM and D + AM are more likely to retain antimicrobials because of the presence of modified starch, thus affecting the taste of coated cauliflowers. This higher retention could result in a slower release of antimicrobials, thus increasing the bioavailability of those bioactive compounds. The taste of samples treated with coatings containing starch and

antimicrobials would then be affected by the interactions between those compounds. Due to sensorial properties similar to the control, the coating E + AM would be more appropriate for industry purposes and has been selected for the following tests.

Table 3.2 Evaluation of odor, texture and taste of cauliflowers treated with antimicrobial coatings[1,2].

	Odor	Texture	Taste
Control	5.6 ± 2.1 ab	7.4 ± 1.5 ab	7.5 ± 1.3 c
C + AM[3]	4.4 ± 2.1 a	6.7 ± 1.8 a	6.4 ± 1.8 ab
D + AM	5.7 ± 2.1 b	7.7 ± 1.1 b	5.6 ± 2.1 a
E + AM	5.9 ± 1.8 c	7.1 ± 0.9 ab	7.0 ± 1.9 bc

[1]Means followed by the same lower-case letter are not significantly different ($p \leq 0.05$).

[2]On hedonic scale for all parameters a score of 9 = like extremely, 5 = neither likes nor dislikes and 1 = dislike extremely.

[3]AM: antimicrobials composed of lactic acid, citrus extract, lemongrass essential oil and Tween 80 (ratio 100:10:1:2) at a final concentration of 3.4%.

3.6.3 **Physico-chemical properties**

3.6.3.1 *Respiration rates*

Results concerning the effect of the coating on the respiration of control and coated cauliflowers (O_2 consumption and CO_2 production) are presented in **Figure 3.1**. Results showed that for both groups, the O_2 consumption significantly decreased ($p \leq 0.05$) during storage, from 0.72 to 0.36 mL L^{-1} h^{-1} for the control and from 1.02 to 0.72 mL L^{-1} h^{-1} for the coating E + AM after 7 d. Similarly, CO_2 production significantly decreased for both groups during storage, from 0.808 to 0.264 mL L^{-1} h^{-1} for the control and from 1.27 to 0.683 mL L^{-1} h^{-1} for the coating after 7 d. Those

results are in accordance with Rahman *et al.* (2013), who also observed a reduction of the respiration rate during storage. It was proposed that this decrease is due to the low amount of oxygen remaining.

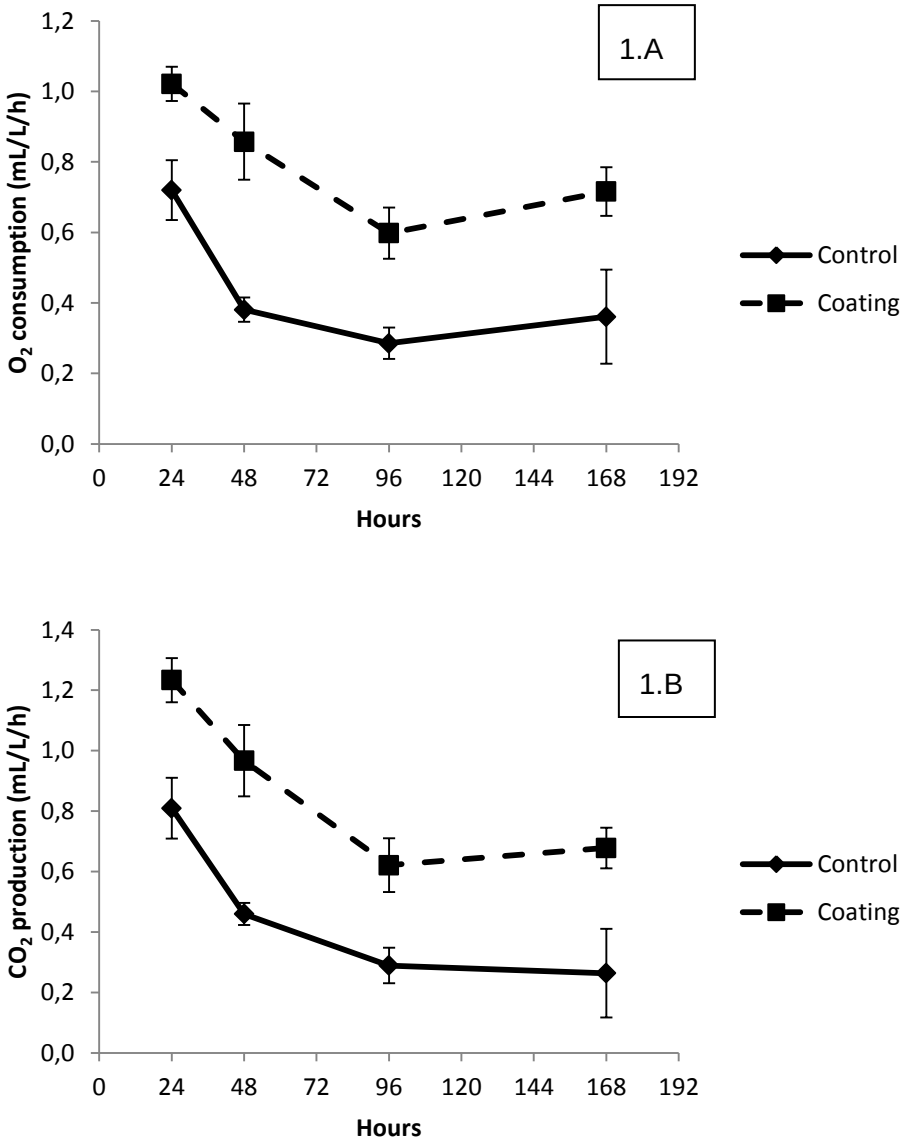


Figure 3.1 Oxygen consumption (Figure 1.A) and carbon dioxide production (Figure 1.B) after treatment of cauliflowers with antimicrobial coating E + AM during storage.

Overall, treatments with the antimicrobial coating E led to an increased respiration as compared to the control. This behavior has already been reported by Salvia-Trujillo *et al.* (2015), who

suggested that the presence of polymers can increase the carbohydrate metabolism and the aerobic respiration.

3.6.3.2 *Color changes*

Results concerning the effect of antimicrobial coating E on the L* values and hue angle of cauliflowers are presented in **Table 3.3**. It can be noticed that both parameters were stable ($p > 0.05$) over time for the control group. Indeed, L* values varied between 84 and 85° while hue angle values ranged between 89 and 90° during a one week storage in control samples. For values on the coated group, L* and hue angle values were not significantly different ($p > 0.05$) from the control between Days 0-4. However, significant decreases ($p \leq 0.05$) were observed at Day 7 for the L* and at Day 4 for the hue angle. Hence, the hue values of coated cauliflowers combined with a lower L* would imply a slight browning of vegetables. Those changes should not have any impact on choices made by consumers since ready-to-eat products are generally used in the days following their purchase. Moreover, L* and hue values decreased of only 3.9% and 3.2% respectively after 7 d of storage, which was not visually noticeable to the human eye. Indeed, as presented in **Figure 3.2**, neither browning nor black spots were observed nor the color of samples was close to white. Thus, cauliflower florets could be considered as visually acceptable for consumption. Those results suggest that a treatment with antimicrobial coating E did not induce major changes on cauliflower florets.

Table 3.3 Color changes of cauliflowers treated with antimicrobial coating E during storage[1].

	L*		Hue (°)	
Days	**Control**	**Coating**	**Control**	**Coating**
0	85.5 ± 0.8 a,A	84.6 ± 1.7 b,A	89.3 ± 0.8 a,A	89.8 ± 1.1 b,A
1	84.1 ± 2.2 a,A	84.4 ± 1.3 b,A	89.7 ± 1.9 a,A	89.4 ± 1.6 b,A
2	84.8 ± 1.7 a,B	82.2 ± 2.1 b,A	89.3 ± 1.9 a,A	90.1 ± 2.7 b,A
4	84.5 ± 2.2 a,A	83.9 ± 1.7 b,A	89.1 ± 2.6 a,A	87.6 ± 1.3 a,A
7	84.8 ± 2.1 a,B	81.3 ± 1.0 a,A	89.8 ± 1.9 a,B	86.9 ± 2.4 a,A

[1] Means followed by the same lowercase letter in each column are not significantly different at the 5% level. Means followed by the same uppercase letter in each row for each property are not significantly different at the 5% level.

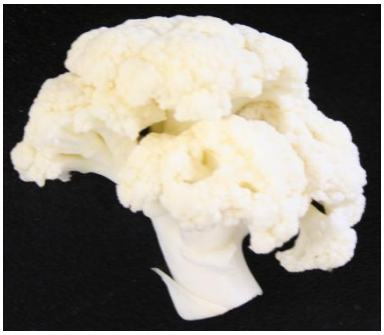
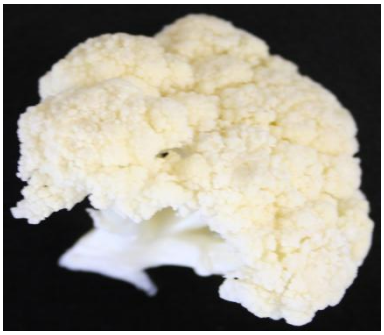
	Day 0	Day 7
Control		
Antimicrobial coating E		

Figure 3.2 Appearance of cauliflowers treated with antimicrobial coating E after storage.

3.6.3.3 *Resistance of penetration and consistency*

The resistance of penetration and consistency results of cauliflowers treated with the antimicrobial coating E are presented in **Table 3.4**. The resistance of penetration represents the force required to puncture the external skin of cauliflowers. For the control cauliflowers, the resistance of penetration did not significantly change ($p > 0.05$) over time (from 611 N at Day 0 to 563 N at Day 7). However, the coating treatment induced a significant increase ($p \leq 0.05$) of the resistance of penetration during storage (from 488 N at Day 0 to 575 N at Day 7). At Day 0, the control cauliflowers had a significantly higher ($p \leq 0.05$) resistance of penetration as compared to the coated cauliflowers (611 and 488 N respectively). From Day 1 to Day 7, no significant differences ($p > 0.05$) were observed between both groups. The application of the

coating reduced the external firmness of cauliflowers. It has already been reported that treatments with coatings containing EOs can lead to a decrease of the firmness. Indeed, Raybaudi-Massilia *et al.* (2008b) have proposed that the presence of EOs might induce a loss of cell wall constituent, thus affecting the firmness.

Table 3.4 Effect of the antimicrobial coating E on the resistance of penetration and the consistency of cauliflowers[1].

	Resistance of penetration (N)		Consistency (N mm)	
Days	**Control**	**Coating**	**Control**	**Coating**
0	611 ± 144 b,B	488 ± 112 a,A	116.2 ± 13.7 c,B	93.8 ± 13.4 a,A
1	530 ± 126 ab,A	505 ± 106 a,A	106.0 ± 11.5 b,A	122.0 ± 14.5 b,B
2	562 ± 141 ab,A	617 ± 135 b,A	105.9 ± 14.8 b,A	119.7 ± 22.0 b,B
4	494 ± 95 a,A	450 ± 56 a,A	95.5 ± 13.5 a,A	112.8 ± 11.8 b,A
7	563 ± 119 ab,A	575 ± 152 b,A	99.8 ± 15.2 ab,A	122.2 ± 18.0 b,A

[1] Means followed by the same lowercase letter in each column for each property are not significantly different at the 5% level. Means followed by the same uppercase letter in each row are not significantly different at the 5% level.

The consistency represents the work needed to tear cauliflower skin and flesh (Mehinagic *et al.*, 2003). The consistency of the control group continuously decreased overtime (from 116 N m at Day 0 to 99.8 N m at Day 7), while it increased for the coated group (from 93 N m at Day 0 to 122 N m at Day 7). At Day 0, the control samples had a significantly higher consistency ($p \leq 0.05$) as compared to the coated samples (116 and 94 N m respectively). During Days 1-2, this trend was inversed and the consistency of cauliflowers was significantly higher ($p \leq 0.05$) after coating (106 N m for the control and 120 N m for the coating at Day 2). At the end of storage, no significant differences were observed between control and coated samples.

It can be hypothesized that a disruption in cauliflower cells occurred immediately after the coating treatment (Raybaudi-Massilia *et al.*, 2008b). However, the presence of MC in the

coating could have helped in reducing changes in firmness. Indeed, Maftoonazad *et al.* (2005) have concluded that MC was effective in limiting loss of firmness. It has also been shown that MC matrices can undergo hydration when stored with vegetables (Boumail, A *et al.*, 2013). Overall, treatments with antimicrobial coating E did not induce major changes since the resistance of penetration and the consistence were similar to the control after a one-week storage.

3.6.4 *In situ* antimicrobial effect of coatings

The effects of the antimicrobial coating on cauliflowers overtime are presented in **Table 3.5**. Results showed a slight decrease of bacteria for the control, from 3.2 log CFU g^{-1} at Day 0 to 2.7 log CFU g^{-1} at Day 7. Treatments with the coating showed an instant antimicrobial effect on *L. innocua,* with a 1.7 log CFU g^{-1} decrease observed at Day 0. This effect was maintained overtime and no apparent colonies were detected after 7 d of storage, suggesting a complete inhibition of *L. innocua*. Those results indicate that the antimicrobial coating is effective against *Listeria* immediately after preparation but also during a long-term storage. This behavior would not only be due to the presence of terpenes, but also to lactic acid and citrus extract. Indeed, Park *et al.* (2011) have studied the effect of lactic acid on the growth of *L. monocytogenes*. They have shown that a 20 g L^{-1} acid lactic treatment induced a 4 log CFU g^{-1} reduction immediately after treatment, and this reduction was increased in the following minutes. The antimicrobial effect for terpenes is well known (Azarakhsh *et al.*, 2014, Lv *et al.*, 2011, Peng *et al.*, 2014) but their high volatility can be a limitation to their use (Bilia *et al.*, 2014). According to results obtained here, it could be hypothesized that the use of MC and MD as a matrix creates a retention of volatiles and a higher diffusion, generating a release of those compounds during time. It has already been mentioned that the presence of humidity can influence the release of EOs (Del Toro-Sánchez *et al.*, 2010, Peng *et al.*, 2014). Indeed, Del Toro-Sánchez *et al.* (2010) have shown that the release of thymol, a main constituent of EOs, is increased when the relative humidity increases. It has been reported by Ayala-Zavala *et al.* (2008) that during storage of fresh food, the relative humidity content increases until saturation is reached. Also, because of their hydrophilic nature, polysaccharide-based films are likely to undergo hydration, as it has already been observed in several studies (Boumail, A *et al.*, 2013, Wyatt *et al.*, 2013). Thus, it could be possible that the absorption of the surrounding humidity by the coating matrix allows continuous release of bioactive compounds in the headspace.

Table 3.5 Effect of the combination of antimicrobial coating E on the growth of *L. innocua* in cauliflower florets.

Treatment	Bacterial count (log CFU/g)[1]				
	Day 0	Day 1	Day 2	Day 4	Day 7
Control	3.23 ± 0.26 c,B	3.07 ± 0.42 c,B	2.30 ± 0.52 a,B	2.80 ± 0.25 b,B	2.71 ± 0.50 b,A
Antimicrobial coating E	1.49 ± 0.16 c,A	1.06 ± 0.22 ab,A	1.24 ± 0.23 b,A	0.99 ± 0.19 a,A	ND[2]

[1] Means followed by the same lowercase letter in each row are not significantly different at the 5% level. Means followed by the same uppercase letter in each column are not significantly different at the 5% level.

[2] ND = not detected (< 0.48 log CFU/g)

3.7 Conclusion

This study showed that EOs can be incorporated in a polysaccharide matrix without affecting their antimicrobial properties. The coating E + AM showed the best *in vitro* antimicrobial properties without affecting the sensorial properties of cauliflowers. Moreover, physicochemical analyses performed showed changes in the respiration rates but no major color modifications. Finally, treatments of cauliflowers with coating E + AM allowed a complete inhibition of *Listeria* after a one-week storage. Therefore, this user-friendly coating could be applied in the food industry as a way to fulfill federal regulation requirements.

3.8 **Acknowledgment**

This research was supported by Bonduelle North America, by the National Sciences and Engineering Research Council of Canada and by the Consortium de Recherche et Innovations en Bioprocédés Industriels au Québec (CRIBIQ). The authors would like to thank BSA Ingredients s.e.c./l.p. and Biosecur Lab Inc. for providing the antimicrobials, and to Winpak Division Ltd. for providing the packaging materials.

4 PUBLICATION 3: COMBINED EFFECT OF ANTIMICROBIAL COATINGS, GAMMA RADIATION AND NEGATIVE AIR IONIZATION WITH OZONE ON *LISTERIA INNOCUA*, *ESCHERICHIA COLI* AND MESOPHILIC BACTERIA ON READY-TO-EAT CAULIFLOWER FLORETS

Publié dans Posthavest Biology and Technology 2016; 118:134-140

Afia BOUMAIL, Stephane SALMIERI, Monique LACROIX*

INRS-Institute Armand-Frappier, Research Laboratories in Sciences Applied to Food, Canadian Irradiation Center, 531 Blvd des Prairies, Laval, Quebec H7V 1B7, Canada

*Corresponding author. Tel.: +1450 687 5010 #4489; fax: +1450 686 5501. E-mail address: monique.lacroix@iaf.inrs.ca

L'article est présenté tel que publié dans le journal. Le numéro des figures et des tableaux ainsi que la présentation des références ont été modifiés pour suivre un ordre continu au travers de la thèse.

4.1 Contribution des auteurs

J'ai préparé le plan expérimental, mis au point puis réalisé toutes les expériences de cette étude. J'ai également rédigé la publication scientifique. Stéphane Salmieri a participé à l'élaboration des protocoles et aux discussions scientifiques. La Pre Monique Lacroix était la responsable scientifique et la coordinatrice du projet de recherche. Elle a supervisé l'élaboration des protocoles, participé aux discussions scientifiques et révisé cet article.

4.2 Résumé en français

Les objectifs de cette étude étaient d'évaluer l'effet d'un enrobage bioactif comestible, à base d'antimicrobiens naturels, combiné à une faible dose d'irradiation γ (0.25 kGy) dans le but d'éliminer les bactéries pathogènes et d'augmenter la conservation de choux-fleurs prêts à manger. Les résultats ont montré que l'enrobage utilisé seul était efficace sur *Listeria innocua* et *Escherichia coli*, avec des réductions de 3.2 et 1.8 log UFC/g respectivement au jour 7. Le traitement par irradiation γ a également permis un effet antimicrobien sur *L. innocua* et *E. coli*, avec des diminutions respectives de 1.8 et 3.6 log UFC/g. Après combinaison de l'enrobage et de l'irradiation γ, aucune bactérie (*L. innocua* ou *E. coli*) n'a été détectée, suggérant des réductions de 3.2 et 3.6 log UFC/g respectivement pendant une conservation de 7 jours. Les résultats ont aussi montré que les bactéries mésophiles étaient affectées par la combinaison de traitements, avec une réduction de 1.8 log UFC/g au jour 0. Ce niveau de bactéries mésophiles a été stable pendant une conservation de 7 jours. Cette étude a démontré que l'enrobage bioactif agit en synergie avec une faible dose d'irradiation γ, provoquant une inhibition de la croissance de *L. innocua* et *E. coli*, ainsi qu'un contrôle des bactéries mésophiles pendant 7 jours.

4.3 **Abstract**

The objective of this study was to evaluate the effect of a bioactive edible coating combined with a low γ-radiation dose or negative air ionization (NAI) with ozone on the microbiological quality of ready-to-eat cauliflowers. Results showed that each treatment alone was effective on *Listeria innocua*, *Escherichia coli* and mesophilic bacteria. After 7 d, treatment with γ-radiation reduced *L. innocua* and *E. coli* of 1.8 and 3.6 log CFU g^{-1} respectively while NAI + ozone reduced *L. innocua* and *E. coli* of 2.0 and 2.8 log CFU g^{-1} respectively. Mesophilic bacteria were reduced of 1.8 log CFU g^{-1} after γ-radiation and 1.4 log CFU g^{-1} after NAI + ozone. This study demonstrated that the bioactive coating acts in synergy with γ-radiation, inducing no bacterial growth of *L. innocua* and *E. coli*, as well as a control of the growth of mesophilic bacteria during 7 d. The combination of bioactive coating and NAI + ozone induced an additive effect on *L. innocua*, *E. coli* and mesophilic bacteria, and suggests potential antioxidant properties of the coating.

Keywords: antimicrobial coating, gamma radiation, ozone, negative air ionization, *Listeria*, *E. coli*

4.4 Introduction

Elimination of pathogenic bacteria is a major concern for food industries. Indeed, Scallan *et al.* (2011) have estimated that 9.4 million illnesses were due to foodborne disease, causing 55,961 hospitalizations and more than 1,300 deaths. Among all the micro-organisms, *Escherichia coli* and *Listeria monocytogenes* are frequently involved in microbial outbreaks. After harvest, fruits and vegetables can be considered as bacteria carriers which is why they undergo disinfection treatments (Ölmez *et al.*, 2009). The use of chemical washing compounds such as peracetic acid can reduce the bacterial load but it is not enough to guarantee safety throughout the entire shelf life of food products (Alvaro *et al.*, 2009, Dai *et al.*, 2012, Siroli *et al.*, 2015). Indeed, according to Erickson (2010), cross-contaminations are likely to occur while food is being processed but also after leaving processing facilities. Despite food safety measures, foodborne outbreaks still happen, leading to health threats and hospitalizations. Fruits and vegetables that are eaten raw can be an easy target, as confirmed by recent outbreaks which occurred on fresh vegetables for salads, cantaloupes or celery, respectively contaminated by *E. coli*, *Salmonella* and *Listeria* (Garner *et al.*, 2016, Kozak *et al.*, 2013)

Gamma-radiation is a cold process that can be used to assure food safety and is usually applied on packaged products. Irradiation provokes DNA double-strand breaks, which can lead to bacterial death (Hussain *et al.*, 2014, Jeong *et al.*, 2010). The maximum dose that can be applied on fresh vegetables should not exceed 1 kJ kg^{-1} (Komolprasert *et al.*, December 2007/January 2008). However, some pathogens such as *L. monocytogenes* can still survive and need higher doses to be eliminated (Bari *et al.*, 2006).

Ozone, an allotrope of oxygen, can be produced by using the corona method. Air is exposed to a high-voltage current, leading electrons to split after their excitation. Single atoms of oxygen will then combine with oxygen molecules, forming a new ozone molecule with a short half-life (Alencar *et al.*, 2013, Kim *et al.*, 1999, Shah *et al.*, 2013). The antimicrobial action of ozone has been studied and several mechanisms have been listed (Kim *et al.*, 1999). Indeed, it has been proposed that ozone induces changes in DNA of microorganisms, leading to their inactivation. Also, according to some authors, ozone would react on cell walls of bacteria by oxidizing major components (Beuchat, 1992). During the last past years, the use of ozone has been paired with negative air ionization. This new air-cleaning technology provides negative charges to particles, resulting in their accumulation on walls and floors (Holt *et al.*, 1999). Challenger *et al.* (1996) showed that the use of negative ions also leads to the generation of hydrogen peroxide. Studies

have been carried out on the survival of microorganisms on cantaloupe, showing that hydrogen peroxide treatments were effective against *L. monocytogenes* and total flora (Sapers *et al.*, 2001b, Ukuku *et al.*, 2002).

