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Mise au point de méthodes antimicrobiennes pour application sur des produits prêts à manger

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Résumé

Malgré le développement technologique dans les industries alimentaires et la mise en place de normes permettant d'assurer la salubrité des aliments telle que la norme d'analyse des dangers et le contrôle des points critiques, la contamination d'aliments reste élevée d'où le nombre de maladies alimentaires qui ne cesse d'augmenter.

Les fruits et légumes sont riches en nutriments tels que les vitamines, les polyphénols, les fibres et les sucres (Anses, 2016). Seulement, ils sont reconnus comme des vecteurs importants de transmission d'agents pathogènes tels qu'*Escherichia coli, Listeria monocytogenes* et Salmonelle qui peuvent proliférer au cours du stockage, du transport ou de la manipulation. Certains pathogènes sont responsables d'intoxications, infections et toxi-infections alimentaires, par exemples : *E. coli* peut causer une colite hémorragique, le syndrome urémique hémolytique et le purpura thrombocytopénique thrombotique, *L. monocytogenes* peut causer la listériose et affecte généralement les femmes enceintes, les personnes immunodéprimées et les personnes âgées.

Les aliments prêts à manger sont également une source importante de contamination, en effet au cours des manipulations de ces fruits et légumes le risque de contamination augmente surtout lors de la découpe de ces aliments, le transport de ces derniers peut être à l'origine d'une contamination ainsi qu'au cours de l'entreposage.

L'utilisation de désinfectants chimiques tel que le chlore pour décontaminer les fruits et légumes peut former des produits cancérigènes en réagissant avec la matière organique. D'où l'intérêt de l'utilisation des méthodes de décontamination non conventionnelle et non chimique. En effet, l'utilisation de chlore pour le traitement des fruits et de végétaux peut induire des saveurs désagréables et altérer le goût.

Les objectifs de ce mémoire seront de tester et de trouver des méthodes de conservation pour les fruits et légumes prêts à manger, sans utilisation de produits chimiques (chlore, benzoate de sodium etc).

Les résultats montrent que le jus de canneberges vaporisé sur les poivrons et canneberges a éliminé significativement les bactéries pathogènes. De plus, les deux formulations étudiées F2 et F6 ont révèlé une activité antibactérienne importante contre *E. coli*, *Listeria* et *Salmonella* dans les differents fruits et légumes testés. Il a aussi été démontré que l'extrait d'agrumes FOODGARD a réduit et inhibé les pathogènes alimentaire, la flore totale et les levures et moisissures dans les garnitures de fruits. La mise au point d'une formulation X1+DF a aussi diminué significativement les microorganismes testés. De plus, l'utilisation de l'ozone gazeux et de l'irradiation a permis une inhibition totale des pathogènes. Ainsi, les résultats obtenus suggèrent que les formulations mises en place ont permis la réduction significative et l'inhibition des pathogènes alimentaires dans les légumes et fruits prêts à manger. Les techniques mises au point au laboratoire seront ainsi appliquées en industrie de transformation alimentaire.

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Table des matières

Sommaire

Liste des Tableaux

Revue de la littérature

Chapitre 1

Chapitre 2

Chapitre 3

Chapitre 4

Liste des Figures

Revue de la littérature

Chapitre 1

Chapitre 2

Chapitre 3

Liste des abréviations

a*	Coordonnée rouge-vert dans l'espace des couleurs CIELAB / *Red-green parameter in the CIELAB color space*
AA	*Ascorbic acid*
AAC	*Agricultural Adaptation Council*
AAC	Agriculture et agroalimentaire Canada
AAO	*Ascorbate oxidase*
AAFC	*Agriculture and Agri-Food Canada*
ACIA	Agence canadienne d'inspection des aliments
AIP	*Agri-Innovation Program*
ANOVA	Analyse de la variance / *Analysis of variance*
ATP	*Adénosine triphosphate*
ATR	*Acid tolerance response*
a_w	Activité de l'eau / *Water activity*
b*	Coordonnée jaune-bleu dans l'espace des couleurs CIELAB / *Blue-yellow parameter in the CIELAB color space*
CDC	*Centers for Desease Control and Prevention*
cfa	*Cyclopropane fatty acyl phospholipid synthase*
CFI	*Canadian Food Innovators*
CFU	*Colony Forming Units*
Cha	Chlorophyll a
Chb	Chlorophyll b
CIC	Centre d'irradiation du Canada / *Canadian Irradiation Center*
CJC	*Cranberry juice concentrate*
CMI	Concentration minimale inhibitrice
COOH	Groupement carboxylique
DCLS	Desoxycholate Lactose Sucrose
DDT	*Dithiothreitol*
DHA	*Dehydroascorbic acid*
EOs	*Essential oils*
EU	*European Union*
FDA	*Food and Drug Administration*
FP	*Food poisoning*
g	gramme
GMP	*Good Manufacturing Practice* / Bonnes pratiques manufacturières

GRAS	*Generally Recognized As Safe* / Généralement reconnu comme sûr
h°	Paramètre de la teinte dans l'espace des couleurs CIELAB / *Hue parameter in the CIELAB color space*
HACCP	Analyse des risques et maîtrise des points critiques / *Hazard Analysis Critical Control Point*
HE	Huiles essentielles
HSP	*Heat shock protein*
HUS	*Hemolytic uremic syndrome*
IC	*Inhibition capacity*
INAF	Institut sur la nutrition et les aliments fonctionnels / *Institute of Nutrition and Functionnal Foods*
INRS	Institut national de la recherche scientifique
L*	Coordonnée de brillance dans l'espace des couleurs CIELAB */ Lighness parameter in the CIELAB color space*
log P	*Partition coefficient*
LPS	*Lipopolysaccharides*
MA	*Modified atmosphere*
MAP	*Modified atmosphere packaging* / Emballages sous atmosphère modifiée
MAPAQ	Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
mL	*millilitre*
MD	Maryland
MHB	*Mueller Hinton Broth*
MIC	*Minimal inhibiting concentration*, voir CMI
min	*Unité de minutes*
MTCs	*Maximum tolerated concentration*
MTT	3(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide
NFB	*Non-food-borne*
NY	New York
OAs	*Organic acids*
OM	*Outer membrane*
OMS	Organisation mondiale de la santé
ON	Ontario
p	*Probability* / Probabilité
PACs	*Pro-anthocyanidins*
PDA	*Potato Dextrose Agar*
PDB	*Potato Dextrose Broth*
Pm	*Permeability coefficient*

PMS	5-methylphenazinium methyl sulphate
psi	*Pound per square inch*
QC	Québec
RESALA	Laboratoire de Recherche en Sciences appliquées à l'Alimentation de Laval
rpm	*Revolutions per minute /* Révolutions par minute
RTE	*Ready-to-eat*
SCFH	*Standard cubic feet per hour*
SF	*Strawberry filling*
SS	*Stainless steel*
TAM	*Total Aerobic Microflora*
TEM	*Transmission electron microscopy*
TSA	*Tryptic Soy Agar*
TSB	*Tryptic Soy Broth*
TSST	Toxine Superantigénique du Syndrome de choc Toxique
UFC	Unités formant des colonies, voir CFU
USA	*United States of America* / États-Unis d'Amérique
USDA	*United States Department of Agriculture*
v/v	*Volume by volume*
VT	Vermont
w/v	*Weight by volume*
WHO	*World Health Organization*, voir OMS
Y/M	*Yeasts/molds*
ΔE	Paramètre de difference de couleur dans l'espace des couleurs CIELAB / *Color difference parameter in the CIELAB color space*

Revue de la littérature

1. Introduction :

Malgré le développement des nouvelles technologies de contrôle de la qualité telle que l'Analyse des risques et maîtrise des points critiques (HACCP), le nombre des maladies d'origines alimentaires n'a pas cessé d'augmenter jusqu'en 2002 (O'Sullivan *et al.*, 2002). D'après le *Centres for Disease Control and Prevention* (CDC) (2014), la majorité des pathogènes d'origines alimentaires inclut *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus* (Collignon et Korster, 2010). *E. coli* O157:H7 peut causer une collite hémorragique, le syndrome hémolytique et un purpura urémique thrombocytopénique. *S.* Typhimurium est l'une des principales souches causant la salmonellose. D'après le CDC US (2005) 73 000 cas d'infection par *E. coli* O157:H7 ont causé la mort de plus de 61 personnes. Jay *et al.* (2005) a rapporté que la listériose est généralement causée par *Listeria monocytogenes,* cette dernière peut affecter les femmes enceintes, les individus immunodéprimés ainsi que les personnes âgées. L'exotoxine sécrétée par *S. aureus* est responsable de l'empoisonnement des aliments (Jay *et al.,* 2005). D'autre part la consommation des fruits et légumes est en augmentation et devient une priorité afin d'équilibrer le manque de vitamines et de minéraux. De plus, certaines études récentes ont prouvé que la consommation de fruits et légumes pourrait réduire le risque de cancer de l'oropharynx, de l'œsophage, des poumons, de l'estomac et du colorectum (Soerjomataram *et al.*, 2010). Selon Statistique Canada (2012), 40.6% des Canadiens âgés de 12 et plus consomment 5 portions de fruits et légumes et plus par jour en 2012. En outre, les préoccupations des consommateurs ont créé une demande pour des aliments plus « naturels » et « peu transformés ». Une augmentation de 88% et de 33% des salades prêtes à manger et des végétaux congelés respectivement est attendue entre 1984 et 2020 (AAFC et Serecon, 2005). Cependant les fruits et légumes prêts à manger sont hautement périssables car ils contiennent entre 80 et 90% d'eau (Dhall, 2013). Etant donné que les aliments prêts à manger ne sont pas traités avant leur consommation, ils constituent ainsi un risque de maladies d'origine alimentaire. Les industries alimentaires ont besoin de procédés innovants dans le but de subvenir aux besoins des consommateurs en produits prêts à manger. Récemment, la demande de poivrons verts a augmenté et surtout son utilisation en tant qu'ingrédient dans les prêts à manger (Gonzalez- Aguilar *et al.*, 2004 ; Ketteringham *et al.*, 2006). En conséquence, l'application de composés antibactériens produits naturellement, comme les huiles essentielles extraites de plantes et les acides organiques utilisés comme conservateurs ou désinfectants ont reçu une grande attention. L'ozone a largement été utilisé en industrie alimentaire pour assurer l'innocuité des aliments. Tous ces antimicrobiens ont été utilisés dans le but de remplacer les traitements chimiques nocifs et les antibiotiques contre lesquels les bactéries ont développé une résistance.

Les maladies d'origine alimentaire

Le taux de maladies d'origine alimentaires est en augmentation. Plusieurs bactéries sont responsables de ces maladies. D'après Scallan *et al.* (2011a) les bactéries les plus responsables d'infections sont les *Salmonella* non-Typhi (28%), *Clostridium perfringens* (27%), *Campylobacter* spp. (23%), *Staphylococcus* spp. (7%), *Shigella* spp. (4%), *Escherichia coli* (3%), *Yersinia enterocolitica* (3%), *Bacillus cereus* (2%), *Escherichia coli* O157:H7 (2%) et *Vibrio parahaemolyticus* (1%). Les maladies d'origines alimentaires sont définies par l'Organisation mondiale de la santé (OMS) par les maladies infectieuses généralement toxiques, causées par des agents qui pénètrent dans le corps par l'ingestion d'aliments (Food Standards Agency du Royaume-Uni, 2011).

On estime qu'environ 2,2 millions de personnes dans le monde meurent chaque année à cause de maladies d'origine alimentaire et hydrique, et ces chiffres continuent à augmenter en raison d'une augmentation de la résistance, mais aussi aux nouvelles apparitions d'agents pathogènes. (Weiss, 2013). Comme les aliments prêts à manger ne sont pas encore traités avant la consommation de manière à réduire de manière significative la charge microbienne, le risque de maladies d'origine alimentaire doit être envisagée. L'inconvénient des légumes fraichement pré-découpés est que la durée de vie soit fortement réduite et cela est due aux procédés mécaniques de découpe (Barriga *et al.*, 1990). L'industrie alimentaire a actuellement besoin de technologies de traitement novatrices afin de répondre à la demande des produits prêts à consommer plus frais et sûrs. *Listeria monocytogenes* peut se développer sous les conditions de réfrigération et peut développer une tolérance à l'acidité. D'après Cosgrove *et al.* (2011) *Listeria monocytogenes* est liée surtout à la contamination des viandes et des produits de la mer et a été associée dernièrement aux produits frais. En plus, *Listeria monocytogenes* est une cause principale de listériose aux États-Unis qui a induit 19% de morts reliées aux maladies alimentaires et coûtant à environs 2.8 milliards de dollars. (Scallan *et al.*, 2011b; US Department of Agriculture Economic Research Service, 2015). Entre 1981 et 1990 des mesures de sécurité alimentaire ont été prises afin de réduire les contaminations de la viande prête à manger et des viandes de volailles en réduisant les cas de maladies de listériose de plus de 50%. (Cartwright *et al.*, 2013).

Il est crucial de mentionner que les personnes âgées, les immunodéprimés, les femmes enceintes et les nouveaux nés présentent un risque élevé de listériose caractérisé par une bactériémie (présence de bactérie pathogène dans le sang circulant), une méningite, une perte du fœtus et la mort (Painter et Stutsker, 2007; Swaminathan Gerner-Smidt, 2007)

L'infection à *E. coli* O157 a causé le syndrome hémolytique urémique chez les enfants (Siegler et Oakes, 2005 ; Banatvala, 2001). Depuis les années 90, *E. coli* O157:H7 a été associée avec plus de 180 maladies d'origine alimentaire aux États-Unis (Rangel *et al.*, 2005).

Rangel *et al.* (2005) et Wendel *et al.* (2006) ont rapporté que la viande hachée, les végétaux et les produits laitiers non pasteurisés sont fréquemment impliqués dans les maladies alimentaires. De plus,

un large spectre de produits alimentaires a été associé à des infections à la *Salmonella*. D'après Sivapalasingam *et al.* (2004), *Salmonella* est la bactérie pathogène la plus reliée à la consommation des fruits légumes. Plusieurs maladies alimentaires ont été liées à la consommation des tomates (Hedberg *et al.*, 1999; Gupta *et al.*, 2007) contaminés à la *Salmonella*. En 2008, les poivrons jalapeño et serrano ont causé plusieurs infections à la *Salmonella* (Center for Disease Control and Prevention, 2008).

Les bacteries pathogènes

Escherichia coli

*C'*est une bactérie Gram négative capable de survivre dans des conditions d'aérobiose ou d'anaérobiose, appartenant à la famille des *Enterobacteriaceae* en forme de bâtonnet, asporulé pouvant se déplacer avec un flagelle. Elle produit des entérotoxines thermolabiles et des entérotoxines thermostables. Cette bactérie peut contaminer l'eau et les aliments et peut être propagée dans plusieurs environnements.

La dose pour infecter une personne adulte est de 10^8 microorganismes. Cependant, à des doses moins importantes, les enfants, les personnes âgées et les personnes handicapées pourraient être infectés. (Agence de la santé publique du Canada 2012).

Salmonella Typhimurium

Salmonella est un bacille à gram négatif, aéro-anaérobie facultatif possédant un flagelle. Elle peut contaminer les viandes, les végétaux, les fruits et l'eau et peut causer des gastro-entérites, des bactériémies et des fièvres entériques. La dose infectieuse varie selon le sérotype, en effet, elle est de 10^3 bacilles dans le cas de la salmonellose non typhique et de 10^5 bacilles dans le cas de la fièvre entérique.

Il est important de mentionner qu'à une dose plus faible, elle peut aussi infecter les personnes agés et les personnes immunodéprimées. (Agence de la santé publique du Canada 2012).

Listeria monocytogenes

Listeria monocytogenes est une bactérie Gram positive en forme de bâtonnet, mobile avec un métabolisme anaérobie facultatif et est responsable de la listériose, qui est une maladie alimentaire qui peut infecter les personnes immunodéprimées et les personnes âgées, elle peut aussi provoquer la mort chez le fœtus. *L. monocytogenes* peut croître à faible température et à un pH entre 4,3 et 9,6 et peut se reproduire à des températures situées entre 1 et 45 °C. Pour les personnes en santé, la dose infectieuse de *L. monocytogenes* est comprise entre 10 et 100 millions d'UFC, par contre pour les individus à

risque élevé d'infection elle est entre 0,1 et 10 millions d'UFC (Farber *et al*., 1996 ; Low et Donachie, 1997; Acha et Szyfres, 2003).

Staphylococcus aureus

Staphylococcus aureus est une bactérie Gram positive, elle est immobile, asporulée et facultativement anaérobique; elle se dispose en grappes. (Muray *et al*., 2003). De nombreuses souches produisent des entérotoxines staphylococciques, la toxine superantigénique du syndrome de choc toxique (TSST-1) et des toxines exfoliatives (Kluytmans *et al*., 1997).

Suite à la consommation d'aliments contaminés par des entérotoxines, cette bactérie cause des toxi-infections alimentaires, résultant de la consommation d'aliments contaminés par des entérotoxines. Les symptômes de cette intoxication alimentaire se manifestent par une apparition brutale de nausées, de vomissements, de douleurs abdominales, de crampes et de diarrhée. La dose infectieuse est d'au moins 100 000 micro-organismes chez les humains (Le Loir *et al*., 2003)

Bacillus cereus

C'est un bacille Gram positif habituellement observés en paires ou en chaînettes courtes, (Muray *et al*., 2007; Logan et Rodrigez 2006). *B. cereus* est anaérobie facultatif, mobile et capable de former des endospores (Ray, 2004). Ce bacille peut produire cinq entérotoxines et une toxine émétique, qui peuvent être thermostables ou thermolabiles, selon les souches (From *et al*., 2005). La dose infectieuse est de 10^4-10^9 bacilles par gramme d'aliment pour la toxine responsable de la forme diarrhéique de l'intoxication qui est produite par le bacille dans l'intestin grêle. Alors qu'environ 10^5 à 10^8 bacilles par gramme d'aliment sont nécessaires à la production d'une quantité suffisante de toxine émétique qui est préformée dans l'aliment ingéré. (Logan et Rodriguez, 2006)

2. Les conservateurs synthétiques et leurs problématiques

De nos jours, les consommateurs sont de plus en plus prudents et exigents. En effet, ils évitent les aliments qui contiennent des conservateurs synthétiques pour éviter les effets néfastes sur la santé et préfèrent plutot les produits naturels (Gutierrez *et al.*, 2009).

2.1. L'ozone :

L'ozone est un puissant agent antimicrobien sous ses deux formes : gazeuse et aqueuse. En effet, l'ozone a permis d'inhiber les bactéries, les moisissures, les levures, les parasites et les virus (Kim *et al.*, 1999**).** De plus, il a permis d'augmenter la durée de vie de différents fruits et légumes comme

par exemple les mûres (Barth *et al.*, 1995), la laitue (Koseki et Isobe, 2006), la tomate entière ou tranchée (Aguayo *et al.*, 2006) et les raisins de tables (Artés-Hernandez *et al.*, 2004).

L'ozonation a été reconnu comme GRAS par la FDA pour le traitement de l'eau potable en bouteille lorsqu'il est utilisé afin d'appliquer les bonnes pratiques de fabrication (FDA, 1995). Dans la plupart des pays, l'ozone a également été utilisé dans différentes applications dans les industries alimentaires, et plus récemment un groupe d'experts a recommandé l'utilisation de l'ozone comme GRAS (*Generally Recognized As Safe*) et a classifié l'ozone comme désinfectant ou désinfectant pour les aliments aux États-Unis (Graham, 1997).

Xu (1999) a rapporté que l'ozone est une fois et demie plus efficace que le chlore et permet d'inhiber et de réduire un plus large spectre de microorganismes que le chlore. L'effet de l'ozone est 3000 fois plus rapide que le chlore sans produire de produits dangereux par décomposition (Graham, 1997). L'utilisation de l'ozone à des concentrations appropriées au cours du stockage sous atmosphère, permet de protéger les fruits et légumes contre les maladies et les dommages physiologiques (Nadas *et al.*, 2003)**.** D'après Guzel-Seydim *et al.* (2004) à faibles concentrations, l'ozone gazeux n'est pas très toxique, par contre à des concentrations élevées, il peut être mortel pour les humains et cela en affectant principalement les voies respiratoires.

Le traitement des poivrons à l'ozone gazeux a permis une réduction importante de population microbienne pathogène et d'altération ainsi que de l'aflatoxine (Inan *et al.*, 2007 ; Akbas et Ozdemir, 2008). D'après Horvitz et Cantalejo (2012) l'exposition des poivrons à une dose d'ozone gazeux de 0.7 ppm pendant 1 à 5 min a réduit de 2.6, 5.8 et de 2.3 log UFC/g les bactéries mésophiles, les bactéries psychrophiles et les levures et moisissures, respectivement.

D'après Selma *et al.* (2004), l'ozone gazeux à 10 000 ppm pendant 30 min a réduit de 4.2 log UFC/g le niveau de salmonelle dans les cantaloups. De plus 5.6 log UFC/g de réduction d'*E. coli* ont été observés dans les épinards à une dose de 750 à 2 000 ppm pendant 5 min (Rahman *et al.*, 2010). L'efficacité du traitement à l'ozone dans la réduction des microorganismes dans les produits frais dépend de la dose qui sera utilisée, du temps d'exposition et de la concentration de l'inoculum initial (Han *et al.*, 2002 ; Das *et al.*, 2006).

Production de l'ozone :

L'ozone est généré par exposition de l'air contenant de l'oxygène à une haute source d'énergie. Comme par exemple la radiation ultraviolette ou la décharge électrique à haut voltage. Cela transforme les molécules de dioxygène en ozone O_3. L'ozone doit être utilisé immédiatement après sa génération car il est instable et se décompose rapidement en O_2. D'après Khadre *et al.* (2001), la durée de la demi-vie de l'ozone dans l'eau distillée à 20°C est de 20 à 30 min. La production de l'ozone peut se faire selon une des méthodes suivantes : par décharge électrique, par radiation électrochimique ou ultraviolet. La méthode la plus utilisée est la décharge électrique mais son inconvénient est qu'elle

consomme beaucoup d'énergie par rapport aux deux autres méthodes et avec une efficacité faible de 2 à 10%.

La **Figure 1** montre le diagramme de la génération d'ozone

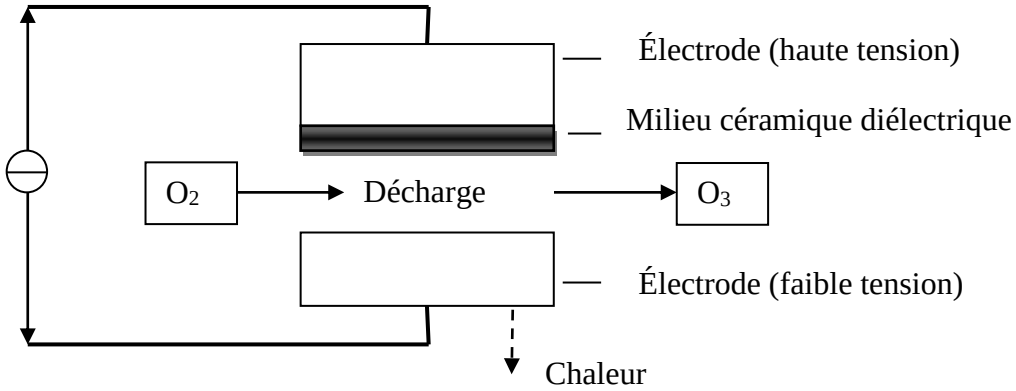

Figure 1- Schéma de diagramme de production de l'ozone par la méthode de corona décharge (Rice *et al.*, 1981).

Il y a deux électrodes dans la décharge corona l'une est à haute tension, l'autre est à faible tension. Elles sont séparées par un milieu céramique diélectrique et par un espace de décharge. Quand les électrons ont suffisamment d'énergie (6 à 7 eV) pour dissocier la molécule d'oxygène. Des collisions se produisent et une molécule d'ozone peut être formée (Rice *et al.*, 1981).

Ozone aqueux

Restaino *et al.* (1995) a étudié les effets antimicrobiens de l'eau ozonée contre les bactéries susceptibles de contaminer les aliments et a conclu que l'ozone a réduit les bactéries Gram (+) telles que *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Enteroccocus faecalis* et aussi les bactéries Gram (-) telles que *Pseudomonas aeruginosa* et *Yersinia enterocolitica*. D'après Restaino *et al*. (1995) l'ozone attaque la membrane glycoprotéique et la membrane glycolipidiques des bactéries. Après avoir comparé l'effet de l'ozone et celui du peroxyde d'hydrogène sur le pathogène alimentaire *Bacillus* ssp. et ses spores, Khadre *et al*. (2001) ont trouvés que l'ozone est plus efficace que le peroxyde d'hydrogène. Le traitement des luzernes à l'eau ozonée à des concentrations entre 4 ppm et 21 ppm a réduit de 0.4 à 1.75 log UFC/g d'*E. coli* O157:H7. (Sharma *et al*., 2002)

Mécanismes d'action de l'ozone :

Il y a deux principaux mécanismes d'action de l'ozone :

1. L'ozone oxyde les groupes sulfhydriles et les acides aminés des enzymes, les peptides et les protéines en peptides plus courts (Victoria, 1992).
2. L'ozone oxyde les acides gras polyinsaturés en peroxydes (Victoria, 1992).

2.2. Le chlore :

Le lavage des fruits et légumes à l'eau chlorée (50-200 ppm) a été largement été utilisé par les industries. Cependant, ce traitement n'a pas éliminé plus de 3 log UFC/g de bactéries comme l'ont rapporté Brackett (1987; 1992), Beuchat (1992; 1999), Beuchat *et al.* (1998) et Taormina *et al.* (1999).

De plus, plusieurs études ont rapporté que le ClO_2 sous ses formes gazeuse ou aqueuse a été efficace pour éliminer les cellules végétatives et les spores des pathogènes alimentaires et des microorganismes d'altération (Han *et al.*, 2001, 2002 ; Lindsay *et al.*, 2002; Lee *et al.*, 2006; Popa *et al.*, 2007). Par contre, les traitements en utilisant des solutions concentrées de chlore peuvent produire des sous-produits nocifs et corrosifs tels que les chloramines et les trihalomethanes (Aieta *et al.*, 1984).

Il est important de noté que Hassenberg et Bender (2008) ont rapporté que l'utilisation de chlore pour le traitement des fruits et de végétaux peut induire des saveurs désagréables et altère le goût.

3. Traitement physique : l'irradiation

D'après Lacroix et Vigneault (2007), l'irradiation est un traitement physique où les aliments sont exposés aux radiations gamma et cela afin d'éliminer les pathogènes et de réduire les microorganismes d'altération et ainsi augmenter la durée de conservation. L'irradiation est un traitement efficace qui peut être utilisé dans le but de réduire les bactéries pathogènes dans la viande crue (Thayer *et al.*, 1995), la viande transformée (Sommers *et al.*, 2004), de même que les fruits et légumes prêts à manger (Niemira, 2003 ; Prakash et Foley, 2004). D'après Park *et al.* (2010), l'irradiation gamma a été testée pour inhiber les microorganismes dans les aliments. Ce procédé non thermique permet de moins affecter la qualité des aliments comme la pasteurisation. Une dose de 5 kGy d'irradiation gamma n'a pas affecté la dureté, la couleur et le goût du pâté de viande de bœuf (Park *et al.*, 2010). Oularbi et Mansouri (1996) ont trouvé que l'irradiation gamma a permis une réduction importante des microorganismes dans les poivrons rouges et poivrons noirs. De plus, en 1997 l'organisation mondiale de la santé (OMS) a indiqué que les aliments traités à une dose de 10 kGy sont sains et leur qualité nutritionnelle n'est pas affectée. D'après Chervin et Boisseau (1994) les carottes râpées traitées à l'irradiation avaient une meilleure qualité sensorielle et nutritionnelle que lorsqu'elles sont traitées à l'eau chlorée.

L'inactivation microbienne est due à deux mécanismes : l'interaction directe de la radiation avec les composés cellulaires, et l'interaction indirecte des produits radiologiques (Farkas, 1997). Concernant la cible de l'irradiation il est important de mentionner que l'ADN chromosomal est la première cible des radiations ionisantes. Il a aussi été rapporté que la radiation affecte la membrane cytoplasmique.

Au Japon en 1996, la consommation de radis contaminés avec *E. coli* O157:H7 a provoqué près de 6 000 cas de maladies (WHO, 1997). Pour remédier à ces problèmes de contamination, des études ont montré que l'irradiation est un mécanisme efficace pour réduire la population microbienne dans le porc et dans la viande hachée. Par exemple, le taux de *Listeria monocytogenes* et 4 autres espèces de *Listeria* a été réduit par des radiation ionisante dans le porc (Tarte *et al.*, 1996).

Un autre cas nous montre aussi la haute efficacité de l'irradiation. En effet, d'après Farkas *et al.* (1997) la radiation ionisante à 1 kGy a réduit le taux de bactéries, a doublé la durée de vie et a amélioré et a préservé la qualité sensorielle des poivrons pré-découpés et des carottes.

