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MISE AU POINT DE TECHNOLOGIES COMBINÉES POUR LA PRÉSERVATION DE LA VIANDE ET DU BROCOLI

Par

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A une famille hors du commun.

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m'a soutenue dans toutes mes folies.

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RÉSUMÉ

Dans les pays industrialisés, les aliments prêts à manger sont en plein essor. Ceci est dû principalement au changement des habitudes des consommateurs demandant encore plus d'aliments faciles à utiliser. Cependant, ces aliments posent un problème sanitaire vu leur instabilité microbienne et physico-chimique durant l'entreposage. Le procédé industriel faisant intervenir le lavage et la coupe favorise l'accélération de la sénescence des végétaux et la contamination croisée. Les bactéries souvent rencontrées sont *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Typhimurium et *Clostridium sporogenes*. Le contrôle de cette contamination passe généralement par l'ajout d'additifs, le chauffage et l'emballage sous atmosphère contrôlée. Cependant, avec la demande croissante d'aliments sans additifs, il devient difficile de contrôler la qualité microbiologique des aliments prêts à manger. L'utilisation d'antimicrobiens naturels est très étudiée, mais très peu sont présentement sur le marché car ils sont instables et leur concentration minimale inhibitrice contre les pathogènes affecte souvent les propriétés sensorielles. Ce projet vise à développer des combinaisons de traitements (d'antimicrobiens naturels, enrobage, prétraitement au calcium, emballage conditionné et irradiation) afin d'assurer la qualité, l'innocuité et la protection des valeurs nutritives, sensorielles et physico-chimiques des légumes et de la viande prêts à manger.

Dans ce projet de maitrise, nous avons développé une formulation antimicrobienne à base d'huile essentielle, d'acide organique et d'un antifongique par la détermination de la concentration minimale inhibitrice et l'étude des interactions possibles. Cette formulation a été par la suite immobilisée dans une matrice polymérique permettant d'avoir une meilleure stabilité et disponibilité des antimicrobiens naturels. L'efficacité antimicrobienne de l'enrobage antimicrobien lorsqu'il est appliqué seul ou combiné à l'irradiation a été évaluée sur le brocoli. Nous avons également évalué l'effet de la combinaison du prétraitement au calcium et l'enrobage à base d'alginate sur la qualité physico-chimique et microbienne du brocoli prêt à manger. D'autre part, une marinade à base d'épices et d'extraits de végétaux a été utilisée pour évaluer son efficacité antimicrobienne *in situ* sur la viande seule ou combinée à l'irradiation gamma.

Nous avons pu sélectionner, *in vitro*, 6 agents antimicrobiens parmi 27. Ces antimicrobiens ont démontré une efficacité à éliminer les pathogènes alimentaires. L'étude de l'interaction entre ces 6 antimicrobiens a permis de mettre au point une formulation

antimicrobienne (huile essentielle lemongrass + diacétate de sodium + natamycine) ayant démontrée un effet additif contre *Escherichia coli*, *Salmonella* Typhimurium et *Aspergillus niger* et un effet synergétique contre *Listeria monocytogenes*. D'autre part, l'analyse *in situ* a permis de mettre en évidence un effet synergique entre l'irradiation gamma à faible dose et l'enrobage antimicrobien pour réduire *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium et *Aspergillus niger* dans le brocoli. Un effet synergique a été observé aussi entre l'irradiation gamma et l'ajout de la marinade pour réduire *Escherichia coli*, *Salmonella* Typhimurium et *Clostridium sporogenes* dans la viande emballée sous vide. Combiner l'ajout de la marinade, l'irradiation gamma et l'emballage sous atmosphère contrôlée semblent être une alternative intéressante pour protéger contre l'oxydation des lipides, la perte des vitamines et la décoloration de la viande durant une conservation à 4 °C. Nous avons pu démontrer aussi que l'application de l'enrobage d'alginate seul ou en combinaison avec un prétraitement de calcium a permis de maintenir la fermeté et la couleur verte du brocoli au cours du stockage. Les traitements utilisés en combinaison ont permis également la réduction du taux de respiration et la perte de masse et l'augmentation de la durée de conservation du brocoli prêt à manger.

Les résultats obtenus lors de cette étude sont d'un grand intérêt pour l'industrie alimentaire permettant ainsi, le développement de nouveaux produits répondant à une demande croissante des consommateurs. Ce travail présente une nouvelle alternative surtout pour les patients immunodéprimés, qui sont condamnés à manger des aliments avec de faibles valeurs nutritionnelles causées par les procédés de décontamination durs en les protégeant des maladies d'origine alimentaire et en augmentant la qualité nutritionnelle des aliments.

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ABSTRACT

In developed countries, the ready-to-cook product category is in continuous growth due the consumer's increasing demand for convenient foods. However, these commodities pose a health problem due to their microbiological and physico-chemical instability during storage. The industrial process involving washing and cutting promotes vegetable senescence and cross-contamination. The often encountered bacteria are *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Typhimurium and *Clostridium sporogenes*. The control of this contamination typically requires the addition of additives, heating and packaging under controlled atmosphere. However, with the growing demand for preservative-free food, it becomes difficult to control the microbiological quality. The use of natural antimicrobial agents for the preservation of food is widely studied in the literature; however, very few are currently used in food industry because of their instability and their ability to affects the sensory properties of food at their determined minimum inhibitory concentration against pathogens. This project aims to develop treatment combinations (natural antimicrobials, edible coating, controlled atmosphere, calcium pretreatment and irradiation) to ensure the quality, safety and the protection of the nutritional value, sensory and physicochemical of ready-to-cook meat and vegetables.

In this project, we have developed an antimicrobial formulation based on essential oil, organic acid and an antifungal by determining their minimum inhibitory concentration and the evaluation of prior synergistic effect between selected antimicrobials by the checker board method. This formulation was subsequently immobilized in a polymer matrix to have better stability and availability of natural antimicrobial. Its antimicrobial efficacy alone or combined with irradiation was evaluated on broccoli floret. Also, we evaluated the effect of combining calcium pretreatment and alginate coating on the physicochemical and microbial quality of broccoli florets. On the other hand, a commercial marinade was used to evaluate its antimicrobial efficacy *in situ* when it was applied alone or in combination with gamma irradiation on the meat. The effect of combining the addition of marinade to irradiation on sensory, nutritional and physicochemical quality of meat was also evaluated.

In this study, we selected *in vitro* 6 antimicrobials among 27. These antimicrobials have demonstrated a high effectiveness in eliminating food pathogens. The study of the interaction between these six antimicrobial agents allowed the development of an antimicrobial formulation (Lemongrass essential oil + sodium diacetate + natamycin) which have

demonstrated an additive effect against *E. coli*, *Salmonella* Typhimurium and *Aspergillus niger* and a synergistic effect against *Listeria monocytogenes*. On the other hand, the analysis *in situ* has demonstrated a synergistic effect between a low dose gamma irradiation and antimicrobial coating to reduce *E. coli*, *L. monocytogenes*, *S.* Typhimurium and *A. niger* on broccoli. A synergistic effect was also observed between gamma irradiation and the addition of the marinade to reduce *E. coli*, *Salmonella* Typhimurium and *Clostridium sporogenes* on meat packed under vacuum. Based on the experimental findings, the combination of the marinade, gamma irradiation and packaging under modified atmosphere treatments was found to be a promising approach to extend the shelf-life of ready-to-cook pork loin as well as to protect against lipid oxidation, the loss of vitamins and meat discoloration.

Applying alginate coating alone or in combination with calcium pretreatment allowed maintaining the firmness and the green color of broccoli floret during storage. Treatments allowed also the reduction of the respiration rate and the loss of weight and the increase of the shelf-life of treated broccoli which is very beneficial for food industry.

The obtained results in this study are of great interest for the food industry allowing the development of new products to meet growing consumer demand. This work presents a new alternative especially for immunocompromised patients who are condemned to eat foods with low nutritional values caused by the harsh decontamination processes, and this by protecting them from foodborne illness and increasing the nutritional quality of food.

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LISTE DES ABRÉVIATIONS

^{60}CO : Cobalt 60

BL: Bactéries lactiques

CMI: Concentration minimale inhibitrice

FDA: Food and Drug Administration

FMT: Flore mésophile totale

g: Gramme

HACCP: Hazard Analysis and Critical Control Point

HE: huile essentielle

Kg: Kilogramme

kGy: kilogray

kGy h^{-1}: Kilogray par heure

pH: Potentiel hydrogène

p/v: poids sur volume

PAM : prêt-à-manger

UFC : Unité Formatrice de Colonies

USDA: United States Department of Agriculture

v/v: Volume/Volume

WHO : World Health Organization

CHAPITRE 1: SYNTHÈSE

1. Généralités

L'incidence des maladies d'origine alimentaire sur la santé humaine peut varier selon le type de microorganisme et selon l'immunité des personnes. Ces maladies peuvent causer des diarrhées mais aussi elles peuvent conduire dans certains cas jusqu'à l'hospitalisation et le décès. Les dépenses nationales en matière de santé causées par les intoxications alimentaires varient d'un pays à un autre mais elles pèsent lourd sur l'économie internationale.

Malgré l'avancement technologique et l'application des normes d'HACCP en industrie alimentaire, l'Agence de la santé publique du Canada estime avoir chaque année environ un Canadien sur huit (soit quatre millions de personnes) qui contracterait une maladie d'origine alimentaire par rapport aux Etats-Unis avec un Américain sur six. Plusieurs pathogènes sont responsables de ces cas d'intoxication cependant, seulement 40% de ces cas sont d'origine connue. On trouve principalement Norovirus, *Clostridium perfringens*, *Campylobacter* spp., *Salmonella* spp. (Figure 1), contre 60% des cas dont l'agent pathogène responsable est inconnu**.**

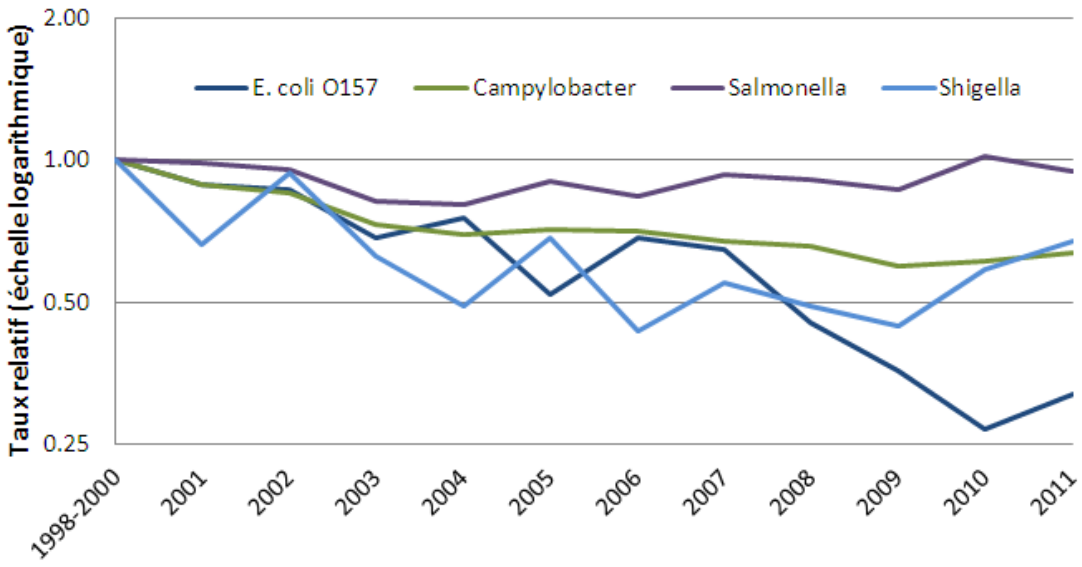

Figure 1 : Taux relatifs d'infections confirmées en laboratoire à *Campylobacter* spp., *Escherichia coli* O157, *Salmonella* spp., et *Shigella* spp. par rapport aux taux de 1998–2000, par année, de 2001 à 2011 (Agence de la santé publique du Canada)

Le tableau 1 récapitule la liste des microorganismes pathogènes les plus souvent rencontrées dans les produits alimentaires.

Tableau 1 . Liste des pathogènes alimentaires

Souches	Aliments	Références
Campylobacter jejuni	Poulet, dinde, porc et bœuf,	(Nørrung *et al.*, 2008)
Salmonella Typhi	viande crue, suya, salades PAM	(Gurler *et al.*, 2015, Tafida *et al.*, 2013)
Verotoxigenic E. coli O157 : H7	Poulet, dinde, porc, viande de bœuf hachée, jus de pomme non pasteurisé, salami, eau non traitée, les bovins et les produits carnés, laitue.	(Nørrung *et al.*, 2008, Woodward *et al.*, 2002)
L. monocytogenes	Salades PAM, roquette, concombre, fraises, viande crue et hachée, saucisses fermentés, hamburger, jambon, viande marinée, boudin noir, céleris et tomates	(Gurler *et al.*, 2015, Hadjilouka *et al.*, 2014, Martín *et al.*, 2014)
Yersinia enterocolitica and *Yersinia pseudotuberculosis*	Viande crue, carcasses de porc, carottes, légumes à feuilles crus et PAM, produits laitiers	(Hultman *et al.*, 2015, Longenberger *et al.*, 2014, Losio *et al.*, 2015, Van Damme *et al.*, 2015, Vasala *et al.*, 2014)
A. niger A. flavi, A. ochraceus	Raisins, riz, épices, haricots noirs, aliments fonctionnels	(Kizis *et al.*, 2014, Kong *et al.*, 2014, Ruadrew *et al.*, 2013)
Bacillus cereus	Plats de riz, pain et produit de pâtisserie, pâtes alimentaires, poulet	(Bennett *et al.*, 2013)
Arcobacter butzleri, *Arcobacter*	Lait de vache frais, les mollusques, poulet, viande de	(Nieva-Echevarria *et al.*, 2013)

cryaerophilus, and *Arcobacter skirrowii*	porc et de bœuf	
Penicillium chrysogenum	riz, épices	(Kizis *et al.*, 2014, Ruadrew *et al.*, 2013)
Clostridium perfringens	Plat de viande (bœuf) et de poulet	(Bennett *et al.*, 2013)
Staphylococcus aureus	Plat de viande (porc) et de poulet	(Bennett *et al.*, 2013)

Toutefois, le changement du comportement du consommateur et sa demande de nouvelles gammes de produits garantissant une durée de vie élevée, des qualités nutritionnelles, sensorielles et microbiologiques semblables aux produits frais à des prix abordables a donc favorisé le développement des aliments prêts à manger. En effet, la consommation des aliments congelés au Québec par exemple a augmenté de 2.6% entre 2005 et 2006. Les produits prêts à l'emploi posent certainement un problème de sécurité sanitaire pour les organismes d'inspection à cause de leur instabilité microbienne et physicochimique au cours de l'entreposage.

De plus, la demande croissante des consommateurs visant à limiter l'ajout d'additifs chimiques dans les produits alimentaires requière de trouver des alternatives à leur utilisation, notamment en favorisant l'incorporation de produits naturels. Ces types de composés bénéficient d'une image positive auprès des consommateurs en quête de produits finis faiblement traités. Le nombre de ces « consommateurs verts » est prévu d'augmenter dans un futur proche, offrant un avenir prometteur à l'utilisation et au développement des produits naturels dérivés de plantes.

2. La viande

La viande est un produit rapidement périssable. Lors de sa chaine de production, la viande peut voir sa qualité physicochimique et microbiologique dégradée à cause de l'état physiologique de l'animal lors de l'abattage, la propagation de la contamination lors de l'abattage et la transformation, la température et les autres conditions de stockage et de distribution (Nychas *et al.*, 2008).

Un des défis importants pour l'industrie est d'appliquer une technologie permettant de maintenir la qualité de la viande au cours de l'entreposage. La flore microbienne participe aussi à l'altération du produit, entraînant ainsi une réduction du temps de conservation et

potentiellement des problèmes de santé pour le consommateur. Les pathogènes qui peuvent contaminer la viande sont nombreux mais leurs incidence diffère. Les entérobactéries, malgré leur contribution non significative à la flore d'altération de la viande et des produits carnés, elles sont considérées comme des indicateurs de la sécurité alimentaire (Nychas *et al.*, 2008). On peut trouver *Staphylococcus aureus* résistante à la méthicilline qui peut aussi contaminer la viande. Selon le procédé utilisé (cuisson, ajout d'additifs…), la MAPAQ spécifie la limite d'acceptabilité de chaque produit final (Tableau 2).

Tableau 2. Normes d'acceptabilité de la viande transformée (MAPAQ, 2009).

Bactéries	**n**	**c**	**m**	**M**
Bactéries aérobies mésophiles	5	3	5.0×10^6	5.0×10^7
Bactérie lactiques	5	3	1.0×10^6	1.0×10^7
E. coli	5	3	1.0×10^2	1.0×10^3

n: nombre d'échantillons analysés

c : nombre maximal d'échantillons pouvant présenter des valeurs se situant entre m at M

m : échantillon acceptable si le résultat est égal ou inférieur à cette valeur

M : seuil limite d'acceptabilité, aucun «échantillon ne doit dépasser cette valeur

2.1. Flore normale de la viande

- **Bactéries lactiques**

Les bactéries lactiques peuvent contaminer la viande à partir de l'abattage avec le contact entre la viande et les déchets de l'animal. Ces bactéries continuent de se développer pendant la durée de vie de l'aliment en raison de leur capacité à croitre à des températures basses. Cette contamination est souvent recherchée pour deux raisons: d'une part la capacité des bactéries lactiques à concurrencer les autres micro-organismes pour les nutriments et par conséquence, l'obtention d'un effet de conservation souhaité par la répression des micro-organismes pathogènes ou leur décomposition. Deuxièmement, pour la saveur générée de la viande qui est désirée dans le cas des saucissons crus. Toutefois, dans certains cas, les bactéries lactiques peuvent produire des transformations indésirables de la viande crue et cuite. Cette modification est due en particulier aux bactéries lactiques hétéro fermentaires qui produisent des sous-produits indésirables tels que le CO_2, l'éthanol, l'acide acétique, l'acide butanoïque et l'acétoïne. En outre, en anaérobiose, les bactéries lactiques homofermentatives

peuvent également produire des quantités importantes d'acide acétique, et conduisent à une coloration verdâtre de la viande (Kröckel, 2013, Leisner *et al.*, 1995). Il a été démontré que cette altération chimique commence après 3 mois de stockage à 2 – 4 °C, ce qui réduit l'importance de cette contamination.

- ***Pseudomonas* spp.**

Pseudomonas est un genre bactérien prédominant souvent associé à la détérioration de la qualité de la viande. Ce sont des bacilles à Gram négatif, aérobies, non sporulés et flagellés. Elles sont capables de pousser même à la température de la réfrigération. La présence de *Pseudomonas* à forte concentration dans la viande induit une mauvaise odeur due à la dégradation des longues chaînes peptidiques des protéines de la viande en acides aminés et en d'autres composés tels que l'ammoniac, les amines, et le sulfure d'hydrogène et une pigmentation jaune-verte due à la production de pyoverdines.

2.2. Espèces bactériennes pathogènes qui contaminent la viande

- ***Campylobacter* spp.**

Campylobacter spp. sont des bactéries Gram négatif, non sporulées qui provoquent des intoxications alimentaires. Elles sont la principale cause de gastro-entérites humaine dans le monde. Les principaux symptômes sont: le malaise, fièvre, douleurs abdominales intenses et de la diarrhée (aqueuse, sanglante). Selon l'EFSA, en 2007, la campylobactériose est la maladie zoonotique la plus fréquemment signalée en santé publique dans l'Union européenne avec 200,507 cas déclarés et confirmés. Pour la viande de porc, les taux déclarés de contamination par les *Campylobacter* spp. varient d'un pays à un autre et c'est de l'ordre 16,9% au Canada par rapport à tous les autres contaminants (Hariharan *et al.*, 1990). Des études portent sur le traitement par des agents chimiques ont été réalisées pour la réduction des *Campylobacter* spp. dans la viande avec l'utilisation de phosphate trisodique, acide citrique (Meredith *et al.*, 2013). D'autres études ont utilisé le lactate de sodium et des fractions de protéine de pomme de terre (González *et al.*, 2011) comme assaisonnement pour la préservation de la viande contre *Campylobacter* spp..

- ***Salmonella* spp.**

Salmonella spp. sont des entérobactéries à bacille, Gram négatif. La salmonellose est classée la seconde plus fréquente maladie enregistrée à l'UE pour 151,995 cas d'humains confirmés. Toutefois, l'incidence des salmonelloses continue de diminuer dans l'Union européenne

(EFSA, 2009). Aux Pays Bas, 15% des cas de d'intoxication causée par la viande de porc sont dues à *Salmonella* dont 50 % sont associés à *Salmonella* Typhimurium.

- ***E. coli* O157 :H7**

E. coli O157 :H7 est une bactérie Gram négatif qui peut contaminer la viande dans son état cru ou cuite et assez souvent la viande hachée. Les symptômes qui peuvent se manifester dans les dix jours qui suivent le contact avec la bactérie sont : vives crampes d'estomac, diarrhée (aqueuse ou sanglante), vomissements, nausées, maux de tête et peu ou pas de fièvre. Des études récentes ont montré que l'irradiation à 1kGy permet de réduire le taux d'*E. coli* dans la viande (Kundu *et al.*, 2014).

- ***Clostridium perfringens***

Clostridium perfringens est une bactérie anaérobie sporulée. Les spores peuvent germer dans certains aliments dont la viande et les volailles. Les principaux symptômes de l'intoxication par *Clostridium perfringens* comprennent la nausée, les douleurs abdominales et la diarrhée. Jackson *et al.* (2011) ont montré que la combinaison de traitement au nitrite et d'agent antimicrobiens naturels offre une amélioration de la sécurité de la viande commercialisée contre les *Clostridium perfringens.*

- ***Listeria monocytogenes***

Listeria monocytogenes est une bactérie Gram positif non sporulée qui se multiplie à des températures comprises entre -0.4 et 50 °C. Elle peut être détectée dans la viande fraîche ou traitée. Les symptômes d'intoxication alimentaire peuvent se manifester subitement sous forme notamment de vomissements, nausées, crampes, diarrhée, violents maux de tête, constipation et une fièvre persistante. Turgis *et al.* (2012) ont démontré l'effet synergique de l'irradiation gamma et l'addition de la nisine sur la réduction de *Listeria monocytogenes* dans la viande hachée. Des travaux similaires ont été réalisés utilisant d'autres combinaisons (Ayari *et al.*, 2012).

3. Les végétaux

Les fruits et les légumes frais sont des éléments importants d'une alimentation saine et équilibrée; leur consommation est encouragée dans de nombreux pays pour se protéger contre une série de maladies telles que les cancers et les maladies cardio-vasculaires. Beaucoup de recherches sur les agents pathogènes humains d'origine alimentaire ont mis l'accent sur leur

transmission par des aliments d'origine animale. Cependant, des études récentes ont identifié des fruits et légumes qui sont la source de nombreuses épidémies. En effet, les fruits et légumes sont de plus en plus reconnus comme des vecteurs importants de transmission des agents pathogènes humains qui étaient traditionnellement associés aux aliments d'origine animale. Malgré l'importance de ce problème pour la santé humaine, actuellement les connaissances sont toujours limités pour les méthodes permettant de préserver les fruits et les légumes contre ces contaminants tout en gardant leur fraicheur.

3.1. Flore normale des fruits et légumes

La flore normale des fruits et des légumes provient principalement de la flore du sol, de l'eau d'irrigation et des insectes. Les fruits et les légumes sont souvent contaminés par les entérobactéries principalement *Enterobacter* spp. (Österblad *et al.*, 1999). Toutefois, on peut trouver aussi d'autre microorganismes comme *Pantoea* spp., *Bacillus* spp., *Cyanobacterium, Pseudomonas, Erwinia* spp., et *Pectobacterium.*

3.2. Les pathogènes qui contaminent les légumes

Les végétaux peuvent être contaminés par plusieurs microorganismes pathogènes. Les plus répandus sont généralement *E. coli spp.*, *Listeria spp.* et *Salmonella spp.* (Moreno *et al.*, 2012, Sant'Ana *et al.*, 2012). Mais d'autres pathogènes peuvent aussi être isolés des produits végétaux comme *Clostridium difficile* (Metcalf *et al.*, 2010). Cette contamination provient principalement des eaux d'arrosage, du sol mais aussi des excréments animales. Avec l'émergence des fruits et légumes prêt-à-manger sur le marché Canadien, le risque de contamination bactérienne augmente vue le procédé industriel suivi à savoir la coupe, le tranchage, l'épluchage et broyage qui peuvent enlever ou d'endommager la surface de protection de la plante ou de fruits et pourrait permettre la contamination et la croissance microbienne. L'étape de lavage des fruits et des légumes permet dans certains cas de propager la contamination aux autres surfaces du végétal. Il est à noter aussi, que le manque général d'efficacité des désinfectants à enlever ou tuer les agents pathogènes sur les fruits et les légumes crus est attribué, en partie, à leur inaccessibilité aux surfaces abritant les pathogènes.

- ***Listeria monocytogenes***

Listeria monocytogenes peut se développer comme précédemment décrit sur les produits de charcuterie mais aussi sur les végétaux. Elle a été identifiée dans les choux, les germes de

soja, céleri, laitue, tomate etc…. Selon la MAPAQ, pour les légumes prêts à l'emploi, aucune colonie n'est tolérable dans 25 g de produit.

- ***E. coli* O157**

E. coli a été détecté dans les choux, céleri, coriandre avec une fréquence respective de 25%, 17.6% et 19.5% (Beuchat, 2002). Au Canada, de 2001 à 2009, trois épidémies dues à l'*E. coli* O157 ont été associées à la laitue contaminée (Kozak *et al.*, 2013). Selon la MAPAQ, la limite d'acceptation pour le compte d'*E. coli* est de 10 UFC g^{-1}.

- ***Salmonella* spp.**

Salmonella spp. peut contaminer les fruits et les légumes avec une incidence de 18 à 20 % dans les aliments pourris et 9 à 10 % dans les aliments sains. Le compte de *Salmonella* spp. dans les légumes prêts à l'emploi doit être zéro UFC dans 25 g selon la MAPAQ.

- **Levures et moisissures**

Les produits d'origine végétale sont très souvent contaminés par des levures et moisissures. Les levures sont des organismes les plus fréquemment trouvés, avec des concentrations allant de moins de 100 à 4.0 $x10^8$ CFU g^{-1}. Une identification des souches a montré que les moisissures les plus communes trouvées dans les légumes frais et peu transformés sont *Cladosporium, Alternaria* et *Penicillium,* et moins fréquemment *Geotrichum* (Tournas, 2005). Selon la MAPAQ, la norme attribuée aux légumes prêts à l'emploi est de 1,0 x 10^4 pour les levures et les moisissures.