Essential oils (EOs) are natural compounds known to possess strong antimicrobial properties due to their content in phenolic compounds and flavonoids (Oussalah *et al.*, 2004). Burt (2004) has suggested that the hydrophobicity of EOs provokes changes in cell membrane permeability, resulting in a loss of ions and other cell contents. Lv *et al.* (2011) showed that a one hour-treatment with EOs such as oregano, bergamot or basil is also enough to damage cell membranes. Oussalah *et al.* (2006a) studied the mechanism of action that occurred after treatment of bacteria with EOs. They showed that EOs are more likely to act on the cytoplasmic membrane, leading to physiological changes in bacteria. Indeed, losses of intracellular ATP and cell constituents were observed, confirming a disruption of bacteria membranes. Other effects such as denaturation of proteins and enzymes have been described to affect cell division mechanisms (Nazzaro *et al.*, 2013). Organic acids can also be used as antimicrobial compounds since they are internalized in the cytoplasm before dissociating into protons and anions, affecting internal pH and cell components (Davidson *et al.*, 2007, Ricke, 2003). Recently, Boumail, A. *et al.* (2016) have developed a bioactive coating which allowed a reduction of *L. monocytogenes* in vegetables.

Treatments are usually combined to induce synergies and increase antimicrobial effects. However, very little research has studied the effect of gaseous ozone and EOs on bacteria. Whangchai *et al.* (2006) studied the effect of ozone combined with citric or oxalic acids. They concluded that gaseous ozone should be followed by another antimicrobial treatment to prevent later contaminations. Thus, the use of negative air ionization with ozone (NAI + ozone) during the whole storage represents an innovative technique to control bacterial growth. The aim of this study was to evaluate and compare the antimicrobial effects of γ-radiation or NAI + ozone in combination with a bioactive coating on ready-to-eat cauliflowers during storage.

4.5 Materials and Methods

4.5.1 Bacterial suspension

Under sterile conditions, 25 g of cauliflower were immersed in 75 mL of peptone water (1 g L^{-1}) and then, homogenized for 1 min using a Stomacher Lab-Blender 400 (Laboratory Equipment, London, UK). A quantity of 1 mL of this resulting mixture was incubated in 9 mL of Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, MI, USA) at 37 °C for 24 h. Those bacteria extracted from cauliflower were used to prepare a stock solution of mesophilic bacteria. *E. coli* ATCC 8739, *L. innocua* ATCC 51742 and mesophilic bacteria were stored at - 80 °C in TSB in presence of glycerol (150 g L^{-1}). Before each experiment, bacteria were propagated through 2 consecutive cycles of 24 h in TSB at 37 °C. The cultivated cultures were centrifuged at 5,000 g for 15 min and the collected pellets were washed twice in peptone water (1 g L^{-1}) to obtain working cultures containing approximately 10^9 CFU (colony forming unit) mL^{-1}.

4.5.2 Preparation of vegetables

Cauliflowers were purchased from a local supermarket, cut into florets (20 to 25 g) and packaged in 12.7 µm metalized polyester-50.8 µm ethylene vinyl acetate copolymer bags (Winpak Division Ltd., Montreal, QC, Canada). Sterilization was done at the Canadian Irradiation Center by γ-radiation, using a UC-15 A (SS canister) underwater calibrator (Nordion Inc., Kanata, ON, Canada) equipped with a ^{60}Co source. A radiation dose of 10 kJ kg^{-1} was delivered at a dose rate of 16.95 kJ kg^{-1} h^{-1}. Vegetables were then stored at 4 °C.

4.5.3 Bioactive coating

The mixture of antimicrobial compounds was prepared according to Boumail, A. *et al.* (2016). The bioactive coating was prepared as described by Boumail, A. *et al.* (2016) and contained 2.5 g L^{-1} of methylcellulose (MC), 7.5 g L^{-1} of maltodextrin (MD), 7.5 g L^{-1} of glycerol (Sigma-Aldrich Ltd) and 34 g L^{-1} of the antimicrobial compounds.

4.5.4 **Bacterial radiosensitization**

The D_{10} is defined as the radiation dose required reducing 90% population (reduction of 1 log CFU g^{-1}) of viable *E. coli* and *L. innocua* on cauliflowers. Control and coated cauliflowers were inoculated with *E. coli* or *L. innocua* in order to reach approximately 10^6 log CFU g^{-1} on vegetables. Samples were stored at 4 °C for 15 h, allowing the bioactive coating to act on bacteria. Irradiation treatment were then performed with doses from 0 to 1 kJ kg^{-1} for *E. coli* and from 0 to 2.4 kJ kg^{-1} for *L. innocua*. For microbiological analysis, cauliflowers samples (25 g) were immersed in 50 g of peptone water (1 g L^{-1}) and homogenized using a stomacher. Serial 10-fold dilutions were made and 1 mL of each dilution was spread on petri dishes before medium was poured. MacConckey culture media supplemented with sorbitol was used for *E. coli* and Palcam supplemented with ceftazidime (20 mg L^{-1}), acriflavin (5 mg L^{-1}) and polymixin B (10 mg L^{-1}) was used for *L. innocua*. Petri dishes were incubated at 37 °C for 48 h before bacterial enumeration. Detection level was calculated as 0.48 log CFU g^{-1} (3 CFU g^{-1}). Bacterial counts were plotted against radiation doses and the D_{10} value was calculated according to the Equation 1.

$$\text{Equation 1 : } D_{10} = \frac{1}{a}$$

where a is the slope of the trendline extracted from the plot.

4.5.5 **Negative air ionization (NAI) with ozone**

The ionizer/ozonator was set to produce minimal amount of ozone (volume of 428 mg m^{-3}) and negative ions (ranging from -0.2 to -0.4 mV) during the whole experiment. The residual ozone content was measured and recorded using a Portable Ozone Analyzer Series 500 (Aeroqual Limited, Auckland, New Zealand) that can detect up to 1070 mg m^{-3}. Data were collected using Aeroqual S500 v6.0 software (Aeroqual Limited). The negative ions content was measured and recorded with an Air Ion Counter (AlphaLab Inc, Salt Lake City, UT, USA) and data were collected using AlphaApp 1.0.19 (AlphaLab Inc.).

4.5.6 **Antimicrobial effect of combined treatments during storage**

Cauliflowers were treated with the bioactive coating and then inoculated by adding 500 µL of a diluted bacterial suspension (10^6 log CFU mL^{-1}) on 20 g of cauliflowers. Final concentrations on cauliflower were 3.2 log CFU g^{-1} for *L. innocua* and 3.6 log CFU g^{-1} for *E. coli* and for mesophilic bacteria. After inoculation, samples were divided into 2 groups. One group was stored at 4 °C for 15 h and then irradiated with a dose of 0.25 kJ kg^{-1} before storage during 7 d. The other group was stored at 4 °C under ionized/ozonated air during 7 d. Microbiological analysis were performed as described in **part 2.4.** at days 0, 1, 2, 4 and 7. Tryptic Soy Agar (TSA, Difco Laboratories, Detroit, MI, USA) was used for the plating of samples inoculated with mesophilic bacteria while Palcam and MacConckey agar supplemented with sorbitol were used for enumeration of *L. innocua* and *E. coli* respectively.

4.5.7 **Synergistic effect of combined treatments**

The relative inactivation value (RIV) of combined treatments was calculated according to the Equation 2 (Severino *et al.*, 2014a). Synergistic effects, occurring after the combination of treatments, were defined when RIV > 1.

$$\text{Equation 2 : } RIV = \frac{logN_{treatment} - logN_{comb}}{logN_{control} - logN_{coat}}$$

where $logN_{control}$ is the microbial population in the control samples, $logN_{treatment}$ is the microbial population after γ-radiation or NAI + ozone treatment, $logN_{coat}$ is the microbial population after coating treatment and $logN_{comb}$ is the microbial population after combined treatments.

4.5.8 **Experimental design and statistical analysis**

All analyses were performed in triplicate (n = 3) and 3 measurements were done for each replicate. The determination of the D_{10} was calculated using a 2 x 3 x 2 x 2 x 10 factorial design: 2 repetitions, 3 replicates, 2 treatments (control or coating), 2 bacteria, 10 γ-radiation doses. For microbial analyses, samples were separated into 6 groups: (i) control; (ii) coating; (iii) γ-

radiation; (iv) NAI + ozone; (v) coating combined with γ-radiation; (vi) coating combined with NAI + ozone. Analyses during storage were done using a 2 x 3 x 6 x 3 x 5 factorial design: 2 repetitions, 3 replicates, 6 treatments, 3 bacteria, 5 d of storage. Analysis of variance, Duncan's multiple-range test and Student test were performed for statistical analysis by using SPSS 16.0 software (IBM Corp., Somers, NY, USA). Differences between means were considered to be significant at a 5% level.

4.6 Results and Discussion

4.6.1 Radiosensitization of bacteria

Figure 4.1 shows the radiosensitization of *L. innocua* and *E. coli* in cauliflowers treated with the bioactive coating. For *L. innocua*, results showed a D_{10} value of 0.25 kJ kg^{-1} for the control, and a D_{10} value of 0.22 kJ kg^{-1} after treatment with the bioactive coating. For *E. coli*, the D_{10} value was reduced from 0.18 to 0.16 kJ kg^{-1} after treatments with the bioactive coating. Those results are in agreement with previous studies (Beauchamp *et al.*, 2012, Lacroix *et al.*, 2009), since Gram-positive bacteria have been described as more resistant to γ-radiation than Gram-negative bacteria (D_{10} values of 0.25 and 0.18 kJ kg^{-1} for *L. innocua* and *E. coli* respectively). A higher decrease of the D_{10} value was observed for *L. innocua* as compared to *E. coli* after treatments with the bioactive coating. This behavior can be explained by the presence of EOs in the bioactive coating. Indeed, hydrophobic compounds such as EOs can easily penetrate the cell wall of Gram positive bacteria, while Gram negative bacteria possess hydrophilic channel in their outer membrane (Nazzaro *et al.*, 2013). Results obtained in this study suggest that cell membranes of *L. innocua* were weakened by the antimicrobial coating pre-treatment, thus exposing DNA to γ-radiation. This would confirm several studies showing that EOs act in synergy with γ-radiation (Caillet *et al.*, 2006a, Lacroix *et al.*, 2009, Lafortune *et al.*, 2005, Severino *et al.*, 2014a, Turgis *et al.*, 2012). Among them, Severino *et al.* (2014a) showed that γ-radiation was more effective when combined with a chitosan-based coating containing EOs since they were able to reduce the D_{10} from 0.32 to 0.24 kJ kg^{-1}, showing an increase of the bacterial radiosensitization of 25 %.

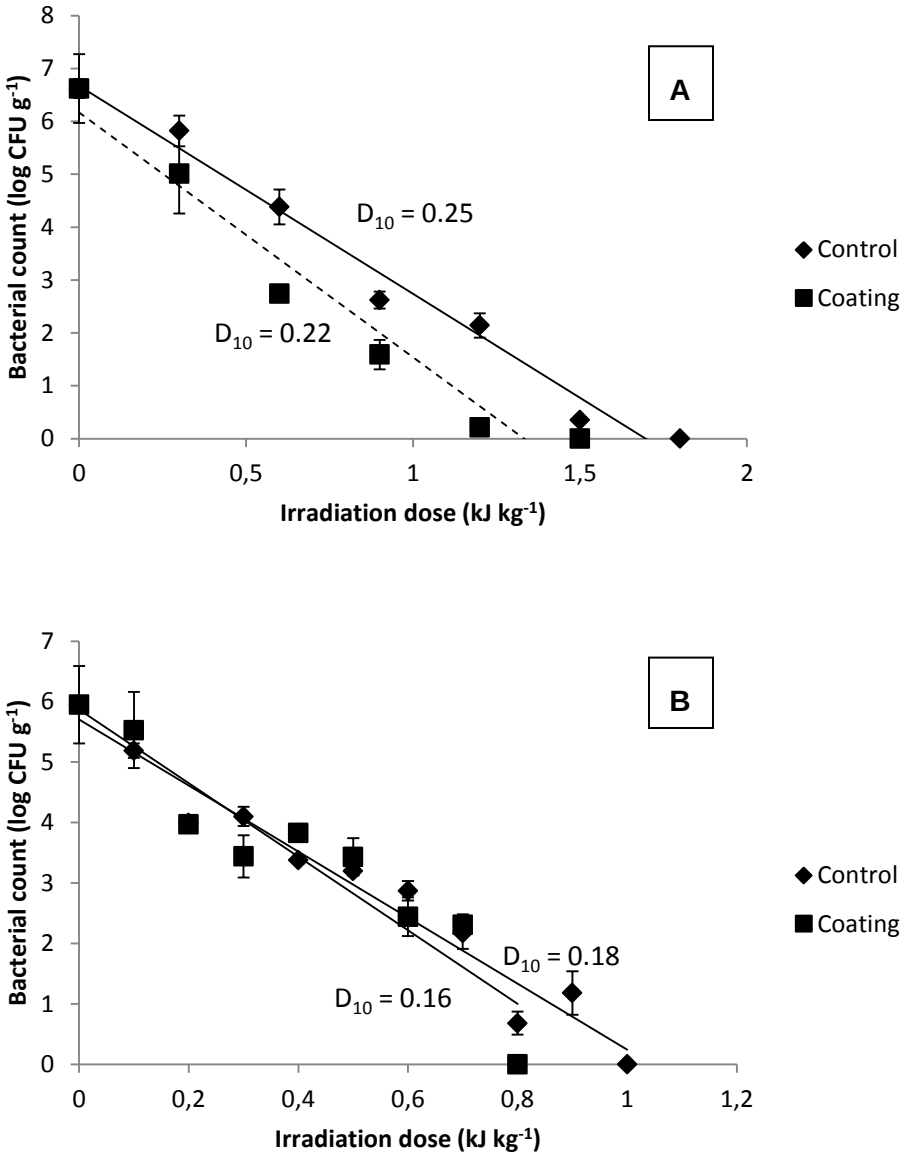


Figure 4.1 Radiosensitization of *L. innocua* (Fig 1.A) and *E. coli* (Fig 1.B) on cauliflowers treated with antimicrobial coating.

4.6.2 **Antimicrobial effect of biactive coating combined with γ-radiation during storage**

The effect of the antimicrobial coating combined with γ-radiation on *E. coli*, *L. innocua* and mesophilic bacteria is presented in **Figure 4.2**. Results showed that the content of *E. coli* in control cauliflowers decreased by 1.5 log CFU g^{-1} in 7 d. Between Day 0 and Day 2, a reduction of bacterial count from 3.6 to 3.0 log CFU g^{-1} was observed. Then, between Day 4 and Day 7, a reduction of bacterial count from 3.0 to 2.1 log CFU g^{-1} was observed. Treatments with γ-radiation also allowed a significant bacterial reduction ($p \leq 0.05$). Indeed, at Day 0, a 1.1 log CFU g^{-1} reduction was observed after γ-radiation treatment and the bacterial load was decreased during storage, until no bacteria were detected at Day 7. This observation would suggest a complete elimination of *E. coli* with a 3.6 log CFU g^{-1} inhibition and could be explained by the fact that *E. coli* is sensitive to γ-radiation (Beauchamp *et al.*, 2012). Treatments with the bioactive coating were also effective in significantly reducing *E. coli* ($p \leq 0.05$) since a 1.3 log CFU g^{-1} reduction was noticed at Day 0. A continuous decrease was observed during storage, from 2.3 log CFU g^{-1} at Day 0 to 1.8 log CFU g^{-1} at Day 7. The combination of the bioactive coating and γ-radiation showed a strong antimicrobial effect since *E. coli* was below the limit of detection during the whole storage.

Concerning *L. innocua*, the control group showed a reduction from 3.2 to 2.7 log CFU g^{-1} after 7 d of storage, characterized by a significant reduction to 2.3 log CFU g^{-1} at Day 2 ($p \leq 0.05$). The γ-radiation treatment induced a significant reduction ($p \leq 0.05$) at Day 0, from 3.2 to 2.3 log CFU g^{-1}. It was followed by a 1.4 log CFU g^{-1} reduction until Day 2 and an increase of bacterial count to 1.5 log CFU g^{-1} from Day 4 to the end of storage. The reduction observed around Day 2 for the control and γ-irradiated samples could be due to environmental stresses. Sela *et al.* (2015) mentioned that the survival can be limited for bacteria located on external parts of plants. Also, it has been reported that the growth of bacteria on the surface of fruits and vegetables would be reduced due to the lack of nutrients and moisture (USFDA, 2015). The coating treatment also showed a significant reduction of 1.7 log CFU g^{-1} ($p \leq 0.05$) of the bacterial load at Day 0. This reduction was continuous over time, until no detection was observed on Day 7. However, the combined treatments had a synergistic effect and the level of *L. innocua* was below the limit of detection during the whole storage. The use of the coating in combination with 0.25 kJ kg^{-1} radiation dose led to a reduction of at least 2.7 log CFU g^{-1}, creating, as for *E. coli*, a synergistic effect between the two treatments.

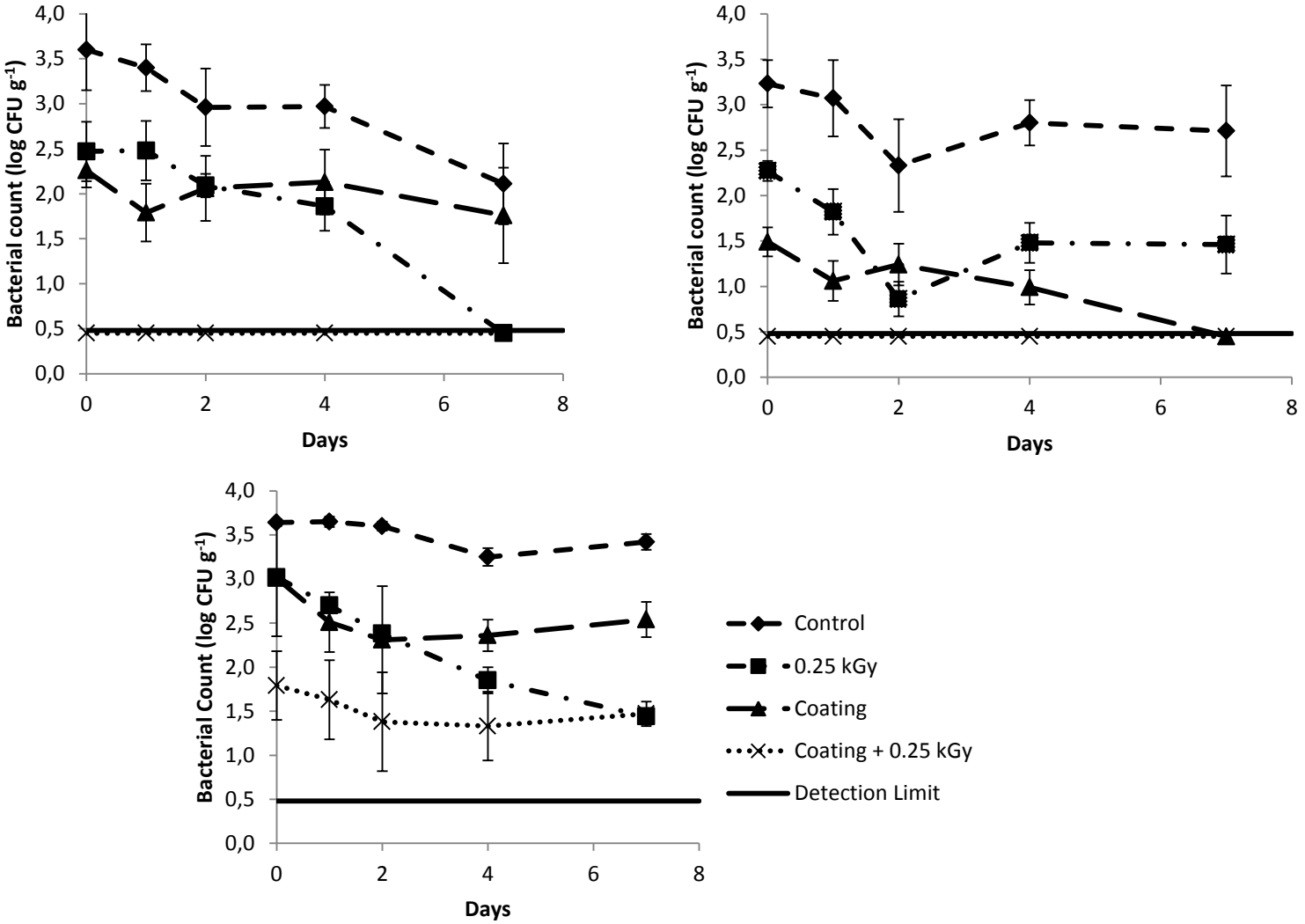


Figure 4.2 Effect of the combination of antimicrobial coating and γ-radiation (0.25 kJ kg^{-1}) on the growth of *E. coli* (Fig 2.A), *L. innocua* (Fig 2.B) and mesophilic bacteria (Fig 2.C) on cauliflower florets.

Finally, results for the mesophilic bacteria showed that the control group was stable during storage, with values between 3.3 and 3.6 log CFU g^{-1}. Results also showed that both irradiation and coating treatments allowed a reduction of bacteria at Day 0, from 3.6 log CFU g^{-1} to 3.0 log CFU g^{-1}. A continuous decrease was also observed during storage on 0.25 kJ kg^{-1} treated samples, showing a reduction from 3.0 log CFU g^{-1} ad Day 0 to 1.4 log CFU g^{-1} at Day 7. Treatment with the coating reduced the mesophilic bacteria from 3.0 log CFU g^{-1} at Day 0 to 2.5 log CFU g^{-1} at Day 1 and showed no variation ($p > 0.05$) until the end of storage. The combination of coating and γ-radiation induced a significant reduction of the mesophilic bacteria load at Day 0 from 3.6 to 1.8 log CFU g^{-1}.

Results obtained after combination of the antimicrobial coating and γ-radiation are consistent with what was previously described. Indeed, Gram-negative bacteria are known to be more sensitive to γ-radiation than Gram-positive bacteria. Lacroix *et al.* (2009) have shown that *L. monocytogenes* required a higher γ-radiation dose than *E. coli* and *Salmonella* Typhimurium (both Gram-negative bacteria) to obtain a reduction of 1 log CFU g^{-1} (0.36, 0.21 and 0.27 kJ kg^{-1} respectively). However, due to the structure of their membrane, Gram-positive bacteria are more likely to be affected by EOs. A previous study has suggested that the use of EOs can increase the sensitivity of bacteria to γ-radiation (Lacroix *et al.*, 2004). Indeed, antimicrobial properties of EOs are known to be due to the presence of phenolic and terpenic compounds. Sikkema *et al.* (1994) have studied the action of terpenes on bacteria. They proposed a mechanism in which terpenes accumulate in the cytoplasmic membrane, increasing its size. Resulting changes induce a reduction of the activity of enzymes (such as the cytochrome c oxidase, responsible for the respiration) and an increase of the proton permeability. Following γ-radiation treatments will target DNA molecules, inducing mutations that can inhibit bacterial growth (Hussain *et al.*, 2014, Jeong *et al.*, 2010). Another important effect, indirect consequence of γ-radiation, is due to reactive oxygen species after radiolysis of water (Beauchamp *et al.*, 2012, Jeong *et al.*, 2010). Results obtained would suggest that the use of the coating followed by a 0.25 kJ kg^{-1}-radiation treatment induced a reduction of at least 3.6 and 3.1 log CFU g^{-1} of *E. coli* and *L. innocua* respectively.