4. Les conservateurs naturels et leurs problématiques

La plupart des pays tentent de rendre les aliments plus sûrs en utilisant les meilleures techniques de conservation des aliments et de production. À cet égard, l'utilisation de composés antimicrobiens naturels est une méthode alternative intéressante à considérer. De plus, une augmentation spectaculaire de résistance aux antibiotiques a induit une crise mondiale en ce qui concerne la sécurité alimentaire, créant ainsi un marché des aliments fonctionnels en plein essor pour les conservateurs naturels (Viuda-Martos *et al.*, 2011). Plusieurs antimicrobiens naturels ont été étudiés en tant que conservateurs potentiels. Des composantes naturelles provenant des plantes telles que les huiles essentielles, les phénols, et d'autres composés apparentés sont reconnues comme antimicrobiens et antioxydants efficaces et ont été étudiées dans diverses matrices alimentaires (Severino *et al.*, 2015; Takala *et al.*, 2013).

D'après Russel (1991), les bactéries Gram (–) sont dans la plupart des cas plus résistantes que les Gram (+) aux huiles essentielles et cela est due aux lipopolysaccharides présents sur la membrane bactérienne externe. L'utilisation de combinaisons d'huiles essentielles permet d'avoir un effet antimicrobien plus efficace et permet aussi de réduire les concentrations utilisées. De plus, d'après Hammer *et al.* (1999), cela permettra de lutter contre les bactéries résistantes aux antimicrobiens naturels tels que *Pseudomonas* sp. En effet, la combinaison des composés majeurs de l'huile essentielle permet une activité antimicrobienne plus importante, dans ce cas il s'agit de synergie (Ultee *et al.*, 2000).

4.1. Les huiles essentielles

Les huiles essentielles sont des mélanges complexes de substances lipophiles qui ont différentes propriétés biologiques bénéficiant d'un statut GRAS « généralement reconnu comme sûr » par la *Food and Drug Administration* (FDA) (Bakkali *et al.*, 2008). Les huiles essentielles sont des mélanges de métabolites secondaires complexes qui contiennent un large éventail de composants aromatiques forts, donnant aux plantes une odeur caractéristique. Ces huiles sont généralement liquides à température

ambiante, de nature lipophile et ont des densités inférieures à l'eau. La plupart d'entre elles hébergent un certain nombre de composés bioactifs ayant des activités antimicrobiennes. De telles propriétés antimicrobiennes semblent provenir d'un mélange de composants actifs tels que les monoterpènes et sesquiterpènes et leurs dérivés oxygénés (alcools, des aldéhydes, des esters, des éthers, des cétones, des phénols et des oxydes) (Boumail *et al.,* 2013). Cependant, l'activité biologique des huiles essentielles dépend de la composition chimique qui varie avec les parties des plantes utilisées, la méthode d'extraction, l'âge, le stade phénologique de la plante, la saison de la récolte et des conditions environnementales (Angioni *et al.*, 2006).

Cependant l'inconvénient le plus important de l'utilisation de ces produits est qu'ils peuvent changer les propriétés sensorielles des aliments. Afin de minimiser ces effets indésirables de nombreux chercheurs ont proposé la combinaison de plusieurs agents antimicrobiens à faibles concentrations afin de contrôler les pathogènes alimentaires (Gutierrez *et al.*, 2008 ; Nazer *et al.*, 2005)

Les activités biologiques de certaines huiles essentielles sont énumérées dans le **Tableau 1**.

Tableau 1. Les principaux composants des huiles essentielles.

Type de l'huile essentielle	Composés majeurs	Bioactivités	Références
Origan	Carvacrol Thymol γ-Terpinène p-Cymène	Antifongique, Antibactérien, Insecticide	Baser and Buchbauer (2010); Djilani and Dicko (2012)
Cannelle	Trans-cinnamaldéhyde	Antifongique, Antibactérien, Insecticide, Anti-Inflammatoire,	Baser and Buchbauer (2010); Djilani and Dicko (2012)
Romarin	α–Pinene Acétate de bornyle Camphre 1,8-Cinéol	Antibactérien, Antifongique, Insecticide	Baser and Buchbauer (2010); Djilani and Dicko (2012)
Thym	Thymol Carvacrol γ-Terpinène p-Cymène	Antibactérien, Antifongique, Insecticide	Vitoratos *et al.* (2013)
Clou de girofle	Eugénol Acétate d'eugénol	Antibactérien, Antifongique, Insecticide	Cardiet *et al.* (2011)

Bien que la bioactivité des huiles essentielles soit principalement attribuable à leurs composés majeurs, la possibilité d'autres phénomènes, comme la synergie ou l'antagonisme avec les composés mineurs ne doit pas être ignorée. La **Figure 2** montre les structures chimiques de composants communs d'huiles essentielles. Lambert *et al.* (2001) ont montré que les huiles essentielles peuvent

causer des changements de structure et cela en diffusant à travers la membrane cellulaire et ce pouvoir est dû à la nature hydrophobe des huiles essentielles, permettant d'endommager la structure des protéines membranaires.

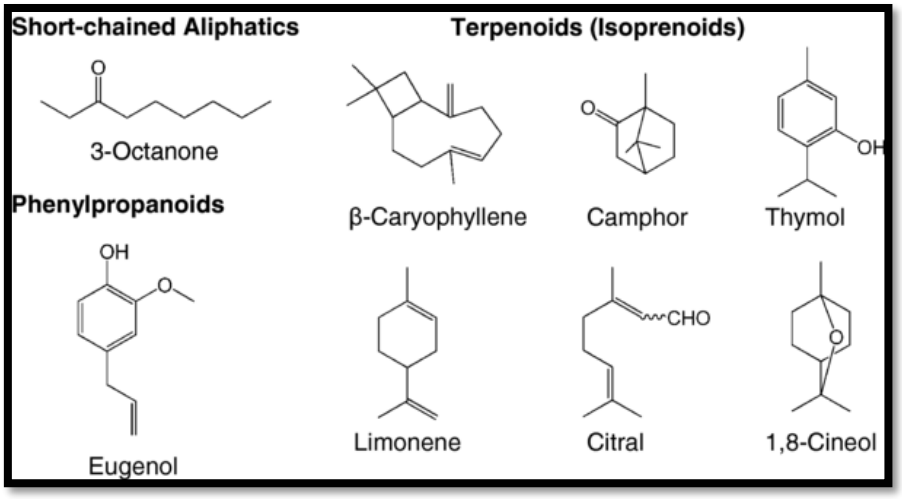

Figure 2. Structures chimiques de certains composés des huiles essentielles (Turek et Stintzing, 2013).

Selon Govaris *et al.* (2010), les principaux composés phénoliques ayant des propriétés antimicrobiennes et antioxydantes sont le carvacrol et le thymol et leur proportion sont de 78 à 85% dans l'huile essentielle d'origan. La concentration de ces deux composés varie selon l'espèce d'origan, dépend du climat, de la saison, de l'altitude et des conditions de culture (Portillo-Ruiz *et al.*, 2012). En raison de leur caractère lipophile, ces composés provoquent l'expansion de la membrane, l'augmentation de la fluidité, inhibe la respiration, et perturbe les protéines (Cristani *et al.*, 2007). Friedman *et al.* (2004) a indiqué que l'application de l'huile essentielle d'origan et de ses principaux composants dans les fruits et légumes a permis d'inhiber la croissance d'*E. coli* dans les jus d'orange, de pomme, de mangue et de tomate.

Au cours des dernières années, les huiles essentielles d'agrumes ont attiré une grande attention en raison de leurs fortes propriétés antimicrobiennes, des rendements élevés, des arômes et des saveurs, et en particulier la présence de flavonoïdes. Les flavonoïdes sont un groupe de composés polyphénoliques qui comprennent les flavanones, les flavones et leurs dérivés. Les flavonoïdes sont un groupe de pigments contenus dans les plantes, les fruits, les légumes, les fleurs, le vin et le miel et ils sont responsables de la coloration des fleurs et des fruits (Yusof *et al.*, 1990).

La pelure et les graines d'agrumes sont très riches en composés phénoliques, tels que les acides phénoliques et flavonoïdes (Ladaniya, 2008). Les flavonoïdes des agrumes comprenant : la naringine, l'hespéridine, l'hespérétine, ont un large spectre d'activité antibactérienne biologique contre une large gamme de bactéries à Gram négatif / positif (Jing *et al.*, 2015). Ils présentent également une activité antivirale contre le rhinovirus et le poliovirus. Les flavonoïdes ont également plusieurs activités

bénéfiques pour la santé, y compris les propriétés d'antioxydants, de protection cardiaque, d'antiallergique et d'anti-cancéreuse (Cormier *et al.*, 2013 ; Kaul *et al.*, 1985).

Les extraits d'agrumes ont été largement étudiés (Fisher et Phillips, 2008 ; Tirado *et al.*, 1995). Certains auteurs ont rapporté que les huiles d'agrumes sont très efficaces, tandis que d'autres ont déclaré que les effets sont variables (Burt, 2004).

4.2. Les acides organiques

On appelle acides organiques les composés organiques qui possèdent des propriétés acidifiantes. L'acide carboxylique est le groupe d'acides organiques le plus répandu et est caractérisé par la présence de plusieurs groupements carboxyliques (COOH). Parmi les acides carboxyliques on cite : les acides acétique, l'acide lactique et l'acide citrique. On note qu'au Canada, la plupart des acides organiques sont permis mais certaines restrictions s'appliquent, car cela dépend de l'aliment et des concentrations permises. La plupart des acides organiques sont synthétiques mais il est possible de faire l'extraction de ces acides organiques de fruits et de légumes. Il faut noter que l'utilisation des acides organiques est permise par Santé Canada et l'Agence canadienne d'inspection des aliments (ACIA). Les acides organiques ont été utilisés dans plusieurs recherches dans le but de réduire la flore microbienne dans les aliments, afin d'assurer des produits sains pour le consommateur, mais leur utilisation se fait selon des concentrations bien définies. Les acides organiques et leurs dérivés ont été souvent utilisés comme conservateurs et cela est dû à leurs propriétés. La diminution du pH défavorise le développement microbien. Cette caractéristique permet de minimiser la contamination initiale en ajoutant des acides durant le processus de fabrication. Il est important de noter que les conditions acides permettent de mieux éliminer les microorganismes au cours d'un traitement thermique (stérilisation) (Lück et Jager, 1997). De plus, la vaporisation des acides organiques a été utilisée comme désinfectant pendant la transformation de viandes. (Cherrington *et al.*, 1991 ; Hardin *et al.*, 1995). Youssef *et al.*, (2012) ont trouvé que la vaporisation de 5% d'acide lactique sur une viande de veau hautement contaminée a réduit le niveau d' *E. coli* et autres pathogènes alimentaires de 0.5 à 1 log UFC/g.

4.3. Les extraits de fruits

Le jus de canneberge

Le jus de canneberge américaine (*Vaccinium macrocarpon*) en particulier a été consommé pour la prévention des infections urinaires. Parmi différentes baies, les canneberges possèdent un effet antimicrobien net contre les agents pathogènes humain (Puupponen-Pimia *et al.*, 2005). Leurs propriétés antibactériennes ont été liées à la présence de nombreux composés phytochimiques, anthocyanines, flavonols, flavan-3-ols, proanthocyanidines, de même que des dérivés phénoliques des acides phénolique (Häkkinen *et al.*, 1999). Marwan et Nagel (1986) ont rapporté aussi que les pro-anthocyanidines et les flavonols provenant des canneberges sont les inhibiteurs les plus importants de

bactéries Gram négative et des levures. Parmi les différentes substances bioactives dans les baies qui ont reçu un intérêt considérable grâce à leurs effets sur les aliments et sur la santé on cite : les composés phénoliques incluant les flavonoïdes, les tannins, les anthocyanines et les acides phénoliques (Heinonen, 2007), en effet, l'activité antimicrobienne de ces composés a été démontrée envers de nombreux groupes de bactéries pathogènes causant des maladies comprenant *Helicobacter pylori*, *Salmonella*, *Staphylococcus aureus*, *Escherichia coli* et *Campylobacter*. Cela expliquerait leur rôle observé dans la prévention de certaines maladies infectieuses, telles que les troubles des voies urinaires, la carie dentaire, ainsi que des ulcères d'estomac et les cancers (Heinonen, 2007). Les tannins polymériques et en particulier les proanthocyanidines consistant principalement en : l'épicatéchine tétramères et les pentamères d'épicatéchine avec au moins une liaison de type A, ceux-ci semblent être les éléments de protection contre les bactéries pathogènes (Heinonen, 2007 ; Seeram et Heber., 2007).

Extrait d'agrumes

Les agrumes sont une source importante de composés bioactifs et en particulier les flavonoïdes et la vitamine C. Les flavonoïdes les plus abondants dans les agrumes sont l'hespéridine, la narirutine, la naringine et l'ériocitrine (Mouly *et al.*, 1994 ; Schieber *et al.*, 2001). D'après Belleti *et al.* (2004) les terpènes, le p-cymène et le citral sont les composants bioactifs les plus efficaces contre plusieurs souches de *Saccharomyces cerevisiae* (en effet, cette souche peut être indésirable dans certains aliments et peut jouer le rôle d'un microorganisme d'altération).

De plus, Kawaii *et al.* (2000) a rapporté que le citron est cultivé pour la présence des alkaloides qui ont des effets anticancer et des effets antimicrobiens dans les extraits bruts des différentes parties de la plante (feuille, fleurs ; racines) du citronnier contre plusieurs espèces bactériennes.

Il est important de noté que d'après Burt (2004) les flavonoïdes présents dans les citrons ont une activité antibactérienne, antifongique, anticancer, antivirale et antidiabétique.

Aussi, il a été rapporté que les feuilles des agrumes sont une source riche en flavonoïdes, coumarines, β et γ sistéroles et en glycosides (Shahnah *et al.*, 2007).

Selon plusieurs études, les extraits d'agrumes ont montré des effets antimicrobiens contre différents pathogènes d'origines alimentaires (*E. coli*, *Listeria monocytogenes*, *Salmonella* sp., *Bacillus* sp. et *Clostridium* sp., *Staphylococcus aureus*), les microorganismes d'altérations (*Lactobacillus* sp. dans les jus), levures et moisissures (*Saccharomyces cerevisiae, Aspergillus parasiticus, Aspergillus flavus, Alicyclobacillus acidoterrestris, Fusarium oxysporum*) (Fisher et Phillips, 2008 ; Friedly *et al.*, 2009 ; Bevilacqua *et al.*, 2012; 2013). En effet, les agrumes contiennent des flavonoïdes ayant des activités biologiques, parmi ces activités on cite : des effets antioxydants, antimicrobiens et anti-inflammatoires (Tripoli *et al.*, 2007).

5. Combinaison des traitements antimicrobiens

Le mélange de plusieurs traitements antimicrobiens permet de réduire plus efficacement les microorganismes d'altération tout en reduisant l'impact sur la qualite sensorielle des aliments. En effet, Ouattara *et al.* (2002) a trouvé que la combinaison de l'irradiation (dose jusqu'à 3 kGy) et la vaporisation d'une formulation antimicrobienne à base de protéines de soja et de l'huile essentielle a réduit le développement de *Pseudomonas putida* et le compte de bactéries aérobies montrant l'effet synergique de l'irradiation et de la formulation antimicrobienne et cela sans affecter la qualité sensorielle des aliments testés. Dans d'autres études, Lin *et al.* (2004 ; 2005) ont rapporté qu'une augmentation de l'effet antimicrobien a été observé après l'addition de l'acide lactique (à pH 6) à un mélange d'huile essentielle d'origan et un extrait de canneberges. De plus, l'étude réalisée par Naveena *et al.* (2006) a montré que le mélange d'huile essentielle de clou de girofle et de l'acide lactique a augmenté significativement la durée de vie de la viande de buffle à 4°C. Tawema *et al.* (2014) a aussi rapporté que deux formulations naturelles F2 et F6 à base de mélange d'acide organique et d'huile essentielle ont exhibé une activité antibactérienne élevée. En effet, ces deux formulations ont réduit *E. coli* O157:H7, *S.* Typhimurium, le compte de bactéries total et les levures et moisissures sous la limite de détection après une inoculation initiale de 4.5 log. Il est important de noter que ces deux formulations n'ont pas affecté la qualité organoleptique des choux-fleurs à un volume de vaporisation de 5 mL par 100 g de légumes.

6. Application en industries alimentaires

Afin d'appliquer l'utilisation des huiles essentielles dans les aliments il faut d'abord commencer par faire des tests *in vitro* pour voir l'effet antimicrobien de ces huiles essentielles ensuite des tests *in situ* confirmeront si ces agents antimicrobiens (à une concentration plus élevée que lors des tests *in vitro*) seront retenus ou non. De plus, des évaluations sensorielles seront importantes afin de voir si l'ajout de ces agents antimicrobiens affectera les paramètres sensoriels de l'aliment ou non. Certaines huiles essentielles sont plus spécifiques à certains aliments en se basant en même temps sur la composition de l'aliment et sur les microorganismes susceptibles de contaminer les aliments, les huiles essentielles d'origan, de thym, de cannelle ou de coriandre sont efficaces contre les microorganismes de viandes, de volailles, de charcuteries et de légumes; l'huile essentielle de menthe contre les microorganismes de produits frais (salades, yaourts…); les huiles essentielles à base de cavarcrol ou de citral contre les microorganismes de poissons; les huiles essentielles de thym, de noix de muscade ou de gingembre pour les céréales (plus particulièrement celles riches en carvacrol contre les microorganismes du riz); et les huiles essentielles à base de carvacrol ou de cinnamaldéhyde contre les microorganismes des fruits (Oussalah et al., 2006, 2007).

D'après Canillac et Mourey (2004), Ismaiel et Pierson (1990) et Juven *et* al. (1994) l'inhibition de l'effet du carvacrol sur le développement microbien est probablement dû à la présence de gras et de

protéines dans les matrices alimentaires.

L'utilisation des huiles essentielles comme agent de conservation dans les aliments peut changer les aspects sensoriels des aliments. Cependant de nouvelles solutions existent afin d'éviter que ces huiles ne changent le goût des aliments, l'un des moyens les plus efficaces est la désaromatisation. Certains goûts indésirables peuvent être évités en sélectionnant l'huile essentielle la plus adéquate pour chaque aliment traité par exemple les huiles d'origan et de thym seront plus adéquates pour les viandes et les charcuteries. Il faut noter aussi que l'utilisation des huiles se fait à des concentrations très faibles et cela n'affectera pas le goût. Il faut aussi faire attention à s'assurer que l'huile essentielle appliquée n'a pas d'incidence sur les bactéries utiles telles que les ferments d'acidification et d'aromatisation. En suivant ces critères, l'incorporation des huiles essentielles dans les aliments pourra éliminer les bactéries pathogènes, aromatiser le produit et assurer le rôle d'un antioxydant.

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Projet, problématique et hypothèses

Projet : Mise au point de méthodes antimicrobiennes afin de protéger les fruits et légumes prêts à manger contre les pathogènes d'origine alimentaire.

Problématique **:** Les fruits et légumes prêts à manger peuvent présenter un risque sanitaire pour le consommateur. Plusieurs cas d'intoxication, d'infections et de toxi-infections alimentaires ont été enregistrés dans le monde. L'utilisation des produits chimiques tels que le chlore ou le peroxyde peuvent provoquer des allergies et des dangers sur la santé de l'être humain. La mise en place de nouvelles solutions afin de protéger les fruits et légumes contre la prolifération des bactéries pathogènes est nécessaire.

Hypothèses :

1- L'utilisation du jus de canneberge en tant que conservateur naturel et sa vaporisation sur les poivrons rouges et les canneberges fraiches réduira et éliminera les pathogènes alimentaires (*Listeria, E. coli* et *Salmonella*) et cela sans affecter les caractéristiques sensorielles.

2- L'extrait d'agrumes Foodgard permettra d'inhiber les bactéries au cours des tests *in vitro* et l'incorporation de cet antimicrobien naturel dans des garnitures de fraises conservera ce produit tout en inhibant les cinq bactéries pathogènes étudiées (*E. coli*, *S.* Typhimurium, *S. aureus*, *B. cereus*, *L. monocytogenes*) sans détériorer la qualité sensorielle des garnitures.

3. Les deux formulations faites à base d'huiles essentielles, d'extraits de fruits et d'acides organiques (F2-F6), permettront d'inhiber les trois pathogènes à étudier au cours des tests *in vitro* et permettront de réduire et d'inhiber les bactéries étudiées sur les poivrons rouges pré-découpés, les canneberges et les pommes de terre pré-frites dans des emballages sous atmosphère modifiée (MAP) et sans affecter les caractéristiques organoleptiques des trois produits alimentaires.

4. La formulation mise en place, l'ozone gazeux et l'irradiation gamma auront un effet antimicrobien synergique sur les poivrons verts sans affecter la qualité.

Objectifs du projet

L'objectif principal du projet était de mettre au point des méthodes de préservation et de décontamination des fruits et légumes prêts à manger.

Les grandes étapes du projet consistaient à :

1. Évaluer *in vitro* la capacité d'inhibition du jus de canneberges seul, de deux formulations antimicrobiennes mise en place précédemment au laboratoire (F2 – F6) et d'un extrait de citrus (Foodgard).
2. Évaluer l'effet antimicrobien *in situ* de F2 et F6 dans les poivrons rouges découpés, dans les canneberges et dans les pommes de terre pré-frites. De même que l'effet antimicrobien *in situ* du jus de canneberges dans les poivrons rouges et les canneberges, de plus évaluer l'effet antimicrobien *in situ* d'une nouvelle formulation contenant plusieurs composés bioactifs (formulation x1+df).
3. Évaluer l'effet antimicrobien de l'ozone gazeux appliqué sur les poivrons verts contre trois bactéries.
4. Évaluer l'effet antimicrobien de l'irradiation sur les poivrons verts prêts à manger.
5. Évaluer l'effet de la combinaison des traitements antimicrobien mise au point sur les poivrons verts.
6. Évaluer l'effet des traitements antimicrobiens sur la qualité sensorielle et nutritionnelle des prêts à manger.

Résumé des méthodes et approches utilisées

Pour les tests d'évaluation *in vitro* de la capacité inhibitrice des différentes formulations testées, la méthode de diffusion sur gélose sera suivie selon une procédure modifiée de Cardiet *et al.* (2012) pour voir l'activité antibactérienne du produit testé en mesurant la zone d'inhibition. La capacité d'inhibition sera ensuite calculée selon l'Équation 1 :

$$Capacité\ d'inhibition = \frac{\text{diamètre d'inhibition}}{\text{diamètre de boîte de Petri}} \times 100 \quad [1]$$

Afin de déterminer les concentrations minimales inhibitrices (CMI) des formulations antibactériennes testées, la méthode de microplaque à 96 puits décrite par Turgis *et al.* (2012) et Hossain *et al.* (2016) sera employée. En effet, après une série de dilution de l'antimicrobien dans le milieu de culture liquide, après l'ajout des bactéries et une incubation à 37°C sous agitation pendant 48 h, la densité optique sera lue à une longueur d'onde de 595 nm. La concentration minimale inhibitrice pourra être ainsi déterminée.

La détermination de la capacité antibactérienne *in situ* des différentes formulations testées sera réalisé selon une méthode développée au cours de nos recherches. En effet, 25 g d'aliments seront inoculés avec 0.1 mL de suspension bactérienne à une concentration de 10^6 UFC/g afin d'obtenir une concentration dans l'aliment de 3 à 4 log UFC/mL, suivi du traitement antimicrobien et une détermination de la viabilité des bactéries au cours de l'entreposage à -20 et 4°C.

Les analyses sensorielles seront réalisées avec 30 personnes pour l'évaluation de la texture, de l'odeur, du goût, de la couleur et de l'appréciation globale en utilisant un test hédonique à 9 chiffres.

La mesure du niveau de chlorophylle sera mesurée dans les poivrons verts afin de voir l'effet des différents traitements antimicrobiens, selon une méthode modifiée d'Arnon (1949).

La mesure de la concentration en acide ascorbique et en acide déhydroascorbique sera mesurée selon une procédure enzymatique, vitafast de r-biopharm.

La mesure des paramètres de couleur des poivrons verts sera réalisée en mesurant les paramètres L* (la clareté), a*(variation de la couleur du vert (-300) au rouge (+299)) et b* (variation de la couleur du bleu (-300) au jaune (+299)) avec un colorimètre (Konica Minolta, Ramsey, NJ, USA) et en déterminant la teinte mesurée en degrés (°) et le ΔE comme mesure de la différence entre 2 couleurs dans l'espace des couleurs CIELAB.

L'irradiation des poivrons verts découpés sera faite dans un irradiateur gamma en utilisant une source de Cobalt 60 (Nordion Inc., Kanata, ON, Canada) à une dose de 0.5 kGy et un débit de 16.74 kGy/h au Centre d'Irradiation du Canada.

Afin d'atteindre nos objectifs, un vaporisateur électrostatique a été commandé pour faire la vaporisation des fruits et des légumes prêts à manger. Une série d'optimisation sera réalisée afin de déterminer la méthode et les paramètres (pression réservoir, distance entre la buse et l'échantillon) optimaux à fixer pour la vaporisation de 0.3 mL de solution sur la totalité de l'échantillon de 25 g.

De plus, un générateur d'ozone gazeux a été aussi commandé avec une cabine en acier inoxydable pour le déroulement des tests antimicrobiens. Une série d'optimisation du temps d'exposition et des concentrations sera réalisée afin de sélectionner la concentration et la durée la plus efficace contre les bactéries testées sans affecter la qualité des légumes.

Les produits naturels tels que le jus de canneberge, l'huile essentielle d'origan, le citral, le dextrose fermenté, acide lactique et le glycerol pour la formulation des solutions antimicrobiennes seront utilisés.

La mise au point des formulations sera réalisée par la sélection des composés antimicrobiens les plus efficaces contre les bactéries étudiées et aussi les plus compatibles avec les aliments testés.

Il est important de noter que l'analyse de la variance (ANOVA) et le test de Duncan seront effectuées pour l'analyse statistique (PASW Statistics 18 ; IBM Corporation, Somers, NY, États-Unis). Les différences entre les moyennes seront considérées significatives lorsque l'intervalle de confiance sera inférieur à 5% ($p \leq 0,05$).

CHAPITRE 1:

ANTIBACTERIAL ACTIVITY OF CRANBERRY JUICE CONCENTRATE ON FRESHNESS AND SENSORY QUALITY OF READY TO EAT (RTE) FOODS

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Mehdi Harich[1], Behnoush Maherani[1], Stephane Salmieri[1], Monique Lacroix[1*] (2017). Antibacterial activity of cranberry juice concentrate on freshness and sensory quality of ready to eat (RTE) foods. *Food control* (75) 134–144

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Contributions des auteurs :

J'ai réalisé les expériences, décrit et interprété les résultats et j'ai rédigé l'article.

Stéphane Salmieri a participé a participé à la planification et au bon déroulement des analyses, et à la rédaction de l'article.

Maherani Behnoush a aidé dans l'interprétation des résultats

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

Résumé

Les aliments prêts à manger ne sont pas traités avant leur consommation pour réduire la charge bactérienne. C'est pour cela que le risque des pathogènes alimentaires doit être pris en considération. Dans ce contexte, l'utilisation des composés antibactériens naturels est une méthode intéressante qui devrait être considérés. Sur ce sujet, les propriétés antibactériennes du jus de canneberge concentré ont été évaluées *in vitro* et *in situ* contre 3 bactéries pathogènes. Les résultats ont montré une activité antibactérienne élevée avec une capacité d'inhibition importante contre *Escherichia coli* O157:H7, *Listeria monocytogenes* et *Salmonella* Typhimurium.

Les résultats des tests de sensibilité des bactéries aux acidesà un pH identique de 2.4 en présence des solutions d'acides organiques (citrique et quinique) ont montré des effets antibactériens. Présumément, grâce à ses composés phénoliques, le jus de canneberge avait une activité antibactérienne plus élevée que les acides organiques testés.

Les résultats des tests *in situ* ont montré une réduction de 2.5, 1.8, et de 5 log UFC/g de *E. coli*, *L monocytogenes* et de *S*. Typhimurium, respectivement, en présence du jus de canneberges concentré sur les poivrons rouges prédécoupés après sept jours de stockage à 4°C. De plus, *L. monocytogenes* a été totalement inhibé au premier jour de stockage dans les canneberges traitées au jus de canneberges concentré. De plus, 3 log UFC/g de réduction de *S*. Typhimurium a été observé après 4 jours de stockage à 4°C sur les canneberges traitées au jus de canneberges concentré.

Les résultats démontrent que le jus de canneberges concentré avec ses composés phénoliques peuvent être une source d'antibactériens naturels pour protéger les aliments prêts à manger des contaminations causées par les bactéries pathogènes sans affecter négativement la qualité sensorielle et nutritionnelle des poivrons rouges.