4. Effet sur les immunodépresseurs

Il est bien connu que la présence des pathogènes dans les aliments pourrait avoir des effets néfastes notamment sur les personnes immunodéprimées qui sont particulièrement vulnérables aux infections opportunistes, en plus des infections normales qui pourraient affecter tout le monde. Une infection par *Listeria monocytogenes*, même si c'est relativement rare au Canada, peut être très dangereuse pour les femmes enceintes, les personnes âgées et les personnes dont le système immunitaire est affaibli. Dans les cas graves, la listériose peut entraîner une fausse couche, une infection du cerveau, un empoisonnement sanguin et même la mort, avec des taux de mortalité de 20 à 30%. La sensibilité par rapport à la listériose est de 7,5 à 2584 fois plus élevée pour les immunodéprimés que pour les personnes saines (WHO, 2004). Ainsi, un régime alimentaire à faible concentration microbienne est recommandé afin

de réduire le risque d'infection d'origine alimentaire (Lund *et al.*, 2009). L'incidence de l'infection par *Salmonella* Typhimurium est plus élevée chez les nourrissons et les jeunes enfants. La mortalité est faible, mais elle augmente chez les personnes âgées et les immunodéprimés.

Ainsi, la responsabilité de l'industrie à fournir des aliments sains pour usage hospitalier répondant aux besoins de cette catégorie de patients est devenue de plus en plus grande (Tableau 3). Pour éviter ces risques pour la santé, la législation a mis en place les bonnes pratiques d'hygiène avant et après avoir manipulé le produit. Toutefois, il reste en permanence la contamination de l'aliment au cours de sa chaîne de vie.

Tableau 3. Les niveaux microbiologiques suggérés pour les aliments destinés aux personnes immunodéprimés (IAEA, 2010)

Microorganismes	**Qualité microbiologique (UFC g^{-1})**
Bactéries aérobies	< 500
Listeria spp.	non détectée dans 25 g
Salmonella spp.	non détectée dans 25 g
Levures et moisissures	< 10
Coliformes totaux	< 10
Staphylococcus aureus	< 10
Spores aérobies	< 10
Spores anaérobiques	< 10

5. Traitements utilisés

Les consommateurs exigent de plus en plus des produits de haute qualité, avec une valeur nutritive élevée, un aspect naturel, qui soient frais avec une saveur et un goût naturels et une durée de vie prolongée. Pour répondre à toutes ces demandes sans compromettre la sécurité alimentaire, plusieurs technologies de conservation non thermique alternatives telles que le High hydrostatic pressure (HHP), l'irradiation, l'impulsions lumineuses, l'emploi d'antimicrobiens naturels dans des emballages actifs ont été proposées et étudiées d'avantages. Ces techniques sont efficaces pour inactiver les micro-organismes végétatifs, le plus souvent liées à des maladies d'origine alimentaire, mais pas de spores. La combinaison de plusieurs technologies non thermiques a également été étudiée afin d'augmenter leur efficacité.

5.1. Irradiation gamma

Les technologies de pasteurisation à froid tels que l'irradiation gamma ont permis d'apporter de nouvelles possibilités pour la décontamination des produits frais en particulier les aliments prêts-à-manger. Approuvée depuis 1981 par les Nations Unies comme traitement de conservation, l'irradiation connait un essor considérable. L'approbation de cette technologie par « Food and Drug Administration » pour le traitement des aliments a accru significativement l'intérêt des industriels. La radiosensibilisation bactérienne par le biais de traitements combinés permettrait de mettre au point des technologies fiables, de donner des outils pour la prise de décisions en matière de contrôle des maladies infectieuses d'origine alimentaire et de réduire significativement les dépenses nationales en matière de santé et d'augmenter le bien-être des Canadiens. Cette nouvelle alternative a l'avantage de conserver l'aliment dans des conditions douces n'affectant pas les qualités sensorielles et nutritives de l'aliment. L'avantage de l'irradiation est de pouvoir l'utiliser sur des aliments frais. Mais des études ont toutefois démontré que comme pour le chauffage, certains pathogènes peuvent être réduits à un niveau non détectable après traitement et réapparaissent durant l'entreposage.

5.2. Emballage sous vide

L'emballage sous vide est considéré comme une alternative intéressante pour la conservation des aliments principalement des produits qui ne respirent pas comme les produits de charcuterie. Les travaux de Cayuela *et al.* (2004) ont montré que l'emballage sous vide de la viande de porc permet d'augmenter la stabilité de la viande à l'oxydation, ce qui entraîne une plus grande stabilité de la couleur par la suite l'augmentation de la durée de conservation. Par contre, les mêmes auteurs ont signalé que ce type d'emballage présente l'inconvénient de l'augmentation de la perte de poids initiale du produit. Les légumes et les fruits restent métaboliquement actifs après la récolte, et cette activité est essentielle pour le maintien de leur qualité; donc un vide poussé n'est pas conseillé pour leur conservation.

5.3. Ajout d'antimicrobiens naturels

Les antimicrobiens naturels sont fortement demandés par le consommateur. Des études antérieures ont mis en évidence l'efficacité de plusieurs extraits naturels, huiles essentielles, bactériocines, acides organiques *in vitro* à inhiber la croissance des pathogènes alimentaires. L'efficacité de l'application de ces antimicrobiens *in situ* (dans une matrice alimentaire) est prouvée aussi pour la protection des aliments contre la contamination bactérienne et fongique

(Bajpai *et al.*, 2013). Le mode d'action des antimicrobiens naturels repose en général sur une altération morphologique considérable de la paroi bactérienne, la libération de l'ATP extracellulaire et la fuite des ions potassium.

5.4. Traitement au calcium

Plusieurs travaux ont montré l'efficacité de l'ajout du calcium dans le traitement des fruits et légumes après leur récolte. En effet, le chlorure de calcium par exemple a été testé sur plusieurs fruits et légumes (melon, pomme, fraise etc) (Misha, 2002). Le prétraitement avec le chlorure de calcium a amélioré la fermeté et la qualité des fruits et des légumes, il a augmenté également leur temps de conservation et a maintenu leur texture et leur fraicheur. Comme source de calcium, on trouve le calcium gluconate, le lactate de calcium et le chlorure de calcium qui sont déjà utilisés dans le domaine alimentaire. Le lactate de calcium semble être une nouvelle alternative pour remplacer le chlorure de calcium. A part son effet antimicrobien, il a été prouvé que le lactate de calcium à 1% n'augmente pas l'amertume du melon et améliore sa texture. Cependant, le chlorure de calcium testé sur les melons à une concentration de 1% et 2% a changé le goût et a augmenté l'amertume du melon.

5.5. Enrobage comestible

Cette nouvelle technologie écologique approuvée par FDA prend de plus en plus de valeur pour les industriels vue son efficacité de préservation des aliments. Une panoplie de bio polymères utilisés en agroalimentaire pour cette fin a permis de mettre au point des formulations d'enrobage diverses selon le type de produit. Que ce soit à base de protéines, de polysaccharides, de lipides ou même des combinaisons de ceux-ci, le choix se multiplie pour offrir une large gamme d'enrobage (Dhall, 2013).

Cette technique est utilisée généralement pour contrôler le transfert de l'eau et des gaz et le processus d'oxydation. L'avantage de l'utilisation de l'enrobage comestible c'est qu'il est possible d'incorporer des composés actifs dans la matrice de polymère et qu'il est consommé avec le produit. Donc, des vertus nutritionnelles peuvent être ajoutées à l'enrobage.

5.6. Combinaison de traitements

Une nouvelle voie dans la recherche est de combiner plusieurs techniques de conservation. Plusieurs études ont prouvé l'efficacité de certaines combinaisons dans la préservation des aliments et l'augmentation de leur durée de conservation. Ces combinaisons ont l'avantage

d'utiliser des techniques dans des conditions plus douces. Ces techniques sont également utilisées sur divers produits (charcuteries, épice, végétaux…). Des études de l'efficacité de l'enrobage comestible seul ou combiné à d'autres traitements thermiques (Moreira *et al.*, 2011), ou non thermiques tels que l'ajout d'antimicrobiens naturels (Mantilla *et al.*, 2013) ou l'irradiation gamma (Vachon *et al.*, 2003) ont été réalisées. D'autres études réalisées sur les légumes et les fruits comme la combinaison de l'enrobage comestible, irradiation δ et l'emballage sous atmosphère modifiée pour la préservation des carottes (Caillet *et al.*, 2006) ont prouvé l'efficacité de ces combinaisons.

6. But, hypothèses, objectifs et résultats

6.1. Problématique

Le brocoli au cours du stockage, souvent à 25 °C, peut faire face à plusieurs modifications physicochimiques. En effet, Finger *et al.* (1999) ont démontré une perte de masse d'environ 7 %, une diminution de 70% de chlorophylle, une réduction des sucres solubles, des sucres réducteurs et non réducteurs, et de la concentration d'amidon, après seulement 72 h de stockage. Ces changements indiquent une détérioration de la qualité physicochimique du brocoli en 3 jours seulement. D'autre part, au niveau microbiologique, des études antérieures ont démontré que la flore microbienne d'un brocoli frais contient 10% de levures et 16.7% de moisissures par rapport aux autres microorganismes. Après 7 jours de conservation, le compte des moisissures passe à 26.7%. Les espèces associées sont *Alternaria*, *Botrytis*, *Cephalcsporium*, *Fusarium*, et *Penicillium* qui constituent la flore totale des levures et des moisissures qui contaminent le brocoli (Mohd-Som *et al.*, 1994). Toutefois, l'altération microbiologique n'a pas été assez étudiée. En effet, il n'existe pas assez de données permettant la préservation du brocoli et la limitation de son altération.

D'autre part, la préservation de la viande passe jusqu'à nos jours par l'addition d'agents conservateurs chimiques ou par traitements thermiques. En effet, les nitrites sont toujours utilisés par les industries. Ils sont capables de contrôler la croissance des bactéries (surtout *Clostridium botulinum* et ses spores) et améliorent la stabilité de la couleur et la texture au cours du stockage. L'interaction du nitrite avec la viande retarde généralement la formation de composés volatiles responsables du mauvais goût. Cependant, les nitrites peuvent aussi réagir avec les amines et former des nitrosamines qui sont considérés comme cancérigènes. Une préoccupation majeure pour les viandes transformées commercialisées comme naturelle/organique, c'est donc qu'elles ne contiennent pas de nitrite. Par conséquents, les

investigations sont orientées vers la substitution de cet agent chimique par d'autres méthodes de préservation. La plupart des études réalisées dans ce contexte n'ont pas pu proposer des solutions fiables ne modifiant pas les qualités organoleptiques du produit final et écartant les produits de la chimie de synthèse.

Dans le cadre de ce projet de recherche, nous voulons utiliser des bactéries pathogènes conventionnelles qui contaminent la viande et les végétaux (*Listeria monocytogenes*, *Salmonella* Typhimurium, *Escherichia coli*, *Clostridium sporogenes* et *Aspergillus niger*) afin d'étudier la radiosensibilité bactérienne suite à l'irradiation-δ, seule ou en présence des substances antimicrobiennes naturelles et sous différentes conditions atmosphériques. Ainsi, combiner deux ou plusieurs techniques de conservation dans leurs conditions douces pourra protéger la qualité et l'innocuité des aliments. Il sera ainsi possible de mettre au point de nouvelles technologies efficaces pour assurer l'innocuité et la qualité des aliments (viande et brocoli) tout en préservant leur fraicheur et sans avoir recours à des additifs chimiques.

6.2. Hypothèses

1- La combinaison de l'irradiation, l'ajout d'un mélange d'antimicrobiens naturels et l'emballage sous vide permet d'assurer l'innocuité et d'augmenter la durée de conservation de la viande stockée à 4 °C.
2- Un prétraitement au calcium suivi d'un enrobage comestible permet de maintenir la qualité et d'augmenter la durée de conservation du brocoli prêt-à-manger (PAM).
3- La combinaison de l'irradiation et l'enrobage du brocoli encapsulant des antimicrobiens naturels permet d'augmenter la radiosensibilité bactérienne.
4- La dose d'irradiation utilisée ne modifie pas les qualités nutritionnelles, sensorielles et physicochimiques de la viande prête-à-cuire.

6.3. Objectifs

Les objectifs de ce projet visaient à :

1- Mettre au point un enrobage comestible du brocoli à base de polysaccharides.
2- Mettre au point des formulations antimicrobiennes d'origine naturelle pour application sur des légumes frais/ congelés PAM et des mets préparés PAM.
3- Immobiliser les formulations les plus efficaces dans la matrice de polymère mise au point.

4- Évaluer la synergie de traitements industriels faisant intervenir l'enrobage comestible contenant les antimicrobiens (végétaux) et la marinade (viande) et l'irradiation gamma.
5- Évaluer l'effet de traitements industriels faisant intervenir l'enrobage comestible et le prétraitement au calcium sur la qualité physico-chimique (couleur, texture, qualité sensorielle, perte de masse) et la durée de conservation des végétaux.
6- Évaluer la qualité nutritive (vitamines B_1 et B_2), sensorielle et physicochimique (oxydation des lipides et la couleur) de la viande ayant subi la combinaison de traitements.

6.4. Résultats

Dans le premier article, nous avons évalué l'effet de la combinaison de l'irradiation gamma et l'ajout d'une marinade à base d'extrait naturel sur l'innocuité, la durée de conservation et les propriétés sensorielles, nutritionnelles et physicochimiques de la viande emballée sous vide. Les résultats de l'effet des traitements combinés obtenus contre *E. coli*, *S.* Typhimurium et *C sporogenes* ont montré qu'une dose d'irradiation de 1 kGy était suffisante pour réduire *E. coli*, *S.* Typhimurium en dessous de la limite de détection contre 1.5 kGy pour *C. sporogenes*. Un effet synergique a été observé quand une irradiation à 1 kGy a été combinée à l'ajout de la marinade pour réduire *C. sporogenes.* Les résultats du compte de la flore mésophile totale (FMT) et des bactéries lactiques (BL) ont démontré un effet synergique des traitements utilisés, soit une augmentation de 9 jours de la durée de conservation de la viande emballée sous vide (**Objectif 4**). Les traitements utilisés ont permis de prévenir l'oxydation des lipides et la perte de thiamine et de la riboflavine au cours du traitement d'irradiation et du stockage. La marinade et l'irradiation ont également augmenté la rougeur de la viande (**Objectif 6**).

Dans le deuxième article, nous avons testé des traitements appliqués au brocoli. Les résultats ont montré qu'un enrobage à base d'alginate pouvait agir en synergie avec un prétraitement de calcium afin de maintenir la qualité du brocoli PAM au cours du stockage. L'application de l'enrobage à base d'alginate seul ou en combinaison avec un prétraitement de calcium a permis de maintenir une texture fraîche et une couleur verte pendant un stockage à 4 °C. Les traitements ont permis également la réduction du taux de respiration et la perte de masse ce qui est très intéressant pour l'industrie alimentaire. La durée de conservation de brocoli traité a été de 11 jours ce qui correspondant à une extension de 6 jours de plus par rapport au témoin (**Objectif 1 & 5**).

Finalement, dans le troisième article, nous avons sélectionné 6 antimicrobiens parmi 27 pour leur potentiel à éliminer *E. coli*, *S.* Typhimurium, *L. monocytogenes*, *A. niger* et la FMT du brocoli. A partir de l'étude de l'interaction entre ces antimicrobiens, la formulation contenant l'huile essentielle de lemongrass, le diacétate de sodium et le natamycine a été sélectionnée comme étant la formulation ayant une activité antimicrobienne qui varie entre additive et synergique contre tous les pathogènes testés (**Objectif 2**).

Les analyses *in situ* sur le brocoli ont montré l'efficacité de l'enrobage encapsulant la formulation antimicrobienne à réduire *E. coli*, *S.* Typhimurium et *L. monocytogenes*. Par contre, pas d'effet significatif a été observé pour *A. niger*. Aussi, les résultats de la combinaison de l'enrobage antimicrobien et l'irradiation gamma à faibles doses a montré un effet synergique à éliminer les pathogènes au cours du stockage à 4 °C (**Objectif 3 & 4**).

CHAPITRE 2:

COMBINED EFFECTS OF MARINATING AND γ-IRRADIATION TO ENSURE THE SAFETY, TO PROTECT THE NUTRITIONAL VALUE AND TO INCREASE THE SHELF-LIFE OF READY-TO-COOK MEAT FOR IMMUNOCOMPROMISED PATIENTS

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Contribution des auteurs

Yosra Ben Fadhel a réalisé les manipulations et la rédaction de l'article.

Valentin Leroy a réalisé les analyses de la durée de conservation de la viande.

Dominic Dussault a fourni la marinade.

France St-Yves et Martine Lauzon ont aidé à faire l'analyse sensorielle.

Stéphane Salmieri a participé à l'analyse sensorielle et à la correction de l'article.

Majid Jamshidian a participé à la correction de l'article.

Dang Khanh Vu a participé à l'analyse statistique et le calcul.

Monique Lacroix: Directrice de recherche, responsable scientifique et coordinatrice du projet de recherche, a participé à la planification des expériences et à la correction de l'article.

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RÉSUMÉ

Le but de cette étude a été d'évaluer l'effet de la combinaison de la marinade et de l'irradiation gamma à des doses de 1, 1,5 et 3 kGy sur *E. coli* O157: H7, *S.* Typhimurium et *C. sporogenes* dans la viande crue emballée sous vide et conservée à 4 °C et d'estimer sa salubrité et sa durée de vie. En outre, l'effet des traitements combinés sur les valeurs nutritionnelles, sensorielles (oxydation des lipides, concentration de thiamine et de riboflavine) et la couleur ont été évalués. L'étude a démontré que l'utilisation de la marinade en combinaison avec une faible dose d'irradiation gamma (1,5 kGy) pourrait agir en synergie pour réduire à un niveau indétectable les bactéries pathogènes et d'augmenter la durée de vie des longes de viande prêt-à-cuire sans affecter leur qualité sensorielle et nutritionnelle.

Mots clés: viande prête-à-cuire; irradiation gamma; marinade ; qualité physico-chimique ; qualité microbiologique; patients immunodéprimés.

ABSTRACT

The aim of this study was to evaluate the effect of combining marinating and γ-irradiation at doses of 1, 1.5 and 3 kGy on *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Clostridium sporogenes* in raw meat packed under vacuum and stored at 4 °C and to estimate its safety and shelf-life. Further, the effect of combined treatments on sensorial, nutritional values (lipid oxidation, concentration of thiamin and riboflavin) and color was evaluated. The study demonstrated that the use of marinade in combination with a low dose of γ-irradiation (1.5 kGy) could act in synergy to reduce to undetectable level of pathogenic bacteria and increase the shelf-life of ready-to-cook meat loin without affecting its sensorial and nutritional quality.

Keywords

Ready-to-cook meat; Gamma irradiation; Marinating; Physicochemical quality; Microbiological quality; Immunocompromised patients

1. Introduction

The industrial food processes involving washing and cutting promote cross-contamination especially with *Escherichia coli*, *Listeria*, *Salmonella* and *Campylobacter* (Dogbevi, Vachon, & Lacroix, 2000). Worldwide foodborne and waterborne diarrheal diseases, taken together, kill about 2.2 million people annually (WHO, 2014). Foodborne zoonotic diseases are a significant and widespread global public health threat with > 320,000 human reported cases each year only in the European Union (EFSA, 2015).

Faced to these health issues, immunocompromised patients are the most concerned category, due to their vulnerability to opportunistic infections, in addition to normal infections that could affect everyone. In fact, the relative susceptibility to develop listeriosis for immunocompromised group increases between 7.5 and 2584 times depending on each person's degree of immune suppression (Rocourt, BenEmbarek, Toyofuku, & Schlundt, 2003) with a fatality rate of 20 to 30%. Salmonellosis is also one of the most frequently reported foodborne diseases worldwide with approximately 40,000 cases reported by the Centers for Disease Control and Prevention (CDC) each year and caused by the ingestion of contaminated poultry, beef, pork, eggs, and milk in 96% of the cases (CDC, 2011). *E. coli* O157:H7 is among the most serious foodborne pathogens, due to the severity of the illness and its low infective dose (< 100 organisms). It caused the death of four children and severe illness of 600 people in the US related to undercooked hamburgers which give serious doubts about food safety (USDA, 1994). Hence, for immunocompromised persons, a low-microbial diet**,** called also neutropenic diet or cooked-food diet, i.e. excluding foods that may contain pathogenic microorganisms, is advisable in order to reduce the risk of foodborne infection (IAEA, 2010, Lund and O'Brien, 2009 and Risi and Tomascak, 1998). Tap water and ice made from tap water, known also as vehicles of infectious disease transmission should be avoided (Risi & Tomascak, 1998). Numerous worldwide health issues were related to meat spoilage due to several microbiological and physicochemical factors often associated, besides meat pathogens, to an abundant development of lactic acid bacteria (LAB) and total mesophilic flora (TMF). The presence of LAB in meat is often sought because of their efficiency to compete microorganisms for nutriments and obtaining a desired preservative effect due to the repression of pathogenic and spoilage microorganisms, and for their desired taste of meat product, such as raw fermented sausage. However, in some case, it can produce undesired transformations such as off-flavors ropy slime and greening of meat due to the synthesis of fermentation derived by-products of both raw and cooked meats. This alteration

is due especially to hetero-fermentative LAB (Kröckel, 2013 and Leisner *et al*., 1995). Notwithstanding the application of good hygiene practices and HACCP principles, microbiological contamination with food pathogens of meat products remains an episodic phenomenon faced by food industries.

In the past, to ensure meat safety, steam autoclaving, prolonged oven baking, and gamma irradiation are required. Food preparation is done aseptically in a laminar air flow hood, to prevent air contamination. All materials used for preparation are sterilized (Risi & Tomascak, 1998). Currently, modern meat processing employs a range of new physical and chemical methods. However, these methods currently used in industry such as high hydrostatic pressure (HHP), generally modify sensorial and nutritional characteristics such as proteins denaturation (globin, myosin and actin) causing meat discoloration (Zhou, Xu, & Liu, 2010). Also, irradiation of meat products at a dose necessary to eliminate pathogens (> 6 kGy) can affect the flavor (Brewer, 2009).

The modified atmosphere packaging (MAP) is often used, but this method of packaging promotes the growth of spore forming bacteria such as *Clostridium* and *Bacillus* that are resistant to conventional treatments and often related to foodborne diseases and some outbreaks in the world. Irradiation can eliminate *Salmonella*, *E. coli* O157:H7 and other non-spore forming pathogens on meat products (Clavero, Monk, Beuchat, Doyle, & Brackett, 1994). But in some cases, as for heating, some pathogens can recur during storage (Le Tien, Lafortune, Shareck, & Lacroix, 2007). Currently, combined methods like MAP, replacement of air by inert gases (nitrogen), addition of protective agents (antioxidants), post-irradiation storage to allow flavor to return to near-normal levels (re-packaging or double packaging in oxygen permeable film), or reduction of irradiation dose by bacterial radiosensitization are increasingly studied (Gould, 2012 and Huq *et al.*, 2015).

Combining irradiation to one or more preservation technique allows increasing the bacterial radiosensitization and decreasing the dose needed to assure food safety (Lacroix *et al*., 2013). Several combinations such as MAP or natural antibacterial compounds or heat treatment increase the bacterial radiosensitization (Lacroix *et al*., 2013). These combined treatments, used at low doses, are able to inactivate pathogens and increase the shelf-life of the commodity without modifying its organoleptic and nutritional properties.

The aim of this study was to evaluate the effect of combined treatments using irradiation with marinating containing vegetable extracts and natural spices on meat pathogens. The shelf-life, the nutritional value, the sensory and physicochemical properties of ready-to-cook meat packed under vacuum during storage at 4 °C were also evaluated.

2. Material and methods

Pork loins were purchased from a local supermarket (IGA, Laval, QC, Canada) and cut into slice of 60 ± 5 g each (± 10 × 8 cm). A commercial marinade based on mango, curry and other ingredients (water, glucose-fructose, onion, garlic, salt, canola oil and vinegar) with a final pH of 3 to 4 was provided by BSA Ingredients S.E.C/L.P. (Montreal, QC, Canada).

Eight different groups were studied (C: untreated meat; M: marinated meat; I1: irradiated meat at 1 kGy; I1.5: irradiated meat at 1.5 kGy; I3: irradiated meat at 3 kGy; MI1: marinated and irradiated meat at 1 kGy; MI1.5: marinated and irradiated meat at 1.5 kGy and MI3: marinated and irradiated meat at 3 kGy) using fresh pork meat.

2.1. Effect of combined treatments on pathogenic bacteria

2.1.1. Preparation of bacterial cultures

E. coli O157:H7, *Salmonella* Typhimurium (SL1344) and *Clostridium sporogenes* were kept at -80 °C in Tryptic Soy Broth (TSB; Becton-Dickinson, Sparks, MD, USA) containing glycerol (10% v/v). Before each experiment, stock cultures were propagated through two consecutive 24 h at 37 °C growth cycles (10^{-1} dilution) in TSB for *E. coli* and *S.* Typhimurium and in Reinforced Clostridial Broth for *C. sporogenes* and then washed in saline solution (0.85% w/v) to obtain working cultures containing approximately 10^9 CFU mL^{-1}. *C. sporogenes* was incubated in anaerobic conditions. *C. sporogenes* was evaluated because it has similar characteristics to *Clostridium botulinum* against irradiation and heat sensitivity and does not need stringent laboratory confinement requirements.

2.1.2. Sample preparation and inoculation procedures

Samples were distributed into Whirl-Pak™ Sterile Filter Bags (one piece of 20 ± 3 g per bag). For the study of pathogenic bacteria, all samples were frozen at -80 °C then irradiated at the Canadian Irradiation Center at 45 kGy in a UC-15A irradiator (Nordion Inc., Kanata, ON, Canada) equipped with a ^{60}Co source at a dose rate of 16.8 kGy h^{-1} at room temperature to sterilize them before inoculation with pathogens and then, kept at 4 °C. All subsequent irradiations were carried out in the same irradiator. Sterile meat samples were inoculated by adding 4 mL of working cultures of *E. coli* or *S.* Typhimurium or *C. sporogenes* at 10^4 CFU mL^{-1}. This concentration allows obtaining after homogenization with peptone water a final concentration of 10^2 CFU g^{-1}. An amount of 10 g of marinade was deposited directly on the surface of the pork slices and were sealed under 96% vacuum in transparent bag

(Winpak Ltd., Vaudreuil-Dorion, QC, Canada) using a packaging machine (model 250 Single Chamber, Sipromac Inc., St-Germain-de-Grantham, QC, Canada) and kept at 4 °C during 24 h to allow the marinade reacting with bacteria. Then, the appropriate samples were irradiated at 1, 1.5 or 3 kGy and refrigerated prior to analyses day. Meat control samples were prepared in the same way as described previously but without irradiation and marinating.

The counts of *E. coli*, *S.* Typhimurium and *C. sporogenes* were performed on days 0, 3, 7, 14 and 28 by adding to meat sample 80 g of 0.1% (w/v) peptone water (Alpha Biosciences Inc., Baltimore, MD, USA). The pork loin samples were mixed during 2 min at high speed in a Lab-blender 400 stomacher (Laboratory Equipment, London, UK), then seeded into Mc sorbitol agar for *E. coli*, Desoxycholate Citrate Lactose Sucrose agar for *Salmonella*, Reinforced Clostridial Medium for *C. sporogenes* and incubated for 24 h at 37 °C. *C. sporogenes* was incubated under anaerobic conditions for 48 h at 37 °C.

2.2. Shelf-life estimation

Initial TMF and LAB concentration in pork loin meat were analyzed during 30 days of storage at 4 °C. In each day of analysis, agar Man, Rogosa and Sharp were used for counting LAB and Tryptic Soy Agar for TMF. Inoculated plates were incubated at 37 °C for 48 h. TMF and LAB growth rate was calculated over the whole storage period. Eq. (1) was used to describe the growth bacteria (*Y*) over time.