4.6.3 **Antimicrobial effect of bioactive coating combined with NAI + ozone during storage**

The effect of the combined treatments on *E. coli*, *L. innocua* and mesophilic bacteria is presented in **Figure 4.3**. For *E. coli*, a continuous decrease was observed for the control group during storage. Indeed, the bacterial load was reduced from 2.8 log CFU g^{-1} at Day 0 to 1.5 log CFU g^{-1} at Day 4. At the end of storage, no bacteria were detected. Treatment with NAI + ozone also induced a significant reduction of *E. coli*. Indeed, a 0.8 log CFU g^{-1} reduction was observed after 1 d of storage and no bacteria were detected from Day 2 to Day 7. The coating as well as the combined treatment showed an immediate reduction of *E. coli*, from 2.8 to 2.0 log CFU g^{-1} at Day 0. Both groups exhibited a similar behavior for the rest of the storage, with a bacterial load of 1.2-1.3 log CFU g^{-1} at Days 1-4 and no bacteria detected at Day 7.

Concerning *L. innocua*, results showed a significant reduction ($p \leq 0.05$) of the bacterial load for the control group during storage, from 3.3 log CFU g^{-1} at Day 0 to 2.1 log CFU g^{-1} at Day 7. All treatments applied on cauliflower also showed antimicrobial effects, with higher reductions. Indeed, a one week-exposition to NAI + ozone resulted in a 2 log CFU g^{-1} reduction, from 3.3 log CFU g^{-1} at Day 0 to 1.3 log CFU g^{-1} at Day 7. Treatment with the coating showed a significant and immediate antimicrobial effect ($p \leq 0.05$), with a reduction of 1.3 log CFU g^{-1}, which is in accordance with results reported in Section 4.6.2. After coating, *L. innocua* was not detected from Day 4 until the end of storage, suggesting a complete inhibition of bacteria. Finally, results showed that the combined treatments (coating followed by storage under NAI + ozone) had a similar antimicrobial effect as compared to the coating alone during the whole storage, with a reduction of bacteria at Day 2, from 2.1 to 1.3 log CFU g^{-1}, and no detection from Day 4 until the end of storage.

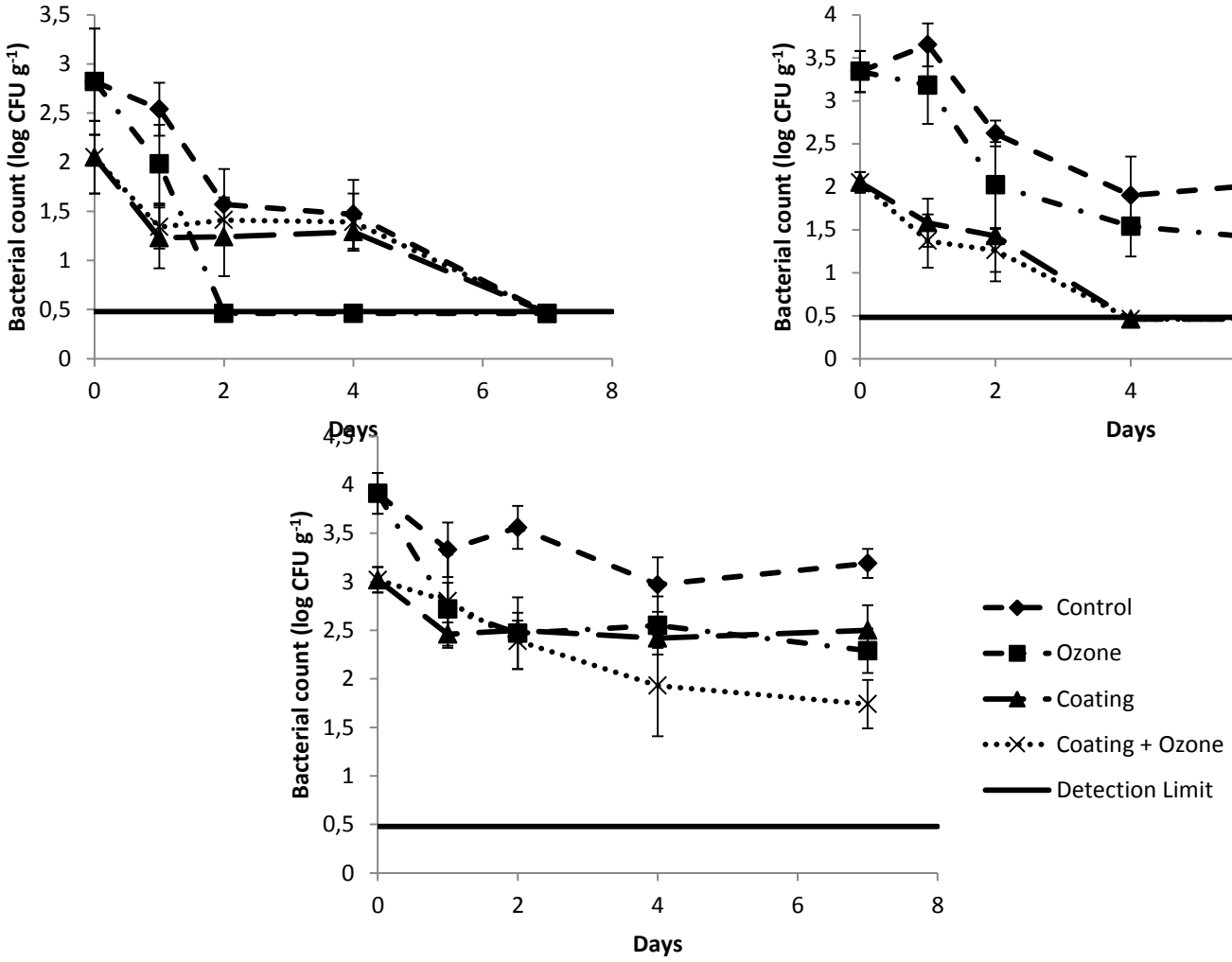


Figure 4.3 Effect of the combination of antimicrobial coating and NAI + ozone on the growth of *E. coli* (Fig 3.A), *L. innocua* (Fig 3.B) and mesophilic bacteria (Fig 3.C) on cauliflower florets.

As for mesophilic bacteria, the control group showed a 0.7 log-reduction ($p \leq 0.05$) over storage, from 3.9 to 3.2 log CFU g^{-1} at Days 0 and 7 respectively. For the other groups, all treatments allowed a significant reduction ($p \leq 0.05$) of bacteria over time. Indeed, the treatment with NAI + ozone reduced the microbial load from 3.9 log CFU g^{-1} at Day 0 to 2.3 log CFU g^{-1} at Day 7, representing a reduction of 1.6 log CFU g^{-1}. The coating treatment resulted in an immediate antimicrobial effect on mesophilic bacteria, with a reduction from 3.9 to 3.0 log CFU g^{-1} at Day 0. The bacterial load slightly decreased at Day 1 and was maintained until the end of storage to a value of 2.5 log CFU g^{-1}. The combined treatments also induced a reduction of bacteria on Day 0, from 3.9 to 3.0 log CFU g^{-1}. The antimicrobial effect was continuously observed until the end of storage, with a final level of 1.7 log CFU g^{-1}, representing a total reduction of 2.2 log CFU g^{-1}.

Treatments with NAI + ozone were effective in reducing the growth of all tested bacteria. Several studies have showed similar results and a mechanism of action has been proposed. According to Mahapatra *et al.* (2005), bacteria cells are electropositive and are likely to attract electrophilic molecules such as ozone. The high oxidation potential of ozone would then allow the deterioration of lipids from the membrane. This disruption of cell would lead to a loss of cell components but also expose nucleic acids, enzymes and protein to oxidation by ozone (Kim *et al.*, 1999, Mahapatra *et al.*, 2005, Miller, F.A. *et al.*, 2013).

Results obtained suggest that the antimicrobial effect of ozone is higher on *E. coli* than on *L. innocua*. This is in agreement with what has been reviewed by Miller, F.A. *et al.* (2013). Indeed, Gram-positive bacteria membranes contain a thick layer of peptidoglycans while Gram-negative bacteria membranes contain a thin peptidoglycans layer and lipids. As a result, Gram-negative bacteria could be considered as a better target in presence of ozone.

Recently, Song *et al.* (2015) have studied the effect of ozone in juices and observed a synergy between ozone and pH. They suggest that ozone increases the permeabilization of bacteria, allowing the diffusion of weak acids. This would result in a dissociation and a release of protons in the cytoplasm, decreasing the internal pH.

4.6.4 **Synergistic effect of combined treatments**

As explained by Bjergager *et al.* (2012), a synergy can be defined as the ability of one treatment (ie antimicrobial coating application) to increase the effect of another treatment (ie γ-radiation or

NAI + ozone treatment). Synergistic effect treatments are observed when RIV (relative inactivation value) > 1 while a RIV of approximately 1 is typical of an additive effect. On the contrary, antagonist effects are observed when RIV < 1. Results presented in **Table 4.1** summarize the RIV of the combined treatments observed on *L. innocua*, *E. coli* and mesophilic bacteria during storage. The combination of antimicrobial coating and γ-radiation induced synergistic effects (RIV > 1) on *L. innocua* and *E. coli* during storage. On the opposite, combining the antimicrobial coating to NAI + ozone led to additive effects on *L. innocua* and *E. coli*, with RIV ≈ 1. Concerning mesophilic bacteria, the combination of the antimicrobial coating with γ-radiation was characterized by a synergy on Day 0 and an additive effect on the following days. Overall, the combination of the antimicrobial coating with NAI + ozone induced an additive effect on mesophilic bacteria.

Table 4.1 Relative inactivation value (RIV) of combined treatments (antimicrobial coating combined with γ-radiation or NAI + ozone) for *E. coli*, *L. innocua* and mesophilic bacteria.

Days	RIV[1]					
	E. coli		***L. innocua***		**Mesophilic bacteria**	
	γ-radiation	**NAI + ozone**	**γ-radiation**	**NAI + ozone**	**γ-radiation**	**NAI + ozone**
0	> 1	1	> 1	1	> 1	1
1	> 1	< 1	> 1	0.9	0.9	< 1
2	> 1	< 1	> 1	0.8	0.7	< 1
4	> 1	< 1	> 1	1	0.7	1.1
7	0	1	1	1	0	0.8

[1] RIV < 1 highlights an antagonist effect, RIV ≈ 1 highlights an additive effect and RIV > 1 highlights a synergistic effect between γ-radiation and the bioactive coating.

From those results, it could be deduced that the use of the bioactive coating increased the antimicrobial effect of γ-radiation on *E. coli* and *L. innocua* from Day 0 to Day 4. The absence of synergy on Day 7 can be explained by the efficiency of the γ-radiation treatment alone for *E. coli* and the bioactive coating treatment alone for *L. innocua*. Similarly, the bioactive coating treatment induced a higher antimicrobial effect of γ-radiation on mesophilic bacteria on Day 0. The absence of synergy in the following days is probably due to the continuous antimicrobial effect of the γ-radiation treatment during storage. Despite the antagonist effect observed, the bacterial load was kept under 2 log CFU g^{-1} during the whole storage (**Fig. 4.2.C**).

The additive effect produced when combining the bioactive coating to NAI + ozone are in agreement with results observed by Severino *et al.* (2014b). Authors combined an antimicrobial coating with ozonated water, leading to an additive effect during the first days of storage. Kavoosi *et al.* (2013) showed that the incorporation of thymol in a bioactive film increased the antioxidant properties. Guerreiro *et al.* (2015) showed that polysaccharide coatings containing citral and/or eugenol had higher antioxidant properties. According to Chiasson *et al.* (2004), antioxidant compounds can interact with free radicals, increasing the resistance of bacteria. Thus, such studies could explain the results we obtained. Indeed, since ozone is a strong oxidizing agent (Artés *et al.*, 2009, Coll Cárdenas *et al.*, 2011, Kim *et al.*, 1999, Mahapatra *et al.*, 2005), it could be hypothesized that the antimicrobial coating also exhibited antioxidant properties towards physical surface treatments. Properties of both treatments could explain the difference in their efficiency since NAI + ozone act on the membrane of cells while γ-radiation has more penetrating properties and would be less affected by the coating applied on the surface of food.

4.7 **Conclusion**

Coating, γ-radiation and NAI + ozone treatments alone were able to decrease significantly the level of *E. coli*, *L. innocua* and mesophilic bacteria in ready-to-eat cauliflower florets. The combination of the bioactive coating induced a synergy and allowed a reduction of the level of these bacteria under the limit of detection while the combination with NAI + ozone induced additive effects. Storage of vegetables in NAI + ozone would be a good technique to reduce and control bacterial growth while the use of γ-radiation could prevent cross-contamination from

turning into microbial outbreaks. It would also answer to federal regulations in order to assure the absence of *E. coli* and *L. innocua* in ready-to-eat food while increasing their shelf-life.

4.8 Acknowledgments

This research was supported by the National Sciences and Engineering Research Council of Canada (NSERC), the International Atomic Energy Agency (IAEA), Bonduelle North America and Ecovie (Boucherville, QC, Canada). Nordion is acknowledged for the irradiation operations. Finally, we are grateful to BSA Ingredients s.e.c./l.p. and Biosecur Lab Inc. for the antimicrobials, and to Winpak Division Ltd. for the packaging materials.

5 DISCUSSION ET CONCLUSION GÉNÉRALES

L'objectif général de ce projet était de développer deux systèmes polymériques, composés d'agents naturels bioactifs, permettant de garantir la qualité des légumes prêts à manger. Les études réalisées ont montré qu'il est possible d'immobiliser différents agents antimicrobiens (HE, acides organiques, extraits naturels) dans des matrices polymériques tout en préservant leurs propriétés antimicrobiennes.

L'étude détaillée dans l'article 1 a montré que les films d'emballage avaient un bon effet antimicrobien *in vitro* sur les bactéries *L. monocytogenes*, *E. coli* et *S.* Typhimurium. Malgré le fait que les bactéries Gram négatif soient plus résistantes aux HE, ce sont celles-ci qui ont été affectées le plus par les films. Ces résultats pourraient suggérer que des mécanismes d'action différents entrent en jeu grâce au contact direct entre les antimicrobiens et les bactéries.

L'étude a montré que le suivi de la libération des composés bioactifs depuis des films d'emballage pouvait se faire selon une méthode conventionnelle (dosage des composés phénoliques) mais également selon une méthode innovante, c'est-à-dire par FTIR. Cette méthode simple et rapide pourrait permettre de suivre plus spécifiquement l'évolution de certains composés selon la longueur d'onde sélectionnée. Un avantage non négligeable de cette méthode est le fait que l'échantillon n'est pas détruit. Utilisée en industrie, cette technique permettrait ainsi un gain de temps considérable lors des étapes de contrôle qualité. Ainsi, après avoir établi une courbe standard, il serait possible d'estimer, en une dizaine de minutes, la concentration en antimicrobiens dans les films. Cette donnée pourrait également être corrélée à l'activité antimicrobienne, aidant ainsi à déterminer les dates de péremption des aliments.

Il a aussi été montré que ces films produisent un effet barrière face à l'humidité et au dioxyde de carbone, ce qui serait principalement dû à la présence de PCL et des agents antimicrobiens respectivement. Enfin, l'évaluation des propriétés mécaniques de ces films a permis de déterminer leur élasticité et leur niveau de résistance face à un stress. Il s'agit d'un critère important lors de la commercialisation car les emballages peuvent être soumis à de nombreux chocs, que ce soit pendant le transport ou en magasin. Il est donc nécessaire de développer un film qui sera assez résistant pour maintenir sa forme et sa structure tout en continuant à libérer les différents composés antimicrobiens pendant toute la durée de conservation. En ce sens, les films ADF-B sembleraient être plus adaptés car plus résistants à long terme.

Cependant, il est important et nécessaire que les films développés produisent un effet antimicrobien contre les principales bactéries pathogènes retrouvées dans l'industrie alimentaire, et en particulier *L. monocytogenes*. Les films d'enrobage pourraient ainsi répondre à cette problématique puisque le contact direct entre l'enrobage et les bactéries permet de se rapprocher des conditions *in vitro* réalisées avec les films d'emballage.

Contrairement aux films d'emballage, les enrobages sont ingérés en même temps que les légumes traités. Il est donc nécessaire que les composés utilisés soient autorisés en industrie alimentaire. Un autre aspect important concernant les antimicrobiens utilisés est le fait qu'ils ne devraient pas altérer le goût des légumes. Ainsi, certains tests peuvent être nécessaires afin de déterminer la concentration en antimicrobiens et l'adapter au besoin. Une diminution de cette concentration peut également réduire l'efficacité de l'enrobage face aux bactéries et il est important d'en tenir compte. L'optimisation du mélange de composés antimicrobiens utilisé dans les articles 2 et 3 ne figure pas ici car sa mise au point a été effectuée précédemment (Tawema *et al.*, 2014). Les tests effectués dans l'article 2 ont montré qu'après immobilisation dans une matrice polymérique, l'effet antimicrobien des composés bioactifs contre *L. monocytogenes* était présent après 24h. Cependant, la composition en polymère semble avoir un effet sur la rétention de ces antimicrobiens, influençant par la suite les propriétés organoleptiques des légumes traités. Les propriétés filmogènes de la méthylcellulose ont permis d'avoir une matrice qui se manipule bien et qui semble également adhérer aux choux-fleurs, permettant d'assurer une répartition de l'enrobage sur toute la surface. La présence d'amidon semble diminuer l'effet antimicrobien des enrobages et altérer le goût des choux-fleurs après traitement. Cela peut s'expliquer par la capacité de rétention de l'amidon, qui semble dépendre de sa concentration (Sosa *et al.*, 2014). Ainsi, les composés bioactifs sont plus retenus dans la matrice, ce qui réduit leur taux de libération et leur efficacité. Les propriétés organoleptiques des légumes peuvent également être affectées (Sánchez-González, L. *et al.*, 2011b), entrainant alors une mauvaise appréciation de l'aliment comme observé dans l'article 2. La maltodextrine, quant à elle, a déjà été utilisée pour encapsuler des composés bioactifs et les protéger contre l'oxydation. Cette protection permettrait ainsi de retarder la dégradation des composés bioactifs et d'augmenter la durée d'efficacité de l'enrobage. D'ailleurs, les analyses *in situ* réalisées par la suite avec l'enrobage final montrent que la charge microbienne diminue au cours du temps, jusqu'à ce que les bactéries ne soit plus détectées après une semaine de conservation. Cela suggère que l'enrobage libère les composés antimicrobiens, qui inhiberaient ensuite la croissance des bactéries. Cet enrobage a montré qu'il n'affecte pas l'odeur, la texture et le goût des choux-fleurs, qui sont globalement appréciés.

Concernant les qualités physico-chimiques des choux-fleurs, la respiration, la couleur et la consistance ont été étudiées au cours du temps. La présence de l'enrobage augmente la consommation d'oxygène de 0.2-0.4 mL et la production de dioxyde de carbone d'environ 0.4 mL par rapport au groupe témoin, suggérant ainsi une augmentation de la respiration. Cependant, l'augmentation de la respiration entre le groupe témoin et le groupe traité ne semble pas importante en comparaison avec d'autres recherches. Qi *et al.* (2011) ont par exemple montré qu'un enrobage à base de chitosane contenant de l'acide ascorbique et du chlorure de calcium permet de réduire la production de CO_2 d'environ 40 mg après 2 jours et 30 mg après 8 jours. Buchner *et al.* (2011) ont quant à eux développé un enrobage qui réduit également la production de CO_2 de 10-15 mg pendant 18 jours. La faible différence au niveau du taux de respiration entre le groupe témoin et le groupe traité présentée à l'article 3 ne semble pourtant pas induire de changements au niveau de la qualité visuelle des choux-fleurs. Pour certains fruits et légumes dits climactériques, la respiration cellulaire va soudainement augmenter lors de l'étape de mûrissement (Perotti *et al.*, 2014). Cela va par la suite entraîner le phénomène de sénescence et la dégradation des fruits et légumes. Le chou-fleur n'étant pas un légume climactérique (Raja *et al.*, 2011), l'augmentation de la respiration ne semble pas provoquer de perturbation majeure.

Les mesures par colorimétrie ont permis de détecter un léger brunissement à la fin de la conservation après traitement avec l'enrobage. La couleur blanche du chou-fleur fait en sorte que le brunissement peut être perceptible dès son apparition. Celui-ci pourrait être dû à la présence de composés phénoliques dans les antimicrobiens, qui produirait des pigments bruns lors de leur dégradation. Cependant, l'évaluation sensorielle menée a montré qu'aucun changement dans la couleur n'était visible à l'œil nu, ce qui répond aux attentes des consommateurs. Concernant la consistance, celle-ci n'est globalement pas modifiée par l'enrobage ni par le temps de conservation.

Par sécurité, toutes les expériences *in situ* des articles 2 et 3 ont été réalisées avec des souches bactériennes non pathogènes. En effet, il est possible de substituer une espèce pathogène par une espèce non pathogène du même genre. L'homologie des génomes induirait un comportement similaire des bactéries face aux antimicrobiens (Silva-Angulo *et al.*, 2015). Le traitement des choux-fleurs avec l'enrobage antimicrobien a permis de réduire *L. innocua* de 1.7 log UFC/g au jour 0 et d'au moins 2.2 log UFC/g après 7 jours, comparativement à des choux-fleurs non traités. Cet enrobage est prometteur puisqu'il induit une bonne réduction de la croissance de *Listeria*. Cependant, les industriels demandent une absence totale de *L.*

monocytogenes dans leurs produits pendant toute la durée de vie, tel que recommandé par le MAPAQ (2009b). L'enrobage développé a donc été testé en combinaison avec l'irradiation γ ou sous air ionisé/ozoné. Les résultats présentés dans l'article 3 ont montré la présence d'une synergie entre l'enrobage antimicrobien et l'irradiation γ, permettant de ne détecter aucune bactérie (*L. innocua* ou *E. coli*) pendant toute la conservation. En revanche, la conservation sous air ionisé/ozoné des choux-fleurs enrobés ne permet pas une telle réduction. Ces résultats peuvent être dus aux modes d'action différents des deux traitements physiques comparés. Contrairement au rayonnement γ qui est très pénétrant et qui produit des espèces réactives de l'oxygène, l'ozone agit en surface et se dégrade en oxygène. Ainsi, l'inflorescence des choux-fleurs pourrait permettre la survie d'une partie des bactéries, limitant ainsi l'utilisation de l'ozonation de l'air comme moyen de désinfection.