Abstract

Ready-to-eat (RTE) foods are not further treated before consumption in such a way that may significantly reduce the microbial load, therefore the risk of foodborne disease must be considered. In this regard, the use of natural antimicrobial compounds is an interesting method to be considered. On this topic, the antibacterial activity of cranberry juice concentrate (CJC) have been evaluated *in vitro* and *in situ* against 3 foodborne pathogenic bacteria. Results showed a high antimicrobial effect with a noticeable inhibition capacity against *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium. Acid sensitivity studies of bacteria indicated that at the same pH level (pH = 2.4) in presence of organic acid solution (citric and quinic acids), cranberry juice concentrate showed greater antibacterial effects than the acids due to their phenolic compounds. *In situ* studies showed 2.5, 1.8 and 5 log reduction of *E. coli*, *L. monocytogenes and S.* Typhimurium, respectively in presence of cranberry juice concentrate, on pre-cut red peppers after 7 days of storage at 4°C. A total inhibition of *L. monocytogenes* on fresh cranberry fruits in primary day of storage, was observed. Cranberries treated with CJC also showed a 3 log reduction of *S.* Typhimurium after 4 days of storage at 4°C. The results suggest that CJC can be an effective preservation, source of natural antibacterial, to protect the RTE foods from foodborne pathogens contamination without effecting on sensorial properties of treated samples and allow to maintain the freshness, sensory and the nutritional quality of RTE foods.

Keywords

Cranberry juice concentrate, Antibacterial activity, Phenolic compounds, Foodborne pathogenic bacteria, and Ready to eat foods.

1. Introduction

In spite of modern technologies and safety concepts such as HACCP, the reported numbers of food-borne illnesses are still increasing (O'Sullivan *et al.*, 2002).

As reported, in the United States (Centers for Disease Control and Prevention, 2014); the major foodborne pathogens include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus* (Collignon and Korsten, 2010).

E. coli O157:H7 can cause hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombocytopenic purpura. *S*. Typhimurium is one of the major stains causing Salmonellosis. According to the US CDC (2005), *E. coli* O157:H7 causes 73,000 illnesses in the United States annually. According to Jay *et al.* (2005), listeriosis generally caused by *Listeria monocytogenes*, can affect pregnant women, immunocompromised individuals and the elderly. Staphylococcal food poisoning is also the result of *S. aureus*'s exotoxin *(*Jay *et al..,* 2005*).* The estimated cost from five bacterial foodborne pathogens was $ 6.9 billion USD/year in 2000 (Anon 1994; USDA 2000).

On the other hand, increasing fruit and vegetable consumption becomes a global priority to equilibrate the diets lacking in vitamins and minerals. Furthermore, recent studies on cancer incidence and the association between fruit and vegetable intake and cancer risk showed the fruit and vegetable consumption can reduce the risk of cancers of the oropharynx, oesophagus, lung, stomach and colorectum (Soerjomataram *et al.*, 2010). According to Statistics Canada (2012), 40.6% of Canadians aged 12 and older consumed fruits and vegetables five or more times per day in 2012. Fresh fruit and vegetables, already a very large category, are forecast to increase marginally, while greater growth in the preprocessed segment is anticipated (AAFC & Serecon, 2005). An increase of 88% and 33% is expected in Canada for Ready-to-eat (RTE) salads and frozen vegetables respectively between 1984 and 2020 (AAFC & Serecon, 2005). Minimally processed fruits and vegetables are economically important commodities due to their convenience, healthiness, and desirable sensory characteristics. However, minimally processed fruits and vegetables are highly perishable as they contain 80-90% water by weight (Dhall, 2013). As RTE-foods are not further treated before consumption in such a way that may significantly reduce the microbial load, therefore the risk of foodborne disease must be considered. The food industry is currently in need of innovative processing technologies in order to meet consumer's demand of fresher and safe RTE products. Most countries try to make the foods safer by using the better food preservation and production techniques. In this regard, the use of natural antimicrobial compounds is an interesting alternative method to be considered. In addition, dramatic increases in antibiotic resistant have fueled a global crisis with regards to food safety, creating a burgeoning functional foods market for natural preservatives (Viuda-Martos *et al.*, 2011).

Numerous natural antimicrobials have been investigated as potential preservatives. Natural compounds derived from plants such as essential oils (EOs), phenolic and related compounds are

recognized as strong antimicrobials and antioxidants and have been investigated in various food systems (Severino *et al.*, 2015; Takala *et al.*, 2013). Juice of the American cranberry (*Vaccinium macrocarpon*) in particular has long been consumed for the prevention of urinary tract infections. Among different berries, cranberry possesses clear antimicrobial effects against human pathogens (Puupponen-Pimia *et al.*, 2005). Its antibacterial properties have been linked to the presence of numerous phytochemicals; anthocyanins, flavonols, flavan-3-ols, proanthocyanindins, and the phenolic acid derivatives (Häkkinen *et al.*, 1999). Among the different bioactive substances in berries, phenolic compounds including flavonoids, tannins, anthocyanins and phenolic acids have received considerable interest due to their effects in food and health (Heinonen, 2007). Marwan and Nagelm (1986) also reported that the proanthocyanidins and flavonols were the major inhibitors on yeast and Gram-negative bacteria. The antimicrobial activity of cranberries phenolic compounds was demonstrated toward numerous groups of illness-causing pathogenic bacteria, including *Helicobacter pylori*, *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, and *Campylobacter* (Côté *et al.*, 2011). This would explain their apparent role in preventing certain infectious diseases, such as urinary tract disorders, dental decay, as well as stomach ulcers and cancers (Heinonen, 2007). The polymeric tannins and in particular, the proanthocyanidins consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage, seem to be the protecting element against pathogenic bacteria (Heinonen, 2007; Seeram & Heber, 2007).

The objective of this study was to evaluate *in vitro*, the inhibition capacity (IC**)** of CJC by agar diffusion bioassay and to determine *in vitro*, the minimal inhibiting concentration (MIC) against foodborne pathogenic bacteria (*Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Listeria monocytogenes*) and also to assess *in situ*, the antimicrobial capacity of CJC on fresh cranberries fruits during 42 days of storage at 4°C and in pre-cut red peppers during 7 days of storage at 4°C. Finally, the sensorial properties of the red peppers treated with CJC were evaluated according to a 9-points hedonic scale.

2. Material and methods

2.1 Raw material

Frozen cranberries and fresh cranberry juice (*Vaccinium macrocarpon*) were used for *in vitro* and *in situ* studies. These samples without any preservative were provided by Atoka Cranberries Inc. (Manseau, QC, Canada) and were stored at -80°C until used. Pre-cut red peppers were provided from Bonduelle Americas Inc. (Saint-Denis-sur-Richelieu, QC, Canada) and were stored at -80°C until used. Sodium benzoate (E211) was kindly provided by Skjodt-Barrett Foods Inc. (QC, Canada).

2.2 Preparation of pathogen cultures

Stock cultures of *E. coli* O157:H7 EDL 933, *S.* Typhimurium SL 1344, *L. monocytogenes* HPB 2812, 2558, 2569, 1043, 2371, 2739, were stored at −80°C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Prior to each experiment, stock cultures were grown through two consecutive 24-48 h growth cycles in TSB at 37°C. Working cultures were diluted in peptone water obtain the bacterial concentration of 10^6 CFU/mL for MIC determination and 10^3-10^4 CFU/mL for *in situ* studies.

2.3 pH effect analysis

The acidic solution was made of citric acid (15%), quinic acid (15%) and their combination based on the cranberry juice composition analysis (Borukh *et al.*, 1972), with pH values equal to cranberry juice (pH = 2.4). For *in vitro* study, the pH of cranberry juice was adjusted to 7 by using NaOH (1 M).

2.4 Determination of the Inhibiting Capacity (IC%) by agar diffusion assay

Agar diffusion assay was carried out according to a modified procedure from Cardiet *et al.*, (2012) to assess bactericide activity of antibacterial agents by measuring microbial growth inhibition zones. Tryptic soy agar (TSA; Alpha Biosciences Inc.) were surface-layered by 100 µL of diluted pathogens at 10^6 CFU/mL. Growth inhibition diameters (mm) were determined by agar diffusion from the deposition of 10 µL of cranberry juice samples on a 12-mm diameter cellulose disc placed onto the surface of TSA. Each agar plate was incubated for 24-48 h at 37°C and the inhibition diameter around the disc (colony-free perimeter) was measured with a Traceable Carbon Fiber Digital Caliper (resolution: 0.1 mm; accuracy: ± 0.2 mm; Fisher Scientific Ltd, Nepean, ON, Canada). All measurements were performed in triplicate (n = 3).

The inhibitory capacity (IC%) was calculated as follow by **Equation 1:**

$$IC\ (\%) = \frac{\text{Diameter inhibition zone}}{\text{Diameter Petri dish}} \times 100 \qquad [1]$$

By considering an internal diameter of Petri dishes equal to 83 mm.

2.5 Determination of the minimal inhibitory concentration (MIC)

The MIC value of antibacterial agents was determined in sterilized flat-bottomed 96-well microplate according to the serial microdilution method as described by Turgis *et al.* (2012) and Hossain *et al.* (2016). Briefly, the cranberry juice samples were added in Mueller Hinton Broth (MHB, DIFCO, Becton Dickinson, USA) pre-added in 96-well microplate, to obtain serial concentrations, such as 63,000 – 60 ppm for CJC.

A sample of 110 µL of the serially diluted cranberry juice was pipetted into 96 wells microplate contain 125 µL MHB. Each well was then inoculated with 15 µL of a pathogenic strain at a concentration of 10^6 CFU/mL. The microplate was incubated aerobically for 24 h at 37℃. Then, the

absorbance was measured at 595 nm in an Ultra Microplate Reader (Biotek instruments, Winooski, VT, USA). The two last column of the microplate were used for a blank containing only a sterile Mueller–Hinton medium as negative control and the positive growth control was a column filled with target bacterium with Mueller–Hinton medium (without cranberry juice samples). The MIC was defined as the lowest concentration of cranberry juice samples showing a complete growth inhibition of the tested bacterial strains (related to a measured absorbance that is equal to the absorbance of the blank). All determinations were performed in triplicate (n = 3).

2.6 Sample treatment with CJC

For samples coating, a volume of 0.3 mL of CJC (pH = 2.4) were uniformly sprayed under sterile conditions on 25 g of fresh pre-cut red pepper and cranberries fruits. The samples were then left to dry for 30 min under laminar flow hood before storage or microbiological analysis. Samples without any treatment (washed with distilled water) were used as negative control.

2.7 Antimicrobial capacity of CJC on Cranberry fruits

The antimicrobial activity of CJC was evaluated *in situ* on cranberry fruits coated with CJC against 3 pathogens: *E coli*, *L. monocytogenes* and *S.* Typhimurium. By considering preliminary results, inoculation with 10^6 and 10^7 CFU/mL, no bacterial growth was observed on petri plate, so that's why the inoculum concentration *in situ* study was raised to 10^8 CFU/g and then the fruits stored at 4°C for 42 days (as recommended by the company). Microbiological analyses were performed at different days. In each day of analysis, the samples were homogenized for 2 min at 260 rpm in 50 mL of sterile peptone water (0.1% w/v) with a Lab-blender 400 stomacher (Laboratory Equipment). From each homogenate, serial dilutions were prepared, plated on the surface of appropriate media. Palcam, MacConckey agar supplemented with sorbitol and DCLS agar, were used for enumeration of *L. monocytogenes*, *E. coli* and *Salmonella*, respectively (Alpha Biosciences Inc., OXOID).

2.8 Antimicrobial capacity in situ of CJC on pre-cut red peppers

Pre-cut red peppers (25 g) coated with CJC were inoculated with pathogens (inoculum concentration of 10^7 CFU/g) to obtain 10^5 to 10^6 CFU/g as final concentration and then stored for 7 days at 4°C. Microbiological analyses were performed during one week (as recommended by the company). In each day, the samples were homogenized for 2 min at 260 rpm in 50 mL of sterile peptone water (0.1% w/v) with a Lab-blender 400 stomacher (Laboratory Equipment). From each homogenate, serial dilutions were prepared, plated on the surface of appropriate media for every bacteria; *Listeria monocytogenes*, *E. coli* and *Salmonella* Typhimurium (Palcam, MacConckey agar supplemented with sorbitol and DCLS agar, respectively) and incubated for 24-48 h at 37°C before bacteria enumeration.

Sample treated with sodium hypochlorite solution (Sigma-Aldrich) was used as commercial method (control) to remove food borne pathogens. Samples were immersed in sodium hypochlorite solution

(200 ppm of free chorine) for 1 minute and rinsed with sterilized delisted-water and then left to dry for 30 min under laminar flow hood before microbiological analysis.

2.9 Sensory analysis of red peppers treated with CJC

The odor, color, global appreciation, flavor and texture of the red peppers treated with CJC were evaluated according to a 9-points hedonic scale : 9 = Like very much; 8 = Like a lot; 7 = Like moderately; 6 = Like a little; 5 = Indifferent; 4 = Dislike a little; 3 = Dislike moderately; 2 = Dislike a lot; 1 = Dislike very much. This method was used in order to measure the degree of acceptance or rejection of samples, and eventually to verify that the addition of CJC as natural antimicrobial formulation has any significant effect ($p > 0.05$) on the organoleptic properties of the food products. Evaluation data were statistically analyzed and comparison tests were performed.

2.10 Statistical analysis

Analysis of variance (ANOVA), Duncan's multiple range test (for equal variances) were performed for statistical analysis (PASW Statistics 18; IBM Corporation, Somers, NY, 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 Determination of the Inhibiting Capacity (IC%) by agar diffusion assay

3.1.1 Listeria monocytogenes

L. monocytogenes is able to grow at different temperature, ranging from 2 to 52°C, and in food having low pH value (4.4) or high salt concentration (14%), while it is easily inactivated by cooking. It is commonly recognized that foodstuffs characterised by extended shelf life at refrigeration temperature, suitable pH and water activity levels and consumed without further cooking (ready-to-eat = RTE) are most frequently involved in outbreaks of listeriosis (Lannetti *et al.*, 2016). The inhibition capacity of different antimicrobial formulations against *L. monocytogenes* is presented in **Fig. 1.** Results showed that citric acid exhibited more inhibition activity (18.5%) in compare to quinic acid (16.7%). The order of antibacterial activity of organic acids against *L. monocytogenes* is citric acid ≥ citric acid + quinic acid > quinic acid > malic acid. The CJC (pH= 2.4) with IC values about 22.2% showed significantly higher antimicrobial capacity against *L. monocytogenes* in compare to other formulations ($p \leq 0.05$). In other side, the pH- neutralized CJC (pH=7) exhibited an IC values of 17.1%, lower than CJC (pH = 2.4). Sodium benzoate was also showed a high antibacterial activity against *L. monocytogenes* as same as citric acid.

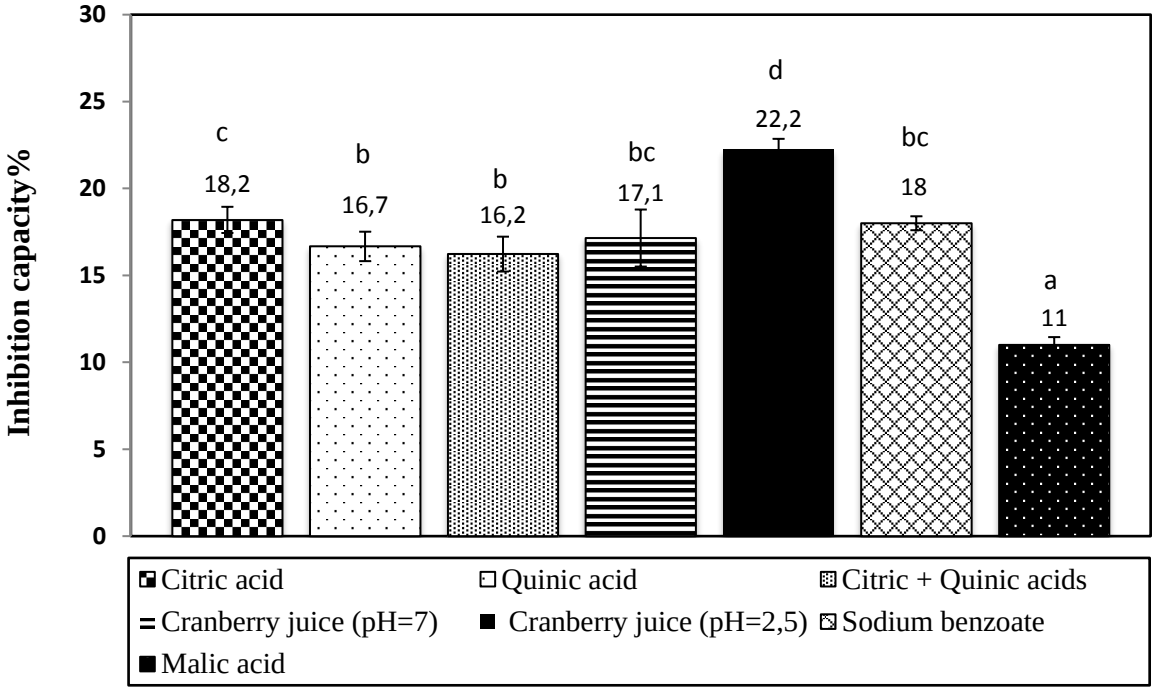

Figure 1. Inhibition capacity of antimicrobial formulations against *Listeria monocytogenes.* Different letters represent significant differences of inhibition capacity ($p \leq 0.05$).

3.1.2 Escherichia coli

Cranberries contain different kinds of organic acids (2.4%). The major organic acids are citric (1.1%), malic (0.26%), and quinic acids (0.5 - 1%), with small amounts of benzoic (0.056%) and glucuronic acids (Borukh *et al.,* 1972). Organic acids were evaluated for their antimicrobial effects on pathogens at the concentration of 15% (eq. of total acid content of cranberry juice concentrate). Results showed an important inhibition capacity against *E. coli* for all formulations (**Fig. 2**). Citric acid, quinic acid and malic acid were used alone as a reference and the results showed an inhibition rate of 16.6, 16.3 and 7.5%, respectively. The formulation containing citric acid + quinic acid (acidity of 15%, pH = 2.4) showed the highest inhibition capacities (18%) in compare to other formulation, which is closer to the inhibition activity (18.9%) of CJC (pH = 2.4). The pH- neutralized cranberry juice concentrate (pH = 7) showed a lower IC value compare to other formulations. Sodium benzoate was also showed antibacterial activity against *E.coli* as same as pH- neutralized cranberry juice concentrate. This phenomenon can be explained by difference in pH of cranberry juice that effect on antimicrobial activity, as in the same formulation by decreasing the pH, antimicrobial activity increased. Almajano *et al.* (2007) observed the same results in presence of caffeic acid in O/W emulsion. The pH and changes in the ionization state and proportion of undissociated molecules of caffeic acid at different pH values modify the antimicrobial activity. It is established that the antimicrobial activity of weak acids is pH dependent due to the influence of this parameter on the proportion of undissociated acid

molecules in solution (Almajano *et al.*, 2007). Furthermore, Côté *et al.* (2011) have demonstrated that the low pH of the juice is not only responsible for the antimicrobial capacity but there is also other components responsible for this antibacterial capacity. According to Côté *et al.* (2011) study, a large variety of bioactive compounds are found in cranberries such as flavanols, flavan-3-ols (catechin and epicatechin), anthocyanins (cyanidin 3-galactoside, cyaniding 3-arabinoside, peonidin 3-galactoside, and peonidin 3-arabinoside), tannins (ellagitannins and proanthocyanidins), and phenolic acid derivatives (ferulic acid, *p*-coumaric acid, coumaryolglucose, feruloylglucose, caffeoyglucose, chlorogenic acid, ellagic acid, and p-hydroxybenzoic acids). These compounds display many promising health properties, such as antioxidant, antimicrobial, anticarcinogen, etc. Caillet *et al.* (2011) found that all the bacterial strains both Gram-positive and Gram-negative, were selectively inhibited by the phenolic compounds of cranberry juice. According to Puupponen-Pimia *et al.* (2001), variation in cell wall structures between Gram-positive and Gram-negative bacteria may cause different damage when the bacteria are subjected to antimicrobial compounds. Caillet *et al.* (2011) reported that all pathogens were very sensitive to at least seven fractions of polyphenols with maximum tolerated concentration (MTCs) below 2 mg phenol/mL and five fractions with MICs below 10 mg phenol/mL.

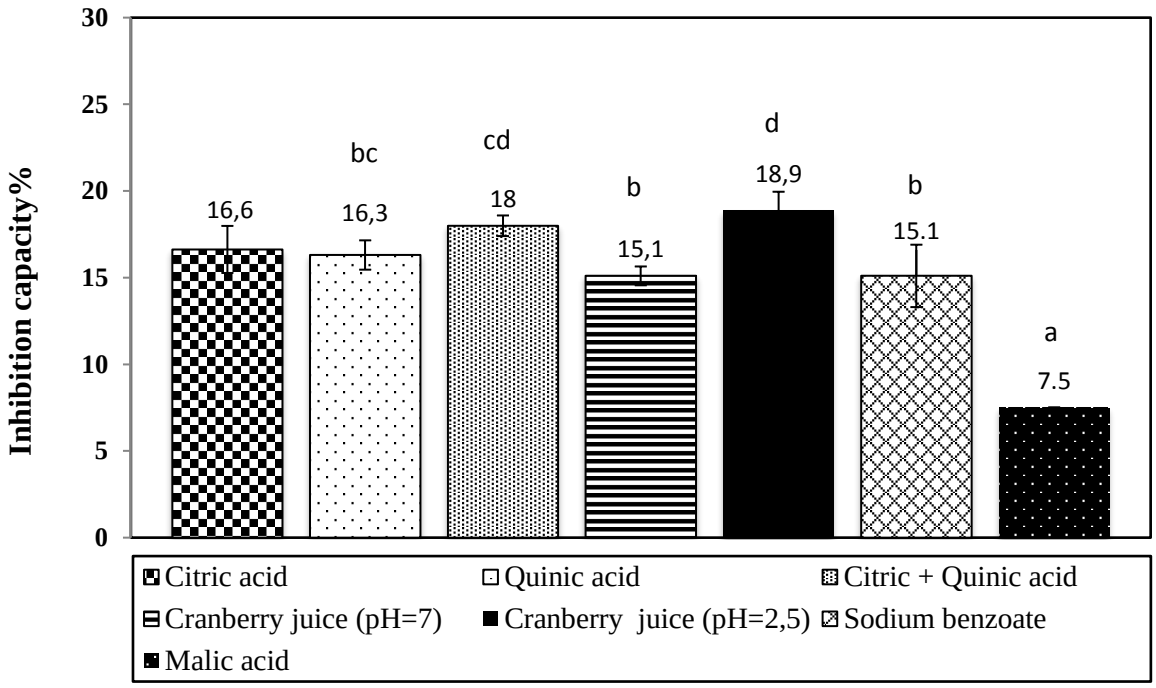

Figure 2. Inhibition capacity of antimicrobial formulations against *Escherichia coli.* Different letters represent significant differences of inhibition capacity ($p \leq 0.05$).

3.1.3 Salmonella Typhimurium

Salmonella serotypes are well-known pathogens that have been implicated in a large number of outbreaks of foodborne disease in world. Although *Salmonella* is not heat resistant and is inactivated by industrial cooking, its presence in non–heat-treated RTE food, or RTE food cross-contaminated after cooking, can pose a health threat for the consumer (Cabedo *et al.*, 2008).

Results showed that IC values of citric acid, quinic acid and citric+quinic acids (pH= 2.4) as a reference exhibited an inhibition effect on *S.* Typhimurium, from 15.6 to 18.2%. The order of antibacterial activity of organic acids against *S.* Typhimurium is quinic acid > citric acid > citric acid + quinic acid > malic acid. Furthermore, the CJC (pH= 2.4) induced an inhibitor capacity of 20% in compare to pH-neutralized CJC (pH= 7) with 17.1% of inhibition capacity (**Fig. 3**). Waterman and Small (1998) was also observed *Salmonella* inoculated onto the surface of pre-acidified ground beef could not survive if the pH on the surface of the beef was 2.61 or lower but was viable if the pH of the surface was 3.27.

Sodium benzoate was also showed antibacterial activity against *S.* Typhimurium significantly lower than other formulations ($p \leq 0.05$). The order of antibacterial activity of sodium benzoate against studied pathogens was *L. monocytogenes* > *E. coli* > *S.* Typhimurium.

The order of resistibility of pathogens to CJC antimicrobial activity was *L. monocytogenes* > *S.* Typhimurium > *E. coli*. In should be also noted that the order of acid sensitivity of pathogens to citric acid + quinic acid as the references, was *E. coli*. > *L. monocytogenes* ≥ *S.* Typhimurium.

Acid sensitivity studies indicated that at the same pH level (≈ 2.4) in presence of organic acids, the CJC showed greater antibacterial effects than the acids because of their phenolic compounds. Antimicrobial compounds in CJC can damage the cell wall, cell membrane and induced cell lysis which facilitates leakage of the cell contents (Wu *et al.*, 2008). Wu *et al.* (2008) also demonstrated that the low pH of the cranberry concentrate plays an important role in inhibiting foodborne pathogens but at the same pH level, cranberry concentrate showed stronger antimicrobial effects than organic acids. The cranberry juice concentrate (CJC) contains some components like polyphenols, anthocyanins, flavanols that can act in synergy with organic acids to reduce the bacterial growth. Vattem *et al.* (2004) investigated the phenolic compounds extracted from cranberry have an antimicrobial activity against *L. monocytogenes* and *Vibrio parahaemolyticus*, and to a lower degree against *E. coli* O157:H7. They also found that the variation in microorganism's sensitivities to the phenolic extracts may indicate different mechanisms of action of the extracts against different microorganisms. Our findings are in good agreement with these results.

Furthermore, Vattem *et al.* (2004) suggested that phenolic compounds with partial hydrophobicity could act efficiently at the bacterial membrane water interface by embedding in the membrane, thereby impairing the cell membrane and the transport process.

Microbial growth inhibition by organic acids can be explained by the ability of these acids to pass across the cell membrane, dissociate in the more alkaline interior and acidify the cell cytoplasm (Kashket, 1987). It is now clear that some bacteria have the ability to let their intracellular pH decline when the extracellular pH becomes highly acidic. This decline in intracellular pH necessitates a metabolism that can tolerate a lower pH, but the strategy appears to be highly adaptive. However, the antimicrobial activity of organic acids on some bacterial species has been correlated with intracellular pH regulation, but bacteria classified as neutrophils seem to be more sensitive than those are acid tolerant (Van Immerseel *et al.*, 2006). Furthermore, the anion model of organic acid toxicity can explain why bacteria differ in their sensitivity to organic acids, but it does not provide information on the antibacterial effect of one acid versus another. This observation indicates that factors such as chain length, side chain composition, *pKa* values and hydrophobicity could affect the antimicrobial activity of organic acids (Hsiao *et al.*, 1999). Lacombe *et al.* (2010) confirmed that the antimicrobial mechanism of organic acids against *E. coli* relies certainly on a low pH. Van Immerseel *et al.* (2006) explained that exposure to low pH can cause sublethal injury to cell membranes, causing disruption of proton motive force owing to loss of H+-ATPase. This damage may make the bacteria more susceptible to the phenolic antimicrobial compounds in the cranberry concentrate.

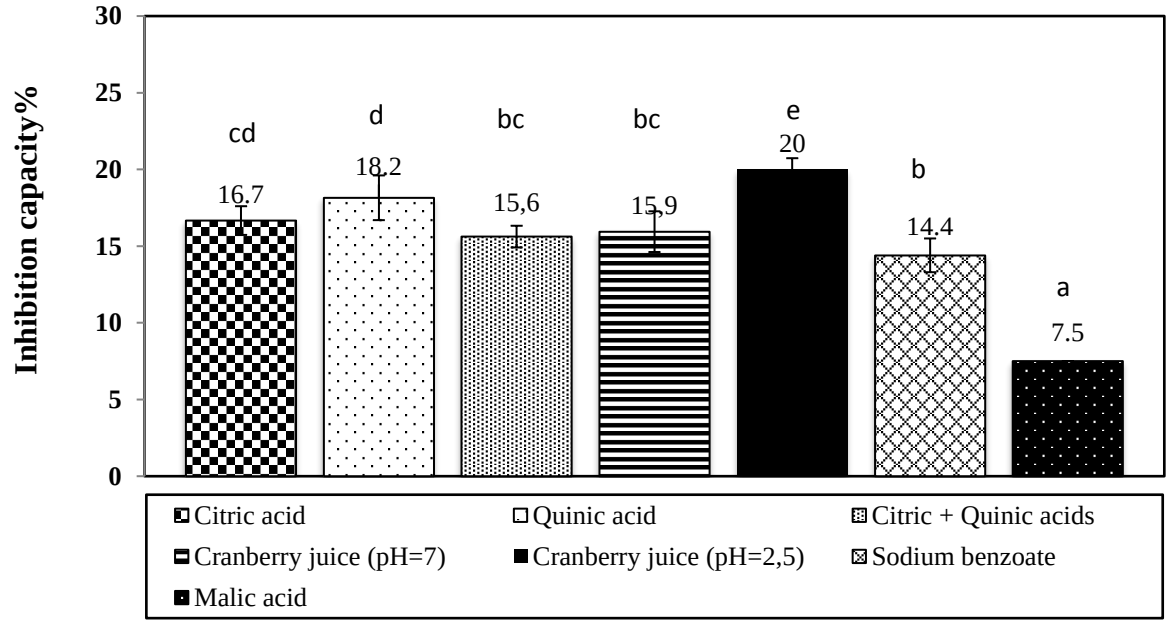

Figure 3. Inhibition capacity of antimicrobial formulations against *Salmonella* Typhimurium. Different letters represent significant differences of inhibition capacity ($p \leq 0.05$).