Equation (1)

$$Y = X \ \ exp.(\mu t)$$

where *X* is the initial population, μ the growth rate of TMF and LAB (Ln CFU g^{-1} day^{-1}) and t the number of storage days.

2.3. Evaluation of nutritional values of irradiated meat

2.3.1. Determination of thiamin (B_1) and riboflavin (B_2) concentrations

Meat samples (5.0 ± 0.3 g) were placed into a transparent bag. A quantity of 2.5 g of marinade was added, and then samples were sealed under 96% vacuum and stored at 4 °C during 24 h. The appropriate samples were irradiated and treated for a total dose of 1, 1.5 or 3 kGy. The analysis of the most unstable vitamins, thiamin and riboflavin, was accomplished after 0, 3, 7, 14 and 28 days of storage at 4 °C.

Vitamin extraction was carried out by acid and enzymatic digestion according to Tang, Cronin, and Brunton (2006). The acid digestion was done with 0.1 M HCl, and the enzymatic digestion was carried out with 50 mg of acid phosphatase (Sigma-Aldrich, ON, Canada).

HPLC determination of thiamin and riboflavin was carried out using an HPLC system (Agilent 1260 Infinity Quaternary LC system) coupled with a Fluorescence Detector (Agilent 1260 Infinity; G1221B FLD). All instrumental parts were automatically controlled by Agilent ChemStation software (Agilent Technologies). The system was equipped with a C_{18} guard column and a Poroshell 120 eclipse Plus EC-18 reversed-phase column (4.6 mm × 75 mm × 2.7 μm) at room temperature. The fluorescence detector operated at an excitation wavelength of 366 nm and 450 nm and an emission wavelength of 434 nm and 510 nm for thiamin and riboflavin respectively. The flow rate was respectively 0.3 mL min^{-1} and 0.4 mL min^{-1} for thiamin and riboflavin. Standard curves were prepared using aqueous standards of thiamin hydrochloride (0.025–0.25 μg mL^{-1}) and standard of riboflavin (0.0625–0.25 μg mL^{-1}). The injection volume was 20 μL.

2.3.2. Lipid oxidation

The lipid oxidation of meat was evaluated by the determination of thiobarbituric acid reactive substances (TBARS) according to Oussalah, Caillet, Salmiéri, Saucier, and Lacroix (2004) after 0, 14 and 28 days of storage at 4 °C. The TBARS values were expressed as micrograms of malondialdehyde (MDA) per 10 g of meat.

2.4. Color

Color determination was carried out on 6 samples, onto 7 preselected locations on the surface of each sample using a Minolta Colorimeter Color reader CR10 (Konica Minolta Sensing, Inc, Mahwah, NJ, USA). L* (lightness, black = 0, white = 100), a* (redness > 0, greenness 0, blue < 0), and total change of color ΔE* were quantified on each sample.

2.5. Sensorial evaluation

Using a hedonic test in nine points (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely), two samples were tested (marinated and irradiated meat at 1.5 kGy, marinated and non-irradiated meat) by 30 panelists during four tasting sessions. A minimum of eight consumer panelists was present for each evaluation session. For each panelist, 3 pieces of meat (± 2 × 2 cm) were served twice to evaluate for the first time the texture and the flavor and for the second time the color, the odor and the global appreciation. Samples of 32 ± 3 g

(± 8 × 8 cm) of tender pork loins were put in each bag containing 50 g of marinade. Then bags were sealed under 96% vacuum and stored for 24 h at 4 °C. Afterwards, samples were irradiated and treated with a total dose of 1.5 kGy and stored at 4 °C, 24 h. Sensory analysis was carried out the following day. All samples were cooked on a grill pan for 3 min (90 s per face).

2.6. Statistical analysis

Each experiment was done in triplicate (n = 3). Analysis of variance (ANOVA), Duncan's multiple range tests for equal variances and Tamhane's test for unequal variances were performed for statistical analysis using SPSS 18.0 software (SPSS Inc., USA). Samples which were not exceeding 2, were statistically analyzed by applying the Student *t*-test. Differences between means were considered significant when the confidence interval was lower than 5% ($P \leq 0.05$).

3. Results and discussion

3.1. Effect of combined treatments on pathogenic bacteria

3.1.1. Effect of combined treatments on growth of *E. coli*

The growth of *E. coli* in meat samples during storage under vacuum at 4 °C is shown in Table 1. Results showed that marinating and vacuum packaging reduced significantly the level of *E. coli* on days 3, 7 and 14 respectively, from 2.26, 3.43 and 2.75 log CFU g^{-1} for untreated meat to only 1.80, 1.90 and 1.89 log CFU g^{-1} for marinated meat. Afterwards, no significant difference was observed between untreated and marinated meat. An irradiation treatment at a dose of 1 kGy was enough to reduce *E. coli* under the detectable level during 28 days. Similar observations were reported by Clavero *et al*. (1994) with irradiated ground beef packed under air. They showed that 0.5 kGy and 1 kGy doses were able to reduce 1.6 log CFU g^{-1} and 3.3 log CFU g^{-1} of *E. coli*, respectively. The resistance of *E. coli* to irradiation depends especially on temperature (Mulder, 1976) and the D_{10} value was higher when irradiation is done at -5 °C as compared to + 5 °C (Thayer & Boyd, 1993). However, vacuum or MAP had no effect on the radiosensitivity of *E. coli* (Kudra *et al*., 2013).

Table 1. Growth of *E. coli* during storage under vacuum at 4 °C.

	Concentration of *E. coli* (log CFU g^{-1})[2]				
Samples	**Day 0**	**Day 3**	**Day 7**	**Day 14**	**Day 28**
C	2.31 ± 0.18 [a]	2.26 ± 0.07 [b]	3.43 ± 0.06 [b]	2.75 ± 0.06 [b]	1.70 ± 0.00 [a]
M	1.98 ± 0.00 [a]	1.80 ± 0.00 [a]	1.90 ± 0.14 [a]	1.89 ± 0.28 [a]	1.70 ± 0.00 [a]
I1	ND[1]	ND	ND	ND	ND
MI1	ND	ND	ND	ND	ND
I1.5	ND	ND	ND	ND	ND
MI1.5	ND	ND	ND	ND	ND
I3	ND	ND	ND	ND	ND
MI3	ND	ND	ND	ND	ND

[1] ND, no colony detected

[2] Within each column, means with the same letter are not significantly different ($P > 0.05$).

3.1.2. Effect of combined treatments on growth of *S.* Typhimurium

The growth of *S.* Typhimurium in meat samples during storage under vacuum at 4 °C is shown in Table 2. Results showed that the application of the marinade alone reduce significantly the growth of *Salmonella* only on day 7 afterwards, no effect was observed during storage (28 days). Similarly to *E. coli*, treatment of meat packed under vacuum with an irradiation dose of 1 kGy was also enough to reduce *S.* Typhimurium below the limit of detection and no CFU was observed. However, Clavero *et al.* (1994) found that 1 kGy was not enough to reduce the level of *S.* Typhimurium packed under air, in polyethylene stomacker bag. This suggests that vacuum packaging increases the radiosensitivity of *S.* Typhimurium. Previous works support this assessment and have demonstrated that the efficacy of irradiation treatment in eliminating *Salmonella* is higher when meat is MAP packed (Turgis, Han, Borsa, & Lacroix, 2008).

Table 2. Growth of *S.* Typhimurium during storage under vacuum at 4 °C.

	Concentration of *S.* Typhimurium (log CFU g^{-1}) [2]				
Samples	**Day 0**	**Day 3**	**Day 7**	**Day 14**	**Day 28**
C	2.52 ± 0.26 [a]	2.11 ± 0.11 [a]	2.61 ± 0.81 [b]	2.88 ± 0.52 [a]	1.70 ± 0.00 [a]
M	2.43 ± 0.28 [a]	1.98 ± 0.20 [a]	2.00 ± 0.14 [a]	2.40 ± 0.50 [a]	1.95 ± 0.01 [a]
I1	ND[1]	ND	ND	ND	ND
MI1	ND	ND	ND	ND	ND
I1.5	ND	ND	ND	ND	ND
MI1.5	ND	ND	ND	ND	ND
I3	ND	ND	ND	ND	ND
MI3	ND	ND	ND	ND	ND

[1] ND, no colony detected

[2] Within each column, means with the same letter are not significantly different ($P > 0.05$).

3.1.3. Effect of combined treatments on growth of *C. sporogenes*

The growth of *C. sporogenes* in meat samples during storage under vacuum at 4 °C is shown in Table 3. The obtained results showed no significant difference ($P > 0.05$) between marinated and untreated meat on day 0. Afterwards, a significant effect ($P \leq 0.05$) was observed and *C. sporogenes* counts in pork meat was reduced by 0.34, 1.18, 1.62 and 2.56 log reduction for marinated meat on days 3, 7, 14 and 28 respectively when compared to untreated meat. Thus, the marinade had an effect in reducing *C. sporogenes* population in pork meat due probably to the high susceptibility of *Clostridium* strain to spices (Nevas, Korhonen, Lindström, Turkki, & Korkeala, 2004) and due also to the low pH of the marinade ($3 < pH < 4$). In fact, *C. botulinum* is unable to grow on $pH < 4.5$ (Leistner & Gould, 2012). Irradiation alone at 1 kGy has no significant effect on day 0, however on day 3, a 1.25 log reduction was observed and from day 7 no CFU was detected.

Table 3. Growth of *C. sporogenes* during storage under vacuum at 4 °C.

	Concentration of *C. sporogenes* (log CFU g^{-1}) [2]				
Samples	**Day 0**	**Day 3**	**Day 7**	**Day 14**	**Day 28**
C	2.87 ± 0.05 [a]	3.26 ± 0.20 [b]	4.10 ± 1.18 [b]	4.36 ± 0.16 [b]	5.29 ± 0.58 [b]
M	2.80 ± 0.18 [a]	2.92 ± 0.11 [a]	2.92 ± 0.02 [a]	2.74 ± 0.18 [a]	2.73 ± 0.08 [a]
I1	2.56 ± 0.02 [a]	2.01 ± 0.28 [a]	ND	ND	ND
MI1	2.51 ± 0.00 [a]	ND	ND	ND	ND
I1.5	ND[1]	ND	ND	ND	ND
MI1.5	ND	ND	ND	ND	ND
I3	ND	ND	ND	ND	ND
MI3	ND	ND	ND	ND	ND

[1] ND, no colony detected

[2] Within each column, means with the same letter are not significantly different ($P > 0.05$).

Therefore, an irradiation dose of 1.5 and 3 kGy allowed reducing *C. sporogenes* to undetectable level during the whole storage. When an irradiation treatment at 1 kGy was combined with marinating, no significant effect was observed on day 0 and then *C. sporogenes* counts remained under the undetectable level during the whole storage. Hence, a synergetic effect was observed between marinating and γ-irradiation treatment at a dose of 1 kGy to eliminate *C. sporogenes* on day 3. The obtained results showed that nevertheless the addition of marinade to meat samples, an irradiation dose ≥ 1.5 kGy is needed to eliminate *C. sporogenes* on meat stored under vacuum.

Results obtained for pathogens (Table 1, Table 2 and Table 3) showed that *C. sporogenes* has a higher resistance to γ-irradiation as compared to *E. coli* and *S.* Typhimurium. These results suggest that *Enterobacteriaceae* are less resistant to γ-irradiation than *C. sporogenes* (Grant & Patterson, 1991). On the basis of these results, it has been shown that to insure meat safety an irradiation treatment at a dose of 1.5 kGy is needed.

3.2. Shelf-life estimation

Shelf-life limit was considered at the limit of acceptability when TMF and LAB counts reached the current authorities regulation level of 5×10^6 CFU g^{-1} and 10^6 CFU g^{-1} respectively (MAPAQ, 2009). For immunocompromised patients, the International Atomic Energy Agency (IAEA) recommended that the total aerobic count should not exceed than 500 CFU g^{-1}. Yeast and mold, coliform, *Staphylococcus aureus* and spores should not exceed 10 CFU g^{-1} and *Listeria* and *Salmonella* should be completely absent (IAEA, 2010).

3.2.1. TMF

The results of TMF counts of meat samples during 30 days are presented in Fig. 1. Results of non-marinated samples (Fig.1a) showed that the limit of acceptability of MAPAQ for untreated and irradiated meat at 1.5 kGy and 3 kGy was reached at days 20, 26 and > 30 respectively. However, a lag phase of 5 and 13 days was observed for samples irradiated at 1.5 kGy and 3 kGy respectively, showing an extension of the lag phase for bacterial growth. The growth rate was also lower in irradiated samples with 0.45 and 0.33 Ln CFU g^{-1} day^{-1} respectively for samples irradiated at 1.5 and 3 kGy as compared to 0.63 Ln CFU g^{-1} day^{-1} for untreated samples (Table 4). When marinade was applied (Fig.2b), the shelf-life of irradiated samples at 1.5 and 3 kGy was determined to 29 days in both cases as compared to 15 days when meat was only marinated. Respective lag phases of 15 and 21 days were observed for marinated samples treated at 1.5 and 3 kGy. Then, the growth rate decreased and reached 0.23 Ln CFU g^{-1} $day^{-1}$for irradiated samples at 1.5 and 3 kGy as compared to 0.58 Ln CFU g^{-1} day^{-1} for marinated and non-irradiated samples. It is interesting to note that adding marinating alone did not increase the shelf-life however, when it was combined to irradiation at 1.5 kGy and vacuum packaging, the shelf-life was increased by 9 more days as compared to untreated meat and by 3 more days as compared to irradiated meat at 1.5 kGy without marinating.

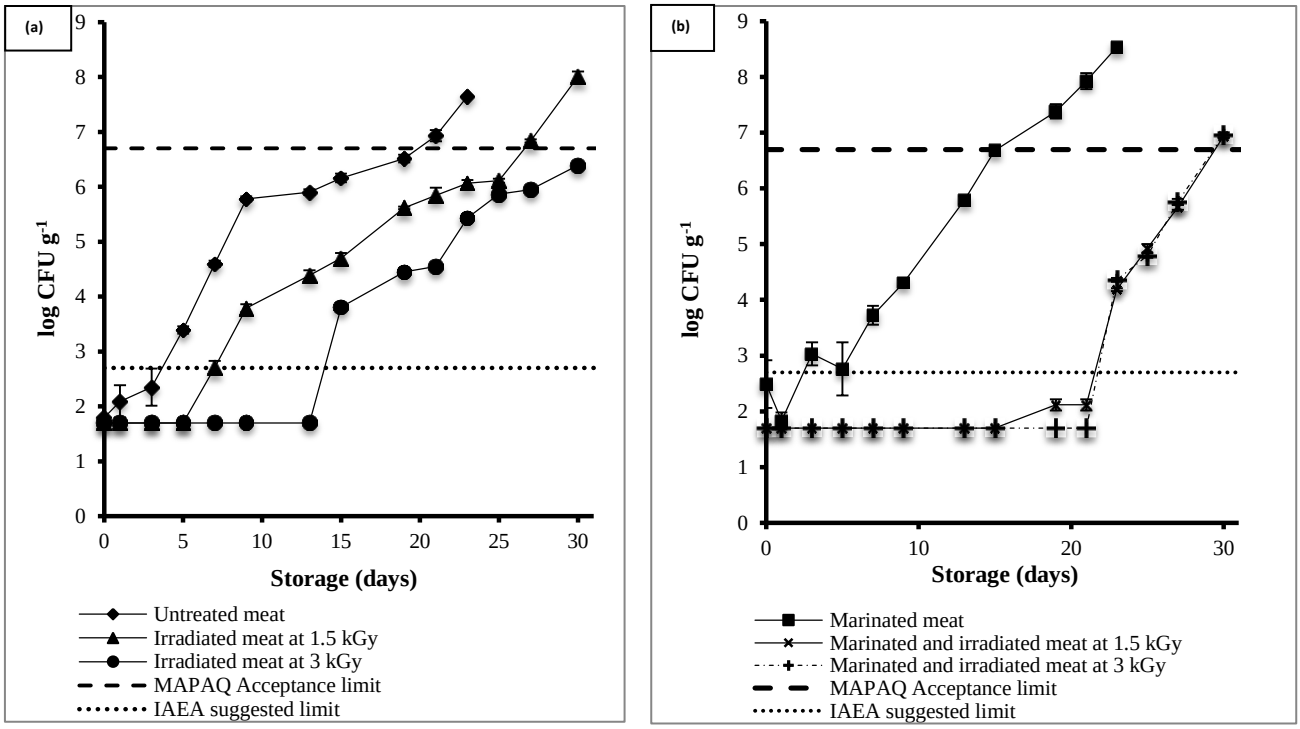

Figure 1. Effect of γ-irradiation on the TMF counts in (a) non-marinated and (b) marinated meat stored under vacuum at 4 °C

Table 4. Growth rate of TMF and LAB in refrigerated pork marinated and submitted to γ-irradiation treatment.

	Growth rate (Ln CFU g^{-1} day^{-1})	
Samples	**TMF**	**LAB**
C	0.63	0.57
M	0.58	0.51
I1.5	0.45	0.32
MI1.5	0.23	0
I3	0.33	0
MI3	0.23	0

For immunocompromised patients, the shelf-life (500 CFU g^{-1}) was reached after 6 and 14 days for treated meat with 1.5 and 3 kGy respectively as compared to only 4 days for untreated meat. When irradiation was combined to marinating, the shelf-life was increased

and reached 15 and 22 days for marinated samples treated with 1.5 and 3 kGy respectively. These observations suggest that the addition of marinade had a synergistic effect when done in combination with γ-irradiation. Dogbevi, Vachon, and Lacroix (1999) demonstrated that even with an irradiation dose of 3 kGy, TMF count on meat packed under air was reduced to undetectable level on day 0 and started growing only from day 3. However, in this study, when combined to marinade addition before irradiation treatment under vacuum, a synergy was observed and the shelf-life was significantly increased. These observations imply that the application of marinade and vacuum packaging increased the sensitivity of TMF against irradiation.

3.2.2. LAB

The effect of γ-irradiation on LAB counts is presented in Fig. 2. Results showed that without marinating (Fig 2a) the limit of acceptability for untreated meat was reached after 17 days. When marinade was applied (Fig 2b), the limit was reached at day 21. For meat irradiated at 1.5 kGy results showed that the shelf-life was increased by 8 more days as compared to untreated meat and the limit of acceptability was reached after 25 days. However, the growth rate of marinated and untreated meat was 0.57 and 0.51 Ln CFU g^{-1} day^{-1} respectively and it decreased to reach 0.32 Ln CFU g^{-1} day^{-1} with an irradiation treatment of 1.5 kGy (Table 4). An irradiation dose of 1.5 kGy on meat without marinating increased the lag phase to day 5 and reduced the growth rate; however, it was not able to keep LAB counts under undetectable level during the whole storage.

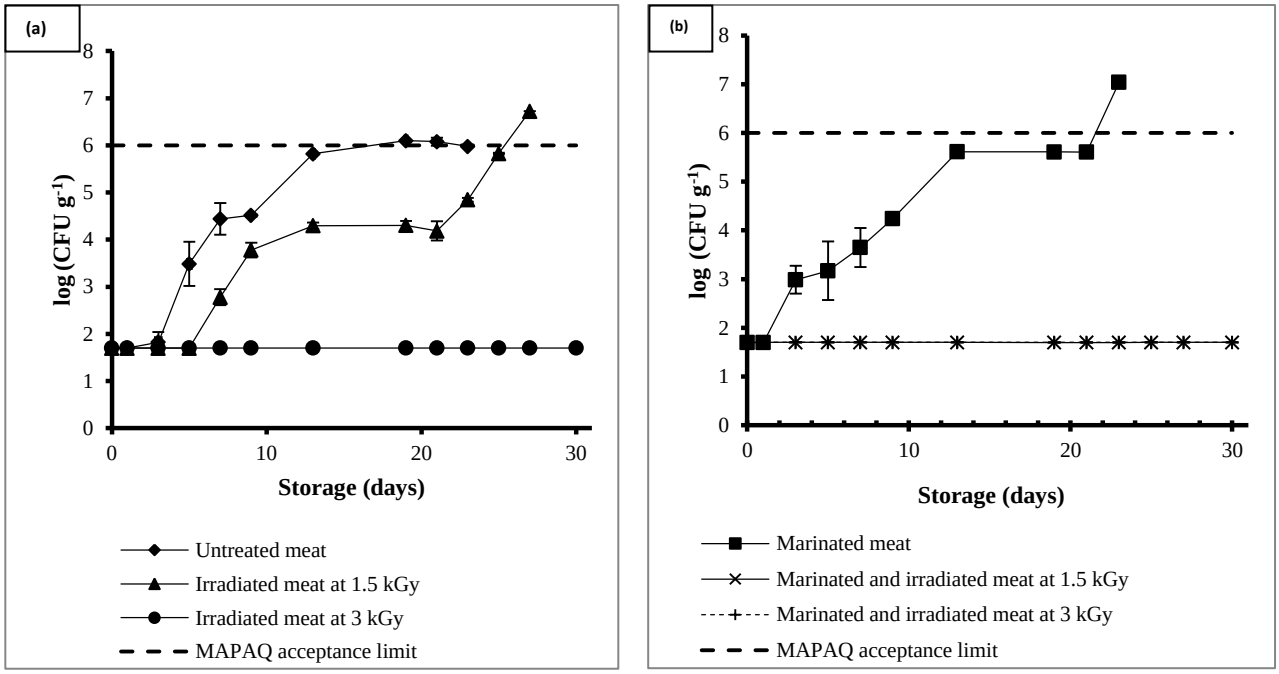

Figure 2. Effect of γ-irradiation on the LAB counts in (a) non-marinated and (b) marinated meat stored under vacuum at 4 °C.

When irradiation at the same dose (1.5 kGy) was combined with marinating (Fig 2b), LAB level was below the detection threshold during the whole storage as well as irradiated samples treated at 3 kGy showing synergistic effects between marinating and irradiation. As reported by previous studies, the irradiation of pork loins at 1.5 and 3 kGy packed under vacuum, reduced LAB counts by respectively 3.4 and 3.7 log CFU g^{-1} on day 0 with an initial count of 5.8 log CFU g^{-1} (Fregonesi *et al*., 2014). The difference between the obtained results and previous works could be explained by the less initial level of LAB which was < 3 log CFU g^{-1} in this study as compared 5.8 log CFU g^{-1} for previous works. On the basis of these results, it can be noted that irradiation treatment alone could reduce the growth rate of LAB. However, when combined to the application of the marinade, a synergy was observed and the dose necessary to eliminate LAB was reduced from 3 to 1.5 kGy.

The obtained results demonstrated that the combination of marination and irradiation at a dose of 1.5 kGy was the most interesting to increase the shelf-life of ready-to-cook meat.

3.3. Evaluation of nutritional values of irradiated meat

3.3.1. Determination of thiamin and riboflavin concentrations

The concentrations of thiamin and of riboflavin determined in pork meat are presented in Table 5. The obtained results show that the amount of thiamin in non-marinated samples was inversely proportional to the dose of irradiation. Indeed, when meat was irradiated at 1, 1.5

and 3 kGy, thiamin losses of 6, 15 and 27% were respectively observed on day 0. Riboflavin was more radioresistant and no significant difference was observed in samples treated at 1 and 1.5 kGy. However, when a dose of 3 kGy was applied, a lost of 11% of riboflavin was observed. This is in agreement with previous works showing that 18% of thiamin loss was observed when fresh meat was treated at a dose of 2 kGy. This study also showed a 47% of thiamin loss in meat treated at 6 kGy. However, only 2.5% of riboflavin was lost on meat treated at doses < 3 kGy (Dionísio *et al*., 2009 and Fox *et al*., 1995). In fact, thiamin is the most radiosensitive water soluble vitamin due especially to the presence of hetero double bonds in the molecule (Molins, 2001). Riboflavin is more resistant to irradiation.

Table 5. Thiamin and riboflavin content in meat treated with different irradiation doses during storage.

	Thiamin, riboflavin content (mg/100 g of meat) [1]							
Samples	**Day 0**		**Day 7**		**Day 14**		**Day 28**	
	B_1	**B_2**	**B_1**	**B_2**	**B_1**	**B_2**	**B_1**	**B_2**
C	0.67 ± 0.02^{aD}	1.23 ± 0.10^{aB}	0.66 ± 0.07^{aB}	1.24 ± 0.07^{aB}	0.66 ± 0.02^{aC}	1.20 ± 0.04^{aC}	0.66 ± 0.02^{aC}	1.19 ± 0.14^{aCD}
I1	0.63 ± 0.00^{aCD}	1.19 ± 0.06^{aB}	0.59 ± 0.06^{aAB}	1.18 ± 0.10^{aB}	0.58 ± 0.05^{aB}	1.15 ± 0.04^{aBC}	0.60 ± 0.04^{aB}	1.11 ± 0.06^{aBC}
I1.5	0.57 ± 0.02^{aABC}	1.16 ± 0.04^{bB}	0.55 ± 0.00^{aAB}	1.14 ± 0.11^{abAB}	0.56 ± 0.02^{aB}	1.08 ± 0.06^{abAB}	0.54 ± 0.04^{aB}	1.06 ± 0.00^{aB}
I3	0.49 ± 0.02^{aA}	1.10 ± 0.02^{bA}	0.45 ± 0.09^{aA}	1.06 ± 0.08^{abA}	0.41 ± 0.09^{aA}	1.03 ± 0.04^{abA}	0.40 ± 0.01^{aA}	0.98 ± 0.01^{aA}
M	0.58 ± 0.01^{aBC}	1.25 ± 0.03^{aB}	0.57 ± 0.04^{aAB}	1.24 ± 0.07^{aB}	0.57 ± 0.03^{aB}	1.24 ± 0.07^{aC}	0.58 ± 0.03^{aB}	1.23 ± 0.05^{aD}
MI1	0.56 ± 0.05^{aABC}	1.21 ± 0.01^{aB}	0.54 ± 0.02^{aAB}	1.22 ± 0.02^{aB}	0.55 ± 0.01^{aB}	1.23 ± 0.03^{aC}	0.55 ± 0.04^{aB}	1.21 ± 0.02^{aCD}
MI1.5	0.53 ± 0.04^{aAB}	1.21 ± 0.06^{aB}	0.53 ± 0.02^{aAB}	1.22 ± 0.09^{aB}	0.53 ± 0.02^{aB}	1.14 ± 0.06^{aBC}	0.54 ± 0.04^{aB}	1.13 ± 0.07^{aBCD}
MI3	0.50 ± 0.04^{aAB}	1.19 ± 0.07^{aB}	0.44 ± 0.05^{aA}	1.16 ± 0.07^{aAB}	0.44 ± 0.02^{aA}	1.16 ± 0.02^{aBC}	0.42 ± 0.00^{aA}	1.14 ± 0.06^{aBCD}

[1] Within each row, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column, means with the same uppercase letter are not significantly different ($P > 0.05$).