Dans le cas d'*E. coli*, l'ionisation/ozonation de l'air permet de réduire la charge microbienne sous le seuil de détection à partir du jour 2. En revanche, la combinaison avec l'enrobage semble faire disparaitre l'effet de l'ionisation/ozonation de l'air pour ne laisser apparaître que celui de l'enrobage. Cette observation pourrait suggérer que l'enrobage agit comme protecteur vis-à-vis des bactéries ou bien que certains composés présents au niveau de l'enrobage annulent l'effet de l'air ionisé/ozoné. L'étude présentée dans l'article 3 indique que l'effet de l'enrobage est constant et reproductible. Le fait que la charge microbienne soit inférieure au seuil de détection au jour 7 dans le cas de l'ionisation/ozonation peut être dû à une inoculation initiale plus faible. En revanche, l'effet de l'ionisation/ozonation sur *E. coli* semble plus rapide que celui de l'irradiation γ à 0.25 kGy. Cela peut s'expliquer par le mécanisme d'action différent entre les deux procédés. En effet, l'irradiation γ agit sur l'ADN en induisant des cassures. Raychaudhuri *et al.* (2000) ont étudié les réparations de l'ADN survenant après irradiation γ. Les auteurs indiquent que, lorsqu'elles ne sont pas réparées, les cassures du double brin d'ADN peuvent être à l'origine de la mort cellulaire. L'utilisation de faibles doses d'irradiation γ induisant moins de dommages, les bactéries pourraient donc enclencher les mécanismes de réparation de l'ADN. Cependant, il semble qu'en cas de cassure du double brin d'ADN, il ne pourrait y avoir plus de 3 à 4 réparations par génome (Krisch *et al.*, 1976, cité dans Raychaudhuri *et al.*, 2000). Également, Sahbani *et al.* (2014) citent plusieurs études suggérant que la présence d'une seule cassure du double brin d'ADN *d'E. coli* induirait la mort cellulaire (Boye *et al.*, 1980, Frankenberg *et al.*, 1981, Kaplan, 1966, Schulte-Frohlinde, 1987, Van Der Schans *et al.*, 1973). En revanche, l'ionisation/ozonation agit sur les membranes bactériennes par oxydation de leurs composés. Les cellules pourraient donc être affectées plus rapidement que par irradiation γ.

D'autre part, Choi *et al.* (2000) proposent que la présence de néral et géranial dans les HE de citrus permet de piéger les radicaux libres, procurant ainsi une protection contre les dommages causés par ces radicaux. L'ozonation de l'air présentée dans l'article 3 est couplée à la production d'ions négatifs, qui peuvent mener à la formation de radicaux $O_2^{\cdot}$ selon le mécanisme présenté précédemment (section **1.4.1.**). Il est donc possible que la présence de ces composés antimicrobiens dans l'enrobage induise des propriétés antiradicalaires. L'effet antimicrobien dû à la présence d'ions négatifs serait ainsi réduit puisque le piégeage des radicaux limiterait la formation de peroxyde d'hydrogène. Lafortune *et al.* (2005) ont noté que la présence d'un enrobage pouvait permettre une croissance bactérienne, ce qui pourrait s'expliquer par l'utilisation des polysaccharides comme source de nutriment.

L'irradiation γ provoque également la formation de radicaux libres par radiolyse de l'eau. Cependant, il ne s'agit pas du mode d'action principal mais d'un effet indirect. Ainsi, les effets directs du rayonnement γ vont empêcher la croissance cellulaire et peuvent également provoquer la mort de la cellule, masquant par la même occasion le potentiel antiradicalaire de l'enrobage.

La culture mixte de bactéries hétérotrophes contient divers micro-organismes normalement présents dans les aliments. Une quantité élevée peut néanmoins être reliée à une contamination et refléter une mauvaise manipulation au cours de la transformation des fruits et légumes (Pilon *et al.*, 2015). Ces micro-organismes pouvant être un mélange de différentes bactéries, il peut être plus difficile de les éliminer ou de réduire leur croissance. Ces bactéries peuvent varier d'une région à l'autre, d'une récolte à l'autre et même d'un chou-fleur à l'autre au sein de la même récolte. Cependant, la croissance de ces bactéries peut mener à l'altération, la dégradation ou le pourrissement des aliments concernés (Barth *et al.*, 2010). Leur contrôle devient donc un des enjeux principaux pour les industries alimentaires. Les résultats présentés dans l'article 3 montrent qu'il est possible de limiter la croissance des bactéries hétérotrophes issues du chou-fleur, que ce soit par l'application de l'enrobage antimicrobien, par irradiation γ, par ozonation de l'air ou par un traitement combiné enrobage/irradiation ou enrobage/ozonation. Ainsi, l'enrobage a permis de réduire la charge microbienne d'environ 1 log CFU/g pendant toute la durée du traitement. Comme il a été observé pour *L. innocua* et *E. coli*, la combinaison enrobage/irradiation γ a démontré la présence d'une synergie. La combinaison enrobage/ozonation a permis d'obtenir un effet additif des traitements pendant l'entreposage, ce qui se traduit par un effet antimicrobien plus important qu'avec un traitement utilisé seul.

En conclusion, ce projet de recherche a montré que des films d'emballage ou d'enrobages bioactifs à base de polymères et d'antimicrobiens d'origine naturelle peuvent être utilisés pour améliorer la salubrité des légumes prêts à manger. En effet, ces deux types de films ont montré leur capacité à réduire, voire empêcher, la croissance de bactéries pathogènes et peuvent donc être utilisés pour empêcher l'apparition d'épidémies microbiennes. Ces films répondent à la fois aux agences de santé mais également aux industriels, qui peuvent se tourner vers de nouveaux marchés en garantissant l'innocuité de leurs produits alimentaires. Les films d'emballage développés au début du projet ont montré des résultats intéressants, au niveau microbiologique mais également au niveau des propriétés mécaniques. L'analyse des composés antimicrobiens par spectroscopie FTIR est une méthode prometteuse de par ses côtés à la fois innovant et rapide. Les enrobages, présentés par la suite, ont été développés de manière à conserver les propriétés organoleptiques et physico-chimiques des choux-fleurs. L'immobilisation des composés antimicrobiens dans une matrice a permis de conserver un effet antimicrobien sur *L. innocua*, *E. coli* et les bactéries hétérotrophes présentes sur le chou-fleur. La combinaison de l'enrobage avec l'irradiation γ a démontré un puissant effet antimicrobien et assurerait aux consommateurs l'innocuité des choux-fleurs. De plus, la présence de l'enrobage permettrait d'augmenter la durée de vie des choux-fleurs en comparaison avec les échantillons non traités.

Le projet présenté ici apporte une innovation au niveau des films ADFs. En effet, l'activité antimicrobienne générée par ce dispositif induit un contrôle des bactéries pathogènes directement à l'intérieur de l'emballage alimentaire. De plus, une nouvelle méthode de mesure de la libération des composés bioactifs a été mise au point par FTIR. Cette méthode permet non seulement de caractériser la structure chimique des films, mais surtout d'établir le profil de diffusion des antimicrobiens depuis le film vers les légumes. Il s'agit d'une méthode rapide, précise, non destructrice et qui est facilement adaptable à l'industrie. Également, les ADFs ont été développés de manière à s'intégrer aisément dans les procédures de fabrication des emballages et permettent ainsi une large application. Les films d'enrobage développés par la suite permettent de réduire puis d'inhiber la croissance des bactéries pathogènes tout en conservant les propriétés physico-chimiques et sensorielles des choux-fleurs. La combinaison de cet enrobage avec l'ionisation/ozonation de l'air est particulièrement innovante puisque peu d'études utilisent ce procédé. En effet, de nombreuses études portent sur les effets de l'ozone en solution aqueuse. Cependant, la génération d'ozone dans l'environnement aérien industriel tout au long de la transformation alimentaire permettrait de réduire davantage les

contaminations microbiennes grâce à une action directe sur les aliments, mais également au niveau des équipements et outils.

Plusieurs perspectives de recherches peuvent faire suite à ce projet. Peu de travaux ont été menés sur l'effet de l'ionisation/ozonation de l'air sur la croissance bactérienne dans les aliments. Les travaux effectués ont démontré un effet antimicrobien prometteur de l'ionisation/ozonation de l'air et il serait intéressant d'intégrer cette technologie à différentes étapes des procédés de désinfection alimentaire. Ainsi, un pré-traitement d'ozonation/ionisation pourrait éventuellement remplacer les différents produits chimiques actuellement utilisés pour la désinfection des fruits et légumes. Dans cette optique, il serait donc nécessaire de déterminer la durée optimale du pré-traitement mais également la concentration optimale d'ozone à utiliser. Il serait également intéressant de mesurer les capacités antioxydantes de l'enrobage dans le but d'adapter les traitements utilisés en combinaison et de produire les meilleures effets antimicrobiens sans provoquer de dommages aux aliments. Aussi, de nombreux légumes, notamment les légumes pré-coupés, perdent leur couleur suite à une oxydation de leurs composés tels que les composés phénoliques. L'effet antioxydant potentiel de l'enrobage pourrait ainsi être directement observé sur des légumes plus sensibles à de telles modifications. D'autre part, l'étude du comportement de l'enrobage au niveau de la surface des légumes permettrait de mieux comprendre les différentes interactions qui ont lieu lors de l'application, dans le but d'adapter l'enrobage à des fruits et légumes ayant une texture différente.

6 ANNEXE : ANTIMICROBIAL EFFECT AND PHYSICOCHEMICAL PROPERTIES OF BIOACTIVE TRILAYER POLYCAPROLACTONE/METHYLCELLULOSE-BASED FILMS IN THE GROWTH OF FOODBORNE PATHOGENS AND TOTAL MIOCROBIOTA IN FRESH BROCCOLI

Publié dans *Journal of Food Engineering* (2013); 116(3):648-655

Perlette N. Takala[1], Stephane Salmieri[1,2], Afia Boumail[1], Ruhul A. Khan[1], Khanh Dang Vu[1], Gregory Chauve[3], Jean Bouchard[3] and Monique Lacroix[1,2*]

[1] Research Laboratories in Sciences Applied to Food, Canadian Irradiation Center (CIC),

INRS-Institut Armand-Frappier, 531 Boulevard des Prairies, Laval, Quebec H7V 1B7, Canada

[2] The Institute of Nutraceuticals and Functional Foods (INAF), 531 Boulevard des Prairies, Laval, Quebec H7V 1B7, Canada.

[3] FPInnovations, 570 Boulevard Saint-Jean, Pointe-Claire, Quebec H9R 3J9, Canada

Corresponding author : Professor Monique Lacroix; Telephone: +1-450-687-5010; Fax: +1-450-686-5501; E-mail: Monique.Lacroix@iaf.inrs.ca

L'article est présenté tel que publié dans le journal. Le numéro des figures et des tableaux ainsi que la présentation des références ont été modifiés pour suivre un ordre continu au travers de la thèse.

6.1 Résumé en français

Des films bioactifs en trois couches ont été préparés avec de la méthylcellulose (MC) et du polycaprolactone (PCL). Deux formulations antimicrobiennes A (acides organiques, extrait d'acide rosmarinique et huile essentielle (HE) Asiatique) et B (acides organiques, extrait d'acide rosmarinique et HE Italienne) ont été ajoutées dans les films MC et les films en trois couches (PCL/MC/PCL) ont été fabriqué par compression. Ces films ont été insérés dans des emballages contenant des brocolis et conservés à 4°C pendant 12 jours pour déterminer le pouvoir antimicrobien et les propriétés physico-chimiques des films. Les films bioactifs ont montré une réduction significative de *Escherichia coli* à partir du jour 4 et une inhibition totale au jour 12. Ils ont également montré une réduction significative de *Salmonella* Typhimurium à partir du jour 2 et une inhibition totale à partir du jour 7. De plus, les films ont permis un contrôle de la croissance de la microflore aérobique totale dans les brocolis pendant 10 jours. L'encapsulation des composés antimicrobiens dans les films a permis une diminution de l'effet barrière des films. La résistance à la traction n'a pas été affectée par la formulation B. Ces résultats ont démontré l'application potentielle des films antimicrobiens pour le contrôle des pathogènes alimentaires et de la flore dans des légumes pré-coupés.

6.2 **Abstract**

Bioactive trilayer films were prepared using methylcellulose (MC) and polycaprolactone (PCL). Two antimicrobial formulations named as A (organic acids, extract of rosmarinic acid and Asian essential oil (EO) mixture) and B (organic acids, extract of rosmarinic acid and Italian EO mixture) were added in MC films during casting and the trilayer composite films (PCL/MC/PCL) were fabricated using compression molding. These films were inserted into packages containing broccoli and the packages were kept at 4^0C during 12 days storage to determine the antimicrobial capacity of the films and the physico-chemical properties of the films. Bioactive films showed a significant reduction of *Escherichia coli* in broccoli from Day 4 and a total inhibition at Day 12. Similarly, these films showed a significant reduction of *S.* Typhimurium from Day 2 and a total inhibition at Day 7. Moreover, the films controlled the growth of total aerobic microbiota (TAM) in broccoli up to 10 days. Encapsulation of antimicrobial compounds in films allowed decrease of barrier properties of films. Tensile strength of films was not affected by the presence of formulation B. These results demonstrated the potential application of trilayer antimicrobial on controlling food pathogens and total flora in pre-cut vegetables.

Keywords: Bioactive films, Methylcellulose, Polycaprolactone, Compression molding, Composite, Broccoli, Antimicrobial effect, Organic acids, Essential oils

6.3 **Introduction**

Broccoli is a highly perishable product due to microbial contamination that remains a major limitation to its shelf life and quality. Therefore, suitable techniques are necessary to maintain its quality levels until consumption (Aiamla-or *et al.*, 2009). Broccoli is considered a highly nutritional vegetable and contains an important level of vitamins, antioxidants and anti-carcinogenic compounds (Lemoine *et al.*, 2009). Fresh products and vegetables have been identified as a transmission mode of bacteria causing the food-borne diseases mainly in Canada and in the United States. Foodborne diseases have a great cost due to loss in productivity and medical expenses. Indeed, public health experts estimate that there are 11 to 13 million cases of foodborne illness in Canada each year, resulting in 12 to 14 billion $ in healthcare costs (Canadian Food Inspection Agency, 2012). Fresh products are now the second leading cause of food-borne illness (Bialka *et al.*, 2008). Commonly, pathogens contained in fresh products include *Escherichia coli, Salmonella* sp*., Listeria monocytogenes and Bacillus cereus* (Gamage *et al*., 2009*)*. Several techniques have been used to extend the postharvest life and maintain the quality of fresh products including food irradiation (Grolichová *et al.*, 2004), high pressure processing, antimicrobial packaging, essentials oils (EOs) etc. (Marcos *et al.*, 2007). The EOs obtained from plant material has been used for centuries as antimicrobial agents, and now most of the studies are carried out on these natural extracted agents to directly preserve food products or to encapsulate them as bioactive agents in antimicrobial packaging (Helander *et al.*, 1998, Suppakul *et al.*, 2003).

In this decade, words like biocompatibility, environment-friendly, biodegradability are commonly in use. Materials contained in the packaging are preferred to be biodegradable. This finds an explanation due to the increasing demand of consumers for high quality foods and environment concerns on limited natural resources, the use of renewable resources to produce edible or biodegradable packaging materials has gained significant attention. The requirements of biodegradable packaging materials include maintaining product quality, extending shelf life, improving the safety and maintaining the sensorial properties (Quintavalla *et al.*, 2002). The use of preservatives in food packaging film offers several advantages compared to their direct application in the food product. Antimicrobial packaging is very attractive and has increased the attention of the food and packaging industries due to a minimal process and preservative free products (Matan *et al.*, 2006). The incorporation of antimicrobial agents into packaging materials are useful to prevent the growth of microorganisms on the product surface and improve

microbial safety of the final product (Appendini *et al.*, 2002, Devlieghere *et al.*, 2004, Suppakul *et al.*, 2003). Application of active packaging is used mainly to release flavour compounds into the product and to add antimicrobial agents from the packaging system to the food in order to control microbial growth during storage (Matan *et al.*, 2006).

PCL is a semi-crystalline synthetic biodegradable aliphatic polyester that is currently being investigated for use in medical devices, pharmaceutical controlled release systems and biodegradable packaging films for food and nonfood applications (Khan *et al.*, 2010a, Khan *et al.*, 2010b). Films prepared from this thermoplastic polymer demonstrate good mechanical properties and are compatible with numerous polymers to be used in polymer blends (Helander *et al.*, 1998, Salmieri *et al.*, 2006). Moreover, PCL is insoluble in water or water-based systems and therefore offers excellent prevention for patch application to be placed in a humid environment.

Methylcellulose (MC) is a cellulosic ether which possesses good mechanical properties and is currently used in the pharmaceutical and food industry for its remarkable gelling but also emulsifying properties as compared to other food-grade cellulose derivatives (Kadla *et al.*, 2000). In this study, nanocrystalline cellulose (NCC) was also used as a reinforcing agent. NCC is recognized as being more effective than micro-sized counterparts to reinforce polymers due to specific interactions between rod-like nanosized particles. Indeed, when homogeneously dispersed, NCC particles create a percolated network within the matrix, thereby providing mechanical reinforcement polymer matrices (Favier *et al.*, 1995). A previous study has already reported siginificant improvements of the physicochemical properties of MC-based films when mixed to NCC reinforcing fillers, generating films with high mechanical properties comparable to conventional petrochemical-based plastic films (Khan *et al.*, 2010a, Khan *et al.*, 2010b).

The inhibition of microorganisms is made possible with specifically designed release systems which allow the migration of encapsulated bioactive agents into the food surface or in the headspace inside the packaging (Coma, 2008). Such class of controlled release packaging is a new generation of active materials that enhance the quality and safety of food products during extended storage (Sanchez-Garcia *et al.*, 2008).

The objective of this study was to develop a bioactive patch-like film to be inserted into a packaging, which would increase the shelf life of fresh broccoli via a controlled release of active compounds into packaging headspace. Compression molding was used to fabricate PCL/MC/PCL trilayer films in order to form an insoluble bioactive agent containing patch film. Two antimicrobial formulations, termed A and B, containing organic acids and EOs were

encapsulated in the MC internal layer, which was itself sandwiched between two layers of insoluble PCL films using compression molding process, and the resulting trilayer composite films were tested in order to verify their controlled release of active compounds during storage. The antimicrobial activity of these diffusion films was evaluated against total aerobic microbiota (TAM) and food-borne bacteria (*E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*) during the storage of broccoli at 4°C up to day 12.

6.4 Materials and Methods

6.4.1 Processing and conditioning of broccoli

Broccoli was purchased from a local supermarket (IGA, Laval, Quebec, Canada). Broccoli heads were cut into florets which were then packaged in 0.5-mil metallized polyester-2-mil ethylene vinyl acetate copolymer bags (205×355 mm; Winpak Division Ltd, Montreal, Quebec, Canada). The bags had a thickness of 62 µm, oxygen transmission rate at 23°C: 0.7 cc/m^2/24 h; water vapor transmission rate at 38.7°C/90% RH: 1.2 g/m^2/24 h. Hence, these bags corresponded to the packaging containing broccoli and in which the trilayer patch film should be inserted subsequently for antimicrobial treatment. The packaged florets were sterilized by gamma-irradiation at 10 kGy (dose rate of 16.74 kGy/h) at the Canadian Irradiation Center, using a UC-15 A (SS canister) underwater calibrator (Nordion Inc., Kanata, Ontario, Canada) equipped with a ^{60}Co source. The sterilized broccoli florets were then stored at 4°C.

6.4.2 Antimicrobial extracts

The antimicrobial formulations were composed of 3 commercial antimicrobial products: i) organic acids mixture, ii) extract of rosmarinic acid and iii) Asian EO (to prepare formulation A) or Italian EO (to prepare formulation B) respectively. The Asian essential oil mixture was mainly composed of nutmeg, lemongrass and citral and the Italian essential oil mixture contained mainly oregano, pimento berry and lemongrass. In a preliminary experiment on the effects of the formulation A containing ‘’Asian" essential oil and formulation B containing "Italian" essential oil on the sensory properties of broccoli using Hedonic test with 20 panelist who tested the color, odor and taste parameters. The answers were based on a 9-point hedonic scale, 9 being “like very much” and 1 “dislike very much” (Larmond, 1979). For the test, broccoli samples were

coated by these formulations. It was found that coated broccoli have acceptable taste and odor (data not shown). All antimicrobial compounds were kindly provided by their respective manufacturers. The organic acids mixture was obtained from Kerry Ingredients and Flavours (Monterey, Tennessee, USA), rosemary extract (containing 40% w/w rosmarinic acid) was obtained from P.L. Thomas & Co., Inc. (Morristown, New Jersey, USA). The Asian and Italian EOs were provided by BSA Food Ingredients s.e.c./l.p. (Montreal, Quebec, Canada). It is relevant to note that all these antimicrobials contain phenolic and/or terpenic compounds as major components.

6.4.3 **Preparation of biopolymer films containing antimicrobial agents**

6.4.3.1 *Film components*

Methylcellulose (M_n ~ 40,000; viscosity 400 cP, 2% in water at 20°C) and polycaprolactone (M_n ~ 80,000) were purchased from Sigma-Aldrich Ltd (Oakville, Ontario, Canada). Tween®80 and glycerol were purchased from Laboratoire Mat (Beauport, Quebec, Canada), vegetable oil (VO) is sunflower oil which was purchased from a local grocery. NCC was provided by FPInnovations (Pointe-Claire, Quebec, Canada).

6.4.3.2 *Preparation of mixture of NCC and antimicrobial compounds*

A 2% (w/l) NCC aqueous suspension was prepared by dispersing the dried NCC powder under vigorous stirring at room temperature, followed by sonication at room temperature for 30 min using a sonicator bath Branson DHA-1000 (Branson Cleaning Equipment Company, Shelton, CT, USA) at a frequency of 40 kHz. The homogenized NCC suspension was then mixed with antimicrobial formulations in order to obtain a concentration of antimicrobials of 6% (w/v) organic acids mixture, 1.35% rosemary extract and 0.6% Asian EO or Italian EO (to prepare formulation A or B, respectively) in the final film suspension. The mixtures were then homogenized at room temperature using an Ultra-Turrax disperser (T25 model; IKA® Works Inc., Wilmington, NC, USA) at 2 cycles of 12,000 rpm for 1 min and 24,000 rpm for 1 min.