3.2 Determination of the Minimal Inhibiting concentration (MIC)

The MIC values of different antimicrobial formulations against three pathogens are presented in **Table 1**. Results showed that CJC exhibited a low MIC values, high antimicrobial activities. The MIC value was 3133 ppm against *E. coli*, *L. monocytogenes* and *S.* Typhimurium. The MIC values of CJC was lower than those of citric acid, quinic acid and citric + quinic acids (7.5% with pH = 2.4), against pathogens. The results of MIC showed that the CJC have also much higher strong inhibiting effect than sodium benzoate.

Table 1. Minimal inhibitory concentration of different antimicrobial formulations against *E. coli, L. monocytogenes and S.* Typhimurium

Samples	MIC (ppm)		
	E. coli	***L. monocytogenes***	***S.* Typhimurium**
CJC	3133	3133	3133
Citric acid	3915	3915	3915
Quinic acid	7831	15662	3915
Citric + Quinic acids	7831	7831	3915
Sodium benzoate *	10730	5185	5185

* Sodium benzoate is used as a synthetic preservative agent in food industry.

By considering the MIC results, we can also conclude that CJC was significantly ($p \leq 0.05$) more effective against all foodborne pathogens as compared to organic acids and sodium benzoate. These findings confirmed that antibacterial activity of CJC is a result of synergic activity of low pH caused by organic acids and phenolic compounds. Our results are consistent with the findings of Alnoman *et al.* (2015). Alnoman *et al.* (2015) evaluated the inhibitory effects of potassium sorbate and sodium benzoate against *Clostridium perfringens* type A food poisoning (FP) and non-food-borne (NFB) disease isolates. No significant inhibition ($p > 0.05$) of spores germination of both FP and NFB isolates was observed in rich medium (pH 7.0). It was also reported that sorbate and benzoate could not inhibit the growth of *C. perfringens* spores in chicken meat stored under extremely abusive condition (Alnoman *et al.*, 2015).

3.3 *In situ* antimicrobial capacity of CJC on pre-cut red peppers

3.3.1 Listeria monocytogenes

The growth inhibition *of L. monocytogenes* on pre-cut red peppers during storage at 4°C is presented in **Fig. 4**. Results showed that the control (without treatment) presented a high level of *L. monocytogenes*, showing a bacterial level between 5 to 5.5 log CFU/g. Sodium hypochlorite treatment also reduced negligibly the numbers of *L. monocytogenes* during the 7 days of storage at 4°C. Samples treated with the CJC showed a rapid reduction of 1.2 log CFU/g at day 1 as compared to control, followed by a decrease from 3.8 to 3.2 log CFU/g between day 1 and 7 days of storage at 4°C. According to Wu *et al.* (2008), *L. monocytogenes* tend to be more resistant in compare to *E. coli* O157:H7 and *S.* Typhimurium. However, the CJC was able to reduce the level of *L. monocytogenes* to 1.8 log CFU/g, after 7 days of storage. Wu *et al.* (2008) reported that *Listeria monocytogenes* and *Staphylococcus aureus* tend to be more rigid when compared with *E. coli* O157:H7 and *S.* Typhimurium. They concluded that it's not easy to destroy *L. monocytogenes* and *Staphylococcus aureus* cells by the formation of lesions or channels on the cell wall. Seltman and Holst (2002) also reported that the peptidoglycan in Gram-positive cells is much more important than in Gram-negative cells and it's responsible for the increasing of the rigidity of cells. *L. monocytogenes* cells were not easily destroyed by formation of lesions or channels on the cell wall (Wu *et al.*, 2008).

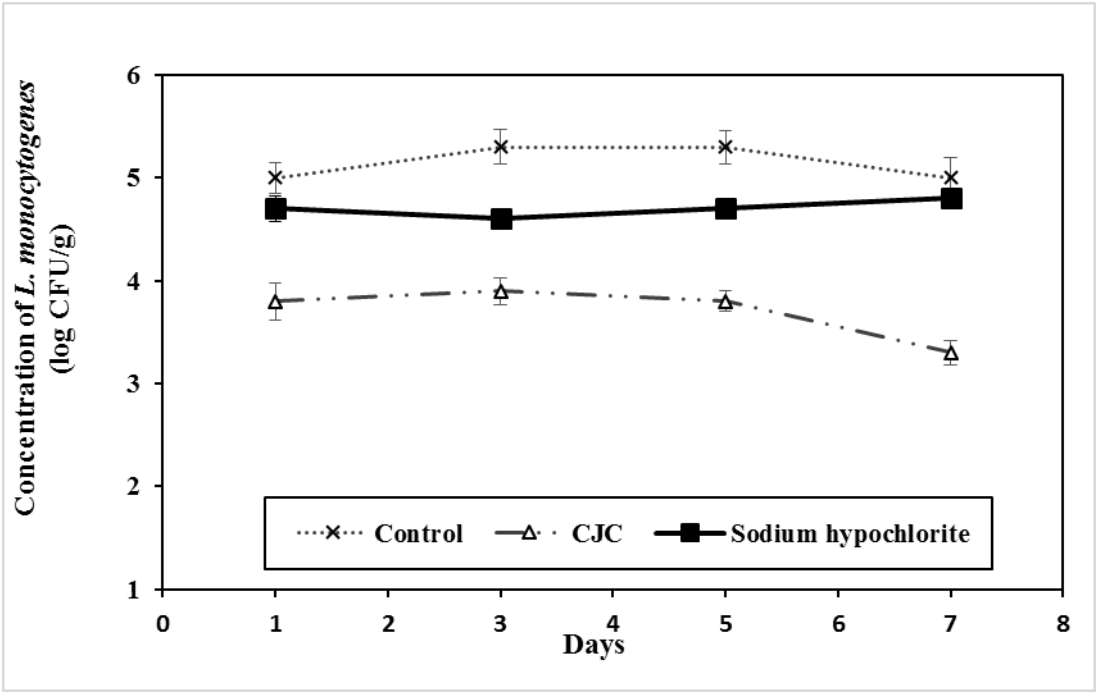

Figure 4. Growth inhibition of *Listeria monocytogenes* on pre-cut red peppers during storage at 4°C. CJC: cranberry juice concentrate

3.3.2 Escherichia coli

The growth inhibition of *E. coli* in pre-cut red peppers during storage at 4°C is presented in **Fig. 5**. The content of *E. coli* in control samples (without treatment) presented a high level, about 5-6 log CFU/g, during the time of storage at 4° C. Sodium hypochlorite treatment reduced negligibly the number of *E. coli* during the 5 days of storage at 4°C and then after an increment was observed such as control samples without any treatment. It is possible during the storage time, the chlorine disintegrated and lost its activity. Sodium hypochlorite (NaOCl; SH) is commonly used to sanitize some kind of fresh-cut vegetables in industry. However, the antimicrobial effectiveness of this chlorinated water is limited and at the consumer level, residual chlorine can be harmful to health. Moreover, the reaction of active hypochlorite with food components resulting in the formation of toxic compounds, and cause tissue damage to some food products, that motivated researchers to find the alternative disinfection agents (Allende *et al.*, 2009). Samples treated with CJC showed a reduction of *E. coli* concentration from 4.4 log CFU/g to 2 log CFU/g after 7 days of storage at 4°C. It was observed the inhibition effect of CJC increased with time. Wu *et al.* (2009) also mentioned time and concentration had a synergistic effect on the reduction of *E. coli* O157:H7 in presence of cranberry concentrate. Côté *et al.* (2011) also observed a strong inhibition of *E. coli* in presence of water-soluble phenolic compounds of neutralized cranberry extract. In addition, according to the findings of Côté *et al.* (2011), *E. coli* was also affected by the presence of neutralized anthocyanin-rich cranberry fraction. According to Wu *et al.* (2009), gene expression levels in *E. coli* O157:H7 were altered by cranberry concentrate (2.5% with pH = 2.2). They found in the ground beef samples treated with cranberry concentrate, the genes associated with cell wall and membrane biogenesis such as cyclopropane fatty acyl phospholipid synthase *(cfa*) was downregulated. The *cfa* could be expressed correspondingly in bacteria to catalyze the synthesis of fatty acid depending on the requirement. Correspondingly, *E. coli* O157:H7 growth was inhibited and maintained a stagnant level in the treated samples. Furthermore, with a strong probability, cranberry concentrate may interact with the cell outer membrane, disrupt the outer membrane, then enter inside the cell and inhibit the transcription of genes. This may prevent the synthesis of proteins that are required for bacterial growth. The inhibition of gene transcription may happen in a short time period (Wu *et al.*, 2009). The transmission electron microscopy (TEM) micrographs also confirmed the membrane damage caused by cranberry concentrate (Wu *et al.*, 2008).

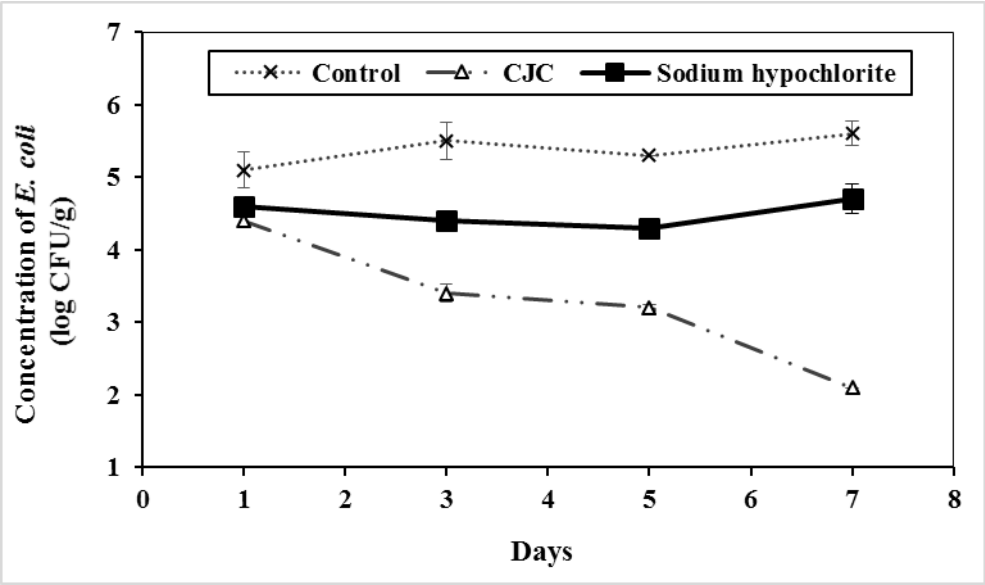

Figure 5. Growth inhibition of *Escherichia coli* on pre-cut red peppers during storage at 4°C. CJC: cranberry juice concentrate.

3.3.3 Salmonella Typhimurium

The growth inhibition *of S.* Typhimuriuim on pre-cut red peppers during storage at 4°C is presented in **Fig. 6**. The control (without treatment) showed a high and increasing level of *S.* Typhimurium between 5.2 to 6 log CFU/g during 7 days of storage. Samples treated with sodium hypochlorite solution showed a 1 log CFU/g reduction of *S.* Typhimuriuim concentration during the 5 days of storage at 4°C and then after concentration increased such as control samples without any treatment.

However, samples treated with CJC showed a rapid and high inhibition activity at day 1 (1.2 log CFU/g reduction), followed by a decrease to 3 log CFU/g at day 3, then, it was reduced to non-detectable levels after 7 days of storage (4 log CFU/g reduction). The antibacterial activity of CJC could be the result of its bioactive compounds such as anthocyanins and non-anthocyanin, polyphenolic compounds (benzoic acid, caffeic acid, *p-coumaric* acid, chlorogenic acid and ursolic acid) in combination with low pH contributed by the organic acids. Wu *et al.* (2009) observed that the exposure of *S.* Typhimurium to cranberry concentrate (5 µL/mL) resulted in morphological damage such as loss of the structural integrity of the wall, membrane and intracellular matrix, and the presence of significant amounts of cytoplasmic material and membrane debris in the cell's surrounding environment, were observed from damaged cells of *S.* Typhimurium.

The findings showed that CJC has antibacterial activate more effective than sodium hypochlorite. Furthermore, the possibility of formation of carcinogenic derivatives of chlorine (chloramines and trichloromethane) during treatment in presence of chlorine in water has raised concerns about its use in food processing.

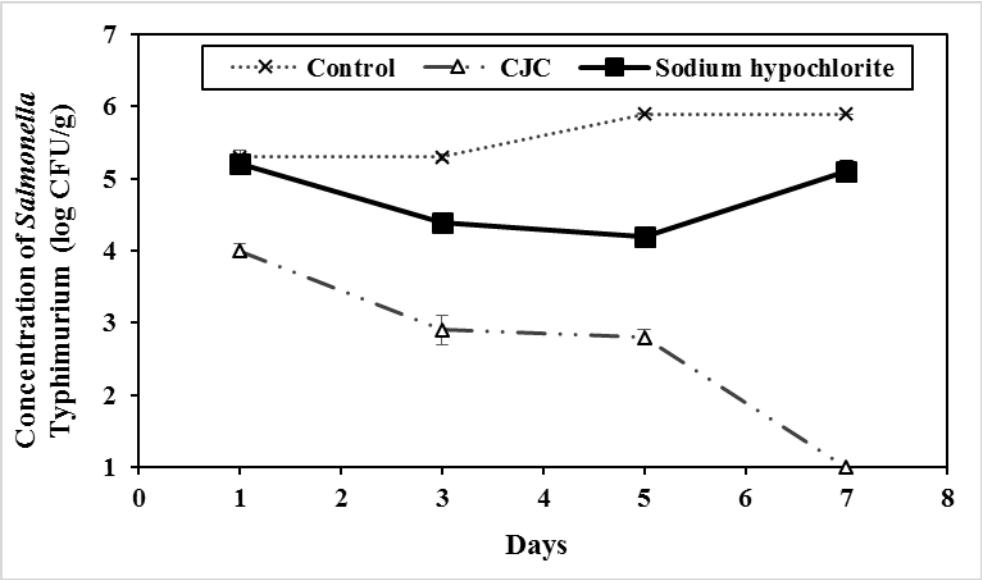

Figure 6. Growth inhibition of *Salmonella* Typhimurium on pre-cut red peppers during storage at 4°C CJC: cranberry juice concentrate.

3.4 *In situ* antimicrobial capacity of CJC on cranberry fruits

3.4.1 Listeria monocytogenes

The growth inhibition of *L. monocytogenes* on cranberries during storage at 4°C is presented in **Fig. 7.** Results showed that the cranberries without treatment (control) showed a bacterial reduction from 3.5 log CFU/g to 2.2 log CFU/g. Then, it was reduced to non-detectable level at day 18. Acceding to Côté *et al.* (2011), this decrement could be due to the presence of polyphenolic compounds, organic acids in cranberries which can effect on the growth of bacteria. On the other hand, samples treated with CJC allowed a total inhibition of *L. monocytogenes* at day 1 of storage. According to results of Robards and Antolovich (1997), the predominant bioactive compounds found in cranberries are the flavan-3-ols, the anthocyanins, the tannins (ellagitannins and proanthocyanidins), and the phenolic acid derivatives. These phytochemicals are commonly associated with the fruit organoleptic (sensory) qualities and have also shown diverse biological properties like antimicrobial properties.

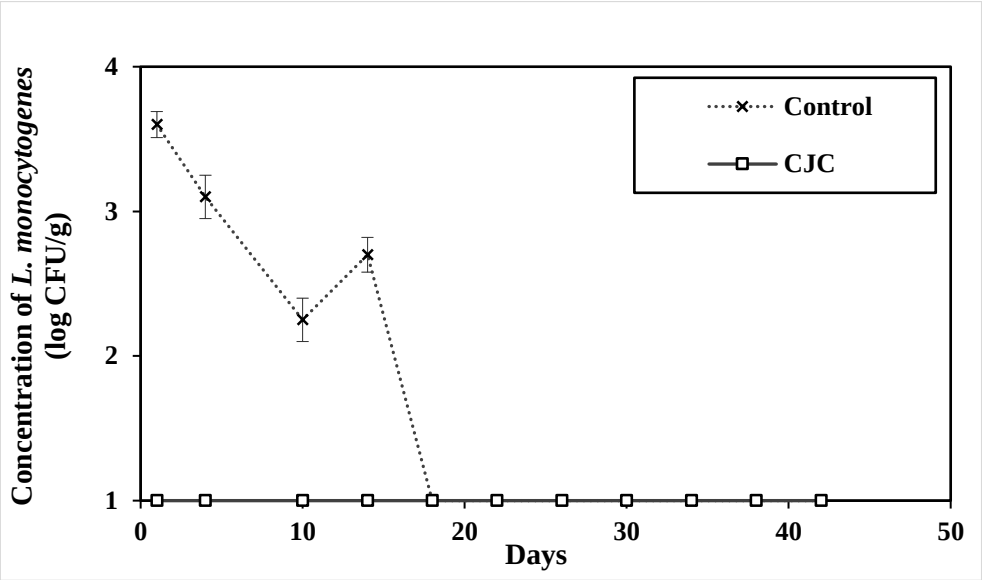

Figure 7. Growth inhibition of *Listeria monocytogenes* on cranberries during storage at 4°C. CJC: cranberry juice concentrate.

3.4.2 Escherichia coli

The growth inhibition of *E. coli* on cranberries during storage at 4°C is presented in **Fig. 8.** Results showed that the cranberry fruits without treatment (control) showed a high level of *E. coli* (2.5-3.3 log CFU/g), then it was reduced to non-detectable level at day 22. This decrease could be due to the presence of polyphenolic compounds, and organic acids present in cranberries. Cranberry fruits treated with CJC showed a rapid and high inhibition of *E. coli* concentration from 3 to 2 log CFU/g at day 1. Then, it was reduced to non-detectable level at day 10. Numerous studies have identified that the procyanidins, consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage, as one of key protecting element of cranberries against the growth of pathogenic bacteria (Heinonen, 2007; Seeram &Heber, 2007).

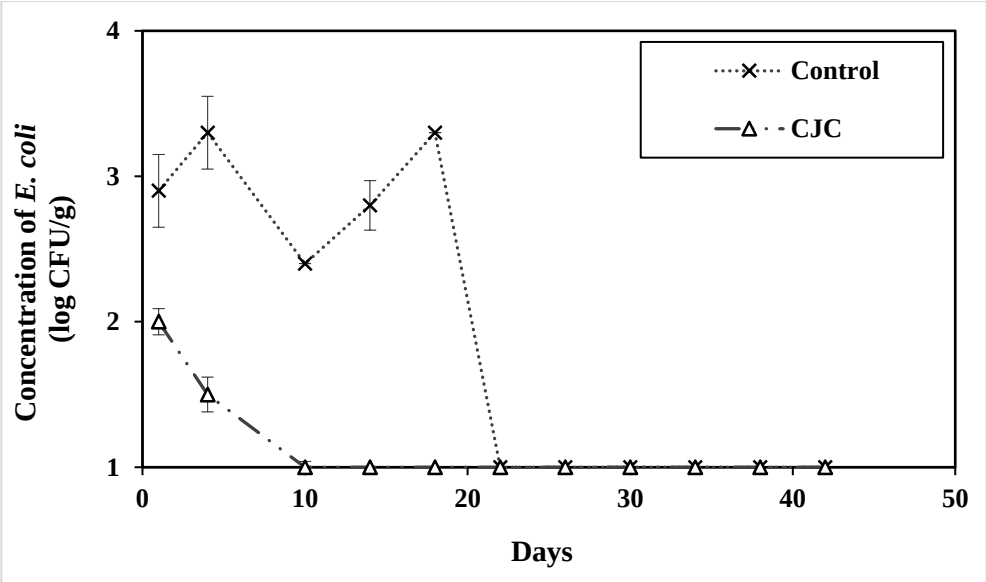

Figure 8. Growth inhibition of *Escherichia coli* on cranberries during storage at 4°C. CJC: cranberry juice concentrate.

3.4.3 Salmonella Typhimurium

The growth inhibition of *S.* Typhimurium on cranberries during storage at 4°C is presented in **Fig. 9.** Results showed that the cranberries without treatment (control) showed a high and variable level of *L. monocytogenes* between 2 and 3 log CFU/g, during the first 20 days of storage. Then, it was reduced to non-detectable level at day 26. Samples treated with CJC allowed a rapid and high inhibition of *S.* Typhimurium. A 0.7 log CFU/g reduction was observed at the day of the treatment and after that, 2.6 log CFU/g reduction was observed in day 4 of storage. According to Wu *et al.* (2008), *S.* Typhimurium was sensitive to the cranberry concentrate at 4°C and they found a significant reduction of *S.* Typhimurium during 24 h in sample treated with 25 µL/mL cranberry-concentrate, and no *S.* Typhimurium was detected in samples treated with 50, 75 and 100 µL/mL cranberry-concentrates.

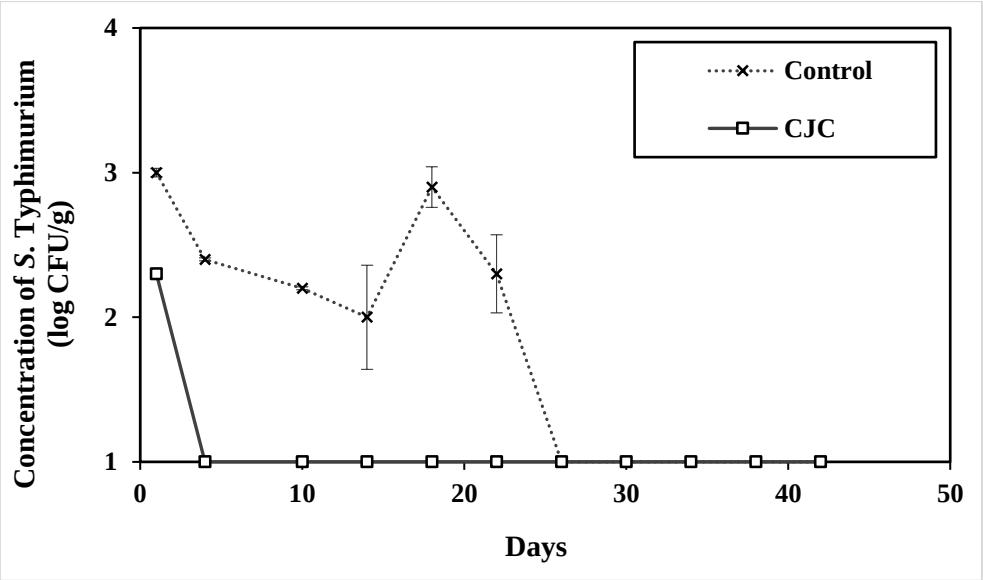

Figure 9. Growth inhibition of *Salmonella* Typhimurium in cranberries during storage at 4°C. CJC: cranberry juice concentrate.

3.5 Sensory Analysis of red peppers

The odor, color, global appreciation, taste and texture of samples were determined according to 9-points scale hedonic levels, resulting in degrees of appreciation, from 1 (Dislike very much) to 9 (Like very much). Results showed that CJC coating did not affect significantly ($p > 0.05$) on sensory properties of red peppers. Indeed, the odor, color, global appreciation, taste and texture of control (untreated red peppers) and red peppers coated with CJC were not significantly different ($p > 0.05$). The results of sensorial analysis are presented in **Table 2**.

Table 2. Effect of CJC coating treatment on the sensory properties of red peppers, by 9-points hedonic evaluation.

Red peppers	Hedonic evaluation of sensory properties[1-3]				
	Odor	**Color**	**Global appreciation**	**Taste**	**Texture**
Untreated (control)	6.4± 1.7[a]	7 ± 1.33[a]	6.2 ± 1.9[a]	5.8 ± 1.6[a]	4.60 ± 2.1[a]
Treated with CJC	7.5 ± 0.97[a]	7.4 ± 0.84[a]	6.6 ± 1.57[a]	7 ± 1.33[a]	5.5 ± 2.5[a]

[1] The hedonic evaluation was scaled as follow: 9 = Like very much; 8 = Like a lot; 7 = Like moderately; 6 = Like a little; 5 = Indifferent; 4 = Dislike a little; 3 = Dislike moderately; 2 = Dislike a lot; 1 = Dislike very much.
[2] The equality of variances was determined by Levene's test. Data were compared according to Duncan's test for assumed equal variances.
[3] Different letters represent significant differences at ($p \leq 0.05$).

For red peppers treated with CJC, score taste (7) was higher than the control (5.8), but there was no significant difference ($p > 0.05$) between the taste, odor, color, global appreciation and texture. These results represented that the trend in appreciation in both samples (treated and untreated) was:

- "Like a little / Like moderately" (between 6-7) for odor, color, global appreciation;
- "Like a little/ Like moderately" (between 6-7) for taste;
- "Indifferent/ Like a little" (5-6) for texture.

Generally, the sensory evaluation results showed that samples treated with CJC were more appreciated compare to untreated samples. Furthermore, the CJC treatment had no negative effect on the odor and taste of peppers, therefore, their sensorial attributes could be accepted for further commercialization.

4. Conclusion

Several investigations demonstrated that the inhibitory effect of natural antimicrobials derived from berries on foodborne pathogens. On the present study, cranberry concentrated juice showed the antibacterial effects on both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*E. coli* O157:H7 and *Salmonella* Typhimurium) bacteria. *In vitro* results indicated that the order of resistibility of pathogens to CJC antimicrobial activity was *L. monocytogenes* > *S.* Typhimurium > *E. coli*. This study suggest that low pH of CJC can play an important role in inhibition of foodborne pathogens growth. Furthermore, acid sensitivity studies indicated that at the same pH level in presence of organic acids, CJC showed greater antibacterial effects than the acidic solution because of their

phenolic compounds. Indeed, the high content of phenolic compounds which constitute a particularly diverse groups in cranberry juice, including low-molecular weight phenolic acids, condensed tannins, proanthocyanidins, and flavonoids such as anthocyanins (in high content) and flavonols, had important role in antimicrobial activities. Recent findings confirmed that the phenolic compounds with partial hydrophobicity could efficiently damage the bacterial membrane.

Furthermore, the results of sensory analysis showed CJC treatment had no effect on sensorial properties of red peppers and it could be accepted for further commercialization.

Based on a broad spectrum of studies that have done on sanitizing methods in food industrials and by considering the MIC results, diluted CJC not also can be used as a natural food sanitizer without the risk of food texture damage and harmful residual components mixtures, but also in concentration of growth inhibition of pathogenic bacteria, it can be considered as a cost-effective natural sanitizer as same as chlorine solution.

Our findings confirmed many additional health benefits can be created by the use of cranberry juice as a natural preservative formulation in food, pharmaceutical and cosmetic applications.

Overall, these findings support the use of cranberry juice at sub inhibitory concentrations as alternative preservatives to guarantee the safety and extend the shelf-life of Ready-to-eat (RTE) fruits. Furthermore, the sensory evaluation results showed that samples treated with CJC were more appreciated compare to untreated samples which makes it more acceptable for further commercialization.

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antimicrobial mechanism related to the downregulated slp, hdeA and cfa. *Food Microbiology*, 26(1), 32-38.

CHAPITRE 2:

COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICIENCY OF FOODGARD F410B ® CITRUS EXTRACT AND SODIUM BENZOATE AGAINST FOODBORNE PATHOGENS IN STRAWBERRY FILLINGS

Article soumis à *Food Science and Nutrition*

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Contributions des auteurs

J'ai réalisé les expériences, décrit et interprété les résultats et rédigé l'article.

Stéphane Salmieri a participé à la planification et au bon déroulement des analyses, et à la rédaction de l'article.

Maherani Behnoush a aidé dans l'interprétation des résultats.

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

Résumé

Dans cette étude, les propriétés antibactériennes *in situ* et *in vitro* d'un extrait d'agrumes FOODGARD F410B ont été évaluées contre cinq bactéries pathogènes (*Eschrerichia coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*), la flore aérobique totale, le compte total des levures et moisissures, une levure *(Zygosaccharomyces rouxii)* et une moisissure (*Xeromyces bisporus*) et ont été comparées au benzoate de sodium (conservateur synthétique).

Les résultats *in vitro* ont montré que FOODGARD était plus efficace contre les bactéries Gram (+) et Gram (-) que le benzoate de sodium.

Les résultats *in situ* ont montré que *S. aureus* et *S.* Typhimurium étaient les plus sensibles. Les résultats *in situ* ont montré que l'incorporation du FOODGARD dans les garnitures de fraises a permis aussi une inhibition totale de *E. coli* et *S.* Typhimurium à 4°C. De plus, Foodgard a permis aussi une inhibition totale de *L. monocytogenes*, 3.5 log de réduction de *B. cereus* et 4 logs de réduction de *S. aureus* dans les garnitures de fraises. De plus, FOODGARD a totalement inhibé la flore aérobique totale et les levures et moisissures.

Les analyses sensorielles ont montré que les échantillons contenant du FOODGARD et ceux contenant du benzoate de sodium étaient similaires aux témoins.