The addition of marinade decreased significantly thiamin content from 0.67 mg/100 g for untreated meat to 0.58 mg/100 g for marinated meat. This is probably due to the presence of flavonoid compounds on the marinade mostly from curry (Ashokkumar, Selvaraj, & KM, 2013). In fact, thiamin is sensitive to irradiation and heating, and can also be destroyed by enzymatic factors such as thiaminase (Dwivedi & Arnold, 1973) and antagonist factors such as sulfur dioxide, alkali and flavonoids (Skurray, Perkes, & Duff, 1986). On the other hand, riboflavin content was not affected by marinade addition, probably due to its smallest water solubility as compared to thiamin (Prodanov, Sierra, & Vidal-Valverde, 2004). In fact, riboflavin is more radio-resistant and its instability is especially related to heating treatment and light exposure (Zhuge & Klopfenstein, 1986).

By combining irradiation treatment to marinating, thiamin content remains stable during irradiation treatment for all irradiation doses. These observations are very important because they highlighted the effect of the marinade to protect thiamine from irradiation due probably to its low pH and its high concentration with carbohydrate (Fox et al., 1995 and Molins, 2001).

Results in this study showed that during the whole storage, thiamin content was stable for both marinated and non-marinated samples. Riboflavin content was also stable for marinated and samples treated or not with irradiation. However, for non-marinated samples, riboflavin remains stable during 14 days afterwards riboflavin content decreased significantly ($P \leq 0.05$) on day 28 for samples treated by γ-irradiation at doses ≥ 1.5 kGy. Thus, the addition of marinade allowed protecting thiamin during irradiation treatment and increased riboflavin stability during storage. Combining marinating and irradiation seems to be a good compromise allowing at the same time insuring meat safety and protecting vitamins.

3.3.2. Lipid oxidation

Concentration of TBARS, which represents the malondialdehyde concentration in meat, is showed in Table 6. Results show that marinating treatment interfered with meat oxidation measurements inducing an increase of TBARS concentration. This is probably due to the presence of acetic acid in the marinade which promotes the oxidation of free polyunsaturated fatty acids present in pork (Toomik, Lepp, Lepasalu, & Püssa, 2012). The presence of carbohydrates in the marinade can also react with TBA inducing an interference with the measurement of TBARS in meat by forming N(carboxymethyl) lysine which is a marker of both glycoxidation and lipoxidation reactions (Fernández, Pérez-Álvarez, & Fernández-

López, 1997). To eliminate this interference, the content of TBARS in irradiated meat was compared to their respective control.

Table 6. Concentration of TBARS in meat samples during storage at 4 °C.

		TBARS values[1] (µg of MDA/10 g of meat)		
	Samples	**Day 0**	**Day 14**	**Day 28**
Group 1 : Non-marinated samples	**C**	66.2 ± 4.3^{aA}	77.6 ± 1.9^{bA}	65.6 ± 6.2^{aA}
	I1	61.4 ± 2.6^{aA}	87.8 ± 5.4^{bAB}	64.6 ± 2.6^{aA}
	I1.5	101.4 ± 5.3^{bB}	99.6 ± 4.2^{bBC}	65.5 ± 5.5^{aA}
	I3	116.2 ± 3.7^{bC}	114.2 ± 2.0^{bD}	62.1 ± 2.4^{aA}
Group 2 : Marinated samples	**M**	369.5 ± 35.4^{aA}	345.2 ± 25.9^{aA}	394.9 ± 15.5^{aB}
	MI1	375.5 ± 29.5^{aA}	352.3 ± 28.2^{aA}	352.5 ± 21.8^{aA}
	MI1.5	371.0 ± 31.7^{aA}	367.0 ± 37.9^{aA}	337.1 ± 34.7^{aA}
	MI3	374.7 ± 39.9^{aA}	369.7 ± 35.7^{aA}	357.5 ± 23.4^{aAB}

[1] Within each row, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column, means of each group (non-marinated or marinated samples) with the same uppercase letter are not significantly different ($P > 0.05$).

Results showed that for non-marinated samples, an irradiation dose of 1 kGy had no significant effect on lipid oxidation on day 0; however an irradiation doses ≥ 1.5 kGy increased significantly the TBARS values ($P \leq 0.05$) showing respectively the value of 101.4 and 116.2 µg of MDA per 10 g of meat on irradiated meat at 1.5 and 3 kGy respectively as compared to 66.2 µg of MDA per 10 g for untreated meat. During 14 days of storage, TBARS values remain stable in irradiated meat at 1.5 and 3 kGy as compared to a significant increase observed in untreated and irradiated meat at 1 kGy. Afterwards, TBARS values decreased for all non-marinated samples and reached 65.6, 64.6, 65.6 and 62.1 µg of MDA per 10 g of meat for treated meat at 0, 1, 1.5 and 3 kGy respectively, as a consequence of the interaction between MDA and proteins such as myosin, amino acids and glycogen (Fernández *et a*l. 1997). Similar observations were found also in previous studies (Babji *et al*., 1998 and de Azevedo Gomes *et al*., 2003).

Results obtained on marinated and irradiated meat showed no significant increase ($P > 0.05$) of the TBARS values nevertheless the irradiation doses. This is probably due to the presence of canola oil and curry in marinade components. An antioxidant activity is related to these

ingredients especially due to flavonoid composition such as myricetin and quercetin present in both of canola oil and curry powder which are effective protectors against lipid oxidation (Chen, Chan, Ho, Fung, & Wang, 1996). On day 28 of storage, marinated and irradiated meat treated at 1 and 1.5 kGy have less TBARS value than marinated meat, this could be related to a protective effect of marinade components during low irradiation doses treatment.

On the basis of these results, it has been shown that the addition of marinade might have protected polyunsaturated fatty acids against oxidation in meat during irradiation treatment and assured their stability during storage. Also, combining irradiation treatment at 1.5 kGy and marinade addition seems to be a good alternative to protect meat during irradiation process.

3.4. Color

The effects of marinating and irradiation on meat color are presented in Table 7. Results showed that for all treatments, an increase of L* value of meat during storage at 4 °C was observed. Nevertheless the treatment applied, L* values for all treatments were > 50 (light level). For untreated meat, an increase of L* value from 50.01 to 55.69 was observed during storage as compared to values from 53.06 to 61.63 for marinated meat. This slight increase was explained by a pH decrease and the accumulation of metabolic by-products during postmortem glycolysis and meat aging (Brewer, 2004). On the other hand, an irradiation treatment increased significantly the L* value on day 0 which reached 55.87, 51.7 and 57.24 for a respective irradiation doses of 1, 1.5 and 3 kGy. When marinade was combined to irradiation treatment, no significant difference ($P > 0.05$) of L* value was observed between marinated and marinated and irradiated samples at almost all irradiation doses during the whole storage (28 days).

Table 7. Color attributes of meat samples.

Samples		Day 0	Day 3	Day 7	Day 14	Day 21	Day 28
C	**L***	50.01 ± 1.81^{aA1}	51.91 ± 1.79^{bAB}	51.90 ± 2.11^{bAB}	54.12 ± 1.97^{cAB}	54.60 ± 2.02^{cdA}	55.69 ± 1.68^{dA}
	a*	3.71 ± 0.64^{aB}	3.87 ± 0.64^{aB}	3.82 ± 0.50^{aB}	3.79 ± 0.55^{aA}	4.23 ± 0.69^{bA}	4.24 ± 0.59^{bAB}
	ΔE*	-	1.72 ± 0.23^{aA}	1.78 ± 0.24^{aA}	3.64 ± 0.42^{bCD}	4.42 ± 0.69^{cB}	5.67 ± 0.99^{dA}
I1	**L***	55.87 ± 2.79^{abDE}	55.25 ± 3.46^{aC}	57.42 ± 2.57^{bD}	59.79 ± 2.09^{cC}	61.42 ± 2.19^{dD}	61.86 ± 2.08^{dE}
	a*	3.32 ± 0.52^{aA}	3.47 ± 0.56^{aA}	3.49 ± 0.62^{aA}	3.46 ± 0.55^{aA}	3.88 ± 0.57^{bA}	3.99 ± 0.54^{bA}

	ΔE*	-	1.55 ± 0.06^{aA}	1.53 ± 0.13^{aA}	3.95 ± 0.73^{bD}	5.76 ± 0.98^{cCD}	6.09 ± 1.09^{cAB}
I 1.5	**L***	51.70 ± 1.43^{aB}	52.46 ± 1.64^{abB}	52.76 ± 1.69^{bBC}	54.57 ± 1.90^{cAB}	56.40 ± 1.48^{dB}	58.01 ± 1.04^{eB}
	a*	4.66 ± 0.55^{abC}	4.28 ± 0.65^{aC}	4.31 ± 0.62^{aC}	4.62 ± 0.74^{abB}	5.06 ± 0.95^{bB}	4.92 ± 0.92^{bBC}
	ΔE*	-	1.56 ± 0.10^{aA}	1.54 ± 0.18^{aA}	3.02 ± 0.50^{bABC}	4.95 ± 0.78^{cBC}	6.33 ± 0.71^{dAB}
I 3	**L***	57.24 ± 1.59^{aE}	56.07 ± 4.27^{aC}	58.62 ± 1.25^{bD}	59.04 ± 1.37^{bC}	60.23 ± 1.53^{cCD}	61.57 ± 1.22^{dDE}
	a*	5.40 ± 0.52^{aD}	5.70 ± 0.67^{aE}	5.51 ± 0.63^{aE}	5.32 ± 0.78^{aCD}	5.60 ± 0.61^{aBC}	5.42 ± 0.91^{aCD}
	ΔE*	-	1.54 ± 0.24^{aA}	1.72 ± 0.23^{abA}	2,25 ± 0.43^{bA}	3.50 ± 0.45^{cA}	5.67 ± 0.68^{dA}
M	**L***	53.06 ± 2.99^{bC}	51.77 ± 2.62^{aAB}	53.57 ± 3.24^{abBC}	58.16 ± 2.56^{cC}	61.36 ± 1.86^{dD}	61.63 ± 1.61^{dDE}
	a*	3.09 ± 0.58^{aA}	3.67 ± 0.78^{abA}	3.81 ± 0.65^{bB}	4.76 ± 1.08^{cBC}	5.83 ± 1.15^{dC}	5.78 ± 1.02^{dD}
	ΔE*	-	1.75 ± 0.21^{aA}	1.78 ± 0.24^{aA}	5.35 ± 0.94^{bE}	9.06 ± 1.34^{cE}	9.33 ± 1.57^{cD}
MI 1	**L***	55.01 ± 2.40^{bcCD}	52.86 ± 3.27^{aB}	53.95 ± 2.62^{abC}	56.09 ± 2.83^{cB}	58.55 ± 2.46^{dBC}	59.94 ± 2.67^{eC}
	a*	3.70 ± 0.59^{aB}	4.30 ± 0.74^{bC}	4.30 ± 0.74^{bC}	5.62 ± 0.93^{cDE}	6.68 ± 1.04^{dD}	6.66 ± 1.08^{dE}
	ΔE*	-	1.72 ± 0.13^{aA}	1.78 ± 0.12^{aA}	3.23 ± 0.53^{bBCD}	6.23 ± 1.21^{cD}	7.97 ± 0.76^{cC}
MI 1.5	**L***	52.14 ± 1.91^{aBC}	51.63 ± 1.26^{aAB}	51.84 ± 1.88^{aAB}	55.03 ± 2.36^{bAB}	59.39 ± 2.92^{cC}	60.14 ± 2.65^{cCD}
	a*	4.63 ± 0.70^{aC}	5.05 ± 0.76^{bD}	5.08 ± 0.68^{bD}	6.14 ± 0.70^{cE}	7.47 ± 0.81^{dE}	7.36 ± 0.7^{dF}
	ΔE*	-	1.74 ± 0.22^{aA}	1.79 ± 0.06^{aA}	4.79 ± 0.86^{bE}	10.31 ± 0.96^{cF}	10.22 ± 0.87^{cD}
MI 3	**L***	52.97 ± 3.09^{aBC}	51.06 ± 3.85^{aAB}	53.02 ± 3.32^{aBC}	52.90 ± 3.29^{aA}	57.09 ± 2.44^{bB}	59.07 ± 2.12^{cBC}
	a*	5.13 ± 0.58^{aD}	5.69 ± 0.62^{bE}	5.63 ± 0.77^{bE}	6.18 ± 1.16^{bcE}	6.98 ± 1.17^{cDE}	7.01 ± 1.32^{cEF}
	ΔE*	-	1.72 ± 0.29^{aA}	1.76 ± 0.31^{aA}	2.76 ± 0.51^{bAB}	6.58 ± 1.11^{cD}	8.48 ± 1.56^{dCD}

[1] Within each row, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column, means with the same uppercase letter are not significantly different ($P > 0.05$).

Results obtained in this study showed that an irradiation treatment at 1 kGy reduced a* value on day 0 which signify greening of meat. When the irradiation dose was increased, a* value increased for both marinated and non-marinated samples and it was dose-dependent. These results suggest that with a low irradiation dose, green pigments are formed. However these pigments are not enough stable and by increasing the irradiation doses to 1.5 and 3 kGy, the green pigment was lost while the red pigment related to the heme pigment-CO ligand formation was formed and was more stable (Brewer, 2004 and Nam and Ahn, 2002). When

marinade was applied without irradiation, the redness of meat decreased from 3.71 for untreated meat to 3.09 for marinated meat from day 0. From day 14, a* values of marinated meat increased more than untreated meat and reached 5.78 on day 28 as compared to 4.24 for untreated meat. When the addition of marinade was combined to irradiation treatment, a significant increase of a* values ($P \leq 0.05$) was observed showing at day 28 a respective a* value of 6.66, 7.36 and 7.01 for marinated and irradiated meat at 1, 1.5 and 3 kGy respectively as compared to 3.99, 4.92 and 5.42 for non-marinated and irradiated meat treated with the same doses. As a consequence, the redness of marinated meat was more important than in non-marinated meat which could be related to the intrinsic color of the marinade and its interaction with meat during storage. The increase of the redness by irradiation is not always perceived as detrimental, because it makes irradiated meat look fresher.

Results of ΔE* showed that regardless of the treatment used, the ΔE* value increased during storage and became significant from day 14. When irradiation was applied alone, no significant difference of ΔE* was observed ($P > 0.05$) between untreated meat and almost of all samples treated at both doses during storage (28 days). It has been shown that combining marinating and irradiation increased the ΔE* values more than in non-marinated and in irradiated samples at the same doses. In fact, for non-marinated samples, ΔE* observed at day 28 was 5.67, 6.09, 6.33 and 5.67 for irradiated meat at doses of 0, 1 1.5 and 3 kGy respectively. However, when marinade was applied, ΔE* increased and reached 9.33, 7.97, 10.22 and 8.48 for respectively treated meat at doses of 0, 1, 1.5 and 3 kGy on day 28. This is due especially to a* values which were higher for marinated than non-marinated samples. Zhou *et al*. (2010) reported that irradiation treatment can alter meat color because of the inherent susceptibility of the myoglobin molecule to energy input by irradiation and then can result in the formation of brown, green and bright red colors. Thus, meat color can be a good indicator to determine the shelf-life of meat products.

3.5. Sensorial evaluation

A pre-test was carried out to evaluate the appreciation of the marinade with 155 untrained panelists. The obtained results have shown that more than 90% of panelists have appreciated the marinade.

Then, sensory analysis of marinated pork loin meat treated or not with irradiation at a dose of 1.5 kGy, was done by evaluating its color, texture, odor, flavor and global appreciation, using a 9-point hedonic scale and results are presented in Fig. 3. The irradiation dose of 1.5 kGy was selected on the basis of shelf-life results. Results show that γ-irradiation did not affect

significantly ($P > 0.05$) the color, the texture and the global appreciation of cooked pork loin meat. Indeed, no significant difference ($P > 0.05$) was observed between marinated meat and marinated and irradiated meat at 1.5 kGy showing a mean hedonic value of 7 "Like moderately" was evaluated for almost of all characteristics. Finally, no negative effect ("Dislike a little" or lower appreciation) was reported on the organoleptic properties of all samples. Previous studies demonstrated also that an irradiation treatment of 3 kGy under vacuum did not affect significantly the sensorial evaluation of pork, beef and turkey meat (Kim, Nam & Ahn, 2002). However, a significant irradiation odor was produced when irradiation treatment of pork meat packed under vacuum exceed 5 kGy. The intensity of irradiation odor was reduced during storage and no negative effect on the acceptance of meat was detected (Ahn, Jo, & Olson, 2000).

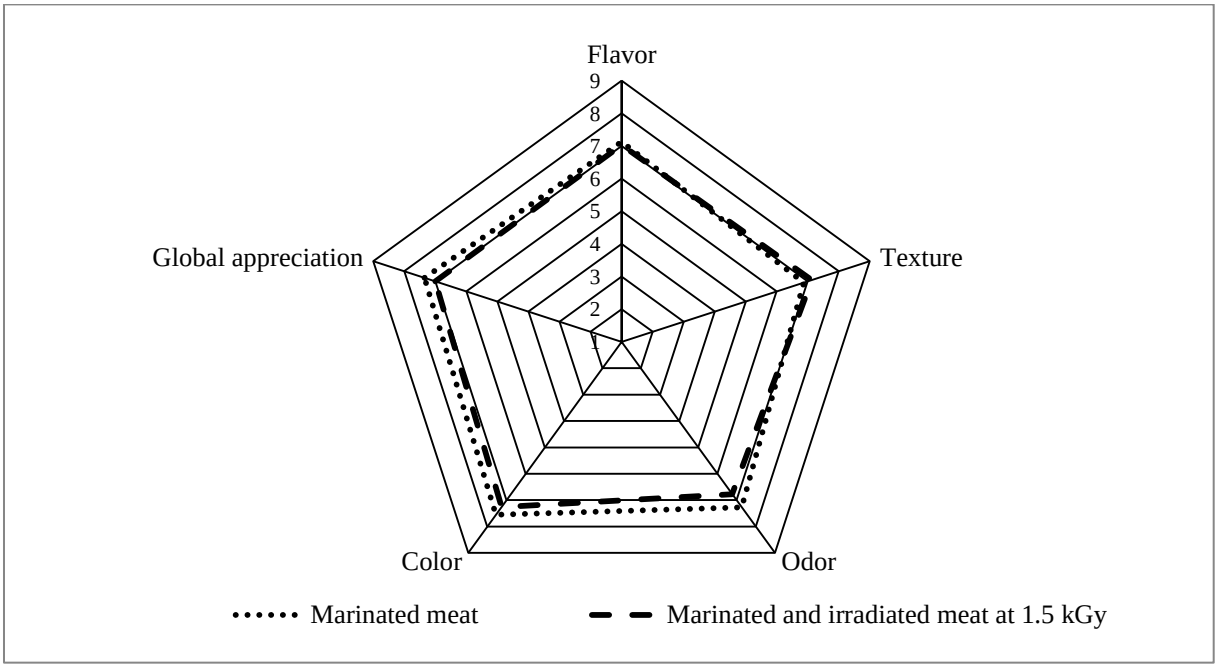

Figure 3. Effect of γ-irradiation on sensory properties of marinated meat refrigerated and packed under vacuum.

These observations confirm the obtained results of lipid oxidation (Section 3.3.2) because lipid oxidation is generally related to off-flavor and off-odor. In this case, an irradiation dose of 1.5 kGy had no effect on lipid oxidation. Hence, these results revealed that an irradiation treatment at a dose of 1.5 kGy provided very acceptable sensory attributes, with a good appreciation of flavor, of color, of texture, of odor and of global quality.

4. Conclusion

The results of this study clearly demonstrated that marinating can act in synergy with γ-irradiation when done under vacuum packaging of pork loins in order to assure food safety. A synergetic effect between irradiation and marinating was highlighted for their bacterial inactivation and shelf-life extension without affecting the nutritional and sensorial quality of pork meat. The use of marinating allowed preventing lipid oxidation during irradiation treatment and storage. Marinating and irradiation were also able to increase the redness of the meat. These results can be of great interest to food industry for food preservation and especially for immunocompromised patients who are condemned to eat foods with low nutritional values caused by the harsh decontamination processes. This work presents a new issue for this category of people by protecting them from foodborne diseases and by increasing the nutritional quality of food.

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CHAPITRE 3:

DEVELOPMENT OF POST-HARVEST TREATMENTS FOR EXTENDING QUALITY AND SHELF-LIFE OF MINIMALLY PROCESSED BROCCOLI FLORET (*BRASSICA OLERACEA L.*)

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Contribution des auteurs

Yosra Ben Fadhel a réalisé les manipulations et la rédaction de l'article.

Nassima Ziane a réalisé les analyses de la texture, perte de masse et couleur.

Stéphane Salmieri a participé au bon déroulement des analyses.

Monique Lacroix: Directrice de recherche, responsable scientifique et coordinatrice du projet de recherche, a participé à la planification des expériences et à la correction de l'article.

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RÉSUMÉ

Cette étude a été entreprise pour évaluer l'effet du prétraitement au chlorure de calcium ($CaCl_2$) combiné à l'enrobage à base d'alginate sur la qualité du brocoli prêt à manger. Les têtes de brocoli ont été prétraitées avec trois concentrations différentes de chlorure de calcium (1, 2.5 and 4 g L^{-1}), puis enrobés d'une formulation d'alginate (13 g L^{-1}). Ensuite, les brocolis PAM ont été stockés à 4 °C pendant 24 jours. L'analyse de la texture, la couleur, la perte de masse, le taux de respiration et du compte de la flore mésophile totale a été effectuée au cours du stockage aux jours 0, 3, 7, 14 et 24. L'étude a démontré que la combinaison de prétraitement avec le calcium et l'enrobage à base d'alginate a permis de réduire la perte de masse, le maintien de la couleur et de la texture et d'augmenter sa durée de vie jusqu'à 11 jours. Toutefois, l'application d'enrobage comestible sans prétraitement de calcium était plus efficace pour réduire le taux de respiration du brocoli conservé à 4 °C ce qui est très intéressant puisque le taux de respiration est directement relié au vieillissement du brocoli et à la perte de sa fraicheur.

Mots-clés: brocoli transformés; enrobage comestible ; prétraitement au calcium ; traitements post-récolte ; durée de conservation.

ABSTRACT

This study was undertaken to evaluate the effect of pretreatment with calcium chloride ($CaCl_2$) and alginate coating on the physico-chemical and microbial properties of ready-to-eat (RTE) broccoli. Broccoli florets were pretreated with three different concentrations of $CaCl_2$ (1, 2.5 and 4 g L^{-1}), then coated with an alginate formulation (13 g L^{-1}) and stored at 4 °C for 24 days. Texture, color, weight loss, respiration rate and total mesophilic flora (TMF) counts were evaluated during storage at days 0, 3, 7, 14 and 24. The study showed that combining pretreatment with calcium and alginate coating led to reducing the weight loss of broccoli, maintaining its color and texture as well as extending its shelf-life by 6 days. However, the obtained results showed that applying edible coating without calcium pretreatment was more effective to reduce the respiration rate of broccoli floret during storage at 4 °C which lead to maintain the broccoli quality for longer storage duration by slowing down ripening metabolism.

Keywords: Minimally processed broccoli floret, Edible coating, Calcium chloride pretreatment, Post-harvest treatments, Shelf-life.

1. INTRODUCTION

In recent years, demand on vegetables is continuously increasing especially in developed countries. According to Island and Scotia (2013), 40.6% of Canadians aged 12 and older, consumed fruits and vegetables five or more times per day. Furthermore, preprocessed fruit and vegetables consumption's is increasing marginally due to the change of consumer behaviours enhanced RTE fruits and vegetables including preprocessed vegetables, RTE salads, mixed vegetables, frozen vegetables and fruit (Ashton, Richards, Galatsanou, & Bollman, 2014). RTE fruits and vegetables are economically important commodities due to their convenience and healthiness. However, they are highly perishable as they contain 80-90% water by weight (Dhall, 2013). Postharvest decay is the major factor limiting the extension of storage life of many fresh harvested commodities because cutting processes enhance the discoloration caused by damaged cells and tissues, and lack of protective skin and dehydration (Watada & Qi, 1999). In addition, the cutting process generates a great loss of nutrients which accelerates the plant dehydration process, the increase in water activity and the accumulation of nutrients in the surface of the vegetables. This phenomenon stimulates bacterial growth and reduces shelf-life of vegetables. The food industry is currently in need of innovative processing technologies in order to meet consumer's demand of fresher and safer RTE vegetables without modifying the sensorial and the nutritional quality. Currently, chilling, addition of chemical preservation (antioxidants, acidulants, ethylene oxide and other synthetic additives) and storage under conventional modified atmosphere packaging (MAP) are the most frequently techniques used for maintaining quality of RTE vegetables (Artés & Allende, 2005). Nevertheless, many of them have shown inconvenient for use due to their potential health hazards (mainly carcinogenic effects). Chemical antimicrobials are able to delay microorganism's growth or to kill them. However, their bactericidal or fungicidal concentration exceed the tolerated concentrations by the regulatory authorities (Davidson & Taylor, 2007). In addition, consumers are concerned about the presence of synthetic chemical residues in the product.

Broccoli is one of the most perishable vegetables. Yellowing caused by either chlorophyll loss or blooming of the buds, is a common problem intimately related to ethylene concentration and temperature of storage. According to Zhuang, Hildebrand, and Barth (1995), chlorophyll loss is linked to lipid oxidation of cell membrane and to the antioxidant enzyme activity of each broccoli varieties especially the ratios of superoxide dismutase and peroxidase. The cut surface and damaged floret stems can turn black during storage due to the oxidation of

polyphenolic substrate in presence of oxygen and polyphenol oxidases. The plant injury induces the decompartimentation of vacuolar phenol and the entry of molecular oxygen into the cell cytoplasm to form quinone compound. Subsequent reactions of quinone lead to melanin accumulation characterized by a brown or black pigment often associated with "browning" in plant tissues. Development of off-odors can be a major concern when broccoli are MAP packed (Gross, Wang, & Saltveit, 2004). Temperature abuse promotes soft rot and mold growth. A storage condition of 0 °C and 98-100% of relative humidity allow the maintain of broccoli freshness for 2-3 weeks (Gross *et al*., 2004). However, when preprocessed, the shelf-life of broccoli becomes shorter and reaches 4 days when stored at 4 °C (Irkin, Degirmencioglu, & Guldas, 2015). The use of edible coatings in wide range of RTE fruits and vegetables is receiving great interest by food industry because of their efficiency to provide a selective barrier to moisture, O_2 and CO_2 which lead to the low respiration, retarding dehydration and loss weight, improving textural quality, helping retain volatile flavor compounds and chlorophyll and reducing microbial growth (Y. Fan *et al*., 2009). Calcium post-harvest treatment is an ancient method proving to have a significant impact on the shelf-life of various fruits and some vegetables. The benefits of applying $CaCl_2$ to fruit and vegetables include delaying aging and ripening, reducing postharvest decay, controlling the development of many physiological disorders and increasing the calcium content, thus improving their nutritional value (Martin-Diana *et al*., 2007). As a secondary messenger, calcium ion mediates a cascade of events leading to balanced growth and development (Aghdam, Hassanpouraghdam, Paliyath, & Farmani, 2012).

The objective of the present study was to investigate how $CaCl_2$ pre-treatment applied in combination with alginate coating can protect the quality and extend the shelf-life of fresh-cut broccoli florets when stored under air at 4 °C.