6.4.3.3 *Preparation of MC-based films*

The MC powder was solubilized into a beaker containing warm deionized water (60°C) to obtain a final concentration of 2 % (w/v) and subjected to constant stirring for MC pre-gelatinization. The suspension was placed in an ice bath, under strong stirring, to ensure a complete solubilization. The addition of 1% VO, 0.5% glycerol and 0.025% Tween®80 was performed at room temperature, under strong stirring for 5 min. The bioactive agents were then incorporated into the MC-based formulation and stored at 4°C prior to application. Composite films were then cast by applying 20 mL of the film-forming suspension onto Petri dishes (50 × 9 mm; VWR International, Ville Mont-Royal, QC, Canada) and allowed to dry for 24 h, at 20°C and 35% relative humidity (RH) before peeling. The matrix composition (dry basis) of MC-based films obtained (thickness ~ 25-30 µm) was 46.1 (w/w) MC, 15.4% VO, 23.1% glycerol, 7.7% NCC and 7.7% Tween.

6.4.3.4 *Preparation of PCL films by compression molding*

PCL films (500 mg) were obtained by melting 500 mg of PCL granules in a compression molding machine (Model-3912, Carver Inc., Wabash, IN, USA). Granules of PCL were placed between two stainless steel plates and heated at 80°C for 1 min under a compression stroke of 2 metric tons. The mold was cooled down in an ice bath for 30 s before the PCL single film was removed and stored at room temperature prior to trilayer composites preparation. Film thickness of PCL films was ~ 100-125 µm.

6.4.3.5 *Preparation of trilayer PCL/MC/PCL films*

For the fabrication of the trilayer composite films, one layer of MC film (with and without antimicrobial formulations) was placed between two layers of PCL films of same dimension. The temporary trilayer construction of MC and PCL films was placed between stainless steel plates in the mold of the compression molding machine. The adhesion of the three layers was obtained at 2 metric tons pressure for 3 min at 80°C. After cooling the mold in an ice bath for 30 s, the

trilayer composite films (thickness ~ 280 µm) were then collected and used immediately for testing.

6.4.4 **Preparation of microbial cultures and inoculation process of tested microorganisms on broccoli florets**

6.4.4.1 *Bacterial strains*

E. coli O157:H7 (EDL 933) and *S.* Typhimurium (SL 1344) were obtained from INRS-Institut Armand-Frappier (Laval, Quebec, Canada). *L. monocytogenes* HPB 2812 serovar 1/2a was isolated from homemade salami by the Health Products and Foods Branch of Health Canada (Ottawa, Ontario, Canada). All the bacterial cultures were maintained at -80^{o}C in tryptic soya broth (TSB) (Difco, Becton Dickinson, Mississauga, Ontario, Canada) containing glycerol (10% v/v).

6.4.4.2 *Isolation of total aerobic microbiota (TAM) from broccoli florets*

For isolation of TAM from broccoli, 25 g of non-sterile broccoli and 100 g of peptone water were placed under sterile conditions in a Whirl-pack® sterile bag (Fisher Scientific, Ottawa, Ontario, Canada). The content was homogenized and mixed thoroughly for 2 min at 2,300 rpm with a 400 Circulator Stomacher® (Seward Laboratory Systems Inc., Bohemia, New York, USA). An aliquot of 1 mL of the mixture was inoculated into 9 mL of TSB and incubated for 24 h at 37^{o}C. Thereafter, 1 mL of this solution was inoculated into 100 mL of TSB and incubated for 24 h at 37 ^{o}C. Glycerol was added to obtain a final concentration of 20% (w/w) and aliquots of 1 mL of the solution were placed in Cryovial® tubes that were then frozen at -80°C for future utilizations. Before each experiment, stock cultures (pathogenic bacteria and TAM) were propagated through 2 consecutive 24 h growth cycles in TSB at 35^{o}C and washed twice in saline solution (0.85% w/v) to obtain working cultures containing approximately 10^{9} CFU/mL.

6.4.4.3 *Inoculation procedure of bacteria on broccoli florets*

An inoculation bath was prepared separately for each pathogenic bacteria (*L. monocytogenes, S.* Typhimurium*, E. coli*) or TAM by adding the bacteria or flora to 3 liters of sterile 0.85% NaCl (w/v) solution to reach a population of approximately 10^6 CFU/mL on broccoli florets. The sterilized packaged broccoli florets were opened under sterile conditions and each broccoli floret was dipped into the inoculation bath and stirred gently for 5 min. After inoculation, the broccoli florets were dried on aluminum paper for 30 min before storage treatment with the bioactive trilayer films.

6.4.5 **Evaluation of the antimicrobial activity of trilayer films on preservation of broccoli**

6.4.5.1 *Experimental design*

In case of PCL/MC/PCL-based film formulations, broccoli florets were separated into 4 groups: (i) control (no treatment); (ii) FWA (film without antimicrobial agents); (iii) PCL/MC/PCL + formulation A; (iv) PCL/MC/PCL + formulation B. The bioactive agents containing trilayer patch films were inserted into Winpak bags which contained approximately 25 g of fresh broccoli florets previously inoculated with different types of microorganisms. Then, these bags were sealed under air and stored at 4^oC during 13 days and the samples were collected and analyzed at Days 0, 2, 4, 7, 10 and 12.

6.4.5.2 *Microbiological analysis*

Each broccoli floret samples was weighed (15 g) and homogenized for 2 min at 2,300 rpm in 45 mL of sterile peptone water (0.1% w/v) with a Lab-blender 400 stomacher (Laboratory Equipment, London, UK). From this homogenate, serial dilutions were prepared, plated onto surface of tryptic soy agar (TSA) (Difco, Becton Dickinson, Mississauga, Ontario, Canada) and incubated for 24 h at 37^oC before bacterial enumeration. The minimal level of detection was 10 CFU/g.

6.4.6 **Mechanical properties of films**

Film thickness was measured using a Mitutoyo digimatic Indicator (Type ID-110E; resolution: 1 µm; Mitutoyo MFG Co. Ltd, Tokyo, Japan), at five random positions around the film. Film width was measured using a Traceable® Carbon Fiber Digital Caliper (resolution: 0.1 mm/0.01''; accuracy: ± 0.2 mm/0.01''; Fisher Scientific). Specimen of films conformed to a thickness comprised between 250 and 310 µm, a length of 60 mm and a width of 12.5 mm before testing. Tensile strength (TS) of the trilayer composite films were measured using a Universal Testing Machine (model H5KT; Tinius Olsen Testing Machine Co., Inc., Horsham, PA, USA), equipped with a 100 N-load cell (type FBB) and 1.5 kN-specimen grips. Measurements were carried out following an ASTM D638-99 method (ASTM, 1999). UTM parameters were set up for "plastics tensile from position" test type with the following selections: 25 mm effective gauge length, flat specimen shape, 1 number of entries, minimum type. The position rate of machine control was fixed to 50 mm/min. Y- and X-axes were assigned to load (100 N-range) and position (500 mm-range) coordinates respectively. TS (maximum stress, MPa) values were automatically collected after film break due to elongation, using Test Navigator® program.

6.4.7 **Barrier properties of films**

6.4.7.1 *Water Vapor Permeability (WVP)*

WVP tests were conducted gravimetrically using an ASTM 15.09:E96 procedure (ASTM, 1983). Films were mechanically sealed onto Vapometer cells (model 68-1; Twhing-Albert Instrument Co., West Berlin, NJ, USA) containing 30 g of anhydrous calcium chloride (0% relative humidity, RH). Cells were initially weighed and placed in a Shellab 9010L controlled humidity chamber (Sheldon Manufacturing Inc., Cornelius, OR, USA) maintained at 25°C and 60% RH for 24 h. The amount of water vapor transferred through the film and absorbed by the desiccant (anhydrous $CaCl_2$) was determined from the weight gain of the cell. The assemblies were weighed initially and after 24 h for all samples and up to a maximum of 10% gain. WVP was calculated according to the combined Fick and Henry's laws of gas diffusion through coatings and films, according to the equation

$$WVP\ (g.mm/m^2.day.kPa) = \Delta w.x/A.\Delta P$$

where Δw is the weight gain of the cell (g) after 24 h, x is the film thickness (mm), A is the area of exposed film (31.67 x 10^{-4} m^2), and ΔP is the differential vapor pressure of water through the film (ΔP = 3.282 kPa at 25°C).

6.4.7.2 *Carbon dioxide transmission rate (CO_2TR)*

The CO_2 transmission rate (CO_2TR) of films was measured using Mocon Permatran-C® model 4/41 (Mocon Inc., Minneapolis, MN, USA). All analyses were carried out under atmospheric conditions, temperature of 23°C and 0% relative humidity. Experiments were done in triplicate and samples (area: 5 cm^2; thickness: 280 µm) were purged with nitrogen for a minimum of 2 h, prior to exposure to 100% carbon dioxide flow of 50 cc/min (pressure of 32 psi). Readings were recorded as cc/m^2.day using WinPerm software.

6.4.8 **Statistical analysis**

All experiments were conducted in duplicate and each measurement was performed in triplicate. Analysis of variance was performed using the PASW Statistics Base 18.0 software (SPSS Inc., Chicago, Illinois, USA) and mean's comparison between each treatment was based on Duncan's multiple range tests ($p \leq 0.05$).

6.5 Results and discussion

6.5.1 **Effect of antimicrobial films on the growth of *L. monocytogenes***

The antimicrobial effects of PCL/MC/PCL trilayer bioactive films on the growth of *L. monocytogenes* in broccoli florets up to 12 days of storage at 4°C are presented in **Figure 6.1**. Films containing formulations A or B did not demonstrate any significant efficacy ($p > 0.05$) on the bacterial growth. Indeed, no antimicrobial effect was observed in all groups (control, FWA, films with formulations A or B). Moreover, bacterial concentrations tended to increase from Day 7 with a concentration of more than 6 log CFU/g as compared to 4 log CFU/g at Day 0. These

results suggest that none of the formulation treatment was able to prevent *L. monocytogenes* growth in broccoli during 13 days of storage.

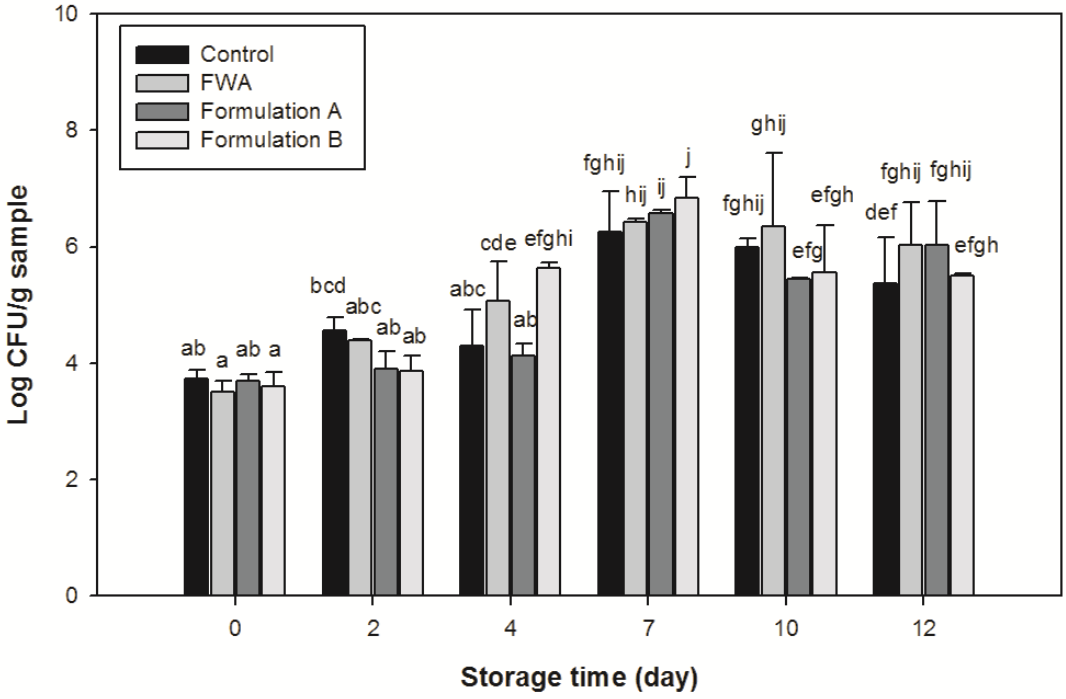


Figure 6.1. Antimicrobial effect of PCL/MC/PCL trilayer patch films on *L. monocytogenes* during storage of broccoli. * Different letters above the error bars of the histogram show the significant differences among treatments (*P* ≤ 0.05)

6.5.2 Effect of antimicrobial films on the growth of *E. coli*

The antimicrobial effects of bioactive films on the growth of *E. coli* in broccoli florets up to 12 days of storage at 4°C are presented in **Figure 6.2**. All samples presented an initial load of bacteria of 3.5 log CFU/g at Day 0. Films containing formulations A or B showed a significant efficacy ($p \leq 0.05$) on the *E. coli* growth during storage, as compared to control and FWA. Indeed, both control- and FWA-treated broccoli samples presented a similar tendency of *E. coli* growth during the whole period of storage with an average bacterial concentration of 2.5-3.5 log CFU/g, from Day 0 to Day 12. In comparison, films containing formulation A allowed decreasing the bacterial content significantly ($p \leq 0.05$) from Day 4 as compared to control and FWA, with a

plateau efficiency (average *E. coli* concentration of 1.5-2 log CFU/g from Day 4 to Day 10, and a total inhibition ($p \leq 0.05$) at Day 12). On the other hand, films containing formulation B were not as efficient as films with formulation A since they generated no significant decrease ($p > 0.05$) of bacterial content as compared to control from Day 4 to Day 10 or from day 7 to day 10 as compared to FWA. However, similarly to bioactive films with formulation A, they allowed a total inhibition ($p \leq 0.05$) of *E. coli* at Day 12.

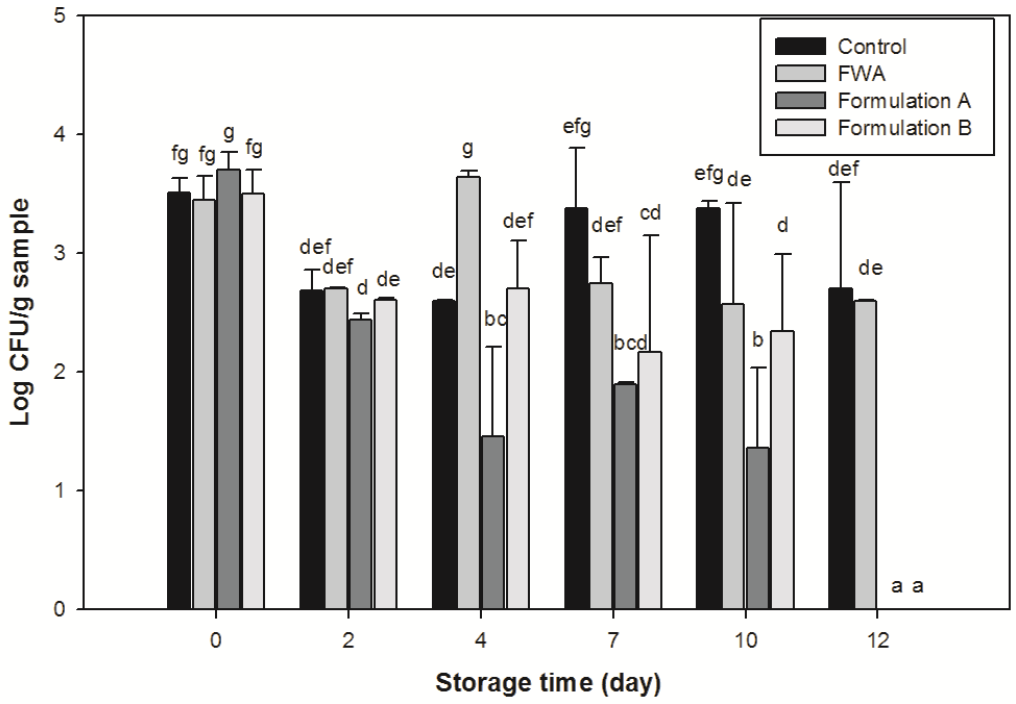


Figure 6.2. **Antimicrobial effect of PCL/MC/PCL trilayer patch films on *E. coli* during storage of broccoli. * Different letters above the error bars of the histogram show the significant differences among treatments ($P \leq 0.05$)**

6.5.3 Effect of antimicrobial films on the growth of *S.* Typhimurium

The antimicrobial effects of bioactive films on the growth of *S.* Typhimurium in broccoli florets up to 12 days of storage at 4°C are presented in **Figure 6.3**. The effect of bioactive films with formulations A or B were the most efficient on *S.* Typhimurium, as compared to other studied pathogenic bacteria. All groups presented an initial load of bacteria of 2.5-3 log CFU/g at Day 0. Films containing formulations A or B showed a significant efficacy ($p \leq 0.05$) on the bacterial

growth during storage, as compared to control and FWA, shortly at Day 2. Indeed, both the control and FWA–treated broccoli samples showed a similar tendency of *S.* Typhimurium growth during the whole period of storage with an average constant bacterial concentration of 2.5 log CFU/g, from Day 0 to Day 12. In comparison, films containing formulations A or B significantly decreased the bacterial content ($p \leq 0.05$) from Day 2 as compared to control and FWA, with a stabilization from Day 2 to Day 4 (average bacterial concentration of 1-1.5 log CFU/g), and a total inhibition ($p \leq 0.05$) of *E. coli* at Day 7. Hence, the bioactive films containing formulation A were as efficient as films B during storage since they generated the same decrease ($p > 0.05$) of bacterial content as compared to control and FWA from Day 2 to Day 12. It is interesting to note that these films led to a total reduction of *S.* Typhimurium much faster than for *E. coli*.

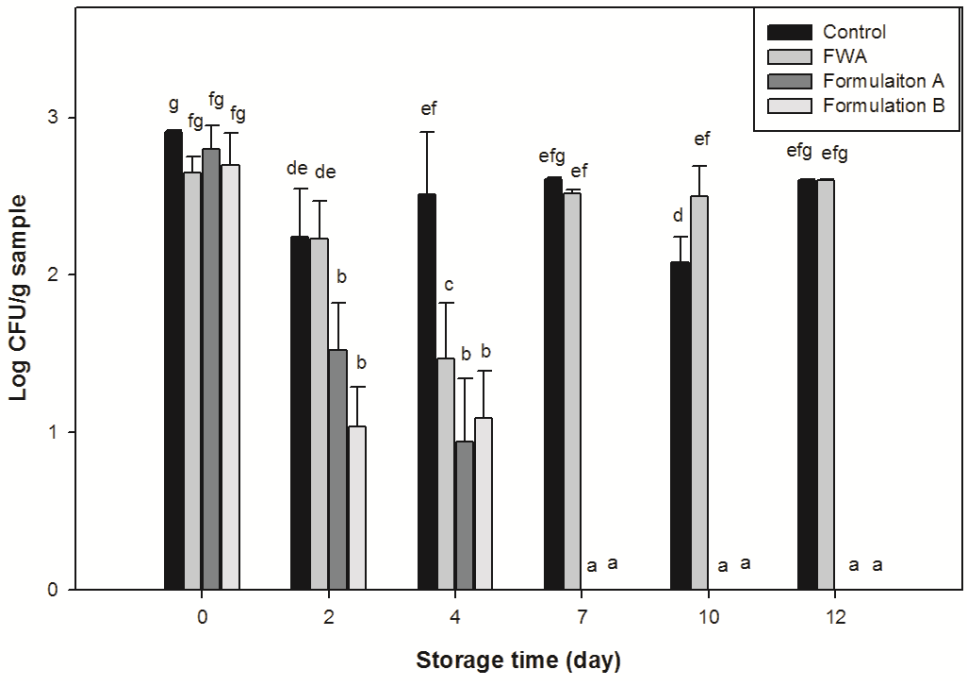


Figure 6.3. Antimicrobial effect of PCL/MC/PCL trilayer patch films on *S.* Typhimurium during storage of broccoli. * Different letters above the error bars of the histogram show the significant differences among treatments ($P \leq 0.05$)

6.5.4 **Effect of antimicrobial films on the growth of TAM**

The antimicrobial effects of bioactive films on the growth of TAM up to 12 days of storage at 4°C are presented in **Figure 6.4**. All groups presented an initial load of microbiota of 3 log CFU/g at Day 0. Films containing either formulations A or B showed no significant efficacy ($p > 0.05$) on the TAM growth during storage, as compared to control and FWA, from Day 0 to Day 2. However, they have allowed reducing the bacterial content significantly ($p \leq 0.05$) from Day 4 as compared to control and FWA, in particular due to a significant increase ($p \leq 0.05$) of TAM concentration in untreated (control) and FWA-treated broccolis, from 2 to 3.5-4 log CFU/g, between Days 2 and 4. Contrary to *E. coli* and *S.* Typhimurium, bioactive films A or B demonstrated a very limited antimicrobial activity, with a maximal efficacy at Day 4. Inversely, from Day 7, the TAM content started increasing or stabilizing for all samples. It was observed that TAM concentration in untreated broccoli increased drastically ($p \leq 0.05$) from 2 to 6 log CFU/g between Days 7 and 12. A similar but less intense tendency was observed in bioactive films containing formulations A or B, with a TAM growth from 3 to 5.5 log CFU/g in the same period whereas the FWA films demonstrated a constant TAM activity of 4 log CFU/g in this period from Day 4 to Day 12.