Abstract

In this study, the *in vitro* and *in situ* antibacterial properties of FOODGARD F410B ® citrus extract (commercial extract) were evaluated compared to sodium benzoate against five pathogenic bacteria (*Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Staphylococcus aureus, Bacillus cereus and Listeria monocytogenes),* Total Aerobic Microflora (TAM), total yeasts/molds and also one yeast (*Zygosaccharomyces rouxi*) and mold (*Xeromyces bisporus*) by agar diffusion assay and minimum inhibitory concentration (MIC) tests.

The *in vitro* study showed that FOODGARD ® was more effective than sodium benzoate against Gram (+), Gram (-) bacteria and Yeast/Molds. The *in situ* study demonstrated that *S. aureus* and *S.* Typhimurium were the most sensitive Gram positive and negative bacteria, respectively.

In situ results showed that the incorporation of FOODGARD® in strawberry filling (SF) also allowed a total inhibition of *E. coli* and *Salmonella* at 4°C in SF. In addition, FOODGARD F410B® allowed a total inhibition of *L. monocytogenes*, 3.5 log reduction of *B. cereus* and 4 log reduction of *S. aureus* in SF at day 7, 14 and 28 of storage, respectively. In addition, FOODGARD allowed inhibiting 90% of yeast population after 7 days of storage. Samples treated with FOODGARD and sodium benzoate also showed a total inhibition of Total Aerobic Microflora (TAM) from the first day of storage.

The sensory analysis was designed to compare SF samples containing FOODGARD F410B®, sodium benzoate and control samples (without preservative). Results showed that samples containing FOODGARD F410B® and those containig sodium benzoate were generally similar to controls (without preservative), with very acceptable sensory attributes.

Practical Application

From a point of view of commercializing natural food preservatives, this study strongly evidenced that FOODGARD F410B® as an effective natural preservative can be used to replace synthetic preservative in acidic food systems, with many additional health benefits to consumers and a high potential market development to food industry.

Keywords

Citrus extract, Antibacterial properties**,** Foodborne pathogens, Sodium benzoate, Sensory analysis

1. Introduction

Despite the existence of different food preservation methods, modern technologies and safety concepts, such as Hazard Analysis and Critical Control Points (HACCP), the reported numbers of foodborne illnesses and intoxications are still increasing (O'Sullivan *et al.*, 2002). Pathogenic bacteria have always been considered harmful for human and they are the major cause of infection. During recent years, there has been an increasing demand for healthy and safe foods, as a consequence of some serious food illnesses caused by consumption of foods contaminated by foodborne pathogens. The World Health Organization (WHO) introduced the Foodborne illness as a kind of diseases which is usually caused by consumption of contaminated food (Food Standards Agency UK, 2011). According to World Health Organization the number associated to death was 420,000 in 2010 in the world (Havelaar et *al.,* 2015). In the United States, major foodborne pathogens include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Staphylococcus aureus*. *E. coli* O157:H7 is an emerging foodborne pathogen which can cause hemorrhagic colitis, hemolytic uremic syndrome and thrombocytopenic purpura. *L. monocytogenes* can cause listeriosis which usually affects pregnant women, immunocompromised individuals and the elderly. *S.* Typhimurium can cause salmonellosis, another common foodborne disease (Jay *et al.*, 2005).

For many years, synthetic preservatives are used as antimicrobial agents to provide the safe foods; however, the consumers' concerns are rising on synthetic compounds with negative health side-effects, which led to a reduce consumer acceptability (Namiki, 1990; Guetierrez *et al.*, 2009).

Furthermore, antibiotic-resistant bacteria represent a major concern worldwide. Therefore, intense research efforts are an urgent need to find new alternatives to prevent foodborne pathogens and develop innovative natural antimicrobial agents. As an alternative sources of bioactive agents, plant extracts have been considered for many years because of their potentially active compounds that can be of interest in food industrial applications. Special attention has been paid to the antimicrobial activity of diverse plant oil extracts and their components, which have been reported to show great inhibitory effects against pathogenic bacteria, yeasts, fungi and viruses (Alvarez-Ordóñez *et al.*, 2013).

Moreover, consumer concern created a demand for more "natural" and "minimally processed" foods. As a result, using naturally originated antibacterial compounds, such as essential oils (EOs) extracted from plants and fruits has received great attention. EOs are complex mixtures of phenolic compounds with a broad spectrum of biological activity and recommended as "Generally Recognized As Safe" (GRAS) by the Food and Drug Administration (FDA) (Bakkali *et al.*, 2008).

In recent years, citrus EOs have been highlighted due to their strong antimicrobial properties, high yields, aromas and flavors and particularly the presence of flavonoids. Flavonoids are a group of polyphenolic compounds that include flavanones, flavones and their derivatives. They are found in the form of pigments contained in plants, fruits, vegetables, flowers, wine, and honey and they are

responsible for flower and fruit coloration (Yusof *et al.,* 1990; Tripoli, *et al.,* 2007). Citrus peel and seeds are very rich in phenolic compounds, such as phenolic acids and flavonoids (Ladaniya, 2008).

Citrus flavanones include: naringin, hesperidin, hesperitin which present a broad spectrum of biological antibacterial activity against a wide range of bacteria (Jing *et al.,* 2015). They also exhibit antiviral activity against rhino- and poliomyelitis viruses. Flavonoids have also several health promoting activities including antioxidant, heart protection, anti-allergic and anti-cancerous (Kaul *et al.,* 1985; Viuda-Martos *et al.,* 2011; Cormier *et al.,* 2013). Pharmacological studies have also confirmed their anti- inflammatory properties. Furthermore, epidemiological studies have approved an inverse relationship between dietary flavonoids intakes and cardiovascular diseases. These beneficial effects are due to their antioxidant and antiradical activity which is related to supplement the body defenses against the development of atherosclerosis and cancer. Studies have shown that regular consumption of flavonoids reduces the risk of coronary diseases in old men (Hertog *et al.,* 1993; Geleijnse & Hollman, 2008).

Moreover, according to Da Cruz Cabral *et al.*, (2013) fungus growth is another responsible for food spoilage and plant disease which induce economic losses. Some reports showed that the Mexican lime essential oils are able to inhibit the growth of *Colletotrichum gloeosporioides* and *Rhizopus stolonifera* in papaya fruit during storage (Bosquez-Molina *et al.*, 2010). In addition, Amusa *et al.* (2003) reported that *Aspergillus niger* was responsible for the bio-deterioration of the African apple during storage. It is important to mention that many researchers reported that *Aspergillus* spp., can grow on a wide range of organic substrates, as a result this microbial growth cause the deterioration of stored food material (Barrios *et al.,* 1997; Misra and Dubey 1994; Paster *et al.*, 1990). Based on the report of the National Academy (Canada) (1986) on pesticides residues on food, carcinogenic risk was more increased by the use of fungicides more than insecticides and herbicides (Research Council, Board of Agriculture, 1987).

Furthermore, two studies reported that yeasts are other significant spoilage microorganisms which are able to grow in foods at low pH, high sugar content, high salt content and also in those containing chemical preservatives such as sorbate or benzoate (Fleet 1992; Tudor and Board 1993). For example, *Zygosaccharomyces bailii* is able to grow in presence of sorbate and benzoate preservatives (Thomas and Davenport 1985).

FOODGARD F410B® citrus extract is a non-toxic, active natural antimicrobial containing citrus bioflavonoids extracted from the *flavedo* and *albedo* layers of various citrus fruits. It is recognized by FDA as GRAS and also approved by Health Canada as a food ingredient.

The objective of this study was to investigate the *in vitro* inhibitory capacity of FOODGARD F410B® (commercial citrus extract) *vs* sodium benzoate (synthetic preservative) against five selected foodborne bacteria (*E.* coli, *S*. Typhimurium, *S. aureus*, *Bacillus cereus* and *L. monocytogenes*), Total

Aerobic Microflora (TAM), total yeasts/molds and one yeast such as *Zygosaccharomyces rouxii* and one mold such as *Xeromyces bisporus*, (considered as spoilage microorganisms) by using the Minimum inhibitory concentration (MIC) and agar diffusion assay. Furthermore, *in situ* study of antibacterial properties of FOODGARD F410B® *vs* sodium benzoate in strawberry fillings against targeted microorganisms (during one month of storage) was also performed. Finally, sensorial analysis was performed to compare SF samples containing FOODGARD F410B®, sodium benzoate and control samples (without preservative).

2. Materials and Methods

2.1. Raw material

FOODGARD F410B® citrus extract was provided by Biosecur Lab Inc. (Mont St-Hilaire, QC, Canada). Strawberry fillings (SF) and sodium benzoate (E211) were kindly provided by Skjodt-Barrett Foods Inc. (Brampton, ON, Canada) and Foodarom Group Inc. (Saint-Hubert, QC, Canada). All antimicrobials were stored at 4°C. All other reagents of analytical grade were obtained from Sigma-Aldrich Ltd (Oakville, ON, Canada).

2.2. Culture preparation

Stock cultures of *E. coli* O157:H7 EDL 933, *S.* Typhimurium SL 1344, *L. monocytogenes* HPB 2812, 2558, 2569, 1043, 2371, 2739, *S. aureus* ATCC 25923 and *B. cereus* LSPQ 2872 were stored at −80°C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Stock cultures of *X. bisporus* ATCC MY-36964 and *Z. rouxii* ATCC (R) 95-12 13356 were stored at -80°C in Potato Dextrose Broth (PDB) medium (Alpha Biosciences Inc.). Prior to each experiment, stock cultures were grown through two consecutive 24-48 h growth cycles in TSB at 37°C and PDB at 28°C, for bacteria and yeast/molds respectively. Working cultures were diluted in peptone water to obtain a concentration of 10^6 CFU/mL for bacteria, and 10^6 conidia/mL for yeast and molds for MIC and inhibition capacity (IC%) determination and 10^3-10^4 CFU/mL for *in situ* studies.

2.3. Agar Diffusion Assay

Agar diffusion assays were carried out according to a modified procedure from Cardiet *et al.* (2012) to assess bactericidal activity of antibacterial agents by measuring the bacterial growth inhibition zones during time. For bacteria inoculation, Tryptic Soy Agar plates (TSA; Alpha Biosciences Inc.) and Potato Dextrose Agar (PDA) (Alpha Biosciences Inc.) were surface-layered by 100 µL of diluted pathogens at 10^6 CFU/mL for bacteria, yeast and mold. Growth inhibition diameters (mm) were determined by agar diffusion from the deposition of 10 µL of antibacterial agent on a pre-sterilized 12-mm diameter cellulose disc placed onto the surface of TSA or PDA. Sodium benzoate was solubilized in pre-adjusted pH distilled water (pH = 2.5 – 3) to provide the desirable condition of activity.

Plates were then incubated for 48-72 h at 37°C or 28°C for bacteria, yeast and mold, respectively and the inhibition diameter around the disc (colony-free perimeter) was measured with a Traceable Carbon Fiber Digital Caliper (resolution: 0.1 mm; accuracy: ± 0.2 mm; Fisher Scientific Ltd, Nepean, ON, Canada).

All measurements were performed in triplicate (n = 3). Inhibition capacity (IC%) was calculated according to the following Equation:

$$IC\ (\%) = \frac{\text{Diameter inhibition zone}}{\text{Diameter Petri dish}} \times 100 \quad [1]$$

2.4. Determination of the Minimal Inhibitory Concentration (MIC) by the broth microdilution method

The MIC values of FOODGARD F410B® citrus extract and sodium benzoate (solubilized in pre-adjusted pH distilled water (pH: 2.5-3) were determined in sterilized flat-bottom 96-well microplates according to the serial microdilution method as described by Turgis *et al.* (2012). Briefly, antibacterial agents were added in Mueller Hinton Broth (MHB, Alpha Biosciences Inc.) pre-added in 96-well microplate, to obtain serial concentrations. Serial dilutions of FOODGARD F410B® citrus extract and sodium benzoate over the decreasing range from 800 to 1 ppm and 41480 to 40.5 ppm were prepared respectively, and individually injected in wells. Each well was then inoculated with 15 µL of a pathogenic strain at a concentration of 10^6 CFU/mL. The microplate was incubated aerobically for 24 h at 37°C or 28℃. Then, the absorbance was measured at 595 nm with an Ultra Microplate Reader (Biotek instruments, Winooski, VT, USA). The last 2 columns of microplates were used as a blank (containing only a sterile MHB or PDB) and the positive control was a column filled with target bacterium with Mueller–Hinton medium or Potato Dextrose Broth (without antibacterial agents). The MIC was determined as the highest concentration generating an absorbance equal to blank absorbance (e.g. total inhibition). All measurements were performed in triplicate (n = 3).

2.5. *In situ* antibacterial capacity evaluation on strawberry filling (SF)

The *in situ* antibacterial activity of FOODGARD F410B® at a concentration of 0.2% and sodium benzoate at a concentration of 1% incorporated in SF (pH 3.1, a_w = 0.953) was evaluated against five foodborne pathogens (*E coli*, *S. aureus*, *B. cereus*, *L. monocytogenes*, *S.* Typhimurium), TAM, total yeast/molds and two spoilage microorganisms: *Z. rouxii* and *X. bisporus* in comparison to control (SF without preservative). SF samples (25 g) were inoculated with a studied microorganism (10^4 CFU/g) and then stored for 28 days at 4°C. Microbiological analyses were performed at different days. At each day of analysis, samples were homogenized for 2 min at 260 rpm in 50 mL of peptone water (0.1% w/v) with a Lab-blender Stomacher® 400 circulator (Seward Laboratory System Inc., Davie, FL, USA) (Laboratory Equipment). From each homogenate, serial dilutions were surface-plated on TSA (Alpha Biosciences Inc.) or PDA and incubated for 24-48 h at 37°C or 28°C before enumeration

(minimum level of detection: 10 CFU/g for bacteria and 30 conidia/mL for mold and yeast).

Water activity is the most important parameter in terms of food safety. Water activity (a_w) represents the amount of free water in foods, this parameter is more important for food chemical and microbial stability, than total water content. A a_w between 0.86 and 0.9 allowed the growth of a wide range of pathogenic bacteria and yeast/ molds.

2.6. Sensory analysis of strawberry filling (SF)

The appearance, color, texture, odor, flavor and global appreciation of the SF were evaluated by a panel comprising 30 persons (Male and Female: 25-40 years old), according to a 9-points hedonic scale test. This method was used in order to measure the degree of acceptance or rejection of samples, and eventually to verify the possible significant effect ($p \leq 0.05$) of natural antibacterial on the organoleptic properties of SF samples. Analysis of variance (ANOVA), Duncan's multiple range test (for equal variances) and Tamhane's test (for unequal variances) were performed for statistical analysis (PASW Statistics 18; IBM Corporation, Somers, NY, 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. Inhibiting Capacity (IC%) of FOODGARD F410B® *vs* sodium benzoate

The *in vitro* IC% of FOODGARD F410B® and sodium benzoate against Gram (-) and Gram (+) bacteria and yeast and mold are presented in **Fig. 1-A, Fig. 1-B**, and **Fig. 1-C**, respectively. The results of agar diffusion assay demonstrated the wide spectrum antimicrobial activity of FOODGARD F410B® compared to sodium benzoate which was effective at very high content that exceeds the limit authorized by FDA. In presence of Gram (-) bacteria (**Fig. 1-A**), FOODGARD F410B® exhibited IC values of 22-23% against *E. coli* and *S.* Typhimurium, whereas sodium benzoate generated significant ($p \leq 0.05$) lower IC values of 15%. In presence of Gram(+) bacteria (**Fig. 1-B**), FOODGARD F410B® exhibited IC values of 24-26% against *L. monocytogenes* and *B. cereus*, and 36% against *S. aureus* whereas sodium benzoate presented IC values in a range of 15-16% against *S. aureus* and *B.* cereus and 18% against *L. monocytogenes*. These results indicated that FOODGARD F410B® has higher inhibiting capacity than sodium benzoate and in particular, Gram (+) bacteria were more sensitive ($IC \geq 24\%$) to FOODGARD F410B® than Gram (-) bacteria ($IC \geq 22\%$). FOODGARD F410B® exhibited IC values of 51.7 and 31.9% against *X. bisporus* and *Z. rouxii* respectively whereas sodium benzoate presented IC values of 22 and 33.8% against *Z. rouxii* and *X. bisporus*, respectively (**Fig. 1-C)**.

Moreover, the average efficiency of FOODGARD F410B® against bacteria, yeast and mold can be confirmed in the decreasing order; *X. bisporus* > *S. aureus* > *Z. rouxii* > *L. monocytogenes* > *B. cereus* > *E. coli* > *S.* Typhimurium. Therefore, it can be concluded that FOODGARD F410B® was significantly ($p \leq 0.05$) more effective against all foodborne pathogens in comparison to sodium benzoate.

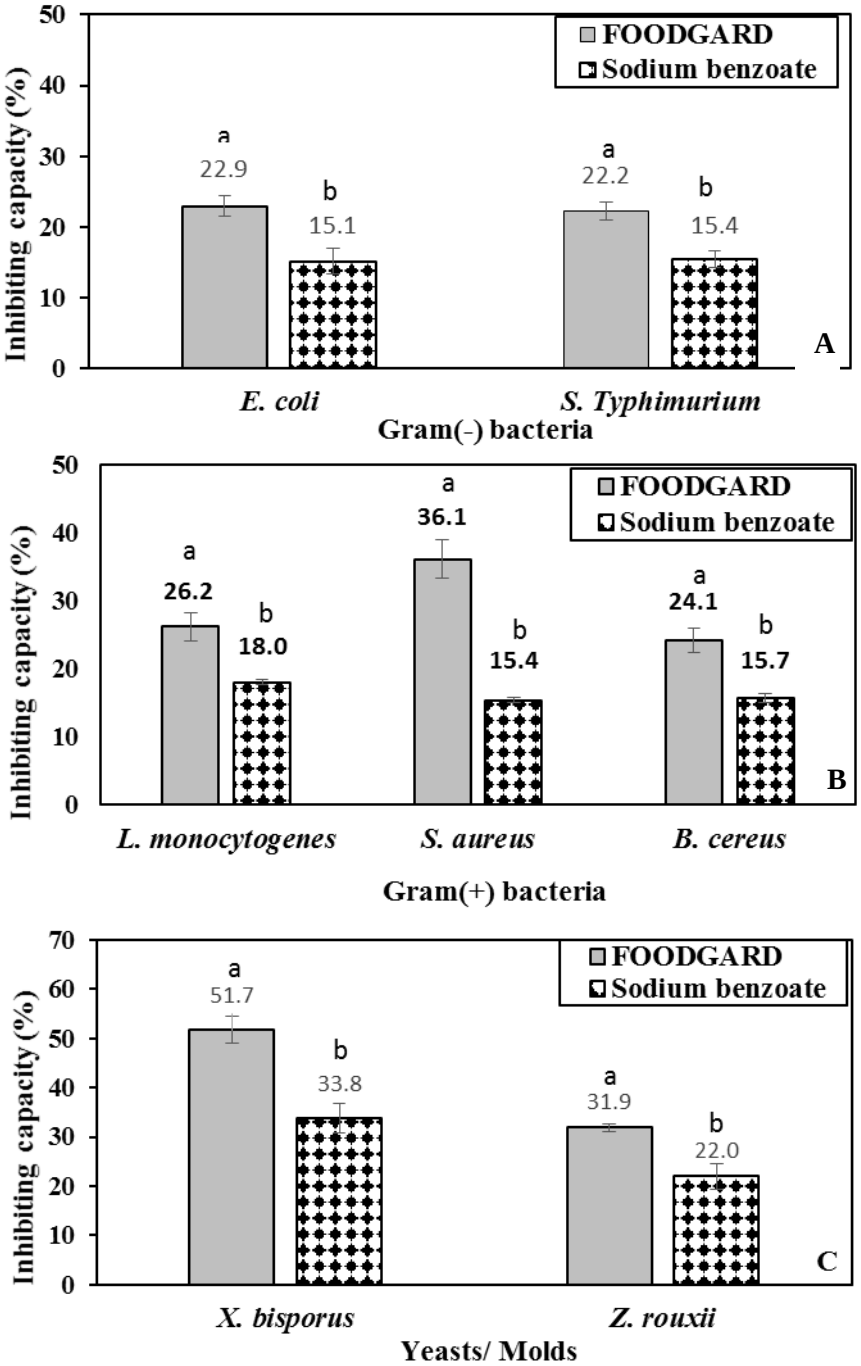

Figure 1. Inhibiting capacity (IC%) of FOODGARD F410B® and sodium benzoate against Gram (-)(**A**), Gram (+) bacteria (**B**) and Yeasts/ Molds (**C**).

3.2. Minimal Inhibitory Concentration (MIC) of FOODGARD F410B® *vs* sodium benzoate

The MIC values of FOODGARD F410B® and sodium benzoate against selected bacteria are presented in **Table 1**. Results showed that the MIC of FOODGARD F410B® were much lower (i.e. strong inhibiting effect) than that of sodium benzoate. FOODGARD F410B® presented a MIC value of 80 ppm against *S. aureus*, and a few hundred ppm (220-260 ppm) against *E. coli*, *S.* Typhimurium, *L. monocytogenes* and *B. cereus.*

Table 1 Minimal inhibitory concentration (MIC) of FOODGARD F410B® and sodium benzoate against selected foodborne pathogens.

Microorganisms	MIC (ppm)	
	FOODGARD F410B®	**Sodium benzoate**
E. coli [1]	220	10,730
S. Typhimurium [1]	260	5,185
L. monocytogenes [2]	220	5,185
S. aureus [2]	80	5,185
B. cereus [2]	220	5,185
Z. rouxii [3]	4	162
X. bisporus [4]	4	81

[1] Gram(-) bacteria; [2] Gram(+) bacteria; [3] Yeasts; [4] Molds.

FOODGARD® showed also an MIC value of 4 ppm against *Zygosaccharomyces rouxii* and *Xeromyces bisporus*. Therefore, *S. aureus* was the most sensitive bacterium to FOODGARD F410B®. On the other hand, sodium benzoate indicated a high MIC value of 10730 ppm against *E. coli*, and 5185 ppm against other bacteria and also MIC value of 162 and 81 ppm against *Z. rouxii and Xeromyces bisporus*, respectively. It was observed that FOODGARD F410B® presented an antibacterial activity about 20-50 times higher than sodium benzoate. These results allowed assuming FOODGARD F410B® as a natural preservative with a broad spectrum antibacterial activity to control and eliminate Gram (+) and Gram (-) bacteria.

FOODGARD F410B® contains bioflavonoids as active ingredients with antimicrobial and preservative

functionalities. It was reported that phenolic compounds, present in citrus extract, generally showed antimicrobial activity against Gram (+) / Gram (-) bacteria (Cosentino *et al.*, 1999). It is interesting to note FOODGARD F410B® was more efficient against Gram (+) bacteria than Gram (-) bacteria. This can be explained by the presence of an outer membrane in Gram (-) bacteria which reduce the accessibility of citrus extract to the bacterial internal cell structure. Several studies have tried to explain the antimicrobial activity of citrus extracts and their implemented components. Most of these studies have attributed this antimicrobial activity to the presence of phenols, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids (Belletti *et al.*, 2004; Helander *et al.*, 1998; Puupponen-Pimia *et al.*, 2004). Citrus extract mainly acts on the membrane and causes conformational and/or compositional damages in some components of the cell membrane (Mata Bilbao *et al.*, 2007). Their effect depends on the quantity of the active components; as at low concentrations, they can interfere with microbial enzymes involved in the production of energy; at higher concentrations, they can denature proteins. The cell wall of Gram (-) bacteria is more complex since it has a peptidoglycan layer with 2-3 nm thickness, which is thinner than the cell wall of Gram (+) bacteria, and is approximately composed of 20% (w/w dry weight) of the cell. An outer membrane (OM) lies outside of the thin peptidoglycan layer, featuring the differentiation between Gram (-) and Gram (+) bacteria, as it is composed of a double layer of phospholipids linked to inner membrane by lipopolysaccharides (LPS). LPS consist of lipid A, the core polysaccharides, whereas the O-side chain, which provides the "quid", induces Gram (-) bacteria to be more resistant to EOs and other natural antimicrobial extracts (Nazzaro *et al.*, 2013; Chanthaphon *et al.*, 2007). Therefore, our findings are in good agreement with recent studies which have approved the antibacterial activities of citrus extract (Frassinetti *et al.*, 2011; Settanni *et al.*, 2012). Some polyphenols such as flavonol and flavan have remarkable antifungal activity (Daglia, 2012).

3.3. *In situ* antimicrobial properties of FOODGARD F410B ® *vs* sodium benzoate

3.3.1. Gram (+) bacterial growth inhibition

In situ investigations of the inhibition properties of FOODGARD F410B® *vs* sodium benzoate incorporated in SF against Gram (+) bacteria are presented in **Fig. 2.**

- ***L. monocytogenes***

The *in situ* inhibiting properties of FOODGARD F410B® and sodium benzoate against *L. monocytogenes* in SF are presented in **Fig. 2-A**. As, it was observed, the SF without preservative (control) presented a high level of *L. monocytogenes* (2.6 - 3.3 log CFU/g) on day 7 whereas FOODGARD F410B® and sodium benzoate reduced the bacterial level to 1.3 and 1 log CFU/g, respectively on 4 day. This growth inhibition profile indicates a rapid elimination of *L. monocytogenes* due to the bactericidal action of FOODGARD F410B® and sodium benzoate in a very similar way. The level of *L. monocytogenes* remained below the detectable level (< 1 log CFU/g) until the last day

of storage, which demonstrated a total growth inhibition of *L. monocytogenes* by FOODGARD F410B® as well as sodium benzoate. A decrease in population of *L. monocytogenes* from 2.6 to < 1 log CFU/g was also noted in control sample from day 7 to day 28 of storage, probably due to the low pH of SF (3.15) or maybe bacteria entering to death phase. Therefore, these results demonstrated the strong inhibition properties of FOODGARD F410B® against *L. monocytogenes* during 1 month, with a rapid total inhibition from day 4.

- ***S. aureus***

The *in situ* inhibiting properties of FOODGARD F410B® and sodium benzoate against *S. aureus* in SF are presented in **Fig. 2-B**. The SF without treatment (control) presented a slight decrease in *S. aureus* level from 3.7 to 2.4 log CFU/g on day 4, followed by a stabilization without any decrement during the last days of storage. Samples containing FOODGARD F410B® presented a rapid and significant ($p \leq 0.05$) inhibition activity by reducing colony counts from 4 log CFU/g to below the detectable level (4 log reduction) on day 4. Sodium benzoate also exhibited a significant inhibition effect ($p \leq 0.05$) on *S. aureus* from 4 to 2 log CFU/g (2 log reduction) on day 4, followed by a total inhibition on day 21. Consequently, FOODGARD F410B® showed an antibacterial activity significantly more effective ($p \leq 0.05$) in comparison to sodium benzoate with a total inhibition of *S. aureus*. These findings are consistent with *in vitro* results, as it was observed that *S. aureus* was the most susceptible Gram (+) bacteria in presence of FOODGARD F410B® (**Table 1**).

- ***B. cereus***

The *in situ* inhibiting properties of FOODGARD F410B® and sodium benzoate against *B. cereus* in SF are presented in **Fig. 2-C**. It was observed that control samples showed an increment in *B. cereus* level after 2 weeks of storage and a reduction from 4 to 2.5 log CFU/g after 3 weeks followed by a plateau until the end of storage. This bacterial count reduction can be explained by the low pH of SF (3.15) or maybe by bacteria entering to death phase. The incorporation of FOODGARD F410B® led to a gradual reduction of *B. cereus* level to 2.5 log CFU/g on day 1, followed by 1 log reduction between day 1 and 7 and a total inhibition on day 14 (4 log reduction). Sodium benzoate induced a reduction of *B. cereus* level from 3.1 log CFU/g to 2.2 log CFU/g after 1 week (0.9 log reduction), followed by a total inhibition on day 14 (4 log reduction). As a result, FOODGARD F410B® exhibited high antibacterial properties and allowed a significant reduction ($p \leq 0.05$) of 4 log CFU/g after 14 days of storage.

Regarding these findings, the susceptibility of selected Gram (+) bacteria to FOODGARD F410B® are in the decreasing order *S. aureus* > *L. monocytogenes* > *B. cereus*, which are consistent with agar diffusion assay results. Thus, these results showed that FOODGARD F410B® presented a similar behavior or a more effective inhibition activity compared to sodium benzoate against Gram (+) foodborne pathogens.