2. MATERIALS AND METHODS

2.1. Samples preparation

Broccolis were purchased from a local supermarket (IGA, Laval, QC, Canada) and cut into florets. Broccoli samples were divided into 5 groups: untreated broccoli (control), coated broccoli (EC), broccoli pretreated with $CaCl_2$ at 1, 2.5 or 4 g L^{-1}, and then coated. Samples were stored in transparent bags (Nasco, Whilpack®, Fort Atkinson, WI, USA) at 4 °C during 24 days (20 g per bag). The weight loss, the color and the texture of broccoli florets were evaluated at days 0, 3, 7, 14, 22 and 24. The evaluation of the respiration rate of broccoli was

also done during storage in desiccator 150 mm, Nalgene®, (VWR, Mont-Royal, QC, Canada) at 4 °C during 24 days. The measurements were performed on day 0, 3, 7, 14, 22 and 24.

2.2. Coating formulation

Alginate (Sigma, Oakville, ON, Canada) 1.3 g, was solubilized in 100 mL of water under vigorous magnetic stirring until total dissolution. Then, glycerol 16 g L^{-1} used as a plasticizer and 0.25 g L^{-1} of tween 80 used to improve the adherence of the polymer were added. The mix was homogenized at 2,300 rpm for 10 min with a high shear mixer (IKA T25 digital Ultra-Turrax disperser, IKA Works Inc., Wilmington, NC, USA).

2.3. Sample treatment

For coated samples, broccoli were dipped in alginate solution for 30 s, kept to dry under laminar flow hood for 30 min, immerged in 15 gL^{-1} of $CaCl_2$ solution for 30 s and dried under laminar flow hood for 15 min. For pretreated samples with $CaCl_2$, broccoli were dipped for 30 s in 1, 2.5 or 4 g L^{-1} of $CaCl_2$ solution, dried under laminar flow hood for 15 min, and coated with 13 g L^{-1} of alginate then 15 g L^{-1} of $CaCl_2$ as described above. For untreated samples, broccoli was put directly in bags or desiccators without any treatments.

2.4. Weight loss determination

Broccoli florets for all groups were weighed during the storage period and weight loss (WL) was calculated from initial weight (Wi) and weights of each storage day (Wt); as detailed in Equation (1):

$$WL\ (\%) = ((Wi-Wt)) / Wi * 100 \qquad (Eq.1)$$

Where:

Wi: initial weight, Wt: weight in day t, WL: weight loss

All results, all weight losses were expressed as WL (%) per 100 g of broccoli.

2.5. Respiration rate

In order to measure the respiration rate, 3 broccoli florets of total weight 60 ± 3 g placed in a hermetically closed desiccator equipped with septum. The measurement was carried out using 6600 Headspace oxygen / carbon dioxide analyzer (Systech Inc., Illinois, USA). Desiccators

were refrigerated prior to analyses day. Results were expressed as respiration rate of the samples in terms of CO_2 generation and O_2 consumption in mg $kg^{-1}day^{-1}$ according to Equation (2) and (3) respectively (Bhande, Ravindra, & Goswami, 2008).

$$RCO_2 = [((GCO_2)t_1 - (GCO_2)t_2)/\Delta t]\ V_{fr} / W \qquad (Eq.2)$$

$$RO_2 = [((GO_2)t_1 - (GO_2)t_2)/\Delta t]\ V_{fr}/ W \qquad (Eq.3)$$

Where:

RO_2: respiration rate, mL $[O_2]$ kg^{-1} day^{-1}

RCO_2: respiration rate, mL $[CO_2]$ kg^{-1} day^{-1}

GO_2: oxygen concentration, decimal

GCO_2: carbon oxide concentration, decimal

V_{fr}: free volume of the respiration chamber, mL

W: mass of broccoli, kg

Δt: time difference between two gas measurements, days

2.6. Texture analysis

The texture analysis of broccoli was performed using texturometer Stevens-LFRA (model TA-1000, Texture Technologies Corp., Scarsdale, NY, USA). The stem of broccoli (10 mm thick with a flat surface) was used for penetration force measurement. A needle probe was used with lowering speed and distance of penetration of 2 mm s^{-1} and 10 mm, respectively. The instrument was calibrated with 500 g before starting the measurements. The maximum penetration force in broccoli stem was recorded. Results were then expressed in Newton.

2.7. Color analysis

Broccoli color analysis was carried out using a Minolta Colorimeter Color reader CR10 (Konica Minolta sensing, Inc., Mahwah, NJ, USA.) during storage at 4 °C. 6 measurements on 4 preselected locations at the floret surface of each sample were assessed. L* (lightness, black=0, white=100), H* (0°= red-purple; 90°= yellow; 180°= bluish-green; and 270°= blue) and C* (chroma) which indicates the intensity or color saturation; were quantified.

2.8. Shelf-life estimation

All broccoli florets (20 ± 1 g each) were prewashed with 0.2 g L^{-1} chlorine, and then dried under laminar hood to reduce the initial TMF. Three different groups were studied (C: untreated broccoli; EC: alginate coated broccoli; 4 g L^{-1} $CaCl_2$+EC: pretreated broccoli with 4 gL^{-1} $CaCl_2$ then alginate coated). Calcium (4 g L^{-1}) pretreatment was selected as it was the most efficient pretreatment in order to preserve the firmness and the weight loss (only 1 % of weight loss at day 24). All samples were distributed into Whirl-Pak™ Sterile Filter Bags (one floret of 20 ± 1 g per bag). Initial broccoli TMF concentration was analyzed during 24 days of storage at 4 °C. On each day of analysis, Tryptic Soy Agar was used and inoculated plates were incubated at 37 °C for 48 h. Results were expressed as bacterial count (log CFU g^{-1}) during storage at 4 °C. TMF growth rate was also calculated over 24 days. Equation (4) was used to describe the growth bacteria (Y) over time.

$$Y = X \exp(\mu t) \quad \text{(Eq.4)}$$

Where X is the initial population, μ; the growth rate of TMF between day 0 and 24 (Ln CFUg^{-1} day^{-1}) and t; the number of storage days.

2.9. Statistical analysis

Each experiment was done in triplicate. Analysis of variance (ANOVA), Duncan's multiple range tests for equal variances and Tamhane's test for unequal variances were performed for statistical analysis using SPSS 18.0 software (SPSS Inc, USA). Differences between means were considered significant when the confidence interval was lower than 5 % ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Respiration rate

The respiration rate RO_2 (Fig.1a) and RCO_2 (Fig.1b) during storage showed that high respiratory activity in broccoli florets, irrespective of treatment, was found during the first day of storage. This effect was attributed to wound response to cutting (Rushing, 1990). Wounding induces a high ethylene production rates, which may stimulate respiration and consequently accelerate deterioration and senescence in vegetative tissues and promote ripening of climacteric fruit and vegetables (Fonseca, Oliveira, & Brecht, 2002). For untreated broccoli, a respiration rate peak was observed on day 5; afterwards, respiration rate decreased significantly. The observed behavior characterizes climacteric vegetables. Moreover,

respiration rates of all coated samples (with and without calcium pretreatments) were significantly lower ($P \leq 0.05$) than untreated samples during the whole storage under air at 4 °C. Therefore, alginate coating has the potential to decrease significantly the respiration rate of fresh-cut broccoli florets ($P \leq 0.05$). This could be related to a formation of an internal modified atmosphere with substantial changes on the CO_2/O_2 balance allowing a reduction in broccoli metabolism and then a reduction of the accumulation of respiratory metabolites occurred in the coated florets during storage. Similarly, X. Fan and Mattheis (2000) observed a reduction on respiration rate of broccoli stored at 10 °C when a 1-Methylcyclopropene treatment was applied. Studies on fresh-cut apples demonstrated that alginate coating allowed at least 53% reduction of RCO_2 as compared to 66% reduction obtained in the current study, on day 14 when broccoli florets were coated with alginate. The effectiveness of the alginate coating can also be due to its high degree of glucuronic residues (67%) which directly affects the reactivity with cations such as Ca^{2+}, and then gives better barrier properties. On the other hand, by combining calcium pretreatment and alginate coating, the effectiveness of alginate coating was reduced and the respiration rate increased as compared to coated samples without calcium pretreatment. The effect of calcium pretreatment on respiration rate depends on vegetables. For apples, calcium pretreatment, applied alone or in combination with coating, was efficient to reduce respiration rate (Zheng, Chun, Hong, & Zang, 2014), however, it has no effect on tomatoes (Aghdam *et al*., 2012).

On the basis of these results, it can be noted that, alginate coating used alone or in combination with calcium pretreatment allowed reducing the respiration rate of broccoli floret during storage at 4 °C. $CaCl_2$ pretreatment increased significantly ($P \leq 0.05$) the respiration rate but it still less than untreated broccoli. The respiration rate is a good indicator of vegetables decay. The shelf-life of vegetables is inversely correlated to their respiration rate: if it is high, vegetables quickly consumes their reserves and evolves into senescence. Broccoli has an extremely high respiratory activity (> 60 mg CO_2 $kg^{-1}h^{-1}$ at 5 °C) (Saltveit, 2004). Thus, to extend its shelf-life, the respiratory activity should be reduced.

3.2. Weight loss

Broccoli weight loss (Fig.2) showed that untreated broccoli had the maximum weight loss as compared to all other treatments. The coated broccoli without calcium pretreatment showed a weight loss of only 1.9 % and 7.9% in day 3 and 24 respectively, as compared to 5.2% and 11.6% weight loss for untreated broccoli. Alginate coating provided a good barrier to water

vapor due to crosslinking of the polymer chain of the alginate by calcium (Zhang, Ma, Critzer, Davidson, & Zhong, 2015). Nevertheless the treatment applied, a significant increase of weight loss was observed during the whole storage ($P \leq 0.05$) due especially to respiration and transpiration. During the respiration process, broccoli release water and carbon dioxide via oxidation of carbohydrates which reduces the dry matter content (Zhan, Hu, Li, & Pang, 2012). However, the rate of weight loss was found to be influenced by the applied treatments. No significant difference ($P > 0.05$) was observed between coated broccoli with or without calcium pretreatment (1 and 2.5 g L^{-1}) on day 7 and 14. Afterward, the weight loss of coated broccoli increased more than calcium pretreatment and coated samples. The treatment with $CaCl_2$ allowed a significant reduction on weight loss of broccoli floret as compared to untreated and coated samples without calcium. 4 g L^{-1} of $CaCl_2$ pretreatment was the most efficient and presented the minimum weight loss on all analyses days as compared to 1 and 2.5 g L^{-1} of $CaCl_2$ pretreatment. These results suggest that applying $CaCl_2$ pretreatment enhance the reduction of the weight loss of broccoli and it was dose-dependent. Hernández-Muñoz, Almenar, Ocio, and Gavara (2006) demonstrated also that combining calcium pretreatment with chitosan coating is efficient to reduce weight loss of strawberries during storage at 20 °C. Weight loss is a good indicator of broccoli quality and it has a direct effect on cost-effectiveness and marketability of the final product. It's intimately related to respiration process. However the obtained results for respiration rate demonstrated that alginate coating allowed the least respiration rate. These observations suggest that the weight loss of coated samples was probably related to the desiccation of edible coating thin films and not related to loss of broccoli inherent weight. Calcium pretreatments allowed protecting the edible coating film from dehydration during storage at 4 °C. Kester and Fennema (1986) explained that polysaccharide polymers act as a sacrificial moisture barrier to the atmosphere, so that the moisture content of the coated food could be maintained. This reduction in weight loss can also be explained by the calcium cross-linking effects in the cellular matrix, which affects the water permeability of the cell structures. Calcium ions can influence on the active water transport through the cell membrane (Castelló, Igual, Fito, & Chiralt, 2009). On the basis of these results, it can be noted that, combining alginate coating with calcium pretreatment allowed reducing the weight loss of broccoli floret during storage at 4 °C.

3.3. Texture

Measurements of the penetration force of broccoli stems (Table.1) showed that on day 0 no significant difference ($P > 0.05$) was observed between untreated, coated and pretreated

broccoli with 1 g L^{-1} of $CaCl_2$. A significant increase of firmness was observed when $CaCl_2$ concentration was ≥ 2.5 g L^{-1} during the first 3 days of storage. Afterwards, $CaCl_2$ do not have effect on firmness during storage. During storage at 4 °C, the firmness of untreated and coated broccoli increased significantly ($P \leq 0.05$) from respectively 3.7 N and 3.9 N on day 0 to 4.5 N for both of treatments on day 24. Similarly, Serrano, Martinez-Romero, Guillén, Castillo, and Valero (2006) found that the shear force of untreated broccoli increased during 28 days due to the increase of the elasticity of the broccoli which signify lower quality. The increase of the elasticity was the result of the weight loss since tissue dehydration and tissue became more fibrous during storage and then more elastic (Serrano *et al*., 2006). Thus, pretreatment with $CaCl_2$ stabilizes the texture during storage. In fact calcium already exists as calcium pectate in the cell wall cementing the structure of the plant cell. The loss of calcium leads to softening of the vegetables. Pretreatment with calcium delay softening and senescence of vegetables by crosslinking between polygalacturonide chains and calcium in cell wall (Rahman, 2007). This is not surprising as a lower respiration rates and weight loss obtained for pretreated broccoli with $CaCl_2$ correlate with firmness and membranes stability. Moreover, the higher respiration rates found in the control, maybe associated with senescence, but also to a slight increase of microbial growth (Silveira, Aguayo, Chisari, & Artés, 2011).

On the basis of these results, it can be noted that, combining alginate coating with calcium pretreatment allowed maintaining the freshness texture of the broccoli floret during the whole storage at 4 °C.

3.4. Color

The effects of alginate coating when applied alone or in combination with calcium pretreatment on broccoli floret color (Table.2) showed that for all treatments, a significant increase of L* value of broccoli floret during storage at 4 °C was observed ($P \leq 0.05$). Nevertheless the treatment applied, L* values for all treatments were < 42. For untreated broccoli, an increase of L* value from 41 to 42 was observed during storage as compared to values from 38 to 40 for coated broccoli. For broccoli pretreated with $CaCl_2$, the L* value increased also from 36 to 41, 37 to 40 and 37 to 39 respectively for 1, 2.5 and 4 g L^{-1} of $CaCl_2$. Similar results were reported by Fernández-León, Fernández-León, Lozano, Ayuso, and González-Gómez (2013). Results of Hue angle showed that for untreated broccoli the Hue angle decreased significantly ($P \leq 0.05$) during storage at 4 °C from a value of 122 on day 0 to 118 on day 24. This reflects the transition from green color to yellow, in parallel with

an increase in chroma from 15 on day 0 to 18 on day 24, reflecting the loss of the grayish green color of these samples, and an increase in lightness. Similar observations were reported by Vasconcelos and Almeida (2002). Yellowness is generally related to senescence which is characterized by chlorophyll degradation during storage (Rai, Jha, Wanjari, & Patil, 2009). The alginate coating, applied alone or in combination with calcium pretreatment on broccoli floret, decreased significantly the Hue angle on day 0 which varied from 122.4 for untreated broccoli to 120.3, 119.9, 119.2 and 119.2 for coated and pretreated broccoli with 1, 2.5 and 4 g L^{-1} of $CaCl_2$, respectively (Table.2). Indeed, as presented in Fig. 3, no yellowing was observed on samples. Thus, broccoli florets could be considered as visually acceptable for consumption. Those results suggest that calcium pretreatment and alginate coating did not induce major changes on broccoli florets. During storage, no significant difference on H* value was observed during the whole storage for all treated broccoli. This suggest that, applying alginate coating allowed protecting greening of broccoli floret and then, reducing the chlorophyll losses. Results of C* value showed a significant increase of C* value for untreated broccoli during storage. A value of 15 on day 0 and 18 on day 24 were observed. Applying alginate coating had permitted the stabilization of C* value during the whole storage. The combination of alginate coating with 1 and 2.5 g L^{-1} of calcium pretreatment allowed to maintain the C* value during storage ($P > 0.05$). However, when $CaCl_2$ concentration was > 2.5 g L^{-1}, C* value decreased significantly during storage ($P \leq 0.05$) and varied from 17 on day 0 to 15 on day 24. On the basis of these results, it can be noted that, applying alginate coating alone or combined to calcium pretreatment at concentration ≤ 2.5 g L^{-1} allowed maintaining the fresh green color of broccoli floret during the whole storage at 4 °C.

3.5. Microbial analysis

Shelf-life limit was considered at the limit of acceptability when TMF count reached the current authorities regulation level of 10^7 CFU g^{-1} (MAPAQ, 2009). TMF growth in broccoli samples during storage at 4 °C (Fig.3) showed that the TMF count on surfaces of broccoli florets increased during storage for all treatments and the rates of increase were greater for the untreated broccoli. The limit of acceptability of MAPAQ for untreated and coated broccoli was reached respectively at day 5 and 6. However, when alginate coating was applied in combination with $CaCl_2$ pretreatment, the shelf-life was increased by 6 more days and showed a shelf-life of 11 days. The growth rate was also lower in treated samples showing 0.30 and 0.43 Ln CFU g^{-1} day^{-1} respectively for coated samples and for pretreated with $CaCl_2$ and

coated samples as compared to 0.51 Ln CFU g^{-1} day^{-1} for untreated samples (Table.3). These observations suggest that when alginate coating was applied alone, no significant effect on the shelf-life duration was observed as compared to untreated broccoli. However, applying calcium pretreatment seems to have a significant effect allowing the extension of RTE broccoli shelf-life. Similarly, Izumi and Watada (1994) demonstrated a significant reduction on TMF on carrot pretreated with $CaCl_2$ at 10 g L^{-1} starting from day 14 of storage nevertheless the storage temperature. The inhibitory effect of calcium was explained as the result of tissue resistance increasing to bacterial infection rather than to a bactericidal action. The inhibitory effect could be also related to the cytosolic calcium concentration increment which enhance the synthesis of phytoalexins and phenolic compound and then a decrease of the activity of pathogenic pectolytic enzymes (Madani & Forney, 2015). Several diseases and disorders are controlled or prevented by calcium and other divalent ion such as bacterial and fungal growth in potato and carrot and the increase of mold incidence in cucumber (Rahman, 2007).

4. CONCLUSION

The results of this study clearly demonstrated that alginate coating can act in synergy with calcium pretreatment of broccoli floret in order to maintain its quality during storage. Applying alginate coating alone or in combination with calcium pretreatment allowed maintaining the firmness and the green color during storage at 4 °C. Treatments allowed also the reduction of the respiration rate and the loss of weight which is very beneficial for food industry. The shelf-life of treated broccoli was extended by 6 days when compared to that of the control. This work can be of great interest to food industry for assuring quality of RTE vegetables during storage.

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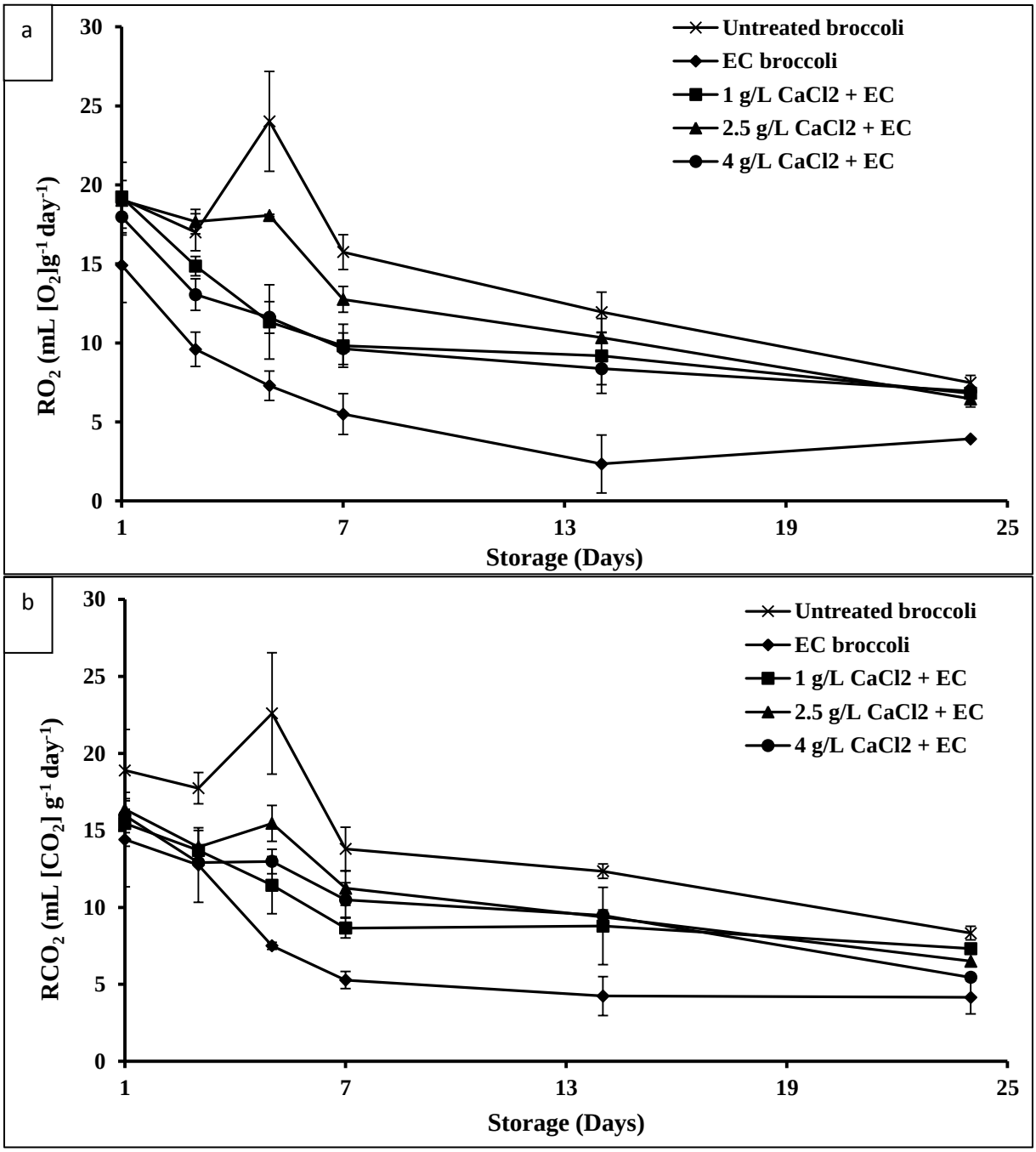

Figure 1. Effect of treatments on RO_2 (a) and RCO_2 (b) of broccoli floret stored at 4 °C[*].

[*] EC: edible alginate based coating.

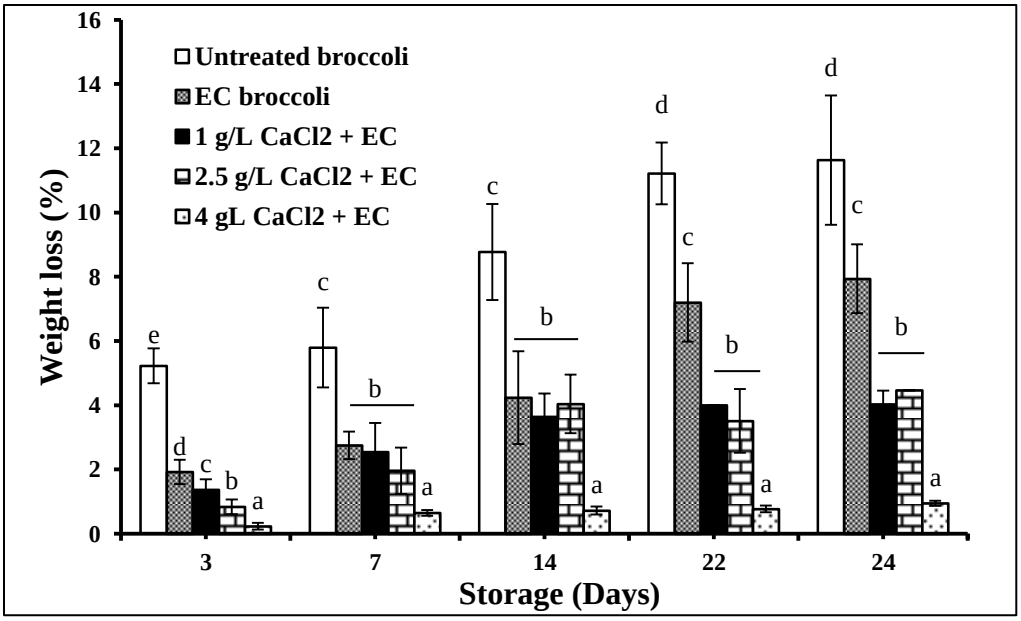

Figure 2. Effect of treatments on weight loss of broccoli florets stored at 4 °C[*].

[*] EC: edible alginate based coating.

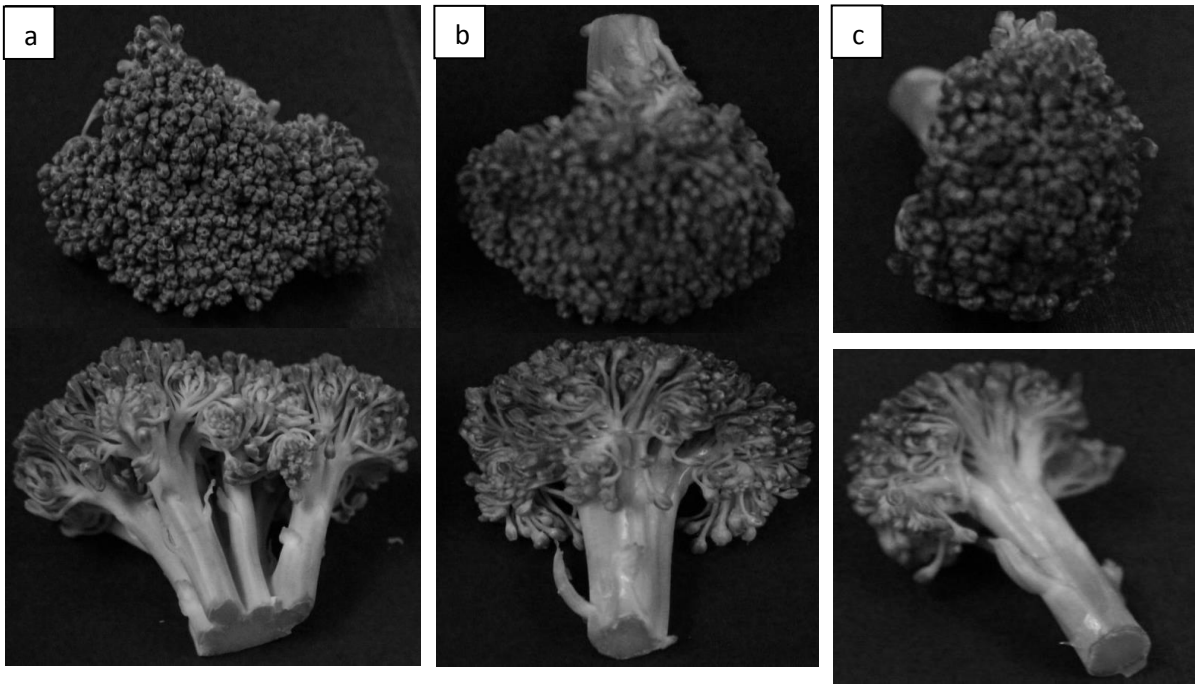

Figure 3. Appearance of broccoli florets before and after treatments: Untreated broccoli (a), EC broccoli (b), 4 g L^{-1} of $CaCl_2$ + EC (c)*.

* EC: edible alginate based coating.