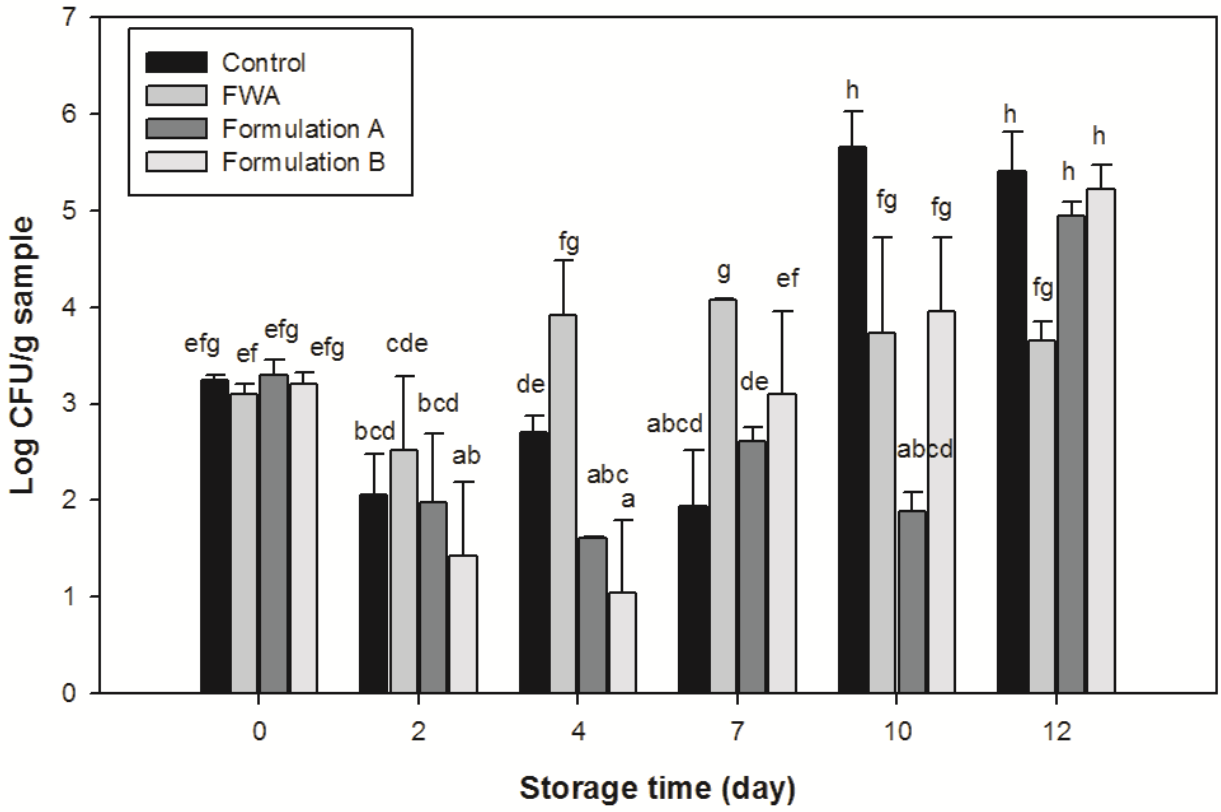


Figure 6.4. Antimicrobial effect of PCL/MC/PCL trilayer patch films on TAM during storage of broccoli. * Different letters above the error bars of the histogram show the significant differences among treatments ($P \leq 0.05$)

From all these results obtained for pathogenic bacteria and TAM, it can be confirmed that films containing both formulations A or B were efficient against *E. coli* and *S.* Typhimurium up to 12 days of storage whereas they had no effect against *L. monocytogenes,* and only a short-time antimicrobial effect (4 days) against TAM. In particular, the antimicrobial capacity of these films allowed a total inhibition of *E. coli* and *S.* Typhimurium after 10 and 7 days of storage, respectively. These data imply that *E. coli* and *S.* Typhimurium are more sensitive than *L. monocytogenes* to the bioactive volatile compounds initially encapsulated in the trilayer films. The bioactive mixture used in this study was essentially composed of organic acids including rosmarinic acid and EOs (Asian EO for formulation-A and Italian EO for -B). Turgis *et al.* (2008) reported a study on the combined effect of EOs, modified atmosphere packaging (MAP), and gamma radiation on the microbial growth on the ground beef. They demonstrated that Gram

positive bacteria were more sensitive against EOs than Gram negative bacteria. Burt (2004) reviewed different studies and concluded that EOs are slightly more active against Gram positive microorganisms, explaining that Gram negative are less susceptible to the action of antibacterial since they possess an outer membrane surrounding the cell wall which restrict the diffusion of hydrophobic compounds, such as EOs, through its liposaccharidic covering. However, our results are opposed to these statements and indicate that the bioactive films had a higher efficiency against Gram negative bacteria (*E. coli* and *S.* Typhimurium) and no effect on *L. monocytogenes* which is a Gram-positive pathogen. Therefore, it can be hypothesized that the action of the bioactive compound is not a singular but a synergetic action of several antimicrobial compounds, especially for indirect action of volatile substances as compared to their diffusion by contact with the food system.

Indeed, in the present study, the bioactive mixtures did not act primarily directly on the bacterial membrane of bacteria since they were diffused from a film that was not applied directly onto the food surface. The results also demonstrated that the bioactive patch film had a limited antimicrobial effect on the growth of TAM during 4 days of storage, followed by a progressive increase in the food matrix up to 13 days. Phillips (1996) reviewed different studies related to MAP effects on the microbiological quality and safety of foods and mentioned that the variation of gas composition had an influence on the bacterial growth. Post-harvest fruits and vegetables possess a metabolic activity due to the endogenous activity such as respiration. He also mentioned that the respiration in anaerobiosis is quickly maintained if the product is sealed and this anaerobic respiration is initiated at very low oxygen concentrations, resulting in the deterioration of organoleptic properties and the growth of bacteria.

It is also interesting to note the increase of the population of bacteria in the FWA group during the storage, suggesting that the films alone (without bioactive agents) had no influence on the bacterial and TAM growth. Ayala-Zavala *et al.* (2008) demonstrated that the water activity (Aw) is directly related to the equilibrium of relative humidity atmosphere that is generated by a food product. Hence, food respiration leads to an increase of Aw that may influence the proliferation of the bacteria population.

6.5.5 **Mechanical properties of films**

TS values of trilayer films in function of storage time are presented in **Table 6.1**. TS is the ultimate strength (or maximum stress) of a material subjected to tensile loading. Results show that all types of films possessed similar resistance ($p > 0.05$) at Day 0, with TS values comprised between 20 and 24 MPa. The TS of FWA did not vary over time ($p > 0.05$), with values ranging from 20.3 to 18.7 MPa between Days 0 and day 12. On the other hand, the TS of antimicrobial films (containing formulations A and B) was reduced significantly ($p \leq 0.05$) during storage. Indeed, the TS values decreased from 23.7 to 7.8 MPa for films containing formulation A (reduction of 67%) and from 24.0 to 17.3 MPa for those containing formulation B (reduction of 28%) after 14 days of storage. However, at day 12, results show no significant difference ($p > 0.05$) between FWA and films containing formulation B with similar TS of 17.3-18.7 MPa whereas the TS of films containing formulation A was significantly lower ($p \leq 0.05$) with a TS value of 7.8 MPa. Hence, these observations imply that films with formulation A were significantly less resistant to tensile stress than FWA and those with formulation B ($p \leq 0.05$) over storage. These behaviours could be explained by the chemical nature of antimicrobials that interacted differently according to the type of antimicrobial formulation. Moreover, it is well-known that the incorporation of EOs into a continuous polymeric matrix tends to decrease its mechanical resistance to fracture because of the structural discontinuities caused by the oil-dispersed phase (Salmieri *et al.*, 2006, Sanchez-Gonzalez *et al.*, 2011).

Table 6.1. Effect of antimicrobial formulation on the tensile strength (TS) of trilayer patch films during storage.

Films	Tensile strength (MPa)[1, 2]		
	Day 0	**Day 6**	**Day 12**
FWA	20.3 ± 4.9 [a,A]	19.1 ± 2.7 [a,A]	18.7 ± 3.7 [b,A]
Formulation A	23.7 ± 2.7 [a,C]	18.0 ± 2.7 [a,B]	7.8 ± 0.7 [a,A]
Formulation B	24.0 ± 3.4 [a,B]	20.4 ± 1.4 [a,A]	17.3 ± 2.0 [b,A]

[1]Means followed by the same lowercase letter in each column are not significantly different at the 5% level. [2]Means followed by the same uppercase letter in each row are not significantly different at the 5% level.

6.5.6 Barrier properties of films

WVP and CO_2TR measurements of trilayer films at Day 0 are presented in **Table 6.2**.

Table 6.2. Effect of antimicrobial formulation on the water vapor permeability (WVP) and carbon dioxide transmission rate (CO_2TR) of trilayer patch films at Day 0.

Films	WVP[1] (g.mm/m².day.kPa)	CO_2TR[1] (cc/m².day)
FWA	3.93 ± 0.43 [b]	118.91 ± 2.47 [b]
Formulation A	3.34 ± 0.34 [a]	0.011 ± 0.007 [a]
Formulation B	3.09 ± 0.22 [a]	0.021 ± 0.009 [a]

[1]Means followed by the same letter in each column are not significantly different at the 5% level

6.5.6.1 *WVP measurements*

The WVP of FWA was significantly higher ($p \leq 0.05$) than those of films containing formulation-A and -B, with a value of 3.93 g.mm/m².day.kPa for FWA as compared to 3.34 and 3.09 g.mm/m².day.kPa for films with formulation-A and -B respectively. Hence, it can be observed that the addition of antimicrobial formulations (containing hydrophobic compounds) allowed reducing the WVP of films. Similar observations were reported by Pelissari *et al.* (2009) and Zivanovic *et al.* (2005). These results also show that the utilization of PCL as external layers of films provided high barrier properties since preliminary measurements of MC internal layer resulted in high WVP values of 5.9 g.mm/m².day.kPa (Khan *et al.*, 2010a, Khan *et al.*, 2010b). Therefore, the formation of composite trilayer films allowed improving barrier properties by 1.5-fold, which is considerable for food application and storage conditions at high RH levels.

6.5.6.2 *CO_2TR measurements*

CO_2TR values were obtained when curves reached an equilibrium after 34, 60 and 64 h for FWA, films with formulation-A and -B respectively. As observed for WVP measurements, it was noticed that the addition of antimicrobial formulations significantly decreased ($p \leq 0.05$) the CO_2TR of trilayers. Indeed, a value of 118.9 cc/m².day was determined for FWA as compared to 0.01 and 0.02 cc/m².day for films with formulation-A and -B respectively, with no significant difference ($p > 0.05$) between both antimicrobial films. Some studies (Ayranci *et al.*, 2001, Donhower *et al.*, 1993, Salleh *et al.*, 2009) demonstrated that gas permeation greatly depends on the diffusion process of the gas through the polymer. The presence of nanocomposites and bioactive compounds interacting with film matrix induces changes in molecular orientation and crystallinity in MC layer, therefore promoting significant decrease of CO_2 transmission through the film. As a result, the diffusive path is made more difficult for permeant gas (Miller, K.S. *et al.*, 1997). Hence, as a corroboration of analysis of mechanical properties with these reported studies, it can be hypothesized that CO_2 barrier was enhanced after addition of bioactive compounds in relation with changes of crystallinity degree and their dispersion/orientation in the nanomorphological structure of internal MC layer of the films.

6.6 Conclusions

The trilayer PCL/MC/PCL bioactive patch films had a strong antimicrobial efficiency against Gram negative bacteria such as *E. coli* and *S.* Typhimurium, with a total inhibition of *E. coli* at Day 12 and *S.* Typhimurium at Day 7. These films containing the formulation A had a stronger effect against *E. coli* (with significant antimicrobial capacity from Day 4) whereas both formulations A or B were efficient against *S.* Typhimurium (with significant antimicrobial capacity from Day 2). Moreover, both films had a good capacity to prevent the growth of TAM in broccoli, for up to 10 days of storage. However, the films had no antimicrobial effect against *L. monocytogenes* during storage, hence demonstrating that the diffusion of volatiles may be supported by a different mechanism of action of antimicrobial volatile compounds, as opposed to the direct diffusion of antimicrobials in contact with foods. Addition of antimicrobials decreased the tensile resistance of the films, but films containing formulation B were determined as more resistant than those with formulation A. Moreover, the encapsulation of antimicrobials in films greatly enhanced their gas barrier properties (water vapor and CO_2).

Consequently, this technology of bioactive films based on a controlled release of volatiles phenolic and terpenic compounds in food packages offers the possibility to control the growth of pathogens in food by using a simple and efficient method that ensures food safety and allows extending the shelf life of products. Another advantage of this technology is the selectivity of bioactive compounds that could also provide enhanced sensorial qualities of the product such as flavour and taste in addition to their antimicrobial capacity.

6.7 Acknowledgements

Le ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) is particularly acknowledged for financial support. FPInnovations is acknowledged for providing nanocrystalline cellulose. Authors are grateful to Nordion Inc. for irradiation procedures and to Winpak Division Ltd for supplying the packaging material used in sterilization process and microbiological analyses. The authors are also thankful to P.L. Thomas & Co. Inc., Kerry Ingredients and Flavours and BSA Food Ingredients s.e.c./l.p. to have graciously provided natural antimicrobials. Finally, authors sincerely thank Simon Duchesne and Allison Heudebourg for their technical contribution.

7 RÉFÉRENCES

Agence de Santé Publique du Canada (2012) *Listéria.*), http://www.phac-aspc.gc.ca/fs-sa/fs-fi/listerios-fra.php (Consulté le 09-09-2015)

Agence de Santé Publique du Canada (2015) *E. coli.*), http://www.phac-aspc.gc.ca/fs-sa/fs-fi/ecoli-fra.php (Consulté le 09-09-2015)

Aiamla-or S., Yamauchi N., Takino S. & Shigyo M. (2009) Effect of UV-A and UV-B irradiation on broccoli (Brassica oleracea L. Italica Group) floret yellowing during storage. *Postharvest Biology and Technology* 54(3):177-179.

Akbas M.Y. & Ozdemir M. (2008) Application of gaseous ozone to control populations of Escherichia coli, Bacillus cereus and Bacillus cereus spores in dried figs. *Food Microbiology* 25(2):386-391.

Alencar E.R.d., Faroni L.R.D.A., Pinto M.d.S., Costa A.R.d. & Silva T.A.d. (2013) Postharvest quality of ozonized "nanicão" cv. bananas. *Revista Ciência Agronômica* 44:107-114.

Alvaro J.E., Moreno S., Dianez F., Santos M., Carrasco G. & Urrestarazu M. (2009) Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *Journal of Food Engineering* 95(1):11-15.

Appendini P. & Hotchkiss J.H. (2002) Review of antimicrobial food packaging. *Innovative Food Science & Emerging Technologies* 3(2):113-126.

Artés F., Gómez P., Aguayo E., Escalona V. & Artés-Hernández F. (2009) Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology* 51(3):287-296.

Artés F., Gomez P., Artés-Hernández F., Aguayo E. & Escalona V. (2007a) Improved strategies for keeping overall quality of fresh-cut produce. in *Acta Horticulturae*), p 245-258.

Artés F., Gomez P.A. & Artés-Hernandez F. (2007b) Physical, physiological and microbial deterioration of minimally fresh processed fruits and vegetables. *Food Science and Technology International* 13(3):177-188.

ASTM (1983) Standard Test Method for Water Vapor Transmission of Materials. *American Society for Testing and Materials*:Method 15.09:E96.

ASTM (1999) Standard Test Method for Tensile Strength of Plastics. *Annual Book of ASTM Standards, ASTM International*:Method D 638-699.

Ayala-Zavala J.F., Del-Toro-Sánchez L., Alvarez-Parrilla E. & González-Aguilar G.A. (2008) High Relative Humidity In-Package of Fresh-Cut Fruits and Vegetables: Advantage or Disadvantage Considering Microbiological Problems and Antimicrobial Delivering Systems? *Journal of Food Science* 73(4):R41-R47.

Ayari S., Dussault D., Jerbi T., Hamdi M. & Lacroix M. (2012) Radiosensitization of Bacillus cereus spores in minced meat treated with cinnamaldehyde. *Radiation Physics and Chemistry* 81(8):1173-1176.

Ayranci E. & Tunc T. (2001) The effect of fatty acid content on water vapour and carbon dioxide transmissions of cellulose-based edible films. *Food Chemistry* 72:231-236.

Azarakhsh N., Osman A., Ghazali H.M., Tan C.P. & Mohd Adzahan N. (2014) Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple. *Postharvest Biology and Technology* 88(0):1-7.

Azeredo H.M.C., Mattoso L.H.C., Avena-Bustillos R.J., Filho G.C., Munford M.L., Wood D. & McHugh T.H. (2010) Nanocellulose Reinforced Chitosan Composite Films as Affected by Nanofiller Loading and Plasticizer Content. *Journal of Food Science* 75(1):N1-N7.

Azeredo H.M.C., Mattoso L.H.C., Wood D., Williams T.G., Avena-Bustillos R.J. & McHugh T.H. (2009) Nanocomposite Edible Films from Mango Puree Reinforced with Cellulose Nanofibers. *Journal of Food Science* 74(5):N31-N35.

Bae Y.-M. & Lee S.-Y. (2012) Inhibitory Effects of UV Treatment and a Combination of UV and Dry Heat against Pathogens on Stainless Steel and Polypropylene Surfaces. *Journal of Food Science* 77(1):M61-M64.

Bagamboula C.F., Uyttendaele M. & Debevere J. (2004) Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards Shigella sonnei and S. flexneri. *Food Microbiology* 21(1):33-42.

Baldwin E.A. (2007) Surface treatments and edible coatings in food preservation. *Handbook of Food Preservation, Second Edition* 21:477-507.

Bari M.L., Todoriki S., Sommers C., Hayakawa F. & Kawamoto S. (2006) Irradiation inactivation of *Listeria monocytogenes* in low-fat ground pork at freezing and refrigeration temperatures. *Journal of Food Protection* 69(12):2955-2960.

Barth M., Hankinson T.R., Zhuang H. & Breidt F. (2010) Microbiological spoilage of fruits and vegetables. *Compendium of the Microbiological Spoilage of Foods and Beverages,* Springer. p 135-183.

Beauchamp S. & Lacroix M. (2012) Resistance of the genome of *Escherichia coli* and *Listeria monocytogenes* to irradiation evaluated by the induction of cyclobutane pyrimidine dimers and 6-4 photoproducts using gamma and UV-C radiations. *Radiation Physics and Chemistry* 81(8):1193-1197.

Berger C.N., Sodha S.V., Shaw R.K., Griffin P.M., Pink D., Hand P. & Frankel G. (2010) Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology* 12(9):2385-2397.

Beuchat L.R. (1992) Surface disinfection of raw produce. *Dairy, Food and Environmental Sanitation* 12(1):6-9.

Bhande S.D., Ravindra M.R. & Goswami T.K. (2008) Respiration rate of banana fruit under aerobic conditions at different storage temperatures. *Journal of Food Engineering* 87(1):116-123.

Bialka K.L. & Demirci A. (2008) Efficacy of Pulsed UV-Light for the Decontamination of Escherichia coli O157:H7 and Salmonella spp. on Raspberries and Strawberries. *Journal of Food Science* 73(5):M201-M207.

Bilia A.R., Guccione C., Isacchi B., Righeschi C., Firenzuoli F. & Bergonzi M.C. (2014) Essential Oils Loaded in Nanosystems: A Developing Strategy for a Successful Therapeutic Approach. *Evidence-Based Complementary and Alternative Medicine* 2014:14.

Bjergager M.B.A., Hanson M.L., Solomon K.R. & Cedergreen N. (2012) Synergy between prochloraz and esfenvalerate in Daphnia magna from acute and subchronic exposures in the laboratory and microcosms. *Aquatic Toxicology* 110-111:17-24.

Boumail A., Salmieri S., Klimas E., Tawema P., Bouchard J. & Lacroix M. (2013) Characterization of Trilayer Antimicrobial Diffusion Films (ADFs) Based on Methylcellulose-Polycaprolactone Composites. *Journal of Agricultural and Food Chemistry* 61(4):811-821.

Boumail A., Salmieri S., St-Yves F., Lauzon M. & Lacroix M. (2016) Effect of antimicrobial coatings on microbiological, sensorial and physico-chemical properties of pre-cut cauliflowers. *Postharvest Biology and Technology* 116:1-7.

Boye E. & Krisch R.E. (1980) Induction and repair of double- and single-Strand DNA breaks in bacteriophage lambda superinfecting escherichia coli. *International Journal of Radiation Biology* 37(2):119-133.

Buchner S., Kinnear M., Crouch I.J., Taylor J. & Minnaar A. (2011) Effect of kafirin protein coating on sensory quality and shelf-life of 'Packham's Triumph' pears during ripening. *Journal of the Science of Food and Agriculture* 91(15):2814-2820.

Burt S. (2004) Essential oils : their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology* 94:223-253.

Caillet S., Millette M., Salmiéri S. & Lacroix M. (2006a) Combined effects of antimicrobial coating, modified atmosphere packaging, and gamma irradiation on *Listeria innocua* present in ready-to-use carrots (Daucus carota). *Journal of Food Protection* 69(1):80-85.

Caillet S., Salmiéri S. & Lacroix M. (2006b) Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. *Food Chemistry* 95(1):1-8.

Caillet S., Ursachi L., Shareck F. & Lacroix M. (2009) Effect of Gamma Radiation and Oregano Essential Oil on Murein and ATP Concentration ofStaphylococcus aureus. *Journal of Food Science* 74(9):M499-M508.

Calo J.R., Crandall P.G., O'Bryan C.A. & Ricke S.C. (2015) Essential oils as antimicrobials in food systems – A review. *Food Control* 54(0):111-119.

Canadian Food Inspection Agency (2012) *Causes of food poisoning.*), http://www.inspection.gc.ca/english/fssa/concen/causee.shtml

Castro-Ibáñez I., López-Gálvez F., Gil M.I. & Allende A. (2016) Identification of sampling points suitable for the detection of microbial contamination in fresh-cut processing lines. *Food Control* 59:841-848.

César J., Sumita T.C., Junqueira J.C., Jorge A.O.C. & do Rego M.A. (2012) Antimicrobial effects of ozonated water on the sanitization of dental instruments contaminated with E. coli, S. aureus, C. albicans, or the spores of B. atrophaeus. *Journal of Infection and Public Health* 5(4):269-274.

Cháfer M., Sánchez-González L., González-Martínez C. & Chiralt A. (2012) Fungal Decay and Shelf Life of Oranges Coated With Chitosan and Bergamot, Thyme, and Tea Tree Essential Oils. *Journal of Food Science* 77(8):E182-E187.

Challenger O., Braven J., Harwood D., Rosen K. & Richardson G. (1996) Indoor Air Quality, Part 1: Negative Air Ionisation and the Generation of Hydrogen Peroxide. *Science of the Total Environment* 177:215-219.

Chiasson F., Borsa J. & Lacroix M. (2005) Combined effect of carvacrol and packaging conditions on radiosensitivity of Escherichia coli and Salmonella typhi in ground beef. *Journal of Food Protection* 68(12):2567-2570.

Chiasson F., Borsa J., Ouattara B. & Lacroix M. (2004) Radiosensitization of *Escherichia coli* and *Salmonella* Typhi in ground beef. *Journal of Food Protection* 67(6):1157-1162.

Chisari M., Todaro A., Barbagallo R.N. & Spagna G. (2010) Salinity effects on enzymatic browning and antioxidant capacity of fresh-cut baby Romaine lettuce (Lactuca sativa L. cv. Duende). *Food Chemistry* 119(4):1502-1506.

Choi H.-S., Song H.S., Ukeda H. & Sawamura M. (2000) Radical-Scavenging Activities of Citrus Essential Oils and Their Components: Detection Using 1,1-Diphenyl-2-picrylhydrazyl. *Journal of Agricultural and Food Chemistry* 48(9):4156-4161.

Chronakis I.S. (1998) On the Molecular Characteristics, Compositional Properties, and Structural-Functional Mechanisms of Maltodextrins: A Review. *Critical Reviews in Food Science and Nutrition* 38(7):599-637.

Chun H., Kim J., Chung K., Won M. & Song K.B. (2009) Inactivation kinetics of Listeria monocytogenes, Salmonella enterica serovar Typhimurium, and Campylobacter jejuni in ready-to-eat sliced ham using UV-C irradiation. *Meat Science* 83(4):599-603.

Code of Federal Regulations (2012) *Title 21, Part 173.315. Secondary Direct Food Additives Permitted in Food for Human Consumption: Chemicals used in washing or to assist in the peeling of fruits and vegetables.*), http://www.ecfr.gov/cgi-bin/text-idx?SID=8f506873afd4ab469240d039a958149e&mc=true&node=pt21.3.173&rgn=div5#se21.3.173_1315 (Consulté le 08-09-2015)

Coll Cárdenas F., Andrés S., Giannuzzi L. & Zaritzky N. (2011) Antimicrobial action and effects on beef quality attributes of a gaseous ozone treatment at refrigeration temperatures. *Food Control* 22(8):1442-1447.