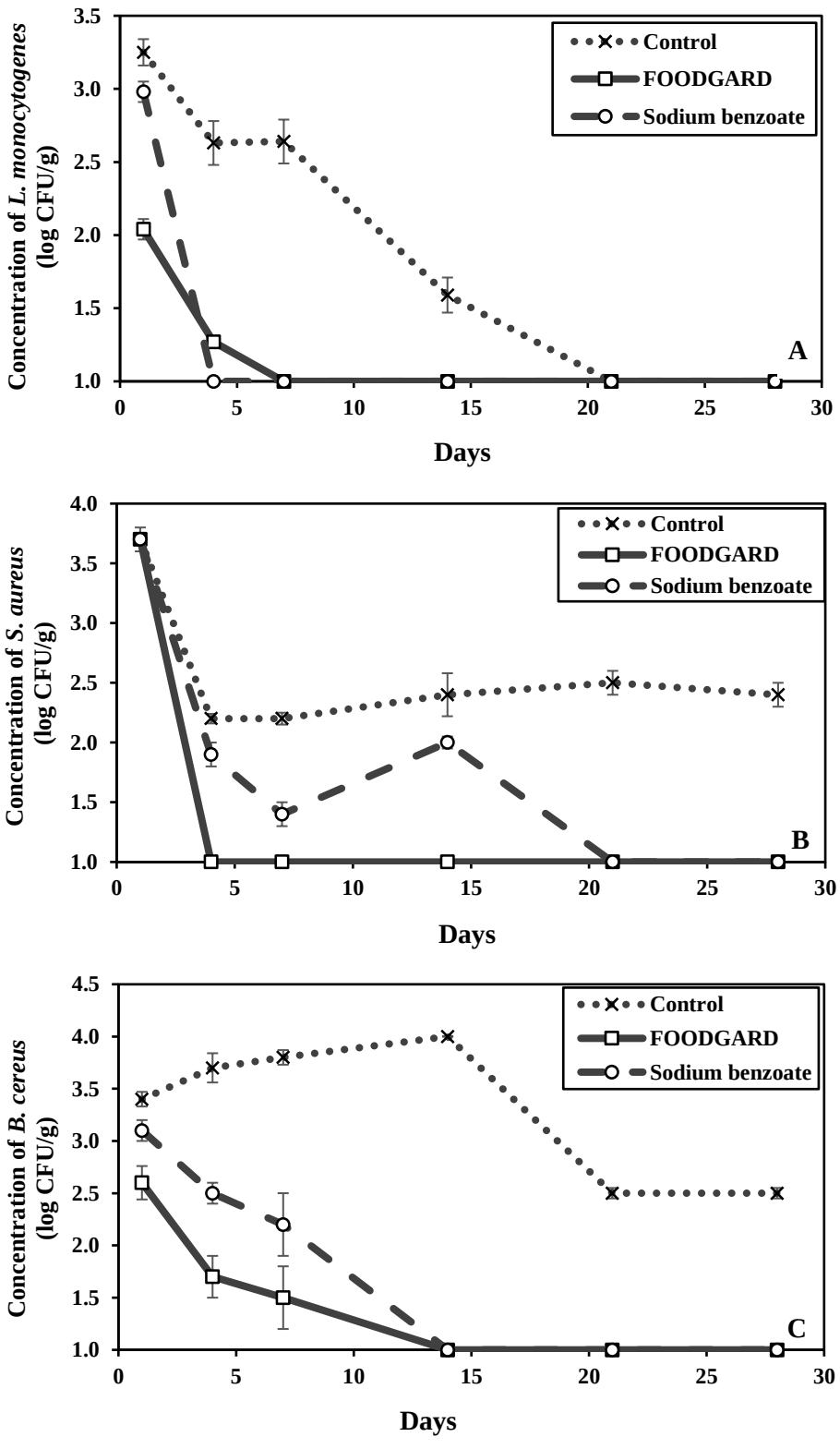

Figure 2 Effect of FOODGARD F410B® *vs* sodium benzoate inoculated in SF on the growth inhibition of *L. monocytogenes* (**A**), *S. aureus* (**B**) and *B. cereus* (**C**) during 1 month of storage at 4°C.

3.3.2. Gram (-) bacterial growth inhibition

The *in situ* inhibition properties of FOODGARD F410B® *vs* sodium benzoate incorporated in SF, against *E. coli*, *S.* Typhimurium and TAM are presented in **Fig. 3**.

- ***E. coli***

In situ inhibition assay results showed that SF without preservative (control) presented a high level of *E. coli* during 7 days of storage (3 log CFU/g), and a constant diminution to non-detectable level during the next 2 weeks was observed (**Fig. 3-A**). This reduction could be due to the acidic pH of SF caused by organic acids (pH= 3.15). On the other hand, the addition of sodium benzoate led to decrease *E. coli* level to < 1 log CFU/g at day 4. Furthermore, FOODGARD F410B® allowed a bacterial reduction to 1.7 log CFU/g at day 4 followed by a rapid decrease to non-detectable level. This inhibition profile indicates a rapid inhibition of *E. coli* by the bactericidal action of FOODGARD F410B® and sodium benzoate in a similar way.

- ***S.* Typhimurium**

In situ results showed that SF without preservative (control) presented a high level of *S.* Typhimurium at day 1 (5 log CFU/g) that decreased to 3.8 log CFU/g at day 3 and then reached to a plateau of 2.4 - 2.6 log CFU/g from day 7 to day 14 before total inhibition at day 21 (probably due to low pH of 3.15 in SF) (**Fig. 3-B)**. In presence of FOODGARD F410B® the bacterial level reduced from 5 to 1 log CFU/g at day 4 followed by a total inhibition (< 1 log CFU/g). In counterpart, sodium benzoate led to reduce the bacterial level from 5 to 1.5 log CFU/g at day 4 and then reach to total inhibition at day 7 (< 1 log CFU/g) and maintain during the storage time.

- **Total Aerobic Microflora**

The level of Total Aerobic Microflora (TAM) was determined in SF **i)** with no preservative (control), **ii)** containing FOODGARD F410® and **iii)** containing sodium benzoate for 28 days of storage (**Fig. 3-C**). Results showed that SF with no preservatives (control) presented a high increase of TAM concentration from 4 log CFU/g to 7 log CFU/g at first 7 days of storage and it was maintained until day 10, then a little decrease to 6.4 log CFU/g was observed at the end of the month.

During all storage time, FOODGARD F410® and sodium benzoate exhibited a similar antimicrobial activity in SF products with a rapid and high reduction of TAM concentration in first days of storage, compared to control. Samples treated with FOODGARD F410® and sodium benzoate showed a total inhibition from the first day of storage and it was maintained until final day of storage time.

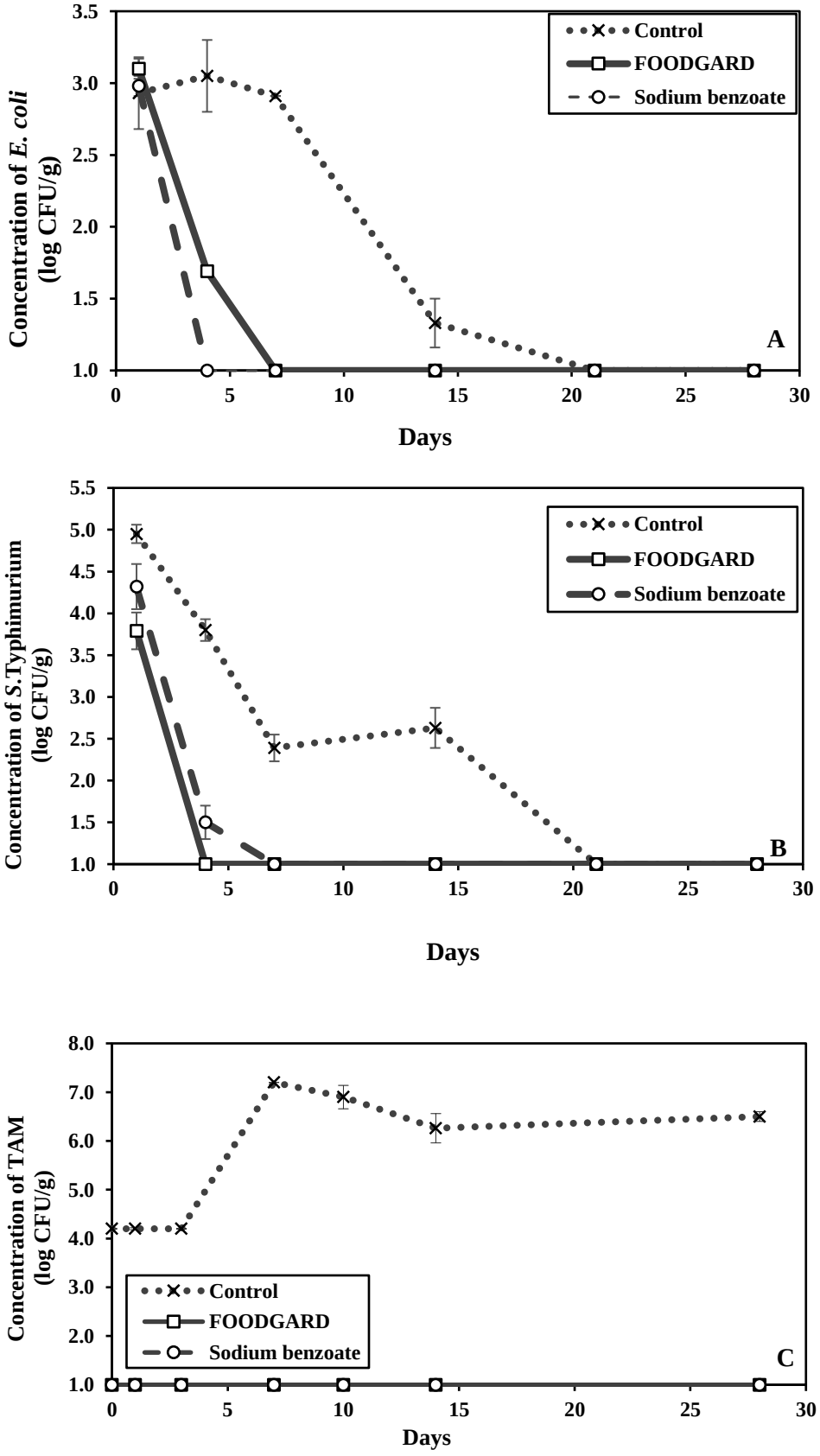

Figure 3 Effect of FOODGARD F410B® *vs* sodium benzoate inoculated in SF on the growth inhibition of *E. coli* (**A**), *S.* Typhimurium (**B**) and Total Aerobic Microflora (**C**) during 1 month of storage at 4°C.

By considering these findings, the susceptibility of selected bacteria to FOODGARD F410B® are in the decreasing order *S.* Typhimurium > *E.coli* > TAM which are in accordance with *in vitro* results with slight differences from MIC results.

Many studies reported that EOs from *Citrus* spp. presented important antimicrobial properties against bacteria and fungi (Lanciotti *et al.*, 2004; Duccio *et al.*, 1998). As reported by Chaisawadi *et al.* (2003), furthermore, EOs extracted from citrus peels displayed antimicrobial activities against *B. cereus*, *S. aureus* and *Salmonella typhi.* Indeed, the antimicrobial activity of EOs depends on their chemical composition. Citrus extract are complex mixtures of approximately 400 active compounds whose content depends on the specific citrus cultivar, extraction and separation methods (Ramakrishna *et al.*, 2009; Espina *et al.*, 2011). Fisher & Phillips (2006) have also expressed that linalool as a major component of lemon, sweet orange and bergamot, exhibited antimicrobial effects against *E. coli* O157, *L. monocytogenes*, *B. cereus* and *S. aureus*. Furthermore, many studies have shown that phenolic compounds present in citrus extract can affect enzyme activity, cause protein denaturation, modify cell permeability, interfere with the bacterial membrane function, induce permeability in the cell membrane causing release of the cell constituents, and decrease the ATP concentration and intracellular pH. Their activity can also be related to the inactivation of membrane-bound proteins (Cushnie *et al.*, 2007; Cushnie and Lamb, 2011). It should be noted that the antimicrobial activity of phenolic compounds depends on their properties such as structure and hydrophobicity.

Several studies have shown the relationship between polyphenols structure and antibacterial activity (Cushnie & Lamb, 2005). Regarding this subject, some authors (Burt, 2004) have demonstrated that oxygenated monoterpenes had a higher antimicrobial activity than hydrocarbons (Espina *et al.*, 2011). Furthermore, comparisons of the activities of different polyphenol components revealed that the presence of a stable galloyl group (3,4,5-trihydroxybenzoyl group) increases antibacterial activity of components (Taguri *et al.*, 2004). Sato *et al.* (1996) also expressed that two isoflavones with hydroxyl groups at the 5, 2 and 4 positions have an intensive inhibitory activity against a wide range of streptococcal species. This may suggest that hydroxylation at position 2 is important for their activity.

Furthermore, the molecular structure determines their mechanism of antibacterial action. Vattem *et al.* (2004) suggested that phenolic compounds with partial hydrophobicity could act efficiently at the bacterial membrane by impairing the cell membrane and the transport process. As another example, the antimicrobial activity of quercetin, has been attributed to inhibition of DNA gyrase (Cushnie & Lamb, 2005).

However, it should be noted that the total antimicrobial activity of FOODGARD F410B® citrus extract can be attributed to its phenolic compounds, and also to a synergistic effect of its constituents (flavonoids, phenolic acids, flavanones such as naringin, hesperidin, hesperitin). It is worth noting that the combination of organic acids (OAs) found in SF such as citric acid, ascorbic acid, malic acid,

ellagic acid and pantothenic acid, and its phenolic compounds such as flavan-3-ols, (+)-catechin and procyanidins can act in synergy with bioflavonoids of FOODGARD F410B® to inhibit bacterial growth. Van Immerseel *et al.* (2006). These findings are in good agreement with the results of Friedly *et al.* (2009) and Aaby *et al.* (2012) who reported synergic effects of combinations between citrus EOs and organic acids.

3.3.2. Yeast and molds growth inhibition

The *in situ* inhibition properties of FOODGARD F410B® *vs* sodium benzoate incorporated in SF, against total yeast and molds, *Z. rouxii* and *X. bisporus* are presented in **Fig. 4.**

- **Yeasts / Molds**

The level of total yeast/molds (Y/M) was determined in SF with and without preservatives for 28 days of storage (**Fig 4. A**). Results showed that SF with no preservatives (control) presented a high and increasing Y/M concentration from 4.1 log CFU/g at day 0 to 4.5 log CFU/g at day one followed by an increase to 7.5 log CFU/g at day 3 then a decrease to 6.8 log CFU/g of Y/M level was observed at day 14 and again the Y/M concentration decreased to 6.5 log CFU/g.

Samples treated with FOODGARD F410® and sodium benzoate showed a total inhibition from the first day of storage and it was maintained until the end of storage with no Y/M growth. Hence, these results demonstrated that FOODGARD was a very efficient antimicrobial with a preservative action similar to sodium benzoate during long-term storage, leading to a rapid inhibition of Y/M as compared to control (SF with no preservatives).

- ***Z. rouxii***

Results in **Fig. 4-B** showed that SF with no preservatives (control) presented a high constant level of *Z. rouxii* about 4 log CFU/g during all storage time. On the other hand, the treatment with FOODGARD F410B ® allowed reducing the yeast level from 4 to 2.9 log CFU/g at day 7, which means that the action of FOODGARD F410B ® allowed inhibiting 90% of yeast population after 7 days of storage. The fungicidal action of FOODGARD F410B ® allowed decreasing continuously the yeast level until the end of storage, with 2.3, 1.75 and 1.5 log CFU/g at days 14, 21 and 28 respectively. In comparison, the action of sodium benzoate was faster with total inhibition of yeasts at day 7.

- ***X. bisporus***

The *in situ* antimicrobial capacity of FOODGARD F410B ® and sodium benzoate incorporated in SF, against *X. bisporus* for 28 days of storage at 4°C is presented in **Fig. 4-C**. Results showed that no fungal growth was observed in control and all treatments during storage time. These results may be explained by the high sensitivity of *X. bisporus* to low pH of SF matrix. It was demonstrated that the ideal environmental of *X. bisporus* is sugary foods with low water activity, optimally at a_w about 0.84

at 30°C (Leong *et al.*, 2011). The high a_w (0.953) and low pH (pH = 3.1) of SF matrix may be responsible for the total inhibition of yeasts after inoculation.

X. bisporus is classified as a fastidious extreme xerophiles fungi that will not grow at all on high a_w media. In addition, *X. bisporus* can even grow at a_w of 0.61 (Pitt and Christian, 1968; Leong *et al.*, 2011),

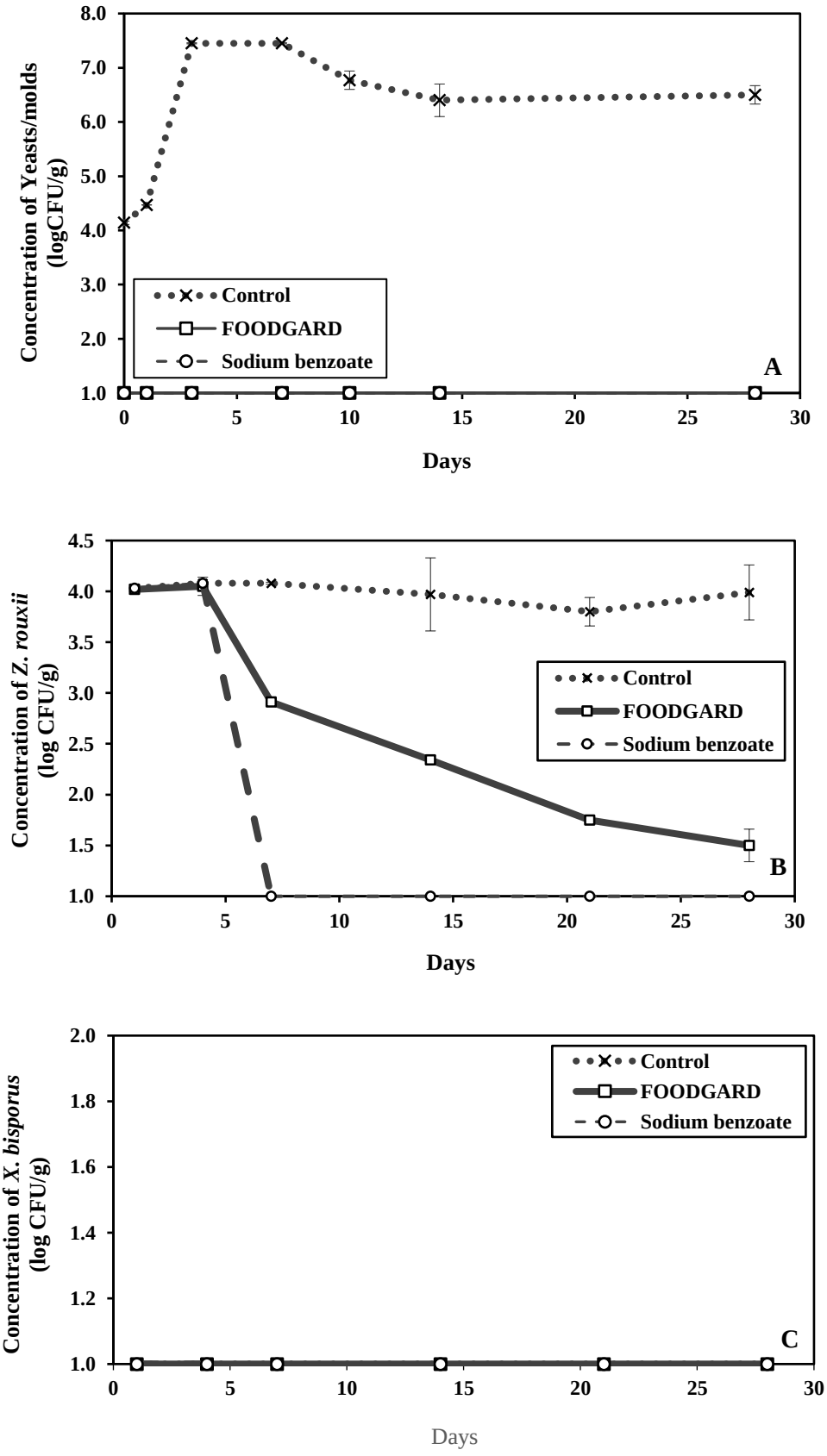

Figure 4 Effect of FOODGARD F410B® *vs* sodium benzoate inoculated in SF on the growth inhibition of yeast and molds (**Y/M**) (**A**), *Z. rouxii* (**B**) and *X. bisporus* (**C**) during 1 month of storage at 4°C.

Del Rio *et al.* (1998) suggested that the antifungal activity of citrus extract is due to the presence of flavonoids. Mencherini *et al.* (2012) reported that citrus extract flavonoids are a group of polyphenolic compounds that include flavone, polymethoxy flavone, flavanone and flavone-O-glycosides and flavone-C-glycosides that can be responsible for antifungal activity.

Praphailong *et al.* (1997) found that *Z. rouxii* was able to grow in acidic condition. It is crucial to note that the growth of yeasts can be affected by the environmental factors but it depend on the yeasts species (Rose 1987; Fleet 1992). As, Praphailong and Fleet (1997) reported *Z. rouxii, Z. bailii,* and *Pichia anomala* were the most tolerant yeast to sucrose, able to grow at concentrations up to 70% sucrose. In addition, Tokuoka (1993) also reported that the high concentration of sugars allowed the growth of *Z. rouxii.*

The high content of flavonoids in FOODGARD 410B was responsible for the inhibition of yeast and molds and bacteria. Many studies reported the antimicrobial properties of flavonoids components. For example, Arima *et al.* (2002) found that quercetin had antibacterial activity against *Bacillus cereus* and *Salmonella* Enteritidis. On another study, seven pure flavonoids including neohesperidioside showed an antibacterial activity against Gram negative bacterial strains (Basile *et al.*, 1999). In addition, Rauha *et al.* (2000) reported that naringenin exhibited bioactive activity against several bacteria. Moreover, naringenin and quercetin exhibited a high antilisterial effect (Proestos *et al.*, 2006).

Wächter *et al.* (1999) reported that the prenylated flavanone showed antifungal activity against the opportunistic pathogen *Candida albicans*. In addition, neohesperidine and naringin inhibited the spore production and modified the *Penicillium digitatum* hyphae (Ortuño *et al.* 2006). Also, Salas *et al.* (2011) reported that, naringin at 0.25 mM inhibited the mycelial growth of all the studied moulds. In the same study *Penicillium expansum* was also sensitive to neohesperidine and pruning. It is important to note that a synergistic activity can be possible when flavonoids are mixed together, for example, according to Silva *et al.* (1998) a synergistic effect was observed after using a flavonoid mixture causing a high inhibition effect.

3.4. Sensory analysis of SF containing FOODGARD F410B®

The sensory evaluation of SF samples containing no preservative (control), FOODGARD F410B® and sodium benzoate is presented in **Table 2**. The color, texture, odor, flavor and global appreciation of SF were determined according to a 9-points hedonic scale, resulting in degrees of appreciation, from 1 (Dislike very much) to 9 (Like very much). Results show that the incorporation of FOODGARD F410B® did not affect significantly ($p > 0.05$) the color, texture and global appreciation of SF. Indeed, the degree of appreciation of sensorial parameters were expressed as “Like moderately” for color (range of 6.7-6.8), “Like a little-Like moderately” for texture (5.7-6.7), “Like moderately-Like a lot” for odor (6.7-7.6) and “Like moderately-Like a lot” for global appreciation (6.7-7.3). By considering these findings, there was no significant difference ($p > 0.05$) between the samples.

Table 2 Effect of FOODGARD F410B® and sodium benzoate on the sensory properties of strawberry fillings (SF).

SF Samples	Sensory properties[1-3]				
	Color	**Texture**	**Odor**	**Taste**	**Global appreciation**
Control	6.83 ± 1.20[a]	6.36 ± 1.79[a]	7.64 ± 0.76[b]	7.32 ± 1.11[b]	7.28 ± 1.21[a]
With FOODGARD F410B®	6.70 ± 1.18[a]	5.69 ± 2.02[a]	7.60 ± 0.91[ab]	5.65 ± 1.73[a]	7.25 ± 1.29[a]
With Sodium benzoate	6.81 ± 1.27[a]	6.68 ± 1.41[a]	6.69 ±1.71[a]	6.69 ± 1.71[b]	6.72 ± 1.06[a]

[1] The hedonic evaluation was scaled as follow: 9 = Like very much; 8 = Like a lot; 7 = Like moderately; 6 = Like a little; 5 = Indifferent; 4 = Dislike a little; 3 = Dislike moderately; 2 = Dislike a lot; 1 = Dislike very much.

[2] Data were compared according to Duncan's test for assumed equal variances or according to Tamhane's test for assumed unequal variances. The equality of variances was determined by Levene's test and Weich and Brown-Forsythe's robust tests were applied to assume the equality of means.

[3] Means with different letters within the same column are significantly different ($p \leq 0.05$).

The odor analysis revealed a good appreciation namely "Like a lot" (7.6) for control and samples treated with FOODGARD F410B® whereas sodium benzoate was significantly less appreciated ($p \leq 0.05$) than control, with a "Like moderately" level (6.7). As a result, the incorporation of FOODGARD F410B® did not affect the sensorial properties of SF, presenting similar values to control in comparison to the slightly lower appreciation of benzoate. On the other hand, the taste analysis showed a good appreciation namely "Like moderately" for control (7.3) and samples treated with benzoate (6.7) whereas FOODGARD F410B® was significantly less appreciated ($p \leq 0.05$) with a hedonic level of 5.7 corresponding to "Like a little" index. Thus, these results revealed that the incorporation of FOODGARD F410B® in SF provided very acceptable sensorial attributes, with a good appreciation of color, texture, odor and global quality, compared to sodium benzoate, but with a very slight depreciation of taste. From a point of view of commercializing natural food preservatives, these results are very promising.

4. Conclusion

FOODGARD F410B® (citrus extract) incorporated in strawberry fillings as an acidic sweet food (pH = 3.1, a_w = 0.953) stored at 4°C allowed an important antimicrobial activity against Gram (-), Gram (+) bacteria and yeasts and molds such as *E. coli*, *S.* Typhimurium, *L. monocytogenes*, *B. cereus*, *S. aureus*, *Zygosaccharomyces rouxi* and *Xeromyces bisporus*. *In vitro* results showed that

FOODGARD F410B® was more effective than sodium benzoate, with higher inhibiting capacities in agar diffusion assay and lower MIC values. *In situ* results showed that FOODGARD F410B® significantly reduced the growth of six foodborne pathogens inoculated in SF, at day 4 or 7 and was particularly more efficient against Gram (+) than Gram (-) bacteria. Also, this study showed that *S. aureus* was the most sensitive Gram (+) bacteria to FOODGARD F410B®. Also, FOODGARD F410B® allowed inhibiting 90% of yeast population after 7 days of storage. A total inhibition of Total Aerobic Microflora growth and total yeast/mold growth was observed from the first day of storage and it can provide a long storage time for samples treated with FOODGARD F410B®. It was also observed that food properties such as composition, water activity, sugar content, pH and semi-liquid medium can influence the antibacterial activity of preservatives. Due to the acidic pH and presence of phenolic compounds in SF, synergic effects can be presumed on the antibacterial activity of FOODGARD F410B® against *S.* Typhimurium and increased its sensitivity.

It is important to mention that sodium benzoate is more efficient at acidic pH, this can explain the difference between *in vitro* and *in situ* results where benzoate showed more efficiency on strawberry fillings than in liquid medium. FOODGARD F410B® was more efficient against the studied microorganisms at a concentration of 0.2% compared to sodium benzoate at 1%.

This study demonstrates that FOODGARD F410B® can be used as a natural antimicrobial preservative in acidic sweet foods in synergic behavior with foods composition, leading to decrease the required effective dose of antibacterial in foods with a high potential market development.

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Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Experimental work and drafting the manuscript and describing the results were done by Mr. Harich and Mr. Stephane Salmieri. Dr. Lacroix designed the study and assisted in interpretation of data and preparation of the manuscript in collaboration with Mrs. B. Maherani.

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CHAPITRE 3:

EVALUATION OF ANTIBACTERIAL ACTIVITY OF TWO NATURAL BIOPRESERVATIVES FORMULATIONS ON FRESHNESS AND SENSORY QUALITY OF READY TO EAT (RTE) FOODS

Article soumis à *Food Control*

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Contributions des auteurs

J'ai réalisé les expériences, décrit et interprété les résultats et rédigé l'article.

Stéphane Salmieri a participé à la planification et au bon déroulement des analyses, et à la rédaction de l'article.

Maherani Behnoush a aidé dans l'interprétation des résultats.

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

Résumé

L'activité antibactérienne de deux formulations naturelles a été étudiée contre les pathogènes alimentaires. Les résultats *in vitro* ont montré que les deux formulations sont efficaces contre *Escherichia coli* O157:H7, *Listeria monocytogenes* et *Salmonella* Typhimurium et trois non-pathogènes: *Listeria innocua, Escherichia coli* ATCC 25922 et *Salmonella enterica* ATCC 53648 chi 4064 avec un intervalle de diffusion de 16 à 21% qui a été mesuré pour toutes les formulations étudiées. Les formulations F2 et F6 ont exhibé de faibles CMI (propriétés antimicrobiennes élevées) de 3 400 ppm (équivalent des actifs totaux). Les résultats *in situ* ont montré que les deux formulations ont permis une réduction significative des bactéries pathogènes dans les canneberges et les poivrons rouges. L'évaluation de la capacité antimicrobienne *in situ* de F2 et F6 a confirmé que les deux formulations ont réduits significativement les bactéries pathogènes dans les canneberges, les poivrons rouges et les pommes de terre. Les résultats obtenus sur des pommes de terre coupées pré frites emballées sous l'atmosphère modifiée (MAP), ont montré que F2 a permis 0.5 et 1.5 log de réduction d'*E. coli* O157 :H7 EDL 933 et *S.* Typhimurium respectivement au jour 10. D'autre part, F6 a permis une réduction de 0.5 et 1.1 log d'*E. coli* O157 :H7 et *S.* Typhimurium respectivement au jour 10.