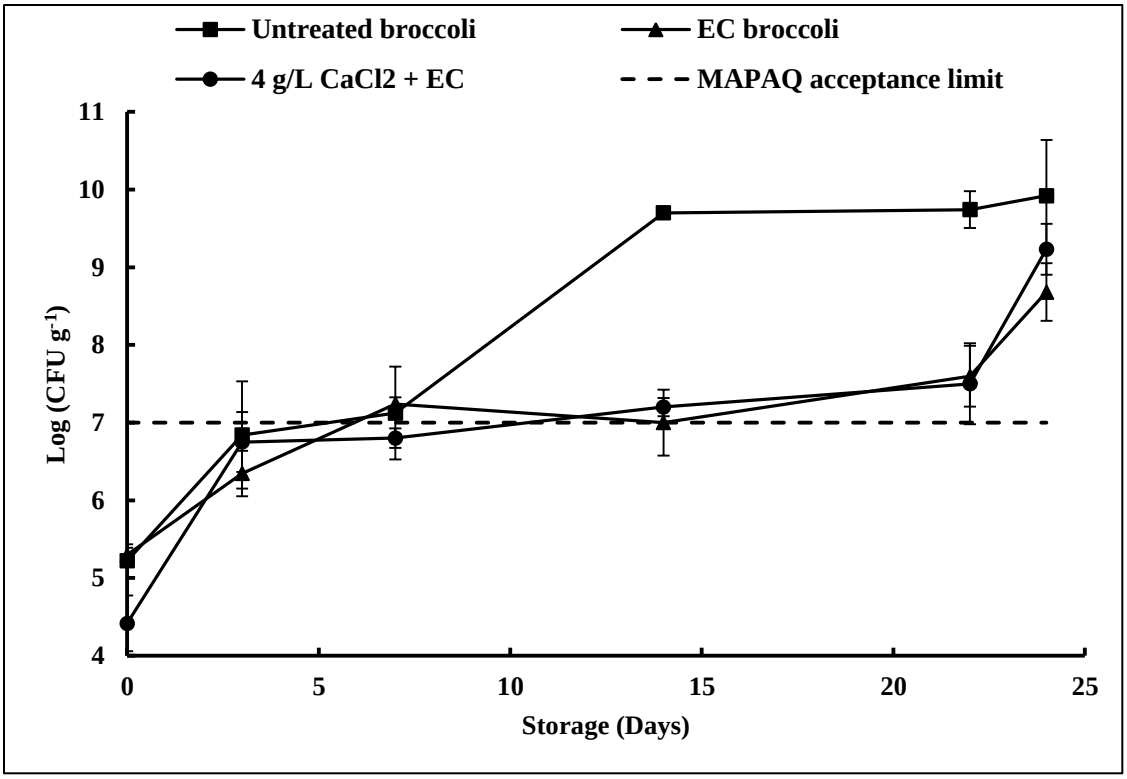

Figure 4. Effect of combined treatments on the TMF counts in broccoli florets stored at 4 °C*.

* EC: edible alginate based coating.

Table 1. Effect of treatments on broccoli florets texture stored at 4 °C.

	Maximum penetration force (N)[1]				
Samples [2]	**Day 0**	**Day 3**	**Day 7**	**Day 14**	**Day 24**
Untreated broccoli	3.74 ± 0.20^{aA}	3.70 ± 0.11^{aA}	4.06 ± 0.24^{bA}	4.10 ± 0.16^{bA}	4.53 ± 0.10^{cB}
EC broccoli	3.89 ± 0.33^{aAB}	3.91 ± 0.31^{aAB}	4.06 ± 0.11^{abA}	4.30 ± 0.35^{bcA}	4.50 ± 0.29^{cB}
1 g L^{-1} $CaCl_2$+ EC	4.00 ± 0.31^{aAB}	4.02 ± 0.23^{aB}	4.20 ± 0.05^{aA}	4.26 ± 0.07^{aA}	4.34 ± 0.24^{aAB}
2.5 g L^{-1} $CaCl_2$+ EC	4.06 ± 0.26^{aB}	4.06 ± 0.20^{aB}	4.10 ± 0.34^{aA}	4.18 ± 0.23^{aA}	4.21 ± 0.13^{aA}
4 g L^{-1} $CaCl_2$+EC	4.12 ± 0.33^{aB}	4.12 ± 0.25^{aB}	4.13 ± 0.20^{aA}	4.12 ± 0.14^{aA}	4.13 ± 0.16^{aA}

[1] Within each row, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column means with the same uppercase letter are not significantly different ($P > 0.05$).

[2] EC: edible alginate based coating

Table 2. Color attributes of broccoli floret samples stored at 4 °C[1].

Samples[2]		Day 0	Day 3	Day 7	Day 14	day 22	Day 24
Untreated broccoli	H*	122.44±3.85^{cdB}	122.73±3.89^{dB}	120.66±2.82^{bcA}	119.49±3.30^{abA}	119.77±2.81^{abA}	118.29±4.11^{aA}
	L*	40.59±1.91^{abC}	40.36±1.57^{aB}	40.82±2.31^{abB}	41.27±2.08^{abB}	41.05±1.68^{abB}	41.58±2.30^{bD}
	C*	14.96±1.50^{aA}	14.77±1.47^{aAB}	15.16±1.46^{abA}	16.21±1.35^{bcB}	17.02±1.68^{cdB}	17.80±1.78^{dB}
EC broccoli	H*	120.33±2.72^{aA}	121.03±2.98^{aA}	119.70±3.47^{aA}	121.31±2.82^{aA}	120.75±1.96^{aA}	120.91±3.45^{aC}
	L*	37.49±2.41^{aB}	38.73±1.87^{abA}	37.68±1.31^{aA}	39.85±1.37^{bcA}	40.06±1.80^{bcAB}	40.22±2.36^{cBC}
	C*	14.81±1.38^{aA}	14.34±1.31^{aA}	14.37±1.25^{aA}	14.42±1.06^{aA}	15.19±0.68^{aA}	15.22±1.59^{aA}
1 g L^{-1} $CaCl_2$+EC	H*	119.92±3.25^{aA}	121.46±3.66^{aA}	119.91±3.44^{aA}	120.68±3.08^{aA}	119.74±4.54^{aA}	119.07±3.36^{aAB}
	L*	35.97±1.71^{aA}	38.22±2.16^{bA}	38.19±1.75^{bA}	39.38±2.07^{bcA}	39.34±1.91^{bcA}	40.67±3.09^{cCD}
	C*	15.80±1.41^{aB}	15.36±1.17^{aBC}	15.06±1.08^{aA}	15.12±1.08^{aAB}	15.25±0.98^{aA}	15.79±1.05^{aA}
2.5 g L^{-1} $CaCl_2$+EC	H*	119.23±4.31^{aA}	120.10±4.53^{aA}	119.83±3.33^{aA}	120.54±3.28^{aA}	119.78±4.37^{aA}	119.15±3.99^{aAB}
	L*	37.03±3.31^{aAB}	38.99±2.40^{bA}	38.91±1.80^{bA}	39.65±1.85^{bA}	39.10±2.92^{bA}	39.46±2.19^{bAB}
	C*	16.16±1.48^{aBC}	15.88±1.49^{aC}	15.43±0.34^{aA}	15.43±1.25^{aAB}	15.31±1.12^{aA}	15.61±1.55^{aA}
4 g L^{-1} $CaCl_2$+EC	H*	119.19±3.32^{aA}	120.34±3.43^{aA}	120.55±3.83^{aA}	120.57±3.34^{aA}	119.32±2.71^{aA}	119.94±3.51^{aBC}
	L*	36.89±2.62^{aAB}	38.48±2.04^{bA}	38.35±2.13^{bA}	39.28±1.62^{bA}	39.53±2.02^{bA}	39.11±2.18^{bA}
	C*	16.83±1.40^{bC}	16.08±1.63^{abC}	15.45±1.44^{aA}	15.28±1.12^{aAB}	15.64±1.00^{abA}	15.36±1.58^{aA}

[1]Within each row, means with the same lowercase letter are not significantly different ($P > 0.05$). Within each column means with the same uppercase letter are not significantly different ($P > 0.05$).

[2] EC: edible alginate based coating.

Table 3. Growth rate of TMF in refrigerated broccoli florets.

Samples	TMF growth rate (Ln CFU g^{-1} day^{-1})
Untreated broccoli	0.51
EC	0.30
4 gL^{-1} $CaCl_2$ + EC*	0.43

* EC: edible alginate based coating.

CHAPITRE 4:

ACTIVE EDIBLE COATING AND γ-IRRADIATION AS COLD COMBINED TREATMENTS TO ASSURE THE SAFETY OF BROCCOLI FLORETS (*BRASSICA OLERACEA L.*)

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Contribution des auteurs

Yosra Ben Fadhel a réalisé les manipulations et la rédaction de l'article.

Sabrina Saltaji a réalisé les analyses de CMI.

Stéphane Salmieri a aidé pour le bon déroulement des expériences.

Dang Khanh Vu a aidé pour le bon déroulement des expériences.

Monique Lacroix: Directrice de recherche, responsable scientifique et coordinatrice du projet de recherche, a participé à la planification des expériences et à la correction de l'article.

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RÉSUMÉ

L'activité antimicrobienne des huiles essentielles (HEs), des acides organiques (AOs) et de la natamycine un antifongique naturel produit pendant la fermentation de la bactérie *Streptomyces natalensis* a été évaluée pour leur pouvoir à inhiber la croissance de quatre agents pathogènes (*E. coli, L. monocytogenes, S.* Typhimurium et *A. Niger*) et flore mésophile totale sur le brocoli. La concentration minimale inhibitrice (CMI) de chaque antimicrobien a été évaluée et caractérisée selon leur efficacité à éliminer chacune des espèces microbiennes testée afin de sélectionner les agents antimicrobiens les plus efficaces. Ensuite, pour évaluer les effets interactifs entre les meilleurs antimicrobiens présentant la plus faible MIC, une étude de synergie a été réalisée. La formulation antimicrobienne la plus efficace montrant des effets synergiques ou additifs a ensuite été encapsulée dans une matrice d'alginate afin de protéger son efficacité antimicrobienne pendant le stockage. L'efficacité de la formulation a été évaluée *in situ* en utilisant le brocoli comme modèle alimentaire. Un traitement combiné (γ-irradiation+ enrobage antimicrobien) a également été réalisé afin d'évaluer l'effet de possible synergie entre les traitements. Les résultats de cette étude ont permis la sélection de 4 huiles essentielles, un acide organique et la natamycine comme un antifongique pour leur faible concentration minimale inhibitrice. L'évaluation des effets interactifs antimicrobiens entre eux a permis la sélection d'une formulation antimicrobienne composée de 300 ppm de huile essentielle lemongrass, 5000 ppm de diacétate de sodium et 40 ppm de natamycine qui présentent un effet additif contre *A. Niger, E. coli et S.* Typhimurium et montrant l'effet synergique contre *L. monocytogenes*. La formulation antimicrobienne a été immobilisée en alginate et évaluée *in situ* sur le brocoli après irradiation à 0,4 ou 0,8 kGy. Les résultats ont montré un effet synergique entre l'enrobage antimicrobien et l'irradiation-γ pour réduire *E. coli, L. monocytogenes, S.* Typhimurium, et *A. Niger* pendant le stockage à 4 °C.

Mots clés: huile essentielle; pathogènes du brocoli ; interaction entre les antimicrobiens ; enrobage comestible antimicrobien ; irradiation-γ.

ABSTRACT

The antimicrobial activity of essential oils (EOs), organic acids (OAs) and natamycin, a natural antifungal produced during fermentation by the bacterium *Streptomyces natalensis*, was assessed against four pathogens (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Aspergillus niger*) and broccoli total mesophilic flora (TMF). The Minimum Inhibitory Concentration (MIC) of each antimicrobial (AM) was assessed to determine their efficiency on tested microbial species in order to select the most efficient. Then, the interactions between different antimicrobial compounds showing the lowest MIC were determined by the checkerboard method. The most effective antimicrobial formulation showing synergistic or additive effects was then encapsulated in an alginate matrix to protect the antimicrobial efficiency during storage. The effectiveness of the formulation was then evaluated in situ using broccoli as a food model. A combined treatment of coating and γ-irradiation was also done in order to evaluate the possible synergistic effect between treatments. The results of this study allowed the selection of 4 EOs, one organic acid (OA) and the natamycin as an antifungal agent exhibiting lower MIC values. The interactive antimicrobial effects between them showed that an antimicrobial formulation composed of 300 ppm of lemongrass EO, 5,000 ppm of sodium diacetate and 80 ppm of natamycin resulted in an additive effect against *A. niger*, *E. coli* and *S.* Typhimurium and showing synergistic effect against *L. monocytogenes*. The antimicrobial formulation was immobilized in alginate-based coating. The in situ tests of combined treatments were carried out on coated floret by applying 0.4 or 0.8 kGy of γ-irradiation. Results showed a synergistic effect between coating and γ-irradiation to reduce the growth of *E. coli*, *L. monocytogenes*, *S.* Typhimurium, and *A. niger* during storage at 4 °C.

Keywords: Essential oil; broccoli; spoilage bacteria; synergy; antimicrobial edible coating; γ-irradiation.

1. INTRODUCTION

Minimal processing fruits and vegetables are perishable products. Their shelf-life is shorter than unprocessed raw materials due especially to the physiology of wounded tissue which is typical to plant tissue under stress conditions. Cutting process increases the respiration rate which uses up the energy reserve and increases the ethylene production. The increase of post-harvest physiological activities makes their softening and senescence faster by the increase of weight and firmness losses (Reilly *et al*., 2004). Also, cutting process induces oxidative reactions, browning, nutrient losses and leaf yellowing (Rahman, 2007). On the other hand, ready-to-eat (RTE) fruits and vegetables are highly affected by postharvest bacterial and fungal diseases, other pathological breakdown processes and insect infestation (Snowden, 2010). Losses are frequently attributable to deterioration during handling, process, transport and storage. To maintain RTE fruits and vegetables quality during shelf-life, it is important to master all process steps starting from harvest conditions, washing, cutting, peeling to packaging and transporting. According to Kokkinakis and Fragkiadakis (2007), vegetables sanitation and the application of critical control points methodology decrease the microbial level and increase their safety. However, even today, fruit and vegetables can be contaminated with pathogenic microorganisms endangering human health. Health Canada (2013) estimates more than 4 million Canadians suffer food poisoning each year. Most people recover, but some die and between 2 and 3% are left with chronic health problems. *L. monocytogenes*, *S.* Typhimurium and *E. coli* contamination are the major causes of food recalls. Food recalls could generates also economic losses and devastating effects on some companies.

The use of chilling can permit the shelf-life extension of RTE vegetables. However, this method is not able to eliminate pathogenic microorganisms (Tian *et al*., 2012). The use of modified atmosphere packaging is also increasing, however, the outbreaks of foodborne diseases and especially the emergence of resistant foodborne give serious doubts about its effectiveness (Caleb *et al*., 2013). The use of natural antimicrobials such as EOs, bacteriocins, bacteriophages, OAs and natural vegetable extracts is seeing now in stronger demand on the part of consumers regarding their safety as compared to synthetic chemical compounds. EOs exhibited a strong antibacterial effect, but their application in foods has been limited by their strong flavor. Also to assure their bioavailability and their stability during storage, these agents should be encapsulated (Liolios *et al*., 2009).

Irradiation is another effective physical method to assure food safety. However, the doses needed to reduce the pathogens to undetectable level pathogenic bacteria are often higher than the doses permitted to fruits and vegetables (≤ 1 kGy) (López *et al*., 2005). Thus, the use of combined treatments is widely suggested as for its ability to act in synergy to reduce the dose of irradiation and the concentration of the antimicrobial compound needed to eliminate pathogens.

The main objective of this study was therefore to assess the antimicrobial activities of 27 different antimicrobial agents against 4 different foodborne pathogens (Gram-negative, Gram-positive and fungi) that could affect the broccoli and on TMF in order to select the best combined antimicrobial compounds, to develop an antimicrobial formulation which will act in synergy or with additive effect; and to evaluate the antimicrobial formulation alone or in combination with γ-irradiation on broccoli florets during storage.

2. MATERIAL AND METHODS

2.1. Antimicrobials

The evaluated EOs (Pranarôm International SA, Ghislenghien, Belgium and Union Nature Aroma-Phyto inc. QC, Canada) and their main constituents are presented in Table 1. EOs were prepared as oil-in-water emulsion before utilization for evaluation of their antimicrobial properties in vitro. The emulsion of EOs was prepared as 2.5 % EO (w/v), 2.5 % Tween 80 (w/v) (Sigma-Aldrich, ON, Canada) in deionized water, and then was homogenized for 2 min at 23,000 rpm using an IKA T25 digital Ultra-Turrax disperser (IKA Works Inc., Wilmington, NC, USA).

OAs of sodium diacetate, sodium acetate, potassium lactate, calcium propionate and sodium citrate (BSA Ingredients s.e.c/l.p., Montreal, QC, Canada) were evaluated. OAs were prepared as 5% (w/v) in deionized water and homogenized with magnetic stir. The natamycin (Profood International, Inc, Naperville, USA) was prepared as 0.008% (w/v) in deionized water. The antimicrobial solutions were filtered through 0.2 µm syringe filter (Sarstedt, Montreal, C, Canada). Solutions were freshly prepared for each repetition.

2.2. Preparation of bacterial cultures

Five strains of *L. monocytogenes* HPB2558, HPB2812, HPB1043, HPB2569 and HPB2371 (Health Canada, Health Products and Food Branch, Ottawa, Canada), *E. coli* O157:H7 (EDL

933, isolated from contaminated meat, provided by Pr. Charles Dozois), *S.* Typhimurium (SL1344, Institut national de la recherche scientifique- Institut Armand Frappier) and broccoli TMF were used in this study. The TMF was obtained according to Vu *et al*. (2011). *E. coli* O157:H7, *S.* Typhimurium (SL1344), broccoli TMF and each strain of *L. monocytogenes* were kept each at -80 °C in Tryptic Soy Broth (TSB; Becton-Dickinson, Sparks, MD, USA) containing glycerol (10%; v/v). Before each experiment, stock cultures were propagated through two consecutive 24 h at 35 °C ± 2 °C growth cycles (10^{-1} dilution) in TSB. Then, all the 5 *Listeria* strains were mixed as 1 mL each and all bacteria were washed in saline solution (0.85% w/v) to obtain a working culture containing approximately 10^9 CFU/mL. *Listeria* strains in mixture, *E. coli*, *S.* Typhimurium and broccoli TMF were diluted until reaching 10^6 CFU/mL. For fungal evaluation, *A. niger* (ATCC 1015, Institut National de la recherché scientifique) were propagated through 72 h growth cycle in potato dextrose agar (PDA, Difco, Becton Dickinson) at 28 °C ± 2 °C. Conidia were isolated from the agar media using sterile platinum loop, suspended in sterile peptone water, and filtrated through sterile cell strainer (Fisher scientific, Ottawa, ON, Canada). The filtrate was adjusted to 10^6 conidia/mL for the MIC determination and the possible synergy between compounds using checkerboard method (Hossain *et al*., 2016).

2.3. MIC determination

The MIC value of each antimicrobial agent was determined in sterilized flat-bottomed 96-well microplate according to the two fold microdilution method (Turgis *et al*., 2012). Briefly, serial two fold dilutions (125:125 µL) of the antimicrobial compounds were made in Mueller Hinton Broth (MHB, Difco, Becton Dickinson) for bacteria and TMF and in Potato Dextrose Broth (PDB, Difco, Becton Dickinson) for fungi and dispensed into 96-well microplates. The concentration ranges were 20-10,000 ppm for EOs, 0.78-400 ppm for natamycin and 50-25,000 ppm for OAs. Then, a volume of 15 µL of bacteria and fungi suspension (106 CFU/mL or conidia/mL) was added to 125 µL of each antimicrobial serial dilution and the microplates were incubated on the shaker at 80 rpm at respectively 37 °C and 28 °C for 24 h and 48 h respectively. The absorbance was measured at 595 nm in a BioTek ELx800® absorbance microplate reader (BioTek Instruments Inc., Winooski, VT, USA). The MIC is the lowest concentration of antimicrobial agent demonstrating the complete inhibition of bacterial and fungal growth and showing equal absorbance as blank. On the basis of the MIC results, the antimicrobial agents with greater efficiency have been selected to evaluate their combined effect.

2.4. Determination of the synergy between antimicrobials agents

The checkerboard method was used in order to evaluate the possible synergy between compounds (Hossain *et al*., 2016; Turgis *et al*., 2012). In this method, each of the two selected AMs was separately two-fold diluted with MHB in microplates of 96 wells. Then, the antimicrobials were transferred into the main microplate which contained a serial concentration of 50 µL of AM_1 along the X axis and the serial concentration of same volume of AM_2 along the Y axis (6 x 6 matrix) to obtain the Fractional Inhibitory Concentration (FIC) index of antimicrobial in combinations. This study was divided into 2 parts. The first part was to evaluate the combined effect between each EO and OA against all broccoli pathogens. The second part was to evaluate the antifungal activity of EO and OA mixture when combined to natamycin against *A. niger*.

Plates were incubated at 37 °C and 28 °C for 24 h and 48 h under agitation at 80 rpm for bacteria and fungi respectively and were read at 595 nm. FIC was calculated according to the following formula:

$FIC_1 = MIC_1$ combined / MIC_1 alone (Eq 1)

$FIC_2 = MIC_2$ combined / MIC_2 alone (Eq 2)

$FIC = FIC_1 + FIC_2$ (Eq 3)

Where:

MIC_1 alone: the MIC value of AM_1 tested alone;

MIC_2 alone: the MIC value of AM_2 tested alone;

MIC_1 combined: the MIC value of AM_1 tested in combination with AM_2;

MIC_2 combined: the MIC value of AM_2 tested in combination with AM_1.

The results are considered as synergistic when $FIC \leq 0.5$, additive when $0.5 < FIC \leq 1$, Not interactive when $1 < FIC \leq 4$ and antagonist when the $FIC > 4$ (Turgis *et al*., 2012).

2.5. Encapsulation of antibacterial formulation in alginate matrix

2.5.1. Preliminary tests

The most efficient antimicrobial formulation showing synergistic effect by the checkerboard test was selected for encapsulation study. Alginate has been used as polymer for encapsulation treatment. Then, a preliminary sensory analysis was carried out to determine the maximal concentration of active compounds that did not affect the sensory properties of the broccoli.

2.5.2. Microemulsion of EOs

Lemongrass EO was used as antimicrobial agent in microemulsion preparation. A mixture of 0.1% (w/v) sunflower oil (IGA, Laval, Quebec, Canada) and 0.03% (w/v) lemongrass EO was dispersed in distilled water containing 0.13% (w/v) of Tween 80: span 20 (Sigma-Aldrich, ON, Canada) with a ratio of 53 : 47. Tween 80 and span 20 were used as they give a hydrophilic-lipophilic balance (HLB) = 12. To stabilize an o/w emulsion, the theoretical HLB is needed to be between 8 and 18. The prepared solution was homogenized using Ultra-Turrax (IKA T25, IKA Works Inc., Wilmington, NC, USA) for 2 min at 23,000 rpm.

2.5.3. Bioactive coating preparation

Alginate (Sigma-Aldrich, ON, Canada, 67% glucuronic residues) 1.3 g was solubilized in 100 mL of warm water (70 °C) under vigorous stirring (130 rpm) until total dissolution. Then, glycerol 1.6% (w/v), sodium diacetate 0.5% (w/v) and natamycin 0.008% (w/v) were added. The microemulsion was then added to the alginate solution and pre-homogenized for 3 h with IKA RW-20 (IKA Works Inc., Wilmington, NC, USA) mechanical homogenizer at 1,500 rpm at room temperature. Subsequently, the primary emulsion was introduced in the inlet reservoir of the microfluidizer (Microfluidics Inc., Newton, MA, USA) and subjected to 6 cycles of high pressure operated at 25,000 psi (150 MPa) to obtain a nanometric size. Between each cycle the alginate solution was cooled in an ice bath.

2.5.4. Effect of bioactive coating and irradiation on broccoli pathogens

Broccoli samples were purchased from a local supermarket (IGA, Laval, QC, Canada) and cut into floret of 20 ± 1 g each then distributed into Whirl-Pak™ Sterile Filter Bags (one floret per bag). All samples were refrigerated and irradiated at a dose of 10 kGy for sterilization at the Canadian Irradiation Center in a UC-15A irradiator (Nordion Inc., Kanata, ON, Canada)

equipped with a 60Co source and having a dose rate of 16.8 kGy/h. Samples were irradiated at room temperature and then stored at 4 °C until used. Sterile broccoli samples were coated with alginate antimicrobial coating by dipping 30 sec then the coated samples were dried under a laminary hood for 30 min. Each coated sample was immerged in sterile $CaCl_2$ 1.5% (w/v) solution for 30 s and dried again in a laminary hood for 15 min. Broccoli samples were then, inoculated by adding 1 mL of working cultures of *E. coli*, *S.* Typhimurium, *L. monocytogenes* or *A. niger* (10^6 CFU/mL) to obtain a final concentration of 10^5 CFU/g. Samples were kept at 4 °C during 24 h to allow the antimicrobial coating reacting with bacteria and fungi. Then, inoculated samples with bacteria or fungi were irradiated respectively at a dose of 0, 0.4 or 0.8 kGy and kept at 4 °C for 14 days. Three broccoli control samples were studied: (1) untreated (no irradiation and coating), (2) coated (no irradiation) and (3) irradiated broccoli (no coating) which were prepared in the same way as described previously.

The microbiological analysis of *E. coli, S.* Typhimurium, *L. monocytogenes* and *A. niger* was done at days 0, 1, 4, 7, 10 and 14. The broccoli florets were homogenized for 2 min in 80 g of 0.1% (w/v) peptone water (Alpha Biosciences Inc., Baltimore, MD, USA) at high speed in a Lab-blender 400 stomacher (Laboratory Equipment, London, UK), then seeded into Mc sorbitol agar for *E. coli*, Desoxycholate Citrate Lactose Sucrose agar for *S.* Typhimurium, PALCAM for *L. monocytogenes* and PDA with chloromphnicol for *A. niger* and incubated for 24 h at 37 °C for bacteria and for 72 h at 28 °C for fungi.

2.6. Statistical analysis

Each experiment was done in triplicate (n = 3). Analysis of variance (ANOVA), Duncan's multiple range tests for equal variances and Tamhane's test for unequal variances were performed for statistical analysis using SPSS 18.0 software (SPSS Inc, USA). Differences between means were considered significant when the confidence interval was lower than 5 % ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. MIC determination

The compounds with their respective MIC values against 3 bacterial strains (*E. coli*, *S.* Typhimurium and *L. monocytogenes*), *A. niger* and broccoli TMF are presented in Table 2.

Results obtained from broth dilution assay showed that lemongrass, *Thymus vulgaris thymol* and winter savory exhibited the highest antimicrobial activity among all the tested EOs. lemongrass and Thymus vulgaris thymol inhibited the growth of *E. coli*, *S.* Typhimurium, *L. monocytogenes*, *A. niger* and broccoli TMF at a concentration of 2,500 ppm. Winter savory was found to be the most effective EO with a lowest MIC value of 1,250 ppm against all the tested species. In view of these results, a MIC of 80 ppm against *A. niger* was observed for natamycin. Respectively against *E. coli*, a MIC of 313 ppm was observed for winter savory and ceylon cinnamon EOs and a MIC of 625 ppm was observed for lemongrass, *Thymus vulgaris thymol*, damask rose and exotic basilica EOs. A MIC of 625 ppm was also observed against *L. monocytogenes* and *S.* Typhimurium for ceylon cinnamon and winter savory EOs.