Coma V. (2008) Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science* 78(1-2):90-103.

Coma V., Martial-Gros A., Garreau S., Copinet A., Salin F. & Deschamps A. (2002) Edible Antimicrobial Films Based on Chitosan Matrix. *Journal of Food Science* 67(3):1162-1169.

Conner D.E. (1993) Naturally occurring compounds. *Antimicrobials in Foods*, Davidson P & Branen ALE (Édit.) Dekker, New York, NY. p 441-468.

Conner D.E. & Beuchat L.R. (1984) Effects of essential oils from plants on growth of food spoilage yeasts. *Journal of Food Science* 49:429-434.

Cosentino S., Tuberoso C.I.G., Pisano B., Satta M., Mascia V., Arzedi E. & Palmas F. (1999) In-vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. *Letters in Applied Microbiology* 29(2):130-135.

Costa M.L., Civello P.M., Chaves A.R. & Meartinez G.A. (2005) Effet of ethephon and 6-benzylaminopurine on chlorophyll degrading enzymes and a peroxidase-linked chlorophyll bleaching during post-harvest senescence of broccoli (*Brassica oleracea* L.) at 20°C. *Postharvest Biology and Technology* 35:191-199.

Croxen M.A., Law R.J., Scholz R., Keeney K.M., Wlodarska M. & Finlay B.B. (2013) Recent advances in understanding enteric pathogenic Escherichia coli. *Clinical Microbiology Reviews* 26(4):822-880.

Dai X., Luo H., Jiang L., Ling L., Xue Y. & Yu Z. (2012) Efficacy of Different Sanitizing Agents and Their Combination on Microbe Population and Quality of Fresh-cut Chinese Chives. *Journal of Food Science* 77(7):M348-M353.

Das D.K., Dutta H. & Mahanta C.L. (2013) Development of a rice starch-based coating with antioxidant and microbe-barrier properties and study of its effect on tomatoes stored at room temperature. *LWT - Food Science and Technology* 50(1):272-278.

Davidson P.M. & Taylor T.M. (2007) Chemical Preservatives and Natural Antimicrobial Compounds. *Food Microbiology: Fundamentals and Frontiers, Third Edition,* American Society of Microbiology, doi:10.1128/9781555815912.ch33.

Davis R. & Mauer L.J. (2010) Fourier tansform infrared (FT-IR) spectroscopy: A rapid tool for detection and analysis of foodborne pathogenic bacteria. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, Mendez-Vilas A (Édit.) Formatex Research Center, Spain Vol 2. p 1582-1594.

Degl'Innocenti E., Guidi L., Pardossi A. & Tognoni F. (2005) Biochemical Study of Leaf Browning in Minimally Processed Leaves of Lettuce (Lactuca sativa L. Var. Acephala). *Journal of Agricultural and Food Chemistry* 53(26):9980-9984.

Dehkharghanian M., Salmieri S., Lacroix M. & Vijayalakshmi M., A. (2009) Antioxidant properties of green tea polyphenols encapsulated in caseinate beads. *Dairy Science & Technology* 89(6):627-485.

Del Toro-Sánchez C.L., Ayala-Zavala J.F., Machi L., Santacruz H., Villegas-Ochoa M.A., Alvarez-Parrilla E. & González-Aguilar G.A. (2010) Controlled release of antifungal volatiles of thyme essential oil from β-cyclodextrin capsules. *J Incl Phenom Macrocycl Chem* 67(3-4):431-441.

Devlieghere F., Vermeiren L. & Debevere J. (2004) New preservation technologies: possibilities and limitations. *International Dairy Journal* 14:273-285.

Digel I., Artmann A.T., Nishikawa K., Cook M., Kurulgan E. & Artmann G.M. (2005) Bactericidal effects of plasma-generated cluster ions. *Med. Biol. Eng. Comput.* 43(6):800-807.

Dima C. & Dima S. (2015) Essential oils in foods: extraction, stabilization, and toxicity. *Current Opinion in Food Science* 5:29-35.

Donhower I.G. & Fennema O. (1993) The effects of plasticizers on crystallinity, permeability and mechanical properties of methylcellulose films. *Journal of Food Processing and Preservation* 17:247-257.

Dussault D., Vu K.D. & Lacroix M. (2014) In vitro evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. *Meat Science* 96(1):514-520.

Erickson M.C. (2010) Microbial Risks Associated with Cabbage, Carrots, Celery, Onions, and Deli Salads Made with These Produce Items. *Comprehensive Reviews in Food Science and Food Safety* 9(6):602-619.

Estrela C., Estrela C.R.A., Barbin E.L., Spanó J.C.E., Marchesan M.A. & Pécora J.D. (2002) Mechanism of action of sodium hypochlorite. *Brazilian Dental Journal* 13:113-117.

European Food Information Council (2012) *Consommation de fruits et légumes en Europe - Les Européens en mangent-ils assez ?*), http://www.eufic.org/article/fr/expid/Consommation-fruits-legumes-Europe/ (Consulté le 15-09-2015)

Falguera V., Pagán J., Garza S., Garvín A. & Ibarz A. (2011) Ultraviolet processing of liquid food: A review. Part 1: Fundamental engineering aspects. *Food Research International* 44(6):1571-1579.

Falguera V., Pagán J., Garza S., Garvín A. & Ibarz A. (2012) Inactivation of polyphenol oxidase by ultraviolet irradiation: Protective effect of melanins. *Journal of Food Engineering* 110(2):305-309.

Falguera V., Pagán J. & Ibarz A. (2010) A kinetic model describing melanin formation by means of mushroom tyrosinase. *Food Research International* 43(1):66-69.

Fan L., Song J., Hildebrand P.D. & Forney C.F. (2002) Interaction of ozone and negative air ions to control micro-organisms. *Journal of Applied Microbiology* 93(1):144-148.

Favier V., Chanzy H. & Cavaillé J.Y. (1995) Polymer nanocomposites reinforced by cellulose whiskers. *Macromolecules* 28:6365-6367.

Fernandes R.V.d.B., Borges S.V. & Botrel D.A. (2014) Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydrate Polymers* 101(0):524-532.

Filho G.R., Assunçao R.M.N., Vieira J.G., Meireles C.S., Cerquiera D.A., Barud H.S., Ribeiro S.J.L. & Messaddeq Y. (2007) Characterization of methylcellulose produced from sugar cane bagasse cellulose: crystallinity and thermal properties. *Polymer Degradation and Stability* 92:205-210.

Francis G.A., Thomas C. & O'Beirne D. (1999) The microbiological safety of minimally processed vegetables. *International Journal of Food Science & Technology* 34(1):1-22.

Frankenberg D., Frankenberg-Schwager M., Bloecher D. & Harbich R. (1981) Evidence for DNA double-strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic or anoxic conditions. *Radiation Research* 88(3):524-532.

Fratini F., Casella S., Leonardi M., Pisseri F., Ebani V.V., Pistelli L. & Pistelli L. (2014) Antibacterial activity of essential oils, their blends and mixtures of their main constituents against some strains supporting livestock mastitis. *Fitoterapia* 96:1-7.

Galus S., Mathieu H., Lenart A. & Debeaufort F. (2012) Effect of modified starch or maltodextrin incorporation on the barrier and mechanical properties, moisture sensitivity and appearance of soy protein isolate-based edible films. *Innovative Food Science & Emerging Technologies* 16(0):148-154.

Gamage G.R., Park H.-J. & Kim K.M. (2009) Effectiveness of antimicrobial coated oriented polypropylene/polyethylene films in sprout packaging. *Food Research International* 42(7):832-839.

Garcia-Ochoa F., Santos V.E., Casas J.A. & Gomez E. (2000) Xanthan gum: production, recovery, and properties. *Biotechnology Advances* 18:549-579.

Garner D. & Kathariou S. (2016) Fresh Produce–Associated Listeriosis Outbreaks, Sources of Concern, Teachable Moments, and Insights. *Journal of Food Protection* 79(2):337-344.

Ge C., Bohrerova Z. & Lee J. (2013) Inactivation of internalized Salmonella Typhimurium in lettuce and green onion using ultraviolet C irradiation and chemical sanitizers. *Journal of Applied Microbiology* 114(5):1415-1424.

Gil M.I., Selma M.V., López-Gálvez F. & Allende A. (2009) Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *International Journal of Food Microbiology* 134(1–2):37-45.

Goldberg R., Lê T. & Catesson A.-M. (1985) Localization and properties of cell wall enzyme activities related to the final stages of lignin biosynthesis. *Journal of Experimental Botany* 36:503-510.

Gonzalez R.J., Luo Y., Ruiz-Cruz S. & McEvoy J.L. (2004) Efficacy of sanitizers to inactivate Escherichia coli O157:H7 on fresh-cut carrot shreds under simulated process water conditions. *Journal of Food Protection* 67(11):2375-2380.

Goupil D. (1996) Sutures. *Biomaterials science*, Ratner BD, Hoffman AS, Schoen FJ & Lemons JE (Édit.) Academic Press, New York Vol 356-360.

Goy R.C., De Britto D. & Assis O.B.G. (2009) A review of the antimicrobial activity of chitosan. *Polimeros* 19(3):241-247.

Grace S.C. (2007) Plant Bioenergetics. *Handbook of Plant Science*, Keith Roberts (Édit.) Vol 2. p 825-831.

Grolichová M., Dvořák P. & Musilová H. (2004) Employing ionizing radiation to enhance food safety - A review. *Acta Veterinaria Brno* 73(1):143-149.

Guerreiro A.C., Gago C.M.L., Faleiro M.L., Miguel M.G.C. & Antunes M.D.C. (2015) The use of polysaccharide-based edible coatings enriched with essential oils to improve shelf-life of strawberries. *Postharvest Biology and Technology* 110:51-60.

Gülçin İ. (2012) Antioxidant activity of food constituents: an overview. *Arch Toxicol* 86(3):345-391.

Han J., Guenier A.-S., Salmieri S. & Lacroix M. (2008) Alginate and Chitosan Functionalization for Micronutrient Encapsulation. *Journal of Agricultural and Food Chemistry* 56:2525-2535.

Helander I.M., Alakomi H.L., Latva-Kala K., Mattila-Sandholm T., Pol I., Smid E.J., Gorris L.G.M. & Von Wright A. (1998) Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *Journal of Agricultural and Food Chemistry* 46(9):3590-3595.

Hill S.A. (2007) Respiration. *Handbook of Plant Science*, Keith Roberts (Édit.) Vol 2. p 880-886.

Hodges D.M. & Toivonen P.M.A. (2008) Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Biology and Technology* 48(2):155-162.

Hollósy F. (2002) Effects of ultraviolet radiation on plant cells. *Micron* 33(2):179-197.

Holt P.S., Mitchell B.W., Seo K.H. & Gast G.K. (1999) Use of Negative Air Ionization for Reducing Airborne Levels of *Salmonella* enterica serovar enteritidis in a Room Containing Infected Caged Layers. *The Journal of Applied Poultry Research* 8(4):440-446.

Hong S.-I. & Kim D.-M. (2001) Influence of oxygen concentration and temperature on respiratory characteristics of fresh-cut green onion. *International Journal of Food Science & Technology* 36(3):283-289.

Hussain P.R., Omeera A., Suradkar P.P. & Dar M.A. (2014) Effect of combination treatment of gamma irradiation and ascorbic acid on physicochemical and microbial quality of minimally processed eggplant (Solanum melongena L.). *Radiation Physics and Chemistry* 103:131-141.

Ivanek R., GrÖHn Y.T., Tauer L.W. & Wiedmann M. (2005) The Cost and Benefit of Listeria Monocytogenes Food Safety Measures. *Critical Reviews in Food Science and Nutrition* 44(7-8):513-523.

Iyer S., Mattinson D.S. & Fellman J.K. (2010) Study of the Early Events Leading to Cassava Root Postharvest Deterioration. *Tropical Plant Biology* 3(3):151-165.

Jafari S.M., Assadpoor E., He Y. & Bhandari B. (2008) Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology* 26(7):816-835.

Jayakumar R., Nwe N., Tokura S. & Tamura H. (2007) Sulfated chitin and chitosan as novel biomaterials. *International Journal of Biological Macromolecules* 40(3):175-181.

Jeong S., Marks B.P., Ryser E.T. & Moosekian S.R. (2010) Inactivation of *Escherichia coli* O157:H7 on Lettuce, Using Low-Energy X-Ray Irradiation. *Journal of Food Protection* 73(3):547-551.

Jin T. & Zhang H. (2008) Biodegradable Polylactic Acid Polymer with Nisin for Use in Antimicrobial Food Packaging. *Journal of Food Science* 73(3):M127-M134.

Joshi K., Mahendran R., Alagusundaram K., Norton T. & Tiwari B.K. (2013) Novel disinfectants for fresh produce. *Trends in Food Science & Technology* 34(1):54-61.

Kadla J.F. & Gilbert R.D. (2000) Cellulose structure: A review. *Cellulose Chemistry and Technology* 34(3-4):197-216.

Kaplan H.S. (1966) DNA-strand scission and loss of viability after x irradiation of normal and sensitized bacterial cells. *Proceedings of the National Academy of Sciences of the United States of America* 55(6):1442-1446.

Kavoosi G., Dadfar S.M.M. & Purfard A.M. (2013) Mechanical, Physical, Antioxidant, and Antimicrobial Properties of Gelatin Films Incorporated with Thymol for Potential Use as Nano Wound Dressing. *Journal of Food Science* 78(2):E244-E250.

Khadre M.A., Yousef A.E. & Kim J.G. (2001) Microbiological Aspects of Ozone Applications in Food: A Review. *Journal of Food Science* 66(9):1242-1252.

Khan R.A., Parsons A.J., Jones I.A., Walker G.S. & Rudd C.D. (2010a) Preparation and characterization of phosphate glass fibers and fabrication of poly(caprolactone) matrix resorbable composites. *Journal of Reinforced Plastics and Composites* 29(12):1838-1850.

Khan R.A., Salmieri S., Dussault D., Uribe-Calderon J., Kamal M.R., Safrany A. & Lacroix M. (2010b) Production and Properties of Nanocellulose-Reinforced Methylcellulose-Based Biodegradable Films. *Journal of Agricultural and Food Chemistry* 58 (13):7878-7885

Kim J.G., Yousef A.E. & Dave S. (1999) Application of ozone for enhancing the microbiological safety and quality of foods: a review. *Journal of Food Protection* 62(9):1071-1087.

Klemm D., Schumann D., Kramer F., Hebler N., Koth D. & Sultanova B. (2009) Nanocellulose materials - Different cellulose, different functionality. *Macromolecular Symposia* 280(1):60-71.

Klemm D., Schumann D., Kramer F., Heßler N., Hornung M., Schmauder H.P. & Marsch S. (2006) Nanocelluloses as innovative polymers in research and application. *Advances in Polymer Science* 205:49-96.

Kohn J. & Langer R. (1996) Bioresorbable and bioerodible materials. *Biomaterials science.*, Ratner Bd HA, Schoen Fj, Lemons Je (Édit.) Academic Press, New York. p 64-72.

Komolprasert V., Bailey A. & Machuga E. (December 2007/January 2008) Irradiation of Food Packaging Materials. *Food Safety Magazine*.

Kozak G.K., MacDonald D., Landry L. & Farber J.M. (2013) Foodborne Outbreaks in Canada Linked to Produce: 2001 through 2009. *Journal of Food Protection* 76(1):173-183.

Kraśniewska K. & Gniewosz M. (2012) Substances with Antibacterial Activity in Edible Films - A Review. *Polish Journal of Food and Nutrition Sciences* 62(4):199-206.

Krisch R.E., Krasin F. & Sauri C.J. (1976) DNA breakage, repair and lethality after 125i decay in rec+ and reca strains of escherichia coli. *International Journal of Radiation Biology* 29(1):37-50.

Kume T., Furuta M., Todoriki S., Uenoyama N. & Kobayashi Y. (2009) Status of food irradiation in the world. *Radiation Physics and Chemistry* 78(3):222-226.

Labas M.D., Martín C.A. & Cassano A.E. (2005) Kinetics of bacteria disinfection with UV radiation in an absorbing and nutritious medium. *Chemical Engineering Journal* 114(1–3):87-97.

Lacroix M. & Chiasson F. (2004) The influence of MAP condition and active compounds on the radiosensitization of *Escherichia coli* and *Salmonella typhi* present in chicken breast. *Radiation Physics and Chemistry* 71:67-70.

Lacroix M., Turgis M., Borsa J., Millette M., Salmieri S., Caillet S. & Han J. (2009) Applications of radiation processing in combination with conventional treatments to assure food safety: New development. *Radiation Physics and Chemistry* 78(11):1015-1017.

Lafortune R., Caillet S. & Lacroix M. (2005) Combined effects of coating, modified atmosphere packaging, and gamma irradiation on quality maintenance of ready-to-use carrots (Daucus carota). *Journal of Food Protection* 68(2):353-359.

Larmond E. (1979) Laboratory Methods for Sensory Evaluation of Food. *Canadian Government Publishing Centre*.

Lavelli V., Pagliarini E., Ambrosoli R., Minati J. & Zanoni B. (2006) Physocochemical, microbial, and sensory parameters as indices to evaluate the quality of minimally processed carrots. *Biology and Technology* 40:34-40.

Le Tien C., Letendre M., Ispas-Szabo P., Mateescu M.A., Delmas-Patterson G., Yu H.L. & Lacroix M. (2000) Development of Biodegradable Films from Whey Proteins by Cross-Linking and Entrapment in Cellulose. *Journal of Agricultural and Food Chemistry* 48(11):5566-5575.

Lemoine M.L., Civello P., Chaves A. & Martínez G. (2009) Hot air treatment delays senescence and maintains quality of fresh-cut broccoli florets during refrigerated storage. *LWT - Food Science and Technology* 42(6):1076-1081.

Lewis O.G. & Fabisial W. (1997) Sutures. *Kirk-Othmer encyclopedia of chemical technology (4th ed.),* Wiley, New York, NY.

Lis-Balchin M., Deans S.G. & Eaglesham E. (1998) Relationship between bioactivity and chemical composition of commercial essential oils. *Flavour and Fragrance Journal* 13(2):98-104.

Llana-Ruiz-Cabello M., Pichardo S., Maisanaba S., Puerto M., Prieto A.I., Gutiérrez-Praena D., Jos A. & Cameán A.M. (2015) In vitro toxicological evaluation of essential oils and their main compounds used in active food packaging: A review. *Food and Chemical Toxicology* 81:9-27.

López P., Sánchez C., Batlle R. & Nerín C. (2007) Development of Flexible Antimicrobial Films Using Essential Oils as Active Agents. *Journal of Agricultural and Food Chemistry* 55(21):8814-8824.

Lv F., Liang H., Yuan Q. & Li C. (2011) In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International* 44(9):3057-3064.

Maftoonazad N. & Ramaswamy H.S. (2005) Postharvest shelf-life extension of avocados using methyl cellulose-based coating. *LWT - Food Science and Technology* 38(6):617-624.

Mahapatra A.K., Muthukumarappan K. & Julson J.L. (2005) Applications of Ozone, Bacteriocins and Irradiation in Food Processing: A Review. *Critical Reviews in Food Science and Nutrition* 45:447-461.

MAPAQ (2009a) Guide d'application du règlement sur les aliments - Formation obligatoire en hygiène et salubrité alimentaires.).

MAPAQ (2009b) Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire.).

Marcos B., Aymerich T., Dolors Guardia M. & Garriga M. (2007) Assessment of high hydrostatic pressure and starter culture on the quality properties of low-acid fermented sausages. *Meat Science* 76(1):46-53.

Martínez-Abad A., Sánchez G., Fuster V., Lagaron J.M. & Ocio M.J. (2013) Antibacterial performance of solvent cast polycaprolactone (PCL) films containing essential oils. *Food Control* 34(1):214-220.

Martins J.T., Cerqueira M.A., Bourbon A.I., Pinheiro A.C., Souza B.W.S. & Vicente A.A. (2012) Synergistic effects between κ-carrageenan and locust bean gum on physicochemical properties of edible films made thereof. *Food Hydrocolloids* 29(2):280-289.

Matan N., Rimkeeree H., Mawson A.J., Chompreeda P., Haruthaithanasan V. & Parker M. (2006) Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *International Journal of Food Microbiology* 107(2):180-185.

Matthews B., Mangalasary S., Darby D. & Cooksey K. (2010) Effectiveness of barrier film with a cellulose coating that carries nisin blends for the inhibition of Listeria monocytogenes. *Packaging Technology and Science* 23(5):267-273.

McClements D.J., Decker E.A. & Weiss J. (2007) Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. *Journal of Food Science* 72(8):R109-R124.

Mehinagic E., Royer G., Bertrand D., Symoneaux R., Laurens F. & Jourjon F. (2003) Relationship between sensory analysis, penetrometry and visible–NIR spectroscopy of apples belonging to different cultivars. *Food Quality and Preference* 14(5–6):473-484.

Mei Y., Zhao Y., Yang J. & Furr H.C. (2002) Using edible coating to enhance nutritional and sensory qualities of baby carrots. *Journal of Food Science* 67(5):1964-1968.

Miller F.A., Silva C.L.M. & Brandão T.R.S. (2013) A Review on Ozone-Based Treatments for Fruit and Vegetables Preservation. *Food Engineering Reviews* 5(2):77-106.

Miller K.S. & Krochta J.M. (1997) Oxygen and aroma barrier properties of edible films: a review. *Trends in Food Science & Technology* 8:228-237.

Mishra V.K. & Gamage T.V. (2007) Postharvest Physiology of Fruit and Vegetables. *Handbook of Food Preservation, Second Edition*, Rahman MS (Édit.). p 19-48.

Moreira M.R., Cassani L., Martín-Belloso O. & Soliva-Fortuny R. (2015) Effects of polysaccharide-based edible coatings enriched with dietary fiber on quality attributes of fresh-cut apples. *J Food Sci Technol* 10.1007/s13197-015-1907-z:1-11.

Nabavi S.M., Marchese A., Izadi M., Curti V., Daglia M. & Nabavi S.F. (2015) Plants belonging to the genus Thymus as antibacterial agents: From farm to pharmacy. *Food Chemistry* 173:339-347.

Nasatto P., Pignon F., Silveira J., Duarte M., Noseda M. & Rinaudo M. (2015) Methylcellulose, a Cellulose Derivative with Original Physical Properties and Extended Applications. *Polymers* 7(5):777.

Nazzaro F., Fratianni F., De Martino L., Coppola R. & De Feo V. (2013) Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals* 6(12):1451-1474.

Ndoti-Nembe A., Vu K.D., Han J., Doucet N. & Lacroix M. (2015) Antimicrobial Effects of Nisin, Essential Oil, and γ-Irradiation Treatments against High Load of Salmonella typhimurium on Mini-carrots. *Journal of Food Science* 10.1111/1750-3841.12918.

Observatoire horticole (2011) Tendances de consommation.).