Les analyses sensorielles des poivrons rouges et des pommes de terre traitées avec F2 et F6 ont prouvé que la qualité sensorielle des légumes testés n'a pas été modifiée et est très acceptables.

Abstract

The antibacterial activity of two natural antibacterial formulations based on lemongrass essential oil / citrus extract / lactic acid for F2 and oregano/citrus extract/lactic acid for F6 was studied against three pathogenic bacteria (*Listeria monocytogenes*, *Escherichia coli* O157:H7 and *Salmonella* Typhimurium) and three non-pathogenic bacteria (*Listeria Innocua*, *Escherichia coli* ATCC 25922, and *Salmonella enterica* ATCC 53648 chi 4064).

The *in vitro* inhibition capacity (IC%) of F2 and F6 formulations demonstrated their high antimicrobial potential against pathogenic and non-pathogenic bacteria. In addition, *in vitro* minimum inhibitory concentration (MIC) of F2 and F6 formulations exhibited a lower MIC (higher antibacterial properties) against pathogenic and non-pathogenic bacteria in compared to sodium benzoate.

Furthermore, *in situ* antimicrobial capacity of two natural formulations (F2 and F6) was assessed on ready to eat vegetables and fruits (pre-cut red peppers, cranberries and pre-cut/pre-fried potatoes) against targeted bacteria.

Results obtained with the *in situ* tests showed that both coatings allowed a significant reduction of pathogenic bacteria in cranberries and red peppers ($p \leq 0.05$). Results obtained on pre-fried sliced potatoes packed under modified atmosphere (MA), showed that F2 allowed a 0.5 and 1.5 log reduction of *E. coli* O157:H7 EDL 933 and *S.* Typhimurium respectively at day 10. On the other hand, F6 allowed a 0.5 and 1.1 log reduction of *E. coli* O157:H7 and *S.* Typhimurium respectively at day 10. The sensory analysis of red peppers and potatoes treated with F2 and F6 formulations suggested that both formulations were acceptable in terms of organoleptic attributes. Therefore, their sensorial attributes could be accepted for further commercialization.

Keywords

Ready-to-eat vegetables, Bio-preservative, Citrus extract, Essential oils, Foodborne pathogens.

1. Introduction

Changes in consumer trends, looking for healthier and convenient products, and also new life styles have brought a noticeable increasing demand (over 30% during the last 10 years) for fresh and ready-to-eat (RTE) food production. This has led to the development of a new range of RTE products. However, it has changed the status of foodborne diseases and had an important economic and social impact in the world (Millan-Sango *et al.*, 2015; Oliveira *et al.*, 2015). As fruits and vegetables can be contaminated with pathogens during all steps from the harvest to the process, and until the consumption, the RTE foods are no longer considered as low-risk foods in terms of safety (De Medeiros Barbosa *et al.*, 2016).

In the United States, the major foodborne pathogens include *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus.* More than 61 deaths per year resulting from 73,000 cases of *E. coli* O157:H7 infections, have been observed according to the US CDC (Rangel *et al.*, 2005). In the United States it was estimated that the cost of disease caused by pathogenic bacteria was $ 77 billion USD/year (Scharff, 2012) Therefore, a new and effective approach to overcome the bacterial survival and growth in RTE food is inevitable.

A variety of disinfectants, including chlorine and hydrogen peroxide have been used to reduce the initial bacterial populations on vegetables but the possibility of formation of carcinogenic derivatives of chlorine (chloramines and trichloromethane) during treatment in presence of chlorine in water has raised concerns about its use in food processing (Harich *et al.*, 2016).

Most of the countries try to proffer new methods to make the food safer for consumption. In this regard, bio-preservation or biocontrol is an interesting alternative to be considered. Bio-preservation or biocontrol refers to the use of natural antibacterial products to extend the shelf life of products and enhance the safety of foods (Daglia, 2012).

Herbs and spices have been recognized to possess a broad spectrum of active constituents that exhibit naturally antibacterial, antifungal, antiparasitic, and/or antiviral activities (Daglia, 2012). Essential oils (EOs) extracted from plant and fruits have been used as bio-functional components for centuries as part of natural traditional medicine. They are aromatic oily liquids with natural antibacterial activity, obtained from plant material (flowers, seeds, leaves, herbs, wood, fruits and roots). The major components that make essential oils effective antimicrobials include polyphenols (flavonoids and acid phenols), terpenes, and their precursors (Daglia, 2012; Manach *et al.*, 2004). Polyphenols are secondary metabolites produced by plants, which have potential healthy properties on human organism, mainly as antioxidants, anti-allergic, anti-inflammatory, anticancer, antihypertensive, and antimicrobial activity (Bakkali *et al.*, 2008; Rivera *et al.*, 2015).

Essential oils can cause structural damages in microorganisms by diffusion through the cellular membrane and this ability is due to the hydrophobic nature of essential oil (Lambert *et al.*, 2001).

Teissedre and Waterhouse (2000) defined essential oils as a complex mixture of cyclic and acyclic monoterpenes responsible for biological properties such as antifungal, antibacterial and antioxidant. The activity of major components, the structure, size and hydrophobicity of functional groups and the possible synergy interactions of essential oils is responsible for the biological function of essential oil (Burt *et al.*, 2007; Koroch *et al.*, 2007; Dorman & Deans, 2000).

Furthermore, Govaris *et al.* (2010) reported that carvacrol and thymol (78-85%) as the major phenolic compounds in oregano, are able to lead membrane expansion and disturbs embedded proteins because of their lipophilic character (Cristani *et al.*, 2007).

Regarding EOs benefits, the objective of this study was to evaluate *in vitro* antimicrobial properties of two natural formulations F2 and F6 based respectively on lemongrass and on oregano by measuring inhibition capacity (IC%) using agar diffusion assay, and determining minimum inhibitory concentration (MIC) against foodborne pathogenic bacteria. Furthermore, the antimicrobial capacity of the two natural formulations (F2 and F6) was assessed on ready to eat vegetables (pre-cut red peppers, pre-cut/pre-fried potatoes) and on cranberries against pathogenic bacteria (*Listeria monocytogenes*, *Escherichia coli* O157:H7 EDL 933 and *Salmonella* Typhimurium*)* and non-pathogenic bacteria (*Listeria innocua*, *Escherichia coli* ATCC 25922, and *Salmonella enterica* ATCC 53648 chi 4064).

Furthermore, the sensory analysis (odor, color, global appreciation, flavor and texture) of the red peppers and potatoes treated with F2 and F6 formulations were evaluated.

2. Materials and methods

2.1 Raw material

Pre-cut red peppers and frozen cranberries (*Vaccinium macrocarpon*) were kindly provided by Bonduelle Americas Inc. (Saint-Denis-sur-Richelieu, QC, Canada) and Atoka Cranberries Inc. (Manseau, QC, Canada), respectively. Both red peppers and cranberries were stored at -20°C until used. Pre-cut/pre-fried potatoes were provided by Michel Saint-ArneaultCo. (St-Hubert, QC, Canada) and were stored at 4°C under modified atmosphere packaging (MAP) before testing.

2.2 Preparation of pathogen cultures

Stock cultures of *E. coli* O157:H7 EDL 933, *S.* Typhimurium SL 1344, *L. monocytogenes* **(**Six strains HPB 2812, 2558, 2569, 1043, 2371, 2739), were stored at −80°C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Prior to each experiment, stock cultures were grown through two consecutive 24-48 h growth cycles in TSB at 37°C. Working cultures were diluted in peptone water to obtain the bacterial concentration of 10^6 CFU/mL for MIC determination or 10^3-10^4 CFU/mL for *in situ* tests.

2.3 Preparation of non-pathogen cultures

Stock cultures of *E. coli* ATCC 25922, *S. enterica* ATCC 53648 chi 4064., *L. innocua* LSPQ 3285 were stored at −80°C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Prior to each experiment, stock cultures were grown through two consecutive 24-48 h growth cycles in TSB at 37°C. Working cultures were diluted in peptone water to obtain the bacterial concentration of 10^6 CFU/mL for MIC determination or 10^3-10^4 CFU/mL for *in situ* tests.

2.4 Preparation of antimicrobial formulations

Formulations F2 and F6 were prepared under sterile conditions, according to the method developed in our laboratories (Tawema *et al..*, 2016). Formulations were prepared in sterile distilled water in presence of previously filtered (0.2 μm) Tween 80 (0.06% w/v) (Sigma-Aldrich Ltd, Oakville, ON, Canada) used as an emulsifying agent. F2 formulation containing lemongrass essential oil, citrus extract and lactic acid at a ratio of 0.01:0.1:1 and F6 formulation containing oregano essential oil, citrus extract and lactic acid at a ratio of 0.01:0.1:1. Lemongrass, oregano essential oils and lactic acid were provided by BSA Food Ingredients Inc. (St-Leonard, QC, Canada) and citrus extract (Biosecur F440D®) was provided by Biosecur lab (Mont St-Hilaire, Quebec, Canada). Sodium benzoate (E211), as synthetic preservative, was kindly provided by Skjodt-Barrett Foods Inc. (QC, Canada).

2.5 Spraying treatment

The emulsified antimicrobial formulations were uniformly sprayed (5 mL per 100 g of sample) under sterile conditions on each sample of cranberries, red peppers and potatoes using a Mastercraft HVLP Air Gravity Spray Gun (Mastercraft Tools™, Johannesburg, South Africa). Samples were sprayed at a distance of 18-24 inches and an inlet pressure of 35 psi for 2 s on each side of sample and then left to dry for 30 min under laminar flow hood before storage and microbiological analyses.

2.6 Inhibition Capacity (IC%) determination by agar diffusion assay

Agar diffusion assay was carried out according to a modified procedure from Cardiet *et al.* (2012) to assess bactericide activity of F2 and F6 formulations by measuring microbial growth inhibition zones. Tryptic soy agar (TSA; Alpha Biosciences Inc., Canada) were surface-layered by 100 μL of diluted bacteria at 10^6 CFU/mL. Growth inhibition diameters (mm) were determined by agar diffusion from the deposition of 10 μL of the antibacterial formulation on a 12-mm diameter cellulose disc placed onto the surface of TSA. Each agar plate was incubated for 24-48 h at 37°C and the inhibition diameter around the disc (colony-free perimeter) was measured with a Traceable Carbon Fiber Digital Caliper (resolution: 0.1 mm; accuracy: ± 0.2 mm; Fisher Scientific Ltd, Nepean, ON, Canada). All measurements were performed in triplicate (n = 3).

The inhibition capacity (IC%) was calculated as follow by **Equation 1:**

$$IC\ (\%) = \frac{\text{Diameter inhibition zone}}{\text{Diameter Petri dish}} \times 100 \quad [1]$$

With an internal diameter of Petri dishes equal to 83 mm.

2.7 Minimum Inhibitory Concentration (MIC)

The MIC value of F2 and F6 samples was determined in sterilized flat-bottom 96-well microplate according to the serial microdilution method as described by Turgis *et al.* (2012). Briefly, serial dilutions of the antimicrobial samples were made in Mueller Hinton Broth (MHB, Difco, Becton Dickinson) pre-added in a 96-well microplate, to obtain serial concentrations.

A sample of 110 µL of the serially diluted antimicrobial formulations was pipetted into a 96 wells microplate containing 125 µL MHB. Each well was then inoculated with 15 µL of a pathogenic/non-pathogenic strain at a concentration of 10^6 CFU/mL. The microplate was incubated aerobically for 24 h at 37°C. Then, the absorbance was measured at 595 nm in an Elx800 Microplate Reader (Biotek instruments, Winooski, VT, USA). The two last columns of the microplate were used for a blank containing only sterile Mueller–Hinton medium as negative control and the positive growth control was a column filled with target bacterium with Mueller–Hinton medium (without antimicrobial formulations). MIC was determined as the highest concentration generating an absorbance equal to blank absorbance (e.g. total inhibition).

2.8 *In situ* antimicrobial capacity of F2 and F6 formulations in red peppers, cranberries and potatoes

- Red peppers and Cranberries

The *in situ* antimicrobial activity of the F2 and F6 formulations was evaluated on red peppers, cranberries and potatoes against foodborne pathogens and non-pathogenic bacteria.

For red peppers and cranberries, the antimicrobial capacity of F2 and F6 was evaluated against *E. coli*, *L. monocytogenes* and *S.* Typhimurium. Red pepper samples (25 g) were inoculated with each bacterial culture (inoculum concentration of 10^7 CFU/mL to obtain 10^4 CFU/g in peppers) and then stored for 15 days (as recommended by Bonduelle) at 4°C. Microbiological analyses were performed at days 1, 3, 5, 7, 10 and 15 of storage.

Cranberry samples (25 g) were inoculated with each bacterial culture (inoculum concentration of 10^8 CFU/mL to obtain 10^4 CFU/g in inoculated cranberries) and then stored for 42 days (as recommended byAtoka). Microbiological analyses were performed during 42 days of storage.

At each day of analysis, samples were homogenized for 2 min at 260 rpm in 50 mL of sterile peptone water (0.1% w/v) with a Stomacher® 400 circulator (Seward Laboratory System Inc., Davie, FL, USA). From each homogenate, serial dilutions were prepared, plated on the surface of appropriate

media for every bacteria; *L. monocytogenes*, *E. coli* and *S.* Typhimurium (Palcam, MacConckey agar supplemented with sorbitol and DCLS agar, respectively) and incubated for 24-48 h at 37°C before bacteria enumeration.

- *Potatoes*

MAP was used by the company for pre-cut/pre-fried potatoes to avoid enzymatically browning reaction during the storage time. In order to respect the limited safety operations regarding MAP processing, the antimicrobial capacity of F2 and F6 formulations on pre-cut/pre-fried potatoes was evaluated against non-pathogenic bacteria, *Escherichia coli* ATCC 25922, *Listeria innocua* LSPQ 3285 and *Salmonella enterica* ATCC 53648 Chi4064. Pre-cut/pre-fried potato samples (25 g) were inoculated with each bacterial culture to obtain 10^4 CFU/g, placed under modified atmosphere packaging (gas mixture containing 46.2% CO_2 and 50.8% N_2) by using a Sipromac® vacuum packaging machine (model 350; Sipromac Inc. St-Germain, QC, Canada) with a gas flush of 89.5% and a sealing time of 1.5 s. Samples were then stored for 10 days at 4°C. Microbiological analyses were performed at days 1, 4, 7 and 10 by following the same procedure as described for the analyses of red peppers and cranberries.

2.9 Sensory analysis of red peppers and potatoes treated with F2 and F6 formulations

The odor, color, global appreciation, flavor and texture of the red peppers and potatoes treated with F2 and F6 formulations were evaluated by a panel comprising 20 persons, according to a 9-points hedonic scale test. This method was used to measure the degree of acceptance of samples treated with antimicrobial formulations, and eventually to verify the effect of F2 and F6 formulations as natural antimicrobials on organoleptic properties of the food products.

2.10 Statistical analysis

Each experiment was done in triplicate and for each replicate, 3 samples were analysed. Analysis of variance (ANOVA), Duncan's multiple range tests (for equal variances) were performed for statistical analysis (PASW Statistics 18; IBM Corporation, Somers, NY, 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 Inhibition Capacity (IC%) determination by agar diffusion assay

- ***L. monocytogenes & L. innocua***

As it was shown in **Fig. 1-A**, F2 formulation induced an IC value of 16.8%, significantly lower than F6 formulation with IC value about 21.4% ($p \leq 0.05$). As it was observed both of the formulations has significantly higher inhibition effect on *L. innocua* compared to *L. monocytogenes*. Sodium benzoate

also showed a high antibacterial activity (18%) against *L. monocytogenes* and *L. innocua*. Hence, F6 formulation can be considered as a stronger inhibiting formulation against *L. monocytogenes* and *L. innocua*.

- ***E. coli pathogen & non-pathogen***

The IC values of different selected antimicrobial formulations against *E. coli* are presented in **Fig. 1-B**. Results showed an important inhibition capacity for all formulations. F2 and F6 formulations exhibited IC values of 17.7 and 16.3%, respectively against *E. coli* pathogen. Although no significant differences was observed between *E. coli* pathogen and non-pathogen regraded to F2 formulation effect but a significantly higher inhibition capacity was observed in presence of F6 against *E. coli* non pathogen. Sodium benzoate was also showed antibacterial activity against *E.coli* pathogen lower than other formulations (15.1%).

- ***S.* Typhimurium and *S. enterica***

The IC values of the antimicrobial formulations against *S.* Typhimurium are presented in **Fig. 1-C**. No significant difference ($p > 0.05$) was observed between F2 and F6 formulations with IC values of 17 and 17.2%, respectively. The same result as observed for *E.coli* pathogen and non-pathogen, was obtained for *S.* Typhimurium and *S. enterica* in presence of F2 and F6 formulations. No significant differences was observed between *S.* Typhimurium and *S. enterica* in presence of F2 formulation but F6 has shown a significantly higher inhibition capacity against *S. enterica*.

Sodium benzoate was also showed antibacterial activity against *S.* Typhimurium and *S. enterica* significantly lower than other formulations (14.4%) ($P \leq 0.05$).

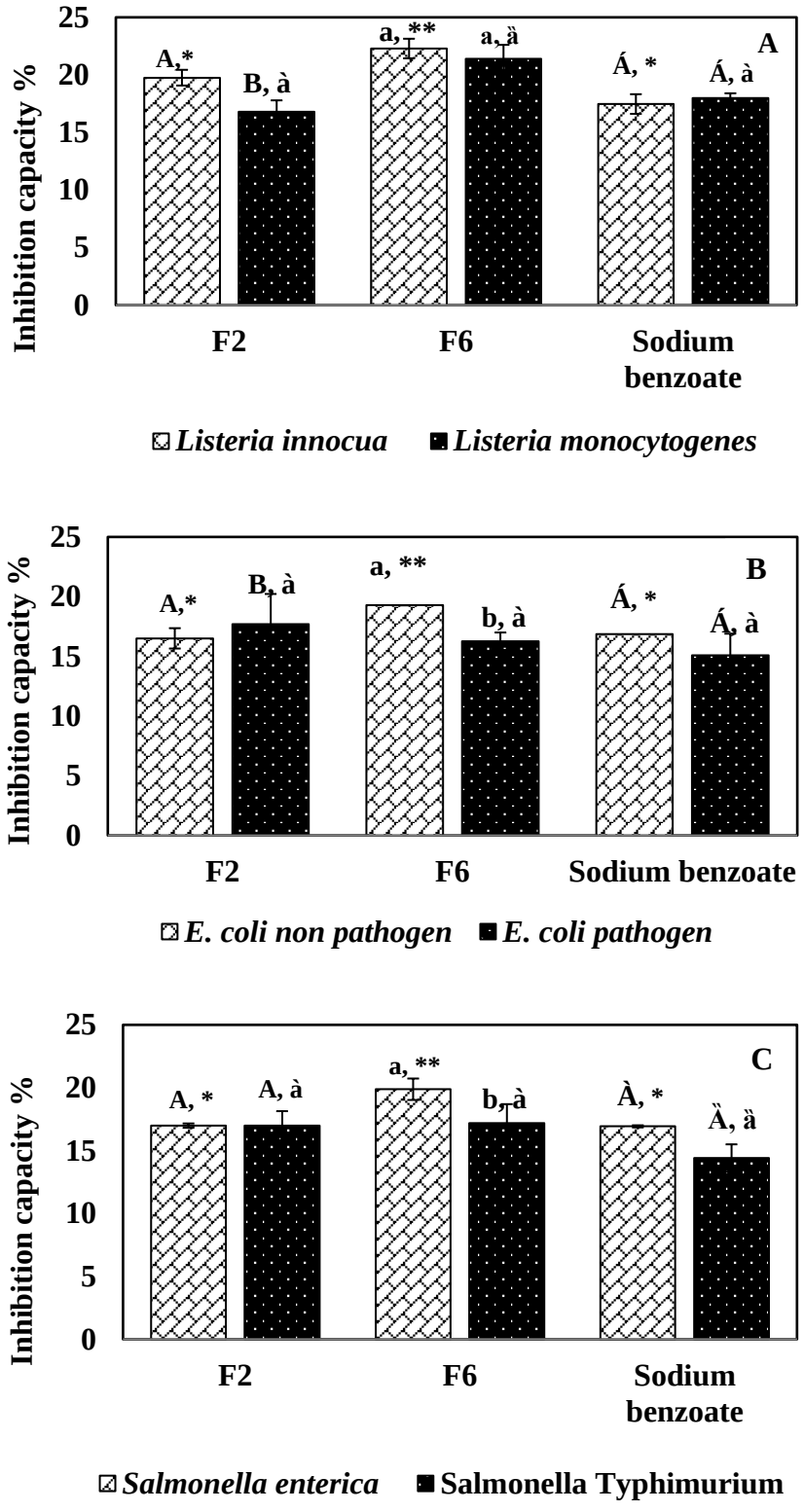

Figure1- Inhibition capacity of antibacterial formulation against *L. monocytogenes* and *L. innocua* (A), *E. coli* pathogen and non-pathogen (B) and *S.* Typhimurium and *S. enterica* (C).

The order of antibacterial activity of sodium benzoate against studied pathogens was *L. monocytogenes* > *E. coli* > *S.* Typhimurium. According to the obtained results, sodium benzoate has antibacterial activity more efficient against Gram-positive bacteria compared to Gram-negative bacteria.

Agar diffusion assays provided interesting results on inhibiting rates of the selected formulations against selected bacteria. By considering the obtained results, the antibacterial activity of F6 against targeted pathogens are in the following order, *L. innocua* > *L. monocytogenes* > *S. enterica* > *S.* Typhimurium > *E. coli* non-pathogen > *E. coli* pathogen. In the presence of F2 formulation, apart from *Listeria* spp. no significative differences in inhibition capacity of F2 formulation against targeted bacteria, pathogens and non-pathogen, was observed. These findings completely related to antibacterial composition of formulations. F6 formulation with carvacrol, as the major constituent of oregano, showed higher inhibition capacity against targeted pathogens in compared to F2 formulation with citral as the major component of lemongrass.

Carvacrol disrupted the cell membrane, causing an increased permeabilization to the nuclear stain EB and also caused leakage of organic ions (Lambert *et al.*, 2001). The presence of lactic acid has also a significant effect on inhibition capacity of formulations.

Recently different kind of organic acids (OAs) such as lactic acid widely used to inhibit the growth of important microbial pathogens. Wang *et al.*, (2015) studied the antimicrobial effect of lactic acid on *S.* Enteritidis, *E. coli* and *L. monocytogenes.* They suggested that the antibacterial activity of lactic acid probably caused by physiological and morphological changes in bacterial cells. They also observed the damaged membrane structure and intracellular structure induced by lactic acid in TEM images (Chenjie *et al.*, 2015).

Lactic acid is able to inhibit the growth of many types of food spoilage bacteria, including Gram-negative bacteria (Alakomi *et al.*, 2000).

Many studies showed that lactic acid is lethal to microorganisms via undissociated molecules that flow through the cell membranes and ionize inside. The acidic pH inside the cell causes deformation and damage to enzymatic activities, proteins and DNA structure, thereby damaging the extracellular membrane. In another mechanism, it leads to changes in the permeability of the cell membrane and electron transport system which leads to the death of the microorganism (Slobodanka *et al.*, 2016). Furthermore, some studies indicated that some parameters such as chain length, side chain composition, *pKa* values and hydrophobicity could affect the antimicrobial activity of organic acids (Hsiao and Siebert, 1999). Van Immerseel *et al.* (2006) also concluded that medium-chain fatty acids are more effective antibacterial against *Salmonella* than short-chain fatty acids. Many studies also confirmed the antibacterial activity of citrus extract (Mandalari *et al.*, 2007; Rafik *et al.*, 2016). Randazzo *et al.* (2016) observed that citrus extract induced a largest spectrum of inhibition against 76

strains of *L. monocytogenes.* Citrus extract contains phenolic and flavonoid compounds that can act as antimicrobial agents (Mandalari *et al.,* 2007).

3.2 Determination of the Minimum Inhibitory Concentration (MIC)

The MIC values of different selected antimicrobial formulations against selected bacteria are presented in **Table 1**. Results showed that F2 and F6 formulations exhibited lower MIC value (higher antimicrobial properties) of 1720 - 3500 ppm against the targeted pathogens compared to sodium benzoate (5180 -10730 ppm). F2 formulation was more efficient than F6 formulation with lower MIC values of 1700 ppm against *L. monocytogenes* and *S.* Typhimurium (**Table 1**). No significant difference ($p > 0.05$) was observed between antibacterial activity of F2 and F6 formulations against *E.coli* pathogen and non-pathogen, and *L. innocua*. F6 formulation has also shown lower MIC values against *S. enterica* compared to F2 formulation. It's important to mention that, F2 and F6 formulations can act as strong antibacterial formulations due to their lower MIC values against selected foodborne pathogens, in a range of 1,700-3,500 ppm in comparison with sodium benzoate where the antibacterial activity was lower than F2 and F6. It is important to emphasize that the significant difference of MIC values between F2/F6 formulations and sodium benzoate as synthetic preservative is related to the presence of bioactive compounds in natural formulations.

Table 1 MIC of different antimicrobial formulations against selected bacteria.

Antibacterial Formulations	MIC (ppm)					
	Pathogen			Non- pathogen		
	L. monocytogenes	***E. coli***	***S.* Typhimurium**	***L. innocua***	***E. coli* ATCC 25922**	***S. enterica***
F2	1,727	3,454	1,727	3,454	1,727	3,454
F6	3,454	3,454	3,454	3,454	1,727	1,727
Sodium Benzoate	5,185	10,730	5,185	10,375	10,375	10,375

Furthermore, the MIC value of sodium benzoate against *L. monocytogenes* is higher than the recommended concentration. These results are in good agreement with the obtained results of Stanojevic *et al.,* (2009). According to EU regulations (92/2/EC) and FDA (2016), the recommended dosage of sodium benzoate is a maximum level of 0.1% in food. It should be also noted that its activity depends largely on pH, a_w and the properties of foods (Guynot *et al.,* 2005; Theron and Rykers Lues, 2010). According to Ray (2004) the inhibitory action of sodium benzoate inhibited the function of the enzymes necessary for oxidative phosphorylation (Ray, 2004).

The combination of lactic acid with EOs present in citrus extract and also pure components such as oregano and lemongrass, lead to a synergic or additive effect on antibacterial activity of formulations.

As the major active components of EOs are phenols, terpenes and aldehydes, they act principally against the cell cytoplasmic membrane, due to their hydrophobic nature, affecting the unsaturated fatty acid on the bacterial membrane and thus altering its structure (Severino *et al.*, 2014).

The mode of action of EOs containing carvacrol as a major component can be resumed on the disruption of cell membrane and increasing of its permeability. (Lambert *et al.*, 2001; Saeed and Tariq, 2009).

The antibacterial activity of EOs is mostly determined by their chemical properties, notably their *pKa* value, hydrophobicity/ hydrophilicity ratio as measured by the partition coefficient (*log P*), solubility and volatility (Stratford and Eklund, 2003).

Organic acids (OAs) are also able to disrupt the outer membrane (OM) of Gram-negative bacteria in both dissociated and undissociated forms. Organic acids differ in lipophilic properties regarding their chain lengths, which affect their cell membrane transfer and consequently their antibacterial activity, as acetic and propionic acids with higher lipophilic properties present more antibacterial activity than lactic acid (Ray, 2004).

Furthermore, the antibacterial activity of lactic acid is not only related to lowering the pH, but also to its ability to penetrate into cytoplasmic membrane as a permeabilizer, resulting in reduced intracellular pH and disruption of the transmembrane of the Gram-negative bacterial outer membrane. This phenomenon amplifies its action. Many studies confirmed that lactic acid is a potent OM-disintegrating agent (Alakomi *et al.*, 2000; Van Immerseel *et al.*, 2006).

Combination of different antibacterial agents with distinct and particular way of inhibition against bacteria, leads to develop an effective antibacterial formulation with a large aspect of activity.

3.3 *In situ* antimicrobial capacity of F2 and F6 formulations in red peppers

- ***L. monocytogenes***

The level of *L. monocytogenes* in red peppers coated with F2 and F6 formulations, during storage at 4°C, was presented in **Fig. 2-A**. Results showed that the growth of *L. monocytogenes* is less affected by F2 and F6 treatments compared to *E. coli*. Indeed, the control indicated an increment of 1.4 log CFU/g from day 1 to day 15. On the other hand, F2 and F6 coatings presented significant inhibitory effects ($p \leq 0.05$) over storage time, with a relatively stable level of *L. monocytogenes* at 3.6-4.0 log CFU/g from day 1 to day 15. However, the effects of F2 and F6 formulations are not significantly different ($p > 0.05$), but this plateau induces 1.3 log reduction ($p \leq 0.05$) of

L. monocytogenes for F2 and 1 log reduction for F6 at the end of storage time (Day 15) in compared to control. These results suggest a bacteriostatic behaviour of F2/F6 formulations against *L. monocytogenes* rather than a bactericidal action.

It was also reported that the use of oregano in sterile liver sausage and in cooked poultry inhibit *L. monocytogenes* (Pandit and Shelef, 1994; Hao *et al.*, 1998).

Sökmen *et al.* (2004) and Arrebola *et al.* (1994) also confirmed that carvacrol is the major component of oregano with noticeable antibacterial activity. According to Aydin *et al.*, (2005), the bacterial membrane is the main target of carvacrol. Edwin *et al.* (2007) observed that the temperature and food texture effect on antibacterial activity of carvacrol against *L. monocytogenes*. Farber & Peterkin (1991) also reported that *L. monocytogenes* can be adapted to lower temperature by the production of phospholipids with shorter and more branched fatty acids which makes it more resistant to antibacterial agents.

- ***E. coli***

The level of *E. coli* in red peppers coated with F2 and F6 formulations, during storage at 4°C, was presented in **Fig. 2-B**. Results showed a relatively strong inhibition effect of F2 formulation that started in the first days of storage and significantly reduced the bacterial growth from day 7 compared to control and F6 formulation. F2 coating generated a 1.25 log reduction at day 7, and in the following 1.6 log reduction at day 10 and finally 2.6 log reductions at day 14, thereby indicating the strongest inhibitory effect. In other side, F6 treatment led to a slight decrease of bacterial level about 1 log at day 15 of storage. Furthermore, it was observed that control (no coating treatment) is characterized by a high constant level of *E. coli* about 4.6-5.0 log CFU/g all over the storage time. According to Burt *et al.* (2007), carvacrol at a concentration of 0.3, 0.5, 0.8 or 1 mM is responsible for the increase of heat shock protein HSP 60 (GroEL) and inhibition of the synthesis of flagelin in *E. coli* O157:H7. Friedman *et al.* (2004) reported that the application of oregano essential oil and their principal components in fruits and vegetables, allowed the inhibition of *E. coli* growth in orange, mango, apple and tomato juices,

Guarda *et al.* (2011) also reported that thymol and carvactrol allowed the disintegration of the outer membrane of Gram- negative bacteria and this is due to release of lipopolysaccharide components and increase of the permeability for the adenosine triphosphate in the cytoplasmic membrane., It was reported that the modification of the passive permeability of the cell, led to destroy the bacteria. According to Rojas-Graü *et al.* (2006; 2007) the major constituents (carvacrol, cinamaldehyde and citral) of oregano, cinnamon and lemongrass essential oils also reduced pathogenic bacteria such as *E. coli* O157:H7.

- ***S.* Typhimurium**

The level of *S.* Typhimurium in red peppers coated with F2 and F6 formulations, during storage at 4°C, was presented in **Fig. 2-C**. Results showed that *Salmonella* is the most sensitive pathogen after F2/F6 treatments. First, the control indicated a 0.7 log reduction in *Salmonella* population during the 7 days of storage and then after the bacterial level were maintained constant from day 7 to day 15. F2 coating showed a strong bactericidal effect from the first day of storage, with a total inhibition (under detection threshold of 1 log CFU/g) at day 5 of storage (2.7 log reduction). In other side, F6 coating presented a strong bactericidal activity from day 3 and then after at day 7 of storage a total inhibition (2.1 log reduction) was obtained. The obvious different activity observed between F2 and F6 treatment in the period time of day 1 and day 7 suggested a significantly faster inhibition ($p \leq 0.05$) with F2 (reached 1.5 log CFU/g at day 3) compared to F6 formulation (reached 3.2 log CFU/g at day 3).

According to Ultee, Bennik & Moezelaar (2002) a major destabilization of the membrane is the consequence of a synergistic activity between carvacrol and cymene. Carvacrol can be accumulated in the cell membrane and its proton-release and hydrogen-bonding abilities can modify the membrane conformation, resulting in cell death (Ben Arfa *et al.*, 2006).

According to Canillac & Mourey (2004); Ismaiel & Pierson (1990) and Juven *et al.* (1994), the inhibition effect of major components of some EOs such as carvacrol on microbial growth is probably due to the presence of fats and proteins in the food matrix.

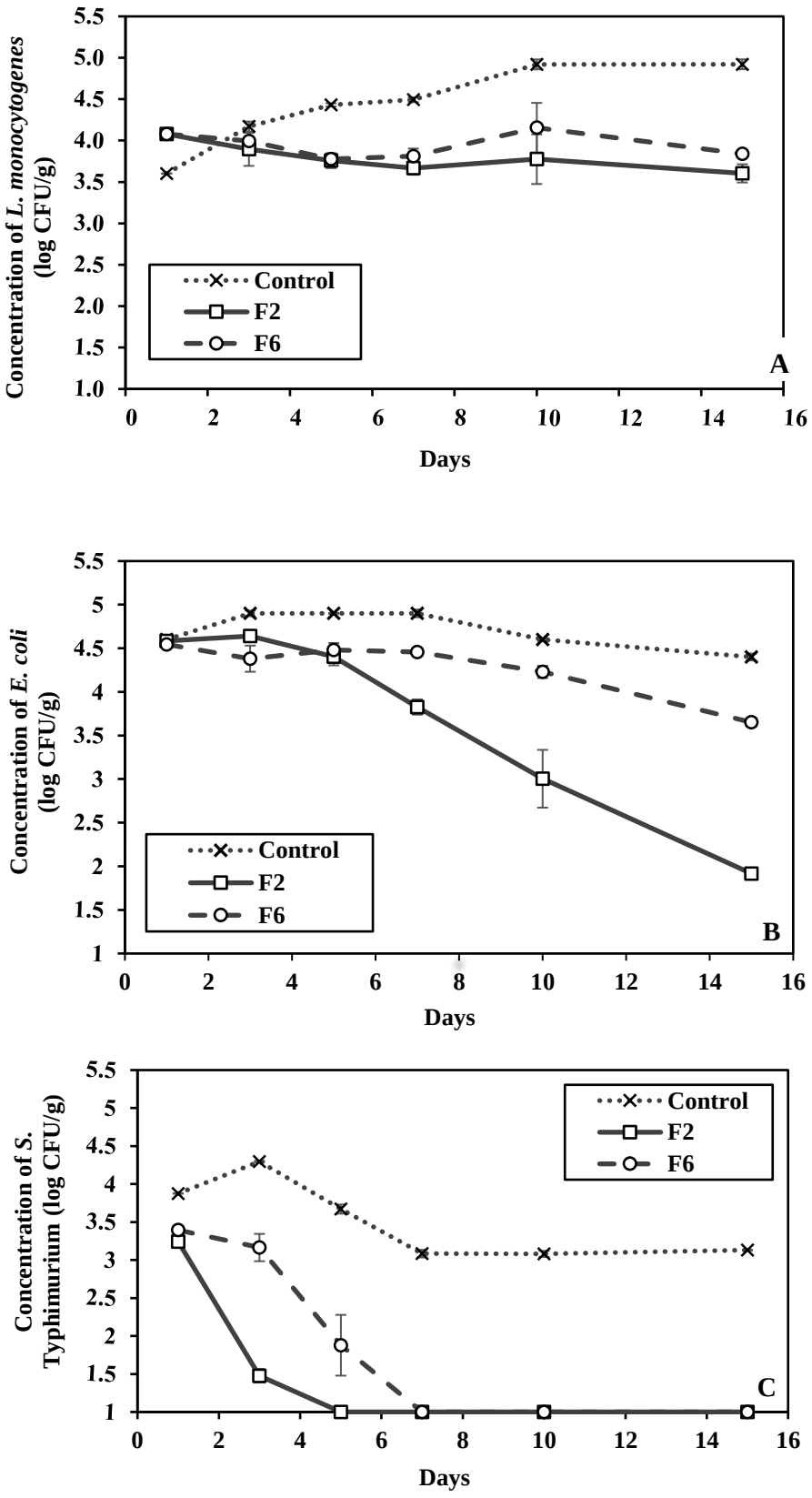

Figure 2 – Growth inhibition of *L. monocytogenes* (**A**), *E. coli* (**B**) and *S.* Typhimurium (**C**) in red peppers coated with F2 and F6 formulation, during storage at 4°C.

F2 and F6 formulations have shown effective *in situ* antibacterial activity against Gram- positive and negative bacteria. Our results are in good agreement with the results obtained by Espina *et al.* (2011). They assessed the antibacterial activity of commercial citrus fruit essential oils against *S. aureus*, L. *monocytogenes, E. faecium*, *S. enteritidis*, *E. coli* O157:H7 and *Pseudomonas aeruginosa*. The obtained results showed that the chemical composition of individual EO constituents affects the mode of action and antibacterial activity of the citrus extracts (Espina *et al.*, 2011).

Oussalah, Caillet & Lacroix (2006) studied the mechanism of the antimicrobial action of Spanish oregano (essential oils) against cell membranes and walls of *E. coli* O157:H7 and *L. monocytogenes.* They observed an increase in the extracellular ATP concentration and cell constituent release of *E. coli* O157:H7 and *L. monocytogenes* in samples treated with Spanish oregano essential oils.

De Medeiros Barbosa *et al*., (2016) assessed the effect of application of oregano and rosemary, alone or in combination, against three pathogenic bacteria *L. monocytogenes, E. coli* and *S. enteritidis* that are associated with fresh leafy vegetables. They found that EOs alone or in tested combinations reduced the viable cell counts of all test strains (De Medeiros Barbosa *et al*., 2016).

Considering the selected pathogenic bacteria, F2 formulation could be employed as a good antibacterial coating against the three pathogens in red peppers. As opposed to F6, F2 formulation allows a faster total inhibition of *E. coli* and *S.* Typhimurium, but its capacity is not sufficient to affect *L. monocytogenes* rapidly. These results are in accordance with the study by Tawema *et al.* (2014) who reported that *Listeria* was found to be the most resistant microorganism to different spraying treatments with natural antimicrobials.

3.4 *In situ* antimicrobial capacity of F2 and F6 formulations in cranberries

- ***L. monocytogenes***

The level of *L. monocytogenes* in cranberries coated with F2 and F6 formulations, during storage at 4°C, was presented in **Fig. 3-A**. It should be noted that the antimicrobial capacity of the coatings in cranberries was investigated for a longer period, due to specific requirements of long-term storage and the acidic/antioxidant nature of this product. An analogous behaviour was observed for F2 and F6 treatments. The control curve displays a continuous decrease of *L. monocytogenes* level, from the initial loading of 3.6 log CFU/g to 2.5 log CFU/g between day 1 and day 14, followed by a rapid total inhibition at day 18 to the end of storage (Day 42), most probably due to the presence of phenolic compounds and acidic nature of cranberries. F2 coating showed a linear strong inhibiting activity from the first day of storage till day 10 and then after, a total inhibition was obtained in day 10 to the end of storage time. F6 coating also presented a noticeable reduction in bacterial level at day 4 and 10 and finally a total inhibition of *L. monocytogenes* was also observed in presence of F6 at Day 14. This total inhibition was maintained in both treatments for long-term storage (up to Day 42).

- ***E. coli***

The level of *E.coli* in cranberries coated with F2 and F6, during storage at 4°C, was presented in **Fig. 3-B**. Results showed that *E. coli* is the most sensitive pathogen in presence of F2/F6 treatments in cranberries. The control curve displayed a plateau of 3.3 log CFU/g from day 1 to day 18, suggesting a stability of initial bacterial loading, then a rapid decline was observed in day 18 till day 22 of storage time. It is worth to mention that the bacterial level of control reached to below the limit of detection from day 22 to the end of storage (Day 42), which can be due to the chemical composition of cranberries.

On the other side, F2 and F6 coatings showed an immediate inhibition effect ($p \leq 0.05$) at day 1, with a 0.8 log reduction by F2 and a 1 log reduction by F6. The bactericidal properties of F2 and F6 are rapidly confirmed at day 4, with a total inhibition of *E. coli* for both treatments (under detection threshold for F2 and F6). This inhibition level was maintained in both treatments for a long-term storage (up to Day 42).

According to Côté *et al.* (2010), a large variety of bioactive compounds are found in cranberries such as flavanols, flavan-3-ols (catechin, epicatechin), anthocyanins (cyanidin-3-galactoside, peonidin-3-galactoside, and derivatives), tannins (proanthocyanidins), and phenolic acid derivatives (ferulic, p-coumaric, chlorogenic, ellagic, p-hydroxybenzoic acids). These phenolic compounds are able to inhibit pathogenic bacteria. Caillet *et al.* (2012) have also demonstrated that cranberry phenolic fractions such as phenolic acids, anthocyanins, flavanols or proanthocyanidins presented effective antimicrobials properties. Côté *et al.*, (2011) also observed that frozen cranberries presented high efficient antibacterial activity against *Pseudomonas aeruginosa*, *S. aureus*, *L. monocytogenes* and *S.* Typhimirium. Furthermore, Robards & Antolovich (1997), stated these phytochemicals are commonly associated with the fruit organoleptic qualities and have also shown strong biological (*e.g.* antimicrobial, antioxidant) properties.

- ***S.* Typhimurium**

The level of *S.* Typhimurium in cranberries coated with formulations F2 and F6, during storage at 4°C, was presented in **Fig. 3-C**. The control samples presented a 0.5 log reduction of *Salmonella* population from day 1 to day 4 (initial loading of 3.2 log CFU/g to 2.7 log CFU/g), followed by a plateau of 2.7-2.9 log CFU/g from day 4 to day 18 and a linear reduction after day 18 to obtain the total inhibition at day 25. Moreover, the bacterial level of control was completely inhibited until the end of storage time (day 42). In other side, F2 and F6 coatings showed significantly effective inhibiting activity ($p \leq 0.05$) of 0.7 log and 1.3 log reduction respectively, compared to control from day 1, as observed against *E. coli*. A continuous reduction in growth of *Salmonella* was observed for both

formulations. The bactericidal properties of F2 and F6 treatment was confirmed at day 14, with a total inhibition of *Salmonella* for both treatments, which indicates an antimicrobial behavior similar to those observed against *E. coli* and *Listeria*.

Therefore, it can be concluded that F2 and F6 formulations acted as strong antimicrobial coatings against the three pathogens *E. coli, L. monocytogenes* and *S.* Typhimurium in cranberries initially loaded with 3.5 log CFU/g with strong, immediate bactericidal effects.

These findings confirmed the possible synergistic lethal effects of F2/F6 formulations in combination with phenolic compounds naturally occurring in cranberries.

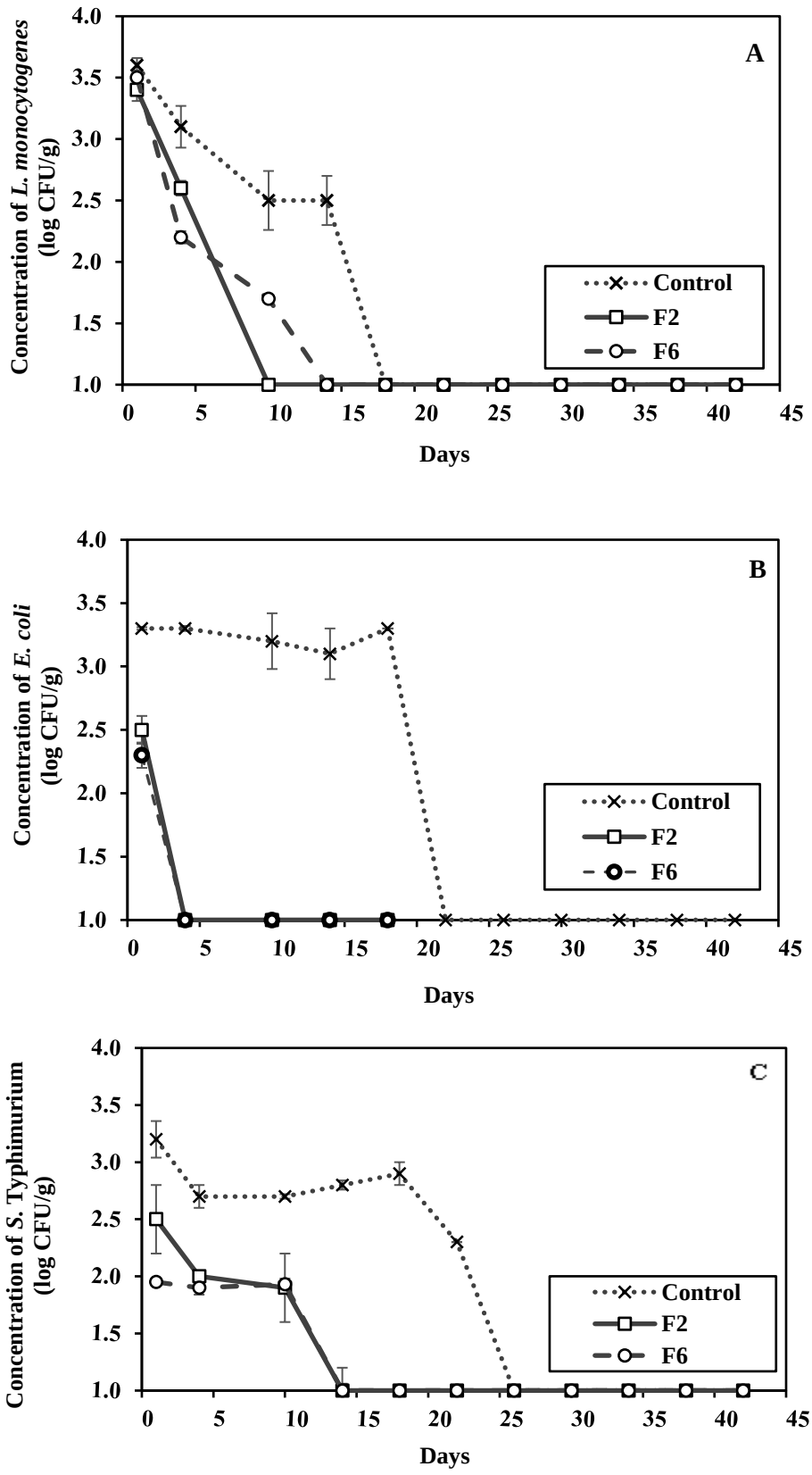

Figure 3 – Growth inhibition of *L. monocytogenes* (**A**), *E. coli* (**B**) and *S.* Typhimurium (**C**) in cranberries during storage at 4°C, in presence of F2 and F6 formulations.

3.5 *In situ* antimicrobial capacity of F2 and F6 formulations in potatoes under MAP condition

- ***L. innocua***

The level of *Listeria* in potatoes coated with F2 and F6 formulations, during storage under MAP at 4°C, was presented in **Fig. 4-A**. No significant difference ($p > 0.05$) was observed between samples treated with F2, F6 formulations and control (without antibacterial treatment), during10 days of storage. Indeed, a plateau of 4.2- 4.5 log CFU/g was noted for control and both F2/F6 treatments from day 1 to day 7. Although a slight decline of *Listeria* level was measured from 4.2 to 3.5 log CFU/g at day 10 in all sample groups. It was concluded that F2 and F6 formulations had no inhibition effect on *L. innocua* inoculated in potatoes packaged under MAP condition compared to control samples (Fig.4A).

As it was observed, MAP, used to avoid the enzymatically browning and extend the shelf life of potatoes, has no reduction effect on bacteria counts in samples.

However, Turgis *et al.* (2008) observed that MAP alone reduced the level of *Pseudomonas* by 1 log CFU/g as compared with the conventional air packaging. Caillet *et al*. (2006) also observed a continuous reduction in populations of *L. innocua* in carrot packaged under modified atmosphere packaging (MAP).

This conflict can be explained by food composition and the combined gas treatment.

In addition, Caillet *et al*. (2006) observed that the antimicrobial coating resulted in a 1.29 log reduction in the concentration of *L. innocua* in carrots packed under air after 21 days of storage and a 1.08 log reduction in carrots packed under MAP (60% O_2, 30% CO_2, and 10% N_2) after 7 days of storage.

According to these results, the MAP condition, such as gas concentration and combination, as an effective factor should be considered for bacterial growth inhibition.

- ***E. coli non-pathogenic***

As it was observed in **Fig. 4-B,** no significant inhibition ($p > 0.05$) was determined with F2 and F6 treatments between day 1 and day 7. However, at day 10, both F2 and F6 treatments induced a similar inhibition, with a slight reduction about 0.5 log of *E. coli*. F2 and F6 demonstrated a moderate inhibition activity against *E. coli* non-pathogenic inoculated in potatoes under MAP after 7 days of storage at 4°C which were less effective in compared to red peppers and cranberries samples. Control sample (without antibacterial treatment) has also shown no significant reduction in of *E.coli* population during the storage time.

Turgis *et al*. (2008) observed that in ground beef, MAP alone reduced the level of *E. coli* by 0.67 log CFU/g. When mustard EO was added to the ground beef in MAP, this treatment kept the *E. coli* population at 3.72 to 3.98 log CFU/g for 14 days.

- ***S. enterica***

The most effective inhibition effect of F2 and F6 treatments on potatoes was observed against *Salmonella* (**Fig. 4-C**). In a first step, a plateau of 3.8-4.3 log CFU/g was observed ($p > 0.05$) in all sample groups (control, F2 and F6 treatments). But in a second step, noticeable inhibitory effects ($p \leq 0.05$) were obtained for F2 and F6 treatments, with 1.5 log and 1.1 log reduction of *Salmonella* level, respectively.

Turgis *et al*., (2008) also observed MAP reduced the *Salmonella* level by 1.72 log CFU/g in ground beef. MAP and oregano EOs combination reduced the level of *Salmonella* by 2.99 log CFU/g at day 1. They concluded when EOs combined with MAP, an additive effect against bacterial growth was observed.

Here again in potatoes like as observed in red peppers, F2 and F6 formulations were more effective against *E. coli* and *Salmonella* (Gram-negative bacteria) compared to *Listeria* (Gram-positive bacteria).

As it was mentioned, MAP was used here to avoid the sensorial alteration of potatoes and to avoid the browning reaction, but we observed no effect on targeted bacteria levels during the storage. According to the findings of Daniels, Krishnamurt & Rizvi (1985), the germicidal effect of MAP is principally attributed to the carbon dioxide. As *Listeria, E. coli*, and *Salmonella* are facultative anaerobic bacteria, it could explain their resistance in MAP conditions.

Another factor that could also explain the low efficiency of F2 and F6 formulations with MAP, is that they were sprayed on the surface of pre-fried sliced potatoes, which could affect the quality of coating adhesion on the surface of potatoes and therefore reduce the contact and migration of antimicrobials to the surface-inoculated bacteria.

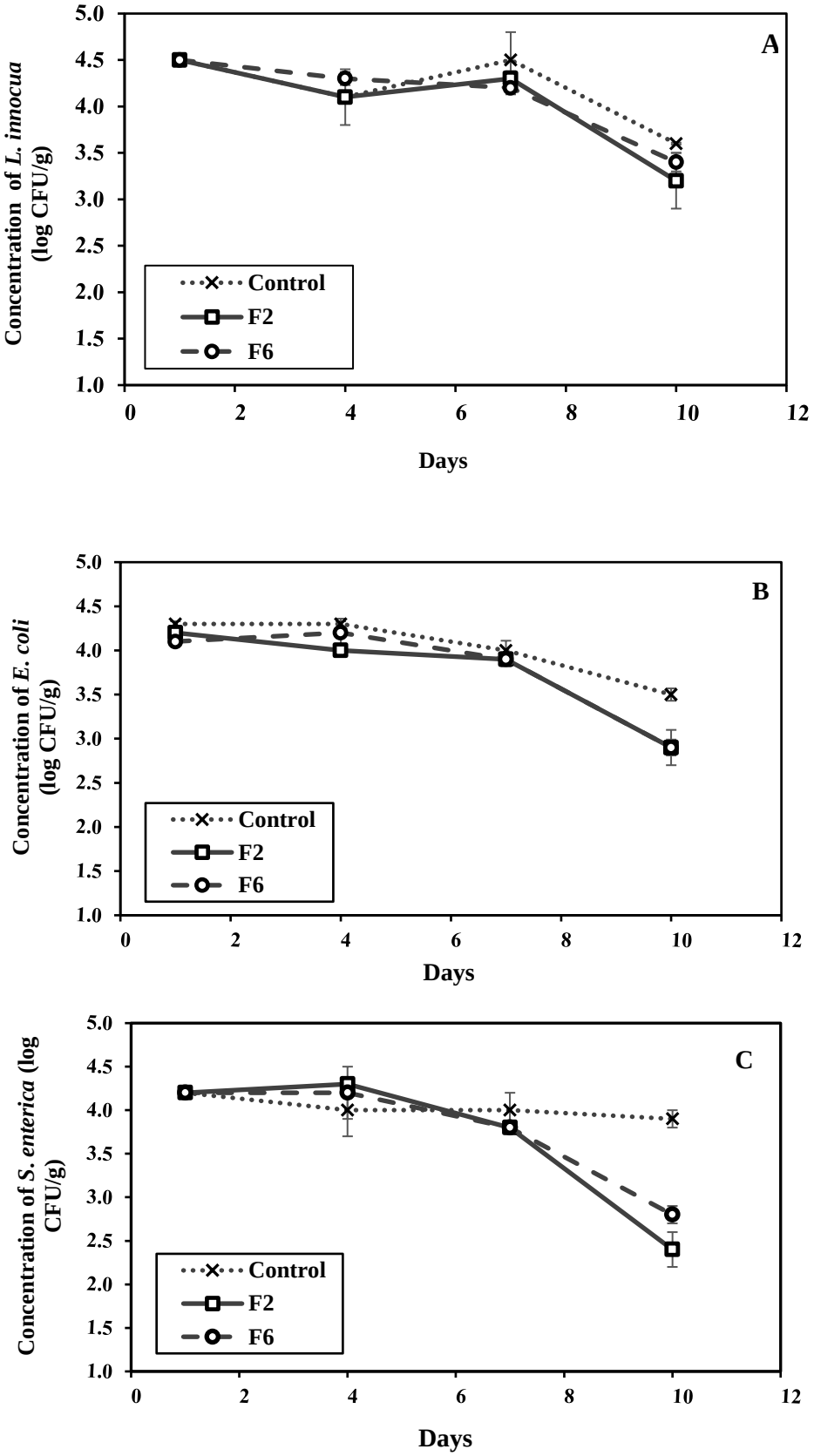

Figure 4 – Growth inhibition of *L. innocua* (**A**), *E. coli* (**B**) and *S. enterica* (**C**) in pre-cut/pre-fried potatoes under MAP during storage at 4°C, in presence of F2 and F6 formulations.

As F2 /F6 formulation contain different antibacterial components that act in various behavior with different rate of action, the obtained results confirmed the hypothesis of a controlled release of bioactive components incorporated in emulsion during the storage time. The molecular size, polarity, hydrophobicity and permeability coefficient (*Pm*) and partition coefficient (*Log P*) of molecules are the most effective factors on molecular transfer of antibacterial through bacterial membranes or interaction with them to act as antibacterial agents. Furthermore, the molecular structure of compounds has a remarkable effect on its antibacterial activity. It has been demonstrated that oxygenated monoterpenes had a higher antimicrobial activity than did hydrocarbons (Espina *et al.*, 2011, Burt, 2004).

Furthermore, Vattem *et al.* (2004) suggested that phenolic compounds with partial hydrophobicity could act efficiently at the bacterial membrane water interface by embedding in the membrane, thereby impairing the cell membrane and transport processes (Vattem *et al*., 2004).

Therefore, these results confirmed the idea of introducing an antimicrobial formulation composed of natural bioactive compounds in order to control foodborne pathogens in fruits and vegetables, but it had little effect on pre-fried potatoes.

Several studies have examined the possible modes of action of EOs on bacteria. The phenolic compounds present in EOs can modify cell permeability in the cell membrane, causing the release of the cell constituents, decrease the ATP concentration and intracellular pH. Moreover, it should be noted that the global antimicrobial activity of EOs are attributable to their phenolic compounds but probably also to synergistic effects of all components such as terpenoids, aldehydes, etc. (Tawema *et al.*, 2014; Tawema *et al.*, 2016). Also, the synergic combination of organic acids and EOs has already been demonstrated by several studies (Aksit *et al.*, 2006; Friedly *et al.*, 2009). Therefore by lowering the pH in presence of OA, the hydrophobicity of EOs increased to affect bacterial membranes. In addition alkaloids, flavoinoids and terpenes as an example of active substances present in essential oils are a great source of antimicrobials (Seoud *et al*., 2005; Dolan *et al*., 2007). According to Ahn, Grun and Mustapha (2007) and Zhang *et al.* (2009) a synergistic antibacterial effect in food products can be the result of the combination between plant extract, low temperature and low pH. Different damage can be caused when the bacteria are subjected to antibacterial compounds by the variation in cell wall structures between Gram-positive and Gram-negative bacteria (Puupponen- Pimia *et al*., 2001). As the cell wall of Gram-positive bacteria contains much more peptidoglycan than that of Gram-negative cells, it provides more rigidity. *L. monocytogenes* cells were not easily destroyed by formation of lesions or channels on the cell wall (Wu *et al.*, 2008). Moreover, Van Immerseel *et al*. (2006) explained that exposure