A MIC of 1,250 ppm was observed against *L. monocytogenes* for lemongrass, Thymus vulgaris thymol, thyme savory, compact oregano and spanish oregano extra EOs. A MIC of 1,250 ppm was observed against *S.* Typhimurium for lemongrass, damask rose, Thymus vulgaris thymol, thyme savory and spanish oregano extra EOs. A MIC of 1,250 ppm was observed against *E. coli* for vulgar thyme geraniol, thyme savory and spanish oregano extra EOs. A MIC of 1,250 ppm was also observed against *A. niger* for only spanish oregano extra EOs. A MIC of 2,500 ppm was observed against *L. monocytogenes* for damask rose and vulgar thyme geraniol EOs. A MIC of 2,500 ppm was observed against *S.* Typhimurium for vulgar thyme geraniol and compact oregano EOs. A MIC of 2,500 ppm was also observed against *E. coli* for marjolaine shell, compact oregano and java citronella EOs. Against *A. niger*, lemongrass and *Thymus vulgaris thymol* EOs had a MIC of 2,500 ppm.

A MIC between 3,130 and 5,000 ppm was observed for marjolaine shell and java citronella EOs against *S.* Typhimurium, for damask rose and thyme savory EOs against *A. niger*, for rosemary verbenon EO against *L. monocytogenes*, *S.* Typhimurium, and *E. coli*, and for sodium diacetate against *E. coli*. 13 of 27 antimicrobial products showed MIC values that were ≥ 10,000 ppm for most of all target bacteria and fungi such as bitter orange, mandarin and rosemary camphor. Hence, *E. coli* seems to be more sensitive to antimicrobial compounds. Broccoli TMF showed the highest MIC values for most of the EOs as compared to *L. monocytogenes* and *S.* Typhimurium. The chemical composition of the tested extracts provided by the manufacturer showed that lemongrass, Thymus vulgaris thymol, winter savory and ceylon cinnamon EOs consist of the associations of geranial, neral, carvacrol, thymol, p-cymene and cinnamaldehyde and are highly effective against all tested microorganisms. These results are consistent with previous works (Dussault *et al*., 2014;

Ghabraie *et al*., 2016). In fact, Eftekhar *et al*. (2009) showed a high antibacterial activity of carvacrol and thymol against *B. subtilis*, *E. faecalis*, *S. aureus* and *E. coli* and γ-terpinene and p-cymene was effective against B. subtilis. The antimicrobial activity of EOs was often related to membrane damage, cytoplasm coagulation and in some case complete alteration of the cells morphology (Nazzaro *et al*., 2013). The two isomers, neral and geranial, are the main compounds of monoterpene citral possess a pronounced antimicrobial activity against several bacteria and fungi (Dorman and Deans, 2000; Silva *et al*., 2008). The antifungal activity of citral and cinnamaldehyde was probably due to their ability to form charge transfer complexes with electron donors of a fungus cell (Kurita *et al*., 1981). Carvacrol is an efficient antimicrobial agent. Its effectiveness was explained by Ultee *et al*. (1999) as the result of the membrane permeability modification for cations like H^+ and K^+. The dissipation of ion gradients leads to impairment of essential processes in the cell and finally to cell death. It's interesting to note that some of these efficient molecules were present in some less efficient EOs such as compact oregano. This suggests that the antimicrobial effect is not only due to the effect of a single molecule, but also to the synergistic effect between different compounds of EOs to enable a high antimicrobial effect. Therefore, winter savory and thyme savory, when combined with carvacrol and p-cymene, were proved to generate a synergistic activity to reduce *B. cereus* (Ultee *et al*., 2000). This suggestion confirm previous studies of Pei *et al*. (2009) who have demonstrated a synergistic effect of combined cinnamaldehyde, thymol, eugenol and carvacrol on reducing *E. coli* growth. Results of MIC for the OAs demonstrated that only sodium diacetate has proved to have a high antimicrobial effect among all tested OAs, at a concentration of 6,250 ppm against *S.* Typhimurium, *L. monocytogenes* and *A. niger* and 3,130 ppm against *E. coli* and broccoli TMF. However, all others OAs tested had MIC $\geq$ 25,000 ppm. Previous studies confirmed the antimicrobial effectiveness of lactic, propionic and acetic acid and their respective salt (Ricke, 2003; Theron and Lues, 2011). However, the antimicrobial efficacy varied between acids and between studied bacterial strains evaluated. It is well known that the effectiveness of OAs is mainly observed when OAs are in their undissociated form (De Keersmaecker *et al*., 2006; Theron and Lues, 2011). OAs and their salts are considered as weak acids and they are not fully dissociated in water. Generally, the undissociated form of OAs are able to penetrate in the microbial cell. The antimicrobial activity of OAs was associated to a succession of reactions starting by penetration of undissociated OAs to bacterial cells inducing an acidification of internal components of cell membranes and disruption. This allow the loss of active transport of nutrients and an increase of membrane permeability, the reduction of water activity, the

inhibition of essential metabolic reactions and the accumulation of toxic anions (Theron and Lues, 2011). In this study, the natamycin was extremely effective in reducing *A. niger* with the lowest MIC (MIC = 80 ppm). Natamycin is a polyene antibiotic used as an antifungal agent because of its broad spectrum of activity and the lack of development of resistance. Its mode of action was related to blocking fungal growth by binding specifically to ergosterol containing membranes (Te Welscher *et al*., 2008).

On the basis of these results, it can be noted that lemongrass, winter savory, Thymus vulgaris thymol and ceylon cinnamon were the most effective EOs that were able to reduce the growth of pathogens and broccoli TMF. Our study also showed that between evaluated OAs, only sodium diacetate was effective to eliminate broccoli pathogens and TMF. In fact sodium diacetate is the mix of sodium acetate and acetic acid which both of them have an antimicrobial activity (Rhee *et al*., 2003; Sallam, 2007). Thus, combining acetic acid and sodium acetate allows an enhanced antimicrobial activity. Natamycin exhibit strong antifungal potential and was more effective in reducing *A. niger* due especially to the growth blocking of the fungi.

3.2. Fractional inhibitory concentration (FIC) of combined antimicrobials against food pathogens

Based on the MIC values against 4 tested species and broccoli TMF, 4 EOs namely lemongrass, *Thymus vulgaris thymol*, winter savory and ceylon cinnamon were combined with sodium diacetate and natamycin. First, each EO was combined to sodium diacetate and evaluated against *L. monocytogenes*, *E. coli*, *S.* Typhimurium and *A. niger*. Then, the optimum concentration of each antimicrobial was selected to be combined to natamycin to evaluate their effect against *A. niger*.

The obtained results showed that the combination of (1) *Thymus vulgaris thymol* and sodium diacetate and (2) winter savory and sodium diacetate had an additive effect against all evaluated strains as compared to cinnamon in combination sodium diacetate which has an additive activity against only *S.* Typhimurium and non-interactive activity against all other bacteria and fungi evaluated (Table 3). It's interesting to note that a synergistic activity was observed between lemongrass and sodium diacetate to reduce *L. monocytogenes*. This combination was effective to reduce *S.* Typhimurium, *E. coli* and *A. niger* with an additive activity (Table 5). The obtained results suggest that combining OA and EO could act in

synergy to reduce pathogenic bacteria and fungi. Some previous studies investigated in this field have demonstrated that lactic acid can act in synergy with thyme, rosemary and oregano EOs to increase the inhibition of *Listeria* (Dimitrijević *et al*., 2007). Also, Zhou *et al*. (2007) demonstrated that a synergistic effect was observed between thymol and acetic acid, thymol and citric acid, carvacrol and acetic acid and carvacrol and citric acid against *S.* Typhimurium.

According to these results, the most effective concentration of each antimicrobial agent was selected and combined to natamycin to evaluate their effectiveness to inhibit the growth of *A. niger*. It is interesting to note that, the addition of the natamycin had different behaviors depending on the formulation. In fact, when natamycin was combined to lemongrass or to ceylon cinnamon and to sodium diacetate, the antifungal activity was improved and varied from non- interactive to additive activity. These combinations could achieve the desired antibacterial effect at concentrations low enough to minimize undesirable changes in flavor. However, no change was observed for *Thymus vulgaris thymol* or winter savory and sodium diacetate.

There is little information on the interaction between EOs and OAs. Oussalah *et al*. (2006) reported the antimicrobial activity of cinnamon and savory EOs to the increase of the cell membrane permeability, the release of the cell constituents, the decrease of the ATP concentration in the cells, and the decrease of the internal pH. When EOs were combined with OAs, Zhou *et al*. (2007) reported the synergistic effect of thymol or carvacrol with acetic acid to the moving thymol or carvacrol in the molecular state in which they become freely permeable across the plasma membrane and thus is able to enter the cell and exert their antimicrobial activity. On the basis of these reports, combining EOs with OAs seems to increase the membrane permeability to EOs and then improve their antimicrobial effectiveness.

3.3. Evaluation in situ of combined effect on pathogenic microorganisms

Results of the growth of pathogens on broccoli during storage at 4 °C are presented in Fig.1. The selected antimicrobial formulation was the combination of Lemongrass, sodium diacetate and natamycin which had proved previously its effectiveness against all bacterial and fungal tested pathogens in vitro. The concentration of bacteria and fungi in untreated broccoli was 5 log CFU/g and 5 conidia/g respectively on the first day of storage for all tested pathogens.

3.3.1. *E. coli*

The growth of *E. coli* in broccoli samples during storage at 4 °C is shown in (Fig.1.a). The obtained results showed that for untreated broccoli (Control), *E. coli* growth was reduced in the first day of storage from 4.8 to 4.1 log CFU/g. Afterwards, *E. coli* count increased and remained stable during storage at 4 °C with an average of 5 log CFU/g. When antimicrobial edible coating (EC+AM) was applied, no significant difference was observed on day 0 as compared to untreated broccoli. On day 7, the antimicrobial coating has permitted a slight reduction of *E. coli* count in broccoli samples with 0.9 log reduction as compared to untreated broccoli. On day 10, a significant reduction on *E. coli* population was observed on coated samples and *E. coli* count reached 2.2 log CFU/g on day 10 as compared to 4.9 log CFU/g for untreated broccoli. Thus, the evaluated antimicrobial edible coating had an effect in reducing *E. coli* population in broccoli samples. The effectiveness of the antimicrobial coating could be related the sensitivity of *E. coli* to Lemongrass and sodium diacetate for which the MIC was the lowest and reached 625 and 3,130 ppm respectively. Thus, Lemongrass at 300 ppm combined to 5000 ppm of sodium diacetate was able to reduce *E. coli* count by 2.7 log reduction on day 10. When broccoli samples were irradiated at a dose of 0.4 kGy (0.4 kGy), *E. coli* count was reduced during 4 days to 3.4 log CFU/g as compared to 5.07 log CFU/g for untreated broccoli, afterward, *E. coli* count still under the undetectable level at day 7. Hence, a dose of > 0.4 kGy was necessary to eliminate *E. coli* counts during storage at 4 °C. The obtained results are in concordance with previous studies. Tawema *et al.* (2016) demonstrated that 0.5 kGy was able to reduce *E. coli* count in fresh cut cauliflower from 3.42 log CFU/g to the undetectable level on day 0. However, after 10 days of storage, *E. coli* was reoccurring and only a dose of 1 kGy irradiation dose was effective to keep *E. coli* count under the undetectable level during the whole storage.

In the current study, when antimicrobial edible coating was applied in combination with γ-irradiation at a dose of 0.4 kGy (0.4 kGy+ EC AM), *E. coli* count was below the undetectable level during the whole storage. Hence, a synergistic effect was observed between antimicrobial edible coating and γ-irradiation treatment to eliminate *E. coli* during the whole storage. Other researchers found other synergistic effects between irradiation and antimicrobial edible coating with different formulations (Lacroix and Ouattara, 2000; Severino *et al*., 2015; Tawema *et al*., 2016). In their works, Severino *et al*. (2015) demonstrated a combined effect of 0.25 kGy irradiation dose and modified chitosan encapsulating carvacrol nanoemulsion in reducing *E. coli* count by 1.68 log reduction. When

applied separately, only 0.53 log reductions was observed for coated samples and only 1.27 log reduction for irradiated samples at a dose of 0.25 kGy.

3.3.2. *L. monocytogenes*

The growth of *L. monocytogenes* in broccoli samples during storage at 4 °C (Fig 1 b) showed that during the first 4 days of storage, no significant difference was observed between untreated and coated broccoli ($P > 0.05$). Afterwards, *L. monocytogenes* population on coated broccoli was reduced and reached 4.2, 3.4 and 2.7 log CFU/g on day 7, 10 and 14 respectively as compared to 5.2, 4.5 and 5.3 for untreated broccoli at the same days which corresponds to a respective 1, 1.1 and 2.6 log reductions.

Thus, the evaluated antimicrobial edible coating had an effect in reducing *L. monocytogenes* population in broccoli florets. Previous studies confirm this observation and showed a 1.8 log reduction of *E. coli* on cauliflower when an antimicrobial coating containing Lemongrass, citrus extract and lactic acid and a second coating containing Oregano, citrus extract and lactic acid were applied. However, in some cases, *L. monocytogenes* can reoccur during storage (Tawema *et al*., 2016).

When irradiation treatment was applied at a dose of 0.4 kGy, *L. monocytogenes* population was reduced and reached 3.4, 3.3 and 2.4 log CFU/g on respectively day 0, 7 and 14 of storage. Hence, a dose of > 0.4 kGy was necessary to reach undetectable level of *L. monocytogenes*. This observation was confirmed by previous studies which demonstrate that even with 1 kGy, *L. monocytogenes* can be reduced to undetectable level and then reoccur again during storage time (Tawema *et al*., 2016). In fact, in response to irradiation stress, *Listeria* could enter in non-cultivable viable cells state (VNC). This stage is defined as a physiological condition in which bacteria are metabolically active but are unable to grow on culture media. However, it is possible that these latent cells reoccur and restart their normal vegetative metabolism (resuscitation) during storage and then cause food poisoning (Oliver, 2005). Previous studies have observed the reoccurrence of *E. coli* on irradiated carrots at 0.3 kGy after the 5th days of storage (Lafortune *et al*., 2005).

When antimicrobial edible coating was applied in combination with γ-irradiation at a dose of 0.4 kGy, *L. monocytogenes* was reduced from 4.5 and 4.3 log CFU/g for untreated broccoli to 2.8 and 3.1 log CFU/g on day 0 and 1. Afterwards no colony was detected during the whole

storage at 4 °C. Hence, a synergistic effect was observed between antimicrobial edible coating and γ-irradiation treatment to eliminate *L. monocytogenes* during the whole storage.

Eliminating *L. monocytogenes* by combined bioactive coating and γ-irradiation was studied previously with different formulations containing nisin, carvacrol, winter savory and their combination (Ndoti-Nembe *et al*., 2015). According to the obtained results, combining natural antimicrobial agent seems to be a good alternative to control *Listeria* growth without modifying the sensory quality of the commodity.

3.3.3. *S.* Typhimurium

The growth of *S.* Typhimurium in broccoli samples during storage at 4 °C (Fig 1c) showed that no significant difference was observed between coated and untreated broccoli during the first day of storage. Afterwards, *S.* Typhimurium count of untreated broccoli reached 5.2 log CFU/g on day 7 and 14 as compared to 3.7 and 3.4 log CFU/g for coated broccoli which corresponds to 1.5 and 1.8 log reduction. Hence, the antimicrobial edible coating was able to reduce significantly by 1.5 log reduction *S.* Typhimurium during storage. Previous studies on plum showed a 0.9, 1.3, 1.4 and > 2.8 log reduction when 2,000, 5,000, 10,000 and 20,000 ppm of lemongrass EO incorporated into carnauba wax-based solution was applied (Kim *et al*., 2013). These results suggest that a synergistic activity was obtained between lemongrass and sodium diacetate to reduce *S.* Typhimurium which lead to a significant reduction ($P \leq 0.05$) with only 300 ppm of lemongrass EO when combined to 5,000 ppm sodium diacetate.

When broccoli samples were irradiated at a dose of 0.4 kGy, *S.* Typhimurium count was reduced significantly and reached the undetectable level on day 1. However, from day 4, *S.* Typhimurium reoccurred and started growing to reach on respectively day 7 and 14, 2.1 and 3.0 log CFU/g. Hence, to inhibit *S.* Typhimurium a dose > 0.4 kGy was needed. In fact Severino *et al*. (2015) showed that with 0.25 kGy irradiation dose only 0.7 log reduction on *S.* Typhimurium in green beans was observed. Also, Lee *et al*. (2006) demonstrated that 2.8 log reduction was observed with irradiated spinach at 1 kGy.

When antimicrobial edible coating was applied in combination with γ-irradiation at a dose of 0.4 kGy, *S.* Typhimurium was reduced and no colony was detected during the whole storage at 4 °C. These results suggest a synergistic activity between irradiation and antimicrobial edible coating. Severino *et al*. (2015) showed also an increase of *S.* Typhimurium reduction

by 0.4 log as compared to irradiated samples when irradiation at 0.25 kGy was combined to edible coating of methyl cellulose encapsulating carvacrol.

3.3.4. *A. niger*

The growth of *A. niger* in broccoli samples during storage at 4 °C (Fig.1d) showed that *A. niger* was the most radioresistant as compared to other evaluated bacteria. In fact, when an irradiation dose of 0.4 kGy was applied on broccoli floret non-significant reduction ($P > 0.05$) was observed on *A. niger* count and only 0.6 log reduction was observed for both day 0 and 14. When broccoli samples were irradiated at a dose of 0.8 kGy, *A. niger* count was reduced significantly ($P \leq 0.05$) and varied from 4.5, 5.0 and 5.1 for untreated broccoli on respectively day 0, 7 and 14 to only 2.8, 2.4 and 2.4 for irradiated broccoli at 0.8 kGy showing a respective 1.7, 2.6 and 2.7 log reduction. Similar results were obtained by El-Samahy *et al*. (2000) on mango where they found a 1 log reduction with an irradiation dose of 0.5 kGy. Some other studies found that an irradiation treatment at a dose of 1 and 2 kGy were able to reduce *A. niger* count by respectively 0.7 and 2.2 log conidia/g in rice (Hossain *et al*., 2014). Aziz *et al*. (2004) demonstrated also 0.98, 1.40, 1.85 and 2.39 log reduction of *Aspergillus* on maize for respectively irradiation doses of 1, 2, 3 and 4 kGy. An irradiation dose of 5 kGy was able to reduce *Aspergillus* under the detectable level for maize, chick-peas and groundnuts. This suggests that the behavior of *Aspergillus* growth differs depending on food model. In fact, *Aspergillus* seems to be more resistant when it contaminated dry food such as cereals, and it seems to be less radio resistant when it contaminated vegetables and fruits with a high aw. More studies need to investigate in the radioresistance of *Aspergillus* to confirm this hypothesis.

Despite the effectiveness of the antimicrobial formulation in vitro, when encapsulated in alginate matrix, applying antimicrobial edible coating on broccoli floret did not have any significant effect on *A. niger* count as compared to untreated broccoli. *A. niger* seems to be more resistant to antimicrobial agents as confirmed by its highest MIC (Table 4). As reported by Raybaudi-Massilia *et al*. (2008), applying alginate coating containing 0.3% lemongrass EO on fresh-cut melon didn't affect yeasts and molds growth during the first week of storage at 4 °C. However, on day 14 of storage, a 1.5 log reduction was observed as compared to untreated samples. Also, the presence of natamycin in the formulation at 80 ppm encapsulated in alginate film did not reduce *A. niger* population. Türe *et al*. (2008) didn't find any antifungal activity of natamycin even at 150 ppm after encapsulation in methyl cellulose

based films. Also, the same authors didn't show any antifungal activity against *A. niger* when natamycin at 1,000 ppm was combined to rosemary extract at 1,500 ppm. However, an antifungal activity was observed only with film containing 1,500 ppm of natamycin combined to 1,500 ppm of rosemary extract. When broccoli floret was irradiated at 0.4 kGy, a significant reduction was observed only day 4 showing 3 log reduction, afterwards no significant reduction was observed between untreated and irradiated broccoli at 0.4 kGy. When bioactive coating was combined to an irradiation dose of 0.4 kGy, a significant reduction was observed during the first week of storage. However, on day 14 no difference was observed between untreated and coated and irradiated samples at 0.4 kGy. Thus, an irradiation treatment at a dose of 0.8 kGy was needed. When antimicrobial edible coating was applied in combination with γ-irradiation at a dose of 0.8 kGy, *A. niger* was reduced to 2.3 and 2.0 log conidia/g on respectively day 0 and 1 and then it decreased to under the detectable level during the whole storage at 4 °C. These observations suggest that the antimicrobial edible coating had a synergistic effect when applied in combination with γ-irradiation. According to Kader (1986), the effectiveness of irradiation as fungicidal and /or fungistatic treatment depend on the pathogen, its level of growth and the count of viable fungal cells on or within the tissue. Generally, a minimum dose of 1.75 kGy is required for effective inhibition of post-harvest fungi. Hence, irradiation treatment alone was able to reduce *A. niger* of RTE broccoli. However, in this study, when combined to antimicrobial edible coating before irradiation treatment, a synergy was observed and the *A. niger* was reduced to undetectable level from 1 day to the end of storage. These observations imply that the application of antimicrobial edible coating increased the sensitivity of *A. niger* against irradiation.

5. CONCLUSION

In this study, EOs of Thymus vulgaris thymol, winter savory, ceylon cinnamon and lemongrass, sodium diacetate and natamycin overly showed high activity in vitro against all tested bacteria and fungi. Also additive and synergistic effect occurred with the number of combination of EO, OA and natamycin. Specifically, the combination of lemongrass, sodium diacetate and natamycin showed high efficiency with a synergistic and additive effect against pathogenic and spoilage bacteria and fungi. The results of this study clearly demonstrated that antimicrobial edible coating can act in synergy with γ-irradiation on broccoli floret in order to eliminate pathogens and then assure food safety.

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Table 1. Selected EOs for antimicrobial activity evaluation against evaluated microorganisms.

Latin name	Common name	Origin	Distilled part	Composition (%)
Origanum majorana[1, 3]	Marjolaine shell	Egypt	Flower top	Terpinene-4-ol+β-Caryophyllene (29.06), γ-Terpinene (13.80), Cis-tuyanol (10.73)…
Citrus reticulata[1, 3]	Mandarin	Brazil	Zest	Limonene (70.74), γ-Terpinene (18.31)…
Cymbopogon citratus[1, 3]	Lemongrass	Guatemala	Aerial part	Geranial (38.87), Neral (30.70)…
Rosa damascena[1, 3]	Damask Rose	Turkey	Flower	Citronellol (31.33), Geraniol (19.78), Nonadecane C19 (16.48)…
Cinnamomum zeylanicum[1, 3]	Ceylon cinnamon	Sri Lanka	Leaves	E-Cinnamaldehyde (63.07), Cinnamylacetate (6.86), β-Phellandrene+1, 8-cineol (4.29), Eugenol (2.40), p-Cymene (2.35)…
Thymus vulgaris[1, 3]	Vulgar Thyme geraniol	France	Flower top	Geranyl acetate (33.32), Geraniol (27.64)…
Thymus vulgaris[1, 3]	Thyme vulgaris thymol	Spain	Flower top	Thymol (38.39), p-Cymene (25.71), γ-Terpinene (9.04)…
Ocimum basilicum ssp basilicum[1, 3]	Exotic Basilica	India	Flower top	Estragol (71.57), linalool (19.59)…
Rosmarinus officinalis[1, 3]	Rosemary cineole	Morocco	Flower top	1-8 Cineol (42.01), α-Pinene (13.72), Camphor (12.85)…
Rosmarinus officinalis[1, 3]	Rosemary verbenone	France	Flower top	α-Pinene+α-Thuyene (43.92), Bornyl acetate (8.65)…
Thymus satureioides[1, 3]	Thyme savory sheet	Morocco	Flower top	Borneol (28.01), α-Terpineol (12.64), Camphene (8.81), Carvacrol (7.04)…
Satureja montana[1, 3]	Winter savory	France	Flower top	Carvacrol (55.14), γ-Terpinene+Trans-β-Ocimene (15.36), p-Cymene (8.54)…

Origanum compactum[1, 3]	Compact oregano inflorescence	Morocco	Flower top	Carvacrol (43.67), Thymol (19.52), γ-Terpinene + Trans-β-Ocimene (16.16), p-Cymene (7.72)
Cymbopogon winterianus[1, 3]	Java citronella	Java	Aerial part	Citronellal (36.42), Geraniol (20.25), Citronellol (12.19)…
Rosmarinus officinalis[1, 3]	Rosemary camphor	Spain	Flower top	α-Pinene (21), 1-8 Cineol (19.05), Camphor (17.28), Camphene (9.42)…
Citrus aurantium[1, 3]	Bitter orange	Tunisia	Zest	Limonene (93.37)…
Rosa x damascena[2, 4]	Rose (Otto) 100% extra	Morocco	Flower	Citronellol (23.81), Geraniol (18.18)
Citrus paradisii[2, 4]	Extra white grapefruit	South Africa	Zest	Limonene (94.10)…
Citrus sinensis[2,4]	Extra blood orange	Costa Rica	Zest	Limonene (95.35)…
Thymus capitatus[2, 4]	Spanish oregano extra	Spain	Plant and flower	Carvacrol (68.34), p-Cymene (6.44)…
Piper nigrum[2, 4]	Black pepper	Madagascar	Dried fruit	α-Pinene (18), Sabinene (15.36), Limonene (13.90), β-Caryophyllene (12.16), β-Pinene (10.30), α-Phellandrene (9.03)

[1] EOs were provided by Pranarom inc. (Ghislenghien, Belgique).

[2] EOs were provided by Union Nature Aroma-Phyto inc. (QC, Canada).

[3] Composition determined by CPG-SM HP; Column: HP Innowax 60-0.5-0.25; Carrier gas Helium: 22 psi.

[4] Composition determined by GC HP 6850; Column: DB-WAX, 20 m x100 µm x0.2 µm; Carrier Gas: Hydrogen: 0.7 mL/min.

Table 2. MIC of antimicrobial compounds evaluated against evaluated microorganisms.