Ölmez H. & Kretzschmar U. (2009) Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT - Food Science and Technology* 42(3):686-693.

Oussalah M., Caillet S. & Lacroix M. (2006a) Mechanism of Action of Spanish Oregano, Chinese Cinnamon and Savory Essential Oils against Cell Membranes and Walls of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Journal of Food Protection* 69(5):1046–1055.

Oussalah M., Caillet S., Salmieri S., Saucier L. & Lacroix M. (2004) Antimicrobial and Antioxidant Effects of Milk Protein-Based Film Containing Essential Oils for the Preservation of Whole Beef Muscle. *Journal of Agricultural and Food Chemistry* 52:5598-5605.

Oussalah M., Caillet S., Salmieri S., Saucier L. & Lacroix M. (2006b) Antimicrobial effects of alginate-based film containing essential oils for the preservation of whole beef muscle. *Journal of Food Protection* 69(10):2364-2369.

Oussalah M., Caillet S., Saucier L. & Lacroix M. (2007) Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes. *Food Control* 18(5):414-420.

Pareta R. & Edirisinghe M.J. (2006) A novel method for the preparation of starch films and coatings. *Carbohydrate Polymers* 63(3):425-431.

Parish M.E., Beuchat L.R., Suslow T.V., Harris L.J., Garrett E.H., Farber J.N. & Busta F.F. (2003) Methods to Reduce or Eliminate Pathogens from Fresh and Fresh-Cut Produce. *Comprehensive Reviews in Food Science and Food Safety* 2:161-173.

Park S.-H., Choi M.-R., Park J.-W., Park K.-H., Chung M.-S., Ryu S. & Kang D.-H. (2011) Use of Organic Acids to Inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* on Organic Fresh Apples and Lettuce. *Journal of Food Science* 76(6):M293-M298.

Pelissari F.M., Grossmann M.V.E., Yamashita F. & Pineda E.A.G. (2009) Antimicrobial, Mechanical, and Barrier Properties of Cassava Starch−Chitosan Films Incorporated with Oregano Essential Oil. *Journal of Agricultural and Food Chemistry* 57(16):7499-7504.

Peng C., Zhao S.-Q., Zhang J., Huang G.-Y., Chen L.-Y. & Zhao F.-Y. (2014) Chemical composition, antimicrobial property and microencapsulation of Mustard (Sinapis alba) seed essential oil by complex coacervation. *Food Chemistry* 165:560-568.

Perera C.O. (2007) Minimal Processing of Fruits and Vegetables. *Handbook of Food Preservation, Second Edition*, Rahman MS (Édit.). p 137-150.

Pérez C.D., Flores S.K., Marangoni A.G., Gerschenson L.N. & Rojas A.M. (2009) Development of a High Methoxyl Pectin Edible Film for Retention of l-(+)-Ascorbic Acid. *Journal of Agricultural and Food Chemistry* 57(15):6844-6855.

Perotti V.E., Moreno A.S. & Podestá F.E. (2014) Physiological aspects of fruit ripening: The mitochondrial connection. *Mitochondrion* 17:1-6.

Perrier-Cornet J.M., Marie P. & Gervais P. (2005) Comparison of emulsification efficiency of protein-stabilized oil-in-water emulsions using jet, high pressure and colloid mill homogenization. *Journal of Food Engineering* 66(2):211-217.

Petri E., Rodríguez M. & García S. (2015) Evaluation of Combined Disinfection Methods for Reducing Escherichia coli O157:H7 Population on Fresh-Cut Vegetables. *International Journal of Environmental Research and Public Health* 12(8):8678.

Phillips C.A. (1996) Review: Modified atmosphere packaging and its effects on the microbiological quality and safety of produce. *International Journal of Food Science and Technology* 31(6):463-479.

Pilon L., Spricigo P.C., Miranda M., de Moura M.R., Assis O.B.G., Mattoso L.H.C. & Ferreira M.D. (2015) Chitosan nanoparticle coatings reduce microbial growth on fresh-cut apples while

not affecting quality attributes. *International Journal of Food Science and Technology* 50(2):440-448.

Prusky D. (2011) Reduction of the incidence of postharvest quality losses, and future prospects. *Food Security* 3(4):463-474.

Public Health Agency of Canada (2004) Severe outbreak of Escherichia coli O157:H7 in health care institutions in Charlottetown, Prince Edward Island, fall, 2002. *Canada Communicable Disease Report* 30(9):81-88.

Qi H., Hu W., Jiang A., Tian M. & Li Y. (2011) Extending shelf-life of Fresh-cut 'Fuji' apples with chitosan-coatings. *Innovative Food Science & Emerging Technologies* 12(1):62-66.

Quintavalla S. & Vicini L. (2002) Antimicrobial food packaging in meat industry. *Meat Science* 62(3):373-380.

Rahman E.A.A., Talib R.A., Aziz M.G. & Yusof Y.A. (2013) Modelling the effect of temperature on respiration rate of fresh cut papaya (Carica papaya L.) fruits. *Food Science and Biotechnology* 22(6):1581-1588.

Raja M.M., Raja A., Mohamed Imran M. & Habeeb Rahman A.H. (2011) Quality aspects of cauliflower during storage. *International Food Research Journal* 18(1):427-431.

Randazzo W., Jiménez-Belenguer A., Settanni L., Perdones A., Moschetti M., Palazzolo E., Guarrasi V., Vargas M., Germanà M.A. & Moschetti G. (2016) Antilisterial effect of citrus essential oils and their performance in edible film formulations. *Food Control* 59:750-758.

Raybaudi-Massilia R.M., Mosqueda-Melgar J. & Martín-Belloso O. (2008a) Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. *International Journal of Food Microbiology* 121(3):313-327.

Raybaudi-Massilia R.M., Rojas-Graue M.A., Mosqueda-Melgar J. & Martin-Belloso O. (2008b) Comparative study on essential oils incorporated into an alginate-based edible coating to assure the safety and quality of fresh-cut Fuji apples. *Journal of Food Protection* 71(6):1150-1161.

Raychaudhuri S., Karmakar P. & Thakur A.R. (2000) γ -Ray-Induced DNA Damage and Repair in Methanosarcina barkeri. *Anaerobe* 6(6):325-331.

Ribeiro F.W.M., Laurentino L.d.S., Alves C.R., Bastos M.d.S.R., Costa J.M.C.d., Canuto K.M. & Furtado R.F. (2015) Chemical modification of gum arabic and its application in the encapsulation of Cymbopogon citratus essential oil. *Journal of Applied Polymer Science* 132(8):n/a-n/a.

Ricke S.C. (2003) Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science* 82(4):632-639.

Rico D., Martín-Diana A.B., Barat J.M. & Barry-Ryan C. (2007) Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology* 18(7):373-386.

Rodgers S.L., Cash J.N., Siddiq M. & Ryser E.T. (2004) A comparison of different chemical sanitizers for inactivating Escherichia coli O157:H7 and Listeria monocytogenes in solution and on apples, lettuce, strawberries, and cantaloupe. *Journal of Food Protection* 67(4):721-731.

Rojas-Grau M.A., Avena-Bustillos R.J., Friedman M., Henika P.R., Martin-Belloso O. & McHugh T.H. (2006) Mechanical, barrier, and antimicrobial properties of apple puree edible films containing plant essential oils. *Journal of Agricultural and Food Chemistry* 54(24):9262-9267.

Rojas-Graü M.A., Tapia M.S., Rodriguez F.J., Carmona A.J. & Martin-Belloso O. (2007) Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. *Food Hydrocolloids* 21:118-127.

Roman M., Dong S., Hirani A. & Lee Yong W. (2009) Cellulose Nanocrystals for Drug Delivery. *Polysaccharide Materials: Performance by Design,* (ACS Symposium Series: 1017) American Chemical Society, Vol 1017. p 81-91.

Rong C., Qi L., Bang-Zong Y. & Lan-Lan Z. (2010) Combined effect of ozonated water and chitosan on the shlef-life of Pacific oyster (*Crassostrea gigas*). *Innovative Food Science & Emerging Technologies* 11:108-112.

Sahbani S.K., Girouard S., Cloutier P., Sanche L. & Hunting D.J. (2014) The relative contributions of DNA strand breaks, base damage and clustered lesions to the loss of DNA functionality induced by ionizing radiation. *Radiation Research* 181(1):99-110.

Salleh E., Muhamad I.I. & Khairuddin N. (2009) Structural Characterization and Physical Properties of Antimicrobial (AM) Starch-Based Films. *World Academy of Science, Engineering and Technology* 55:432-440.

Salmieri S., Islam F., Khan R., Hossain F., Ibrahim H.M., Miao C., Hamad W. & Lacroix M. (2014) Antimicrobial nanocomposite films made of poly(lactic acid)-cellulose nanocrystals (PLA-CNC) in food applications: part A—effect of nisin release on the inactivation of Listeria monocytogenes in ham. *Cellulose* 21(3):1837-1850.

Salmieri S. & Lacroix M. (2006) Physicochemical Properties of Alginate/Polycaprolactone-Based Films Containing Essential Oils. *Journal of Agricultural and Food Chemistry* 54(26):10205-10214.

Salvia-Trujillo L., Rojas-Graü M.A., Soliva-Fortuny R. & Martín-Belloso O. (2015) Use of antimicrobial nanoemulsions as edible coatings: Impact on safety and quality attributes of fresh-cut Fuji apples. *Postharvest Biology and Technology* 105:8-16.

Sanchez-Garcia M.D., Ocio M.J., Gimenez E. & Lagaron J.M. (2008) Novel Polycaprolactone Nanocomposites Containing Thymol of Interest in Antimicrobial Film and Coating Applications. *Journal of Plastic Film and Sheeting* 24(3-4):239-251.

Sánchez-González L., Pastor C., Vargas M., Chiralt A., González-Martínez C. & Cháfer M. (2011a) Effect of hydroxypropylmethylcellulose and chitosan coatings with and without bergamot essential oil on quality and safety of cold-stored grapes. *Postharvest Biology and Technology* 60(1):57-63.

Sanchez-Gonzalez L., Vargas M., Gonzalez-Martinez C., Chiralt A. & Chafer M. (2011) Use of Essential Oils in Bioactive Edible Coatings. *Food Engineering Reviews* 3:1-16.

Sánchez-González L., Vargas M., González-Martínez C., Chiralt A. & Cháfer M. (2011b) Use of Essential Oils in Bioactive Edible Coatings: A Review. *Food Engineering Reviews* 3(1):1-16.

Santé Canada (2006) *Liste des additifs alimentaires autorisés au Canada.*), http://www.hc-sc.gc.ca/fn-an/securit/addit/diction/dict_food-alim_add-fra.php#g (Consulté le 2015-09-24)

Santé Canada (2007) *Quelles quantités d'aliments avez-vous besoin chaque jour?*), http://www.hc-sc.gc.ca/fn-an/food-guide-aliment/basics-base/quantit-fra.php (Consulté le 15-09-2015)

Santé Canada (2011) *Politique sur la présence de Listeria monocytogenes dans les aliments prêts-à-manger.*), http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2011-fra.php#criteresconform (Consulté le 2015-05-22)

Santé Canada (2012) Rôle de Santé Canada.).

Santé Canada (2013) *Projet de décision d'homologation PRD2013-22, Peroxyde d'hydrogène.*), http://www.hc-sc.gc.ca/cps-spc/pest/part/consultations/_prd2013-22/prd2013-22-fra.php#a3 (Consulté le 2015-09-07)

Sapers G.M., Miller R.L., Pilizota V. & Kamp F. (2001a) Shelf-Life Extension of Fresh Mushrooms (Agaricus bisporus) By Application of Hydrogen Peroxide and Browning Inhibitors. *Journal of Food Science* 66(2):362-366.

Sapers G.M., Miller R.L., Pilizota V. & Mattrazzo A.M. (2001b) Antimicrobial Treatments for Minimally Processed Cantaloupe Melon. *Journal of Food Science* 66(2):345-349.

Scallan E., Hoekstra R.M., Angulo F.J., Tauxe R.V. & Widdowson M.A. (2011) Foodborne Illness Acquired in the United States - Major Pathogens. *Emerging Infectious Diseases* 17(1):7-15.

Scavia G., Baldinelli F., Busani L. & Caprioli A. (2012) The burden of self-reported acute gastrointestinal illness in Italy: A retrospective survey, 2008-2009. *Epidemiology and Infection* 140(7):1193-1206.

Scharff R.L. (2012) Economic Burden from Health Losses Due to Foodborne Illness in the United States. *Journal of Food Protection* 75(1):123-131.

Schulte-Frohlinde D. (1987) Biological consequences of strand breaks in plasmid and viral DNA. *British Journal of Cancer* 55(SUPPL. 8):129-134.

Sela S. & Manulis-Sasson S. (2015) What else can we do to mitigate contamination of fresh produce by foodborne pathogens? *Microbial Biotechnology* 8(1):29-31.

Selma M.V., Allende A., López-Gálvez F., Conesa M.A. & Gil M.I. (2008) Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry. *Food Microbiology* 25(6):809-814.

Severino R., Vu K.D., Donsì F., Salmieri S., Ferrari G. & Lacroix M. (2014a) Antibacterial and physical effects of modified chitosan based-coating containing nanoemulsion of mandarin essential oil and three non-thermal treatments against *Listeria innocua* in green beans. *International Journal of Food Microbiology* 191(0):82-88.

Severino R., Vu K.D., Donsì F., Salmieri S., Ferrari G. & Lacroix M. (2014b) Antimicrobial effects of different combined non-thermal treatments against *Listeria monocytogenes* in broccoli florets. *Journal of Food Engineering* 124(0):1-10.

Shah N.N.A.K., Rahman R.A. & Hashim D.M. (2013) Changes in physicochemical characteristics of ozone-treated raw white rice. *J Food Sci Technol* 10.1007/s13197-013-1111-y:1-9.

Shargawi J.M., Theaker E.D., Drucker D.B., MacFarlane T. & Duxbury A.J. (1999) Sensitivity of Candida albicans to negative air ion streams. *Journal of Applied Microbiology* 87(6):889-897.

Sharmin N., Khan R., Salmieri S., Dussault D. & Lacroix M. (2012) Fabrication and Characterization of Biodegradable Composite Films Made of Using Poly(caprolactone) Reinforced with Chitosan. *J Polym Environ* 10.1007/s10924-012-0431-8:1-8.

Sikkema J., de Bont J.A. & Poolman B. (1994) Interactions of cyclic hydrocarbons with biological membranes. *Journal of Biological Chemistry* 269(11):8022-8028.

Silva-Angulo A.B., Zanini S.F., Rosenthal A., Rodrigo D., Klein G. & Martínez A. (2015) Comparative Study of the Effects of Citral on the Growth and Injury of *Listeria innocua* and *Listeria monocytogenes* Cells. *PLoS ONE* 10(2):e0114026.

Simões A.d.N., Ventrella M.C., Moretti C.L., Carnelossi M.A.G. & Puschmann R. (2010) Anatomical and physiological evidence of white blush on baby carrot surfaces. *Postharvest Biology and Technology* 55:45-52.

Siroli L., Patrignani F., Serrazanetti D.I., Tappi S., Rocculi P., Gardini F. & Lanciotti R. (2015) Natural antimicrobials to prolong the shelf-life of minimally processed lamb's lettuce. *Postharvest Biology and Technology* 103(0):35-44.

Sivakumar D. & Bautista-Baños S. (2014) A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. *Crop Protection* 64:27-37.

Skowron K., Bauza-Kaszewska J., Dobrzański Z., Paluszak Z. & Skowron K.J. (2014) UV-C Radiation as a Factor Reducing Microbiological Contamination of Fish Meal. *The Scientific World Journal* 2014:928094.

Song W.J., Shin J.Y., Ryu S. & Kang D.H. (2015) Inactivation of *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* in apple juice at different pH levels by gaseous ozone treatment. *Journal of Applied Microbiology* 119(2):465-474.

Sosa N., Zamora M., van Baren C. & Schebor C. (2014) New Insights in the Use of Trehalose and Modified Starches for the Encapsulation of Orange Essential Oil. *Food and Bioprocess Technology* 7(6):1745-1755.

Statistique Canada (2011) *Aliments disponibles selon les principaux groupes au Canada.*), http://www5.statcan.gc.ca/cansim/a26?lang=fra&retrLang=fra&id=0020019&pattern=020010%2C0020011%2C0030035%2C0030036%2C0030037%2C0020019%2C0030080&tabMode=dataTable&srchLan=-1&p1=-1&p2=-1 (Consulté le 15-09-2015)

Suppakul P., Miltz J., Sonneveld K. & Bigger S.W. (2003) Active Packaging Technologies with an Emphasis on Antimicrobial Packaging and its Applications. *Journal of Food Science* 68(2):408-420.

Takala P.N., Vu K.D., Salmieri S. & Lacroix M. (2011) Effect of antimicrobial coatings on the radiosensitization of *Escherichia coli*, *Salmonella Typhimurium* and *Listeria monocytogenes* in fresh broccoli. *Journal of Food Protection* 74:1065-1069.

Talibi I., Boubaker H., Boudyach E.H. & Ait Ben Aoumar A. (2014) Alternative methods for the control of postharvest citrus diseases. *Journal of Applied Microbiology* 117(1):1-17.

Tawema P., Han J., Salmieri S., Vu K. & Lacroix M. (2014) Application of selected natural antimicrobial formulations for the control of food pathogens in freshcut cauliflower. *Journal of Food Science and Engineering* 6:261-270.

Terefe N.S., Buckow R. & Versteeg C. (2014) Quality-Related Enzymes in Fruit and Vegetable Products: Effects of Novel Food Processing Technologies, Part 1: High-Pressure Processing. *Critical Reviews in Food Science and Nutrition* 54(1):24-63.

Thomsen M.R. & McKenzie A.M. (2001) Market incentives for safe foods: An examination of shareholder losses from meat and poultry recalls. *American Journal of Agricultural Economics* 83(3):526-538.

Toivonen P.M.A. & Brummell D.A. (2008) Biochemical bases of appearance and texture changes in fresh-cut fruit and vegetables. *Postharvest Biology and Technology* 48(1):1-14.

Turgis M., Han J., Borsa J. & Lacroix M. (2008) Combined effect of natural essential oils, modified atmosphere packaging, and gamma radiation on the microbial growth on ground beef. *Journal of Food Protection* 71(6):1237-1243.

Turgis M., Millette M., Salmieri S. & Lacroix M. (2012) Elimination of *Listeria* inoculated in ready-to-eat carrots by combination of antimicrobial coating and γ-irradiation. *Radiation Physics and Chemistry* 81(8):1170-1172.

Tyagi A.K., Malik A., Gottardi D. & Guerzoni M.E. (2012) Essential oil vapour and negative air ions: A novel tool for food preservation. *Trends in Food Science and Technology* 26(2):99-113.

Tyagi A.K., Nirala B.K., Malik A. & Singh K. (2008) The effect of negative air ion exposure on Escherichia coli and Pseudomonas fluorescens. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering* 43(7):694-699.

Uchechukwu-Agua A., Caleb O. & Opara U. (2015) Postharvest Handling and Storage of Fresh Cassava Root and Products: a Review. *Food and Bioprocess Technology* 8(4):729-748.

Ukuku D.O. & Fett W. (2002) Behavior of *Listeria monocytogenes* inoculated on cantaloupe surfaces and efficacy of washing treatments to reduce transfer from rind to fresh-cut pieces. *Journal of Food Protection* 65(6):924-930.

United States Department of Agriculture - Economic Research Service (2014) *Cost Estimates of Foodborne Illnesses.*), http://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx#48448 (Consulté le 2015-05-02)

USFDA (2000) Irradiation in the production, processing, and handling of food.), p 71056-71058.

USFDA (2015) *Chapter IV. Outbreaks Associated with Fresh and Fresh-Cut Produce. Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce.*), http://www.fda.gov/Food/FoodScienceResearch/SafePracticesforFoodProcesses/ucm091265.htm (Consulté le Consulted on 2015/07/16)

Välimaa A.-L., Tilsala-Timisjärvi A. & Virtanen E. (2015) Rapid detection and identification methods for Listeria monocytogenes in the food chain – A review. *Food Control* 55(0):103-114.

Van Der Schans G.P., Bleichrodt J.F. & Blok J. (1973) Contribution of various types of damage to inactivation of a biologically-active double-stranded circular DNA by gamma-radiation. *International Journal of Radiation Biology* 23(2):133-150.

Velasquez G., Herrera-Gomez A. & Martin-Polo M.O. (2003) Identification of bound water through infrared spectroscopy in methylcellulose. *Journal of Food Engineering* 59:79-84.

Vu K.D., Hollingsworth R.G., Leroux E., Salmieri S. & Lacroix M. (2011) Development of edible bioactive coating based on modified chitosan for increasing the shelf life of strawberries. *Food Research International* 44:198-203.

Wang X., Yuan Y. & Yue T. (2015) The application of starch-based ingredients in flavor encapsulation. *Starch - Stärke* 67(3-4):225-236.

Warriner K. & Namvar A. (2009) What is the hysteria with Listeria? *Trends in Food Science & Technology* 20(6–7):245-254.

Wen A., Delaquis P., Stanich K. & Toivonen P. (2003) Antilisterial activity of selected phenolic acids. *Food Microbiology* 20:305–311.

Whangchai K., Saengnil K. & Uthaibutra J. (2006) Effect of ozone in combination with some organic acids on the control of postharvest decay and pericarp browning of longan fruit. *Crop Protection* 25(8):821-825.

Wyatt V.T. & Yadav M. (2013) A multivariant study of the absorption properties of poly(glutaric acid-glycerol) films. *Journal of Applied Polymer Science* 130(1):70-77.

Yamauchi N., Funamoto Y. & Shigyo M. (2004) Peroxidase-mediated chlorophyll degradation in hortocultural crops. *Phytochemistry Reviews* 3:221-228.

Yoshihara K. & Kumazaki S. (2000) Primary processes in plant photosynthesis: photosystem I reaction center. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* 1(1):22-32.

Yoshii F., Darwis D., Mitomo H. & Keizo M. (2000) Crosslinking of poly(ε-caprolactone) by radiation technique and its biodegradability. *Radiation Physics and Chemistry* 57:417-420.

Zilelidou E.A., Tsourou V., Poimenidou S., Loukou A. & Skandamis P.N. (2015) Modeling transfer of Escherichia coli O157:H7 and Listeria monocytogenes during preparation of fresh-cut salads: Impact of cutting and shredding practices. *Food Microbiology* 45, Part B:254-265.

Zivanovic S., Chi S. & Draughon A.F. (2005) Antimicrobial Activity of Chitosan Films Enriched with Essential Oils. *Journal of Food Science* 70(1):M45-M51.

Zou P., Yang X., Wang J., Li Y., Yu H., Zhang Y. & Liu G. (2016) Advances in characterisation and biological activities of chitosan and chitosan oligosaccharides. *Food Chemistry* 190:1174-1181.

Zsivánovits G., Marudova M. & Ring S. (2005) Influence of mechanical properties of pectin films on charge density and charge density distribution in pectin macromolecule *Colloid and Polymer Science* 284(3):301-308.