	MIC (ppm)				
	L. monocytogenes	***S.* Typhimurium**	***E. coli***	**Broccoli TMF**	***A. niger***
Winter savory	625	625	313	1,250	625
Lemongrass	1,250	1,250	625	1,250	2,500
Thymus vulgaris thymol	1,250	1,250	625	1,250	2,500
Ceylon cinnamon	625	625	313	1,250	10,000
Damask Rose	2,500	1,250	625	2,500	5,000
Thyme savory	1,250	1,250	1,250	2,500	5,000
Vulgar Thyme geraniol	2,500	2,500	1,250	1,250	5,000
Spanish oregano extra	1,250	1,250	1,250	5,000	1,250
Compact oregano	1,250	2,500	2,500	10,000	> 10,000
Java citronella	10,000	5,000	2,500	2,500	> 10,000
Marjolaine shell	10,000	5,000	2,500	10,000	> 10,000
Rosemary cineole	5,000	5,000	5,000	5,000	> 10,000
Mandarin	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Exotic Basilica	> 10,000	> 10,000	625	> 10,000	> 10,000
Rosemary verbenone	10,000	> 10,000	10,000	> 10,000	> 10,000
Rosemary camphor	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Bitter orange	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Rose (Otto) 100% extra	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Extra white grapefruit	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Extra blood orange	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Black pepper	> 10,000	> 10,000	> 10,000	> 10,000	> 10,000
Sodium diacetate	6,250	6,250	3,130	3,130	6,250
Potassium lactate	> 25,000	> 25,000	> 25,000	> 25,000	> 25,000
Calcium propionate	> 25,000	> 25,000	> 25,000	> 25,000	12,500
Sodium citrate	25,000	> 25,000	> 25,000	25,000	> 25,000
Sodium acetate	> 25,000	> 25,000	> 25,000	> 25,000	25,000
Natamycin	-	-	-	-	80

Table 3. FIC of combined EOs, OA and antifungal against evaluated microorganisms.

	***S.* Typhimurium**		***L. monocytogenes***		***E. coli***		***A. niger***	
Mixture	**FIC**	**Act[1]**	**FIC**	**Act[1]**	**FIC**	**Act[1]**	**FIC**	**Act[1]**
Ceylon cinnamon +SD	0.69±0.08	AD	1.04±0.03	NI	1.06±0.00	NI	1.00±0.00	NI
Thymus vulgaris thymol+SD	0.88±0.18	AD	0.81±0.00	AD	0.75±0.00	AD	0.88±0.00	AD
Winter savory+SD	0.88±0.09	AD	0.75±0.00	AD	1.00±0.00	AD	1.00±0.00	AD
Lemongrass+SD	0.75±0.00	AD	0.44±0.00	S	0.81±0.26	AD	1.00±0.00	AD
Ceylon cinnamon +SD+Nat	-	-	-	-	-	-	0.69±0.09	AD
Thymus vulgaris thymol +SD+Nat	-	-	-	-	-	-	1.00±0.00	AD
Winter savory +SD+Nat	-	-	-	-	-	-	1.00±0.00	AD
Lemongrass +SD+Nat	-	-	-	-	-	-	0.75±0.00	AD

[1]Act= Activity: FIC≤0.5: synergistic effect (S); 0.5<FIC≤1: additive effect (AD); 1<FIC≤4: no interactive effect (I); FIC>4: antagonistic effect (A).

[2] SD: Sodium diacetate; Nat: natamycin.

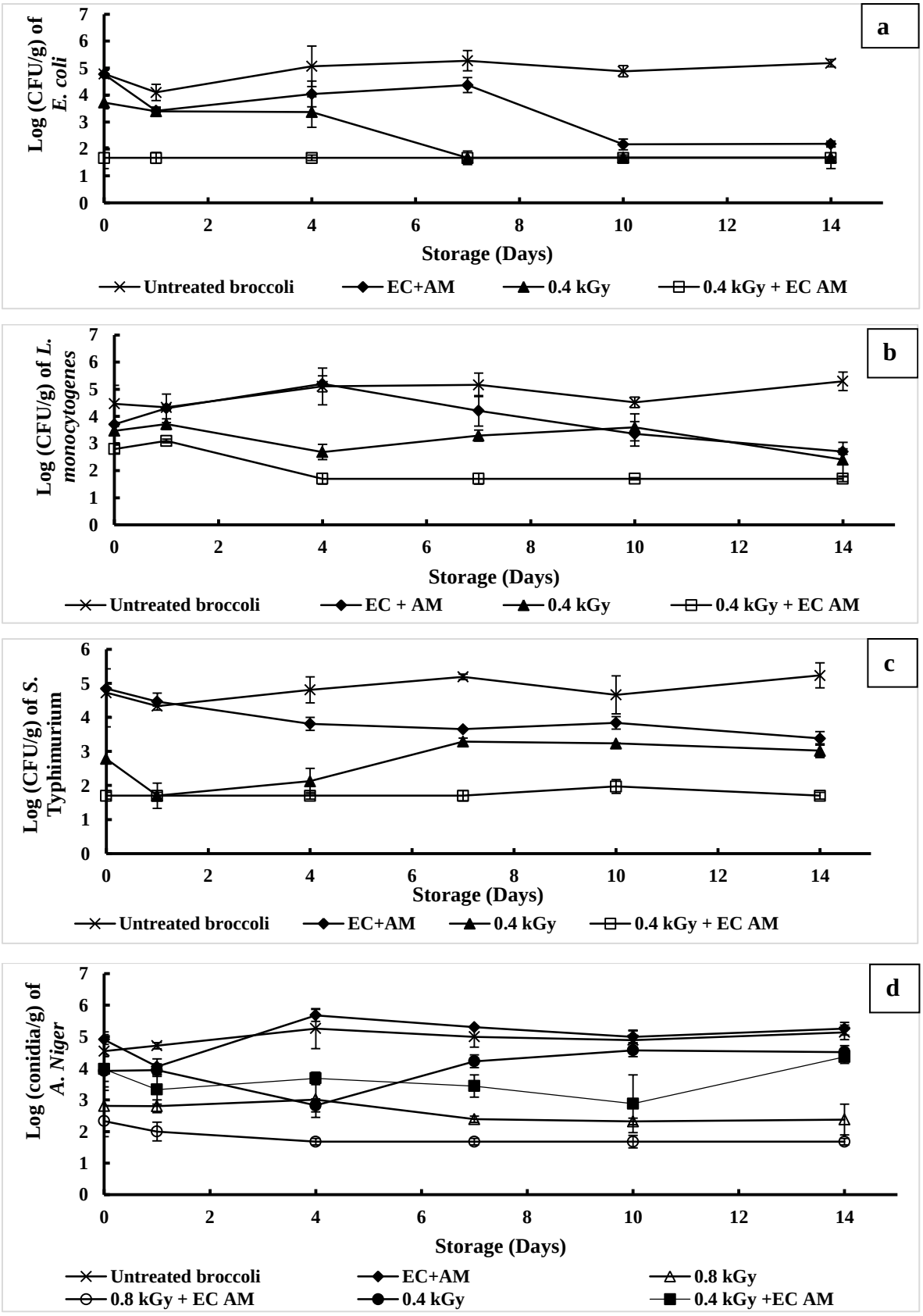

Figure 1. Effect of antimicrobial coating application combined to γ-irradiation on population of (a) *E. coli,* (b) *L. monocytogenes,* (c) *S.* Typhimurium and (d) *A. niger* on broccoli florets during storage at 4 °C.

CHAPITRE 5: DISCUSSION GÉNÉRALE

Encore aujourd'hui, les denrées alimentaires peuvent se retrouver contaminées par des microorganismes pathogènes mettant en péril la santé humaine voire animale. Des techniques de préservation utilisant des additifs de synthèse pour assurer la sécurité alimentaire sont utilisées. Cependant nous assistons à l'heure actuelle à une demande de plus en plus forte de la part des consommateurs concernant l'utilisation d'agents conservateurs naturels (Roller, 1995). Les antimicrobiens naturels ont été utilisés depuis très longtemps pour leur pouvoir inhibiteur des pathogènes alimentaires et leur capacité à prolonger la durée de conservation des aliments. Les huiles essentielles, les bactériocines et les antifongiques naturels semblent être une bonne alternative dans cette démarche de préservation naturelle des denrées alimentaires.

1- SÉLECTION DE LA FORMULATION ANTIMICROBIENNE

1.1. Détermination de la concentration minimale inhibitrice

L'objectif de cette étude a été de sélectionner les antimicrobiens capables inhiber plusieurs bactéries et moisissures pathogènes par la détermination de leur CMI. Parmi les 27 antimicrobiens, quatre huiles essentielles (Sarriette des montagnes, lemongrass, cannelle de ceylon et thym à thymol), le diacétate de sodium et le natamycine ont démontré une CMI de 1,250 ppm, 2,500 ppm, 10,000 ppm, 6,250 ppm et 80 ppm respectivement.

L'efficacité des HEs sélectionnées est reportée à certaines molécules, majoritaires dans la composition des HEs, qui ont un effet antimicrobien. C'est le cas du géraniale, néral, carvacrole, thymol, p-cymene et E-Cinnamaldehyde présents à des concentrations élevées dans HE lemongrass, thyme vulgaris thymol, sarriette des montagnes et cannelle de ceylon**.** L'activité antimicrobienne des HEs est souvent liée à des dommages de la membrane, de la coagulation du cytoplasme et dans certains cas en des modifications complètes de la morphologie des cellules (Nazzaro *et al.,* 2013). L'activité antifongique du citral et cinnamaldehyde était probablement dû à leur capacité à former des complexes de transfert de charge avec des donneurs d'électrons des cellules de moisissures (Kurita *et al.,* 1981). L'efficacité du carvacrol est due également à la modification de la perméabilité de la membrane aux cations tels que H^+ et K^+ ce qui conduit à une altération des processus essentiels de la cellule et engendre la mort cellulaire (Ultee *et al.*, 1999).

Parmi les 7 AO, seulement le diacétate de sodium a démontré un pouvoir antimicrobien avec une CMI= 6,250 ppm. Il est bien connu que l'efficacité des AOs est observée principalement lorsque ces derniers sont dans leur forme non dissociée. Sous cette forme, les AOs sont

capables de pénétrer dans la cellule microbienne. L'activité antimicrobienne des AOs a été associée à une succession de réactions commençant par la pénétration des AOs dans la cellule bactérienne induisant une acidification des composants internes et perturbation de la membrane cellulaire. Cela permet la perte du transport actif des nutriments, une augmentation de la perméabilité membranaire, la réduction d'activité de l'eau, l'inhibition de réactions métaboliques essentielles et l'accumulation d'anions toxiques (Theron *et al.*, 2010). En industrie alimentaire, les AOs sont utilisés non seulement pour leur pouvoir antimicrobien, mais aussi comme acidulant, antioxydants, agent de saveur, ajustement de pH et aussi pour leur valeur nutritive (Theron *et al.*, 2010)

D'autre part, la natamycine a montré une grande efficacité pour éliminer *A. niger* avec une CMI = 40 ppm. En fait, la natamycine est un antibiotique polyene utilisé comme agent antifongique en raison de son large spectre d'activité et l'absence de développement de résistance (Te Welscher *et al.*, 2008). La natamycine bloque la croissance fongique en se liant spécifiquement à l'ergostérol des membranes (Te Welscher *et al.*, 2008).

1.2. Étude des interactions

Les résultats obtenus ont montré que la combinaison de (1) thym thymol et le diacétate de sodium et (2) sarriette de montagne et diacétate de sodium ont un effet additif contre toutes les souches évaluées. Par contre, l'HE cannelle de Ceylan combinée au diacétate de sodium avait une activité additive seulement contre *S.* Typhimurium et une activité non-interactive contre toutes les autres bactéries et les moisissures évaluées. Une activité synergique a été observée pour la combinaison de EO lemongrass et le diacétate de sodium pour réduire *L. monocytogenes*. Cette combinaison était aussi efficace pour réduire *S.* Typhimurium, *E. coli* et *A. niger* démontrant une activité additive. Les résultats obtenus suggèrent que la combinaison d'AO et HE pourrait agir en synergie pour réduire les bactéries et les moisissures. D'autres combinaisons ayant une activité synergétique ont été mis en évidence par Zhou *et al.* (2007) et Dimitrijević *et al.* (2007). L'effet synergétique entre les HEs et les AOs a été expliqué par Zhou *et al.* (2007), qu'en présence d'AO, les HEs sont sous leur forme moléculaire et donc, elles sont plus perméables via la membrane cytoplasmique des bactéries ce qui engendre une activité antimicrobienne améliorée.

2. TEST SUR LES VÉGÉTAUX

Dans cette étude, nous avons étudié deux combinaisons de traitements possibles sur le brocoli: 1- le prétraitement au calcium et l'enrobage d'alginate 2- l'enrobage antimicrobien et l'irradiation gamma.

2.1. Combinaison du prétraitement au calcium et l'enrobage comestible

L'objectif de cette étude a été d'évaluer l'effet d'un prétraitement au calcium appliqué en combinaison avec un enrobage à base d'alginate sur la qualité physicochimique et microbiologique du brocoli PAM. Le suivi du taux de respiration (bon indicateur de la senescence des légumes) a été réalisé. En effet, la durée de conservation de légumes est inversement corrélée à leur taux de respiration: s'il est élevé, les légumes consomment rapidement leurs réserves et évoluent vers la sénescence. Le brocoli a une activité respiratoire extrêmement élevée (> 60 mg kg^{-1} CO_2 h^{-1} quand il stocké à 5 °C) (Saltveit, 2004). Ainsi, pour prolonger sa durée de conservation, il est nécessaire de réduire son activité respiratoire.

Les résultats obtenus lors de cette étude ont démontré que l'enrobage d'alginate semble avoir un grand potentiel à réduire significativement le taux de respiration du brocoli PAM. Cela pourrait être lié à la formation d'une atmosphère interne modifiée et une réduction du métabolisme du brocoli et ainsi toutes les réactions relatives d'oxydo-réduction entre l'environnement et le brocoli traité. Ceci induit des modifications importantes sur l'équilibre CO_2 / O_2 dans l'atmosphère interne et par la suite une réduction de l'accumulation de métabolites respiratoires produits dans les brocolis enrobés pendant le stockage.

Les résultats du suivi de la perte de masse ont démontré qu'indépendamment du traitement appliqué, une augmentation de la perte de masse a été observée pendant toute la durée du stockage dû principalement au processus de respiration et la transpiration (Maguire *et al.*, 2001). En fait, au cours de la respiration de brocoli, une libération d'eau et de dioxyde de carbone par l'oxydation des glucides réduit la teneur en matière sèche (Zhan *et al.*, 2012). Toutefois, lorsque l'enrobage d'alginate a été appliqué seul ou combiné à un prétraitement au calcium, une réduction significative ($P \leq 0,05$) de la perte de masse a été observée. Le prétraitement avec 3 différentes concentrations de $CaCl_2$ a permis une réduction significative de la perte de masse du brocoli par rapport aux échantillons non traités et seulement enrobés. Les résultats obtenus suggèrent que l'application du prétraitement au chlorure de calcium permet de protéger contre la perte de masse du brocoli et son efficacité était dose-dépendante. Toutefois, les résultats obtenus pour le taux de respiratoire ont démontré que l'enrobage d'alginate a permis de réduire d'une façon moindre le taux de respiration. Ces observations suggèrent que la perte de masse dans les échantillons enrobés était probablement liée à la dessiccation de films de l'enrobage comestible et non pas liée à la perte de masse inhérente au brocoli. Ainsi, le prétraitement de calcium a permis de protéger le film d'alginate contre la

déshydratation pendant le stockage à 4 °C. Les résultats obtenus lors de cette étude sont très intéressant car la perte de masse est un bon indicateur de la qualité de brocoli et il a un impact direct sur le coût-efficacité et la valeur marchande du produit final.

Les résultats obtenus de la texture du brocoli que la force de pénétration du brocoli non traitée et enrobé a augmenté de façon significative par rapport au brocoli prétraité au calcium. Ceci ne signifie pas qu'une amélioration de la fermeté mais plutôt une augmentation de l'élasticité est le résultat de la déshydratation du tissu. Ainsi, le tissu de la tige du brocoli devient de plus en plus fibreux au cours du stockage et par la ensuite il devient plus élastique (Serrano *et al.*, 2006). Un prétraitement au $CaCl_2$ stabilise la texture au cours du stockage et maintient ainsi sa fermeté. En effet, le calcium existe déjà comme pectate de calcium dans la paroi cellulaire en tant qu'agent de maintien de la cellule végétale. La perte de calcium au cours du stockage conduit à un ramollissement des légumes. Un prétraitement au calcium retarde la sénescence et le ramollissement des légumes par réticulation entre les chaînes et polygalacturonide et le calcium dans la paroi cellulaire (Rahman, 2007).

Les résultats obtenus pour le suivi de la couleur ont montré que l'angle Hue de la tête du brocoli non traitée diminue significativement ($P \leq 0,05$) au cours du stockage à 4 °C couplée à une augmentation des valeurs de C*. Ceci reflète la transition de la couleur verte au jaune la perte de la couleur verte grisâtre de ces échantillons. Ceci n'a pas été observé pour les échantillons traités. Donc, l'application de l'enrobage d'alginate seul ou combiné au prétraitement au calcium a permis de maintenir la couleur verte du brocoli en réduisant les pertes de chlorophylle. Une diminution des valeurs de C* a été observée seulement lorsque la concentration du $CaCl_2$ > 0.25%. Indépendamment du traitement appliqué, les valeurs de L* ont augmenté au cours du stockage. Le jaunissement est généralement lié à la sénescence, qui est caractérisé par la dégradation de la chlorophylle au cours du stockage (Rai *et al.*, 2009). Pendant le stockage, aucune différence significative sur les valeurs de l'angle Hue * n'a été observée pendant toute la durée de stockage pour tous les brocolis traités. Cela suggère que, appliquer un enrobage d'alginate, a permis de protéger la couleur verte du brocoli en réduisant les pertes de chlorophylle.

Les résultats du compte du FMT ont montré que l'application de l'enrobage d'alginate seul n'a pas d'effet signification sur la prolongation de la durée de conservation du brocoli. Cependant en combinant avec le prétraitement au calcium, la durée de conservation a été augmentée de 6 jours par rapport au brocoli non traité. Le taux de croissance était également

plus faible dans les échantillons traités. Ainsi, l'application de prétraitement de calcium semble avoir un effet significatif permettant l'extension de la durée de conservation du brocoli PAM. L'effet inhibiteur du calcium est le résultat de l'augmentation de la résistance du tissu à une infection bactérienne et non à une action bactéricide. L'effet inhibiteur a été également expliqué par Madani *et al.* (2015) comme le résultat de l'augmentation de la concentration en calcium cytosolique qui augmente la synthèse des phytoalexines, des composés phénoliques, puis une diminution de l'activité des enzymes pectolytiques pathogènes.

2.2. Combinaison de l'irradiation gamma et de l'enrobage antimicrobien

L'objectif de cette étude consiste à évaluer l'efficacité *in situ* de l'enrobage encapsulant la formulation antimicrobienne lorsqu'il est appliqué seul ou en combinaison à l'irradiation gamma sur le brocoli. Les résultats obtenus montrent que l'enrobage antimicrobien était efficace pour réduire significativement le compte d'*E. coli*, *S.* Typhimurium et *L. monocytogenes* pendant le stockage à 4 °C. Cependant, aucune réduction significative n'a été observée avec *A. niger*. Donc pour éliminer *A. niger* une concentration plus élevée en antimicrobien devrait être appliquée. Ces résultats confirment aussi la résistance d'*A. niger* aux AMs observée lors de l'étude des CMI. Un effet synergétique a été observé en combinant l'enrobage antimicrobien et l'irradiation gamma à 0.4 kGy pour réduire significativement *E. coli, Listeria* et *Salmonella* durant 14 jours de stockage. Pour *A. niger*, une dose d'irradiation à 0.4 kGy était insuffisante pour l'éliminer. Une dose de 0.8 kGy était nécessaire pour éliminer *A. niger*. L'efficacité de l'irradiation comme un traitement fongicide et/ou fongistatique dépend du pathogène étudié, son stade de croissance et le nombre de cellules viables de moisissures sur ou dans le tissu. En général, une dose minimum de 1,75 kGy est requise pour une inhibition efficace des moisissures (Kader, 1986),

3. TEST SUR LES CHARCUTERIES

L'objectif de cette étude a été d'évaluer l'activité antimicrobienne d'une marinade commerciale lorsqu'elle est appliquée seule ou en combinaison avec l'irradiation gamma. L'effet des traitements combinés sur les propriétés sensorielles et nutritionnelles et physicochimiques a aussi été évalué. Les résultats ont montré que *C. sporogenes* est plus radiorésistante que *E. coli* et *S.* Typhimurium. Pour éliminer *E. coli* et *S.* Typhimurium durant 28 jours de stockage à 4 °C, une dose d'irradiation de 1 kGy est nécessaire contre 1.5 kGy pour éliminer *C. sporogenes*. Ces résultats suggèrent que des *Enterobacteriaceae* sont moins radiorésistants que *C. sporogenes* (Grant *et al.*, 1991). Un traitement d'irradiation à 1.5 kGy

sur une viande marinée a également permis de retarder la croissance de FMT et des BL au cours du stockage. Ainsi, une prolongation de la durée conservation a été observée. Cependant, Dogbevi *et al.* (1999) ont démontré que même avec une dose d'irradiation de 3 kGy, le compte de FMT est réduit à un niveau indétectable au jour 0 et recommence à croitre à partir du jour 3. Ainsi, une synergie a été observée entre l'ajout de la marinade, l'irradiation à 1.5 kGy et l'emballage sous vide. La durée de conservation a également augmenté de manière significative. Donc, l'application de la marinade et l'emballage sous vide a augmenté la sensibilité de FMT et des BL à l'irradiation.

L'étude de l'impact des traitements appliqués sur les vitamines (thiamine et riboflavine) ont montré que la thiamine est particulièrement la vitamine hydrosoluble la plus radiosensible en raison de la présence de hétéro doubles liaisons dans la molécule (Molins, 2001). La riboflavine, par contre, est plus radiorésistante et seulement une irradiation à une dose de 3 kGy a induit une réduction significative de sa concentration dans la viande.

La présence de la marinade durant le traitement d'irradiation a permis de protéger la thiamine et la riboflavine contre l'irradiation. Ceci est dû au faible pH de la marinade et sa forte concentration en carbohydrates. Il est important de noter que la thiamine est plus stable en présence de glucose et dans des conditions acides plutôt que sous des conditions neutres ou alcalines où sa destruction devient beaucoup plus rapide (Fox *et al.*, 1995, Molins, 2001).

Les résultats de l'effet des traitements sur l'oxydation des lipides ont montré qu'une irradiation des doses ≥ 1,5 kGy a augmenté significativement les valeurs de TBARS ($P \leq 0,05$). Lorsque l'irradiation est appliquée sur la viande marinée, aucune augmentation significative ($P > 0,05$) des TBARS n'a été observée indépendamment de la dose d'irradiation appliquée. Le rôle protecteur de la marinade contre l'oxydation des lipides dans la viande est probablement dû à la présence de l'huile de canola et la poudre de curry dans la marinade. En effet, une activité antioxydante est liée à ces ingrédients en particulier des flavonoïdes telles que myricétine et la quercétine présents à la fois dans l'huile de canola et la poudre de curry qui sont des protecteurs efficaces contre l'oxydation des lipides (Chen *et al.*, 1996, Ningappa *et al.*, 2008).

La combinaison de l'ajout de la marinade et l'irradiation gamma a donné une viande plus rouge due à la formation de ligand d'hème pigment-CO même avec de la viande emballés sous vide (Nam *et al.*, 2002). Les résultats ont montré aussi que pour tous les traitements, une augmentation de valeur L * de la viande durant le stockage à 4 ° C a été observée due à la

diminution du pH et l'accumulation de sous-produits métaboliques au cours de la glycolyse post-mortem et vieillissement de la viande (Brewer, 2004, Rahman, 2007). Les résultats de l'analyse sensorielle n'ont dévoilé aucun effet significatif de l'irradiation à une dose de 1.5 kGy sur le goût, la saveur, la couleur, la texture et sur l'appréciation globale de la viande cuite. Ces observations confirment les résultats obtenus de l'oxydation des lipides. En effet, l'oxydation des lipides est l'un des indicateurs les plus importants de la détérioration de la viande et elle est généralement liée à la production de mauvaise flaveur et odeur.

CHAPITRE 6 : CONCLUSION GÉNÉRALE

Dans ce mémoire nous nous sommes intéressés au développement de nouvelles combinaisons de traitements permettant d'assurer la salubrité des aliments PAM sans pour autant modifier leurs propriétés physicochimiques et nutritionnelles du produit fini. Nous avons fait le criblage de 27 antimicrobiens naturels afin de sélectionner la formulation la plus efficace capable d'éliminer les pathogènes alimentaire. Pour le brocoli, l'effet de la combinaison du prétraitement au calcium et l'enrobage comestible a été évalué sur les propriétés physicochimiques et microbiologiques du brocoli PAM. Aussi, l'efficacité de la combinaison de l'irradiation gamma et de l'enrobage antimicrobien à éliminer les pathogènes du brocoli a été évaluée. Pour la viande emballée sous vide, la combinaison de l'irradiation gamma et de l'ajout d'une marinade à base d'épice et d'extraits de végétaux a été évaluée pour leur efficacité à réduire les pathogènes de la viande et à prolonger la durée de conservation. L'effet de cette combinaison sur la qualité sensorielle, nutritionnelle et physicochimique a aussi été évalué.

Nous avons essayé de trouver et de sélectionner une formulation antimicrobienne à base d'AN efficace à éliminer les bactéries et les moisissures susceptibles à contaminer le brocoli PAM. La formulation sélectionnée a démontré une interaction synergique contre *L. monocytogenes*. Nous avons pu constater également une synergie entre la formulation antimicrobienne encapsulée dans une matrice de polymère et l'irradiation gamma pour la réduction d'*E. coli, L. monocytogenes, S.* Typhimurium et *A. niger* dans les bouquet de brocoli stockés à 4°C.

Cette étude a aussi démontré qu'il est possible de conserver de la viande à une température de 4 °C en contrôlant les bactéries pathogènes en présence de marinade combiné à un traitement d'irradiation à une dose de 1.5 kGy tout en conservant les propriétés d'un produit frais. Il a aussi été démontré que combiner un prétraitement au calcium à une concentration > 0.4% à un enrobage d'alginate permet de maintenir une qualité fraiche du brocoli PAM durant 11 jours de stockage 4 °C.

La combinaison de traitements appliqués sur le brocoli peut être sujette à des modifications et améliorations ce qui peut être très intéressant à faire comme travaux futurs. Il sera possible de combiner le prétraitement au calcium à l'enrobage antimicrobien pour évaluer leur potentiel à inhiber les pathogènes du brocoli. Aussi une étude plus approfondie

sur l’effet de ces traitements sur la morphologie et l’intégrité microbienne peut être aussi très intéressant à exploiter afin de comprendre et de trouver des solutions efficaces pour la résurrection des bactéries dans les aliments durant le stockage. D’autres travaux peuvent chercher dans ce sens et trouver une solution à ce problème. Aussi, il sera intéressant de travailler sur les formes végétatives (spores) vu leur grande résistance et trouver des traitements efficaces à les éliminer sans déclencher les mécanismes de résistances.

Tous ces résultats nous révèlent qu’effectivement les traitements combinés peuvent être une alternative intéressante pour assurer la salubrité des aliments PAM. Des traitements utilisés en combinaison peuvent agir en synergie réduisant ainsi la dose ou le temps de traitement pour garantir l’innocuité et la stabilité physicochimique sans modifier les propriétés nutritionnelles. Ces résultats peuvent être d'un grand intérêt pour l'industrie alimentaire pour la conservation des aliments et en particulier pour les patients immunodéprimés qui sont condamnés à manger des aliments avec de faibles valeurs nutritionnelles causées par les procédés de décontamination rudes. Ce travail présente une nouvelle alternative pour cette catégorie de personnes en les protégeant des maladies d'origine alimentaire et en augmentant la qualité nutritionnelle des aliments.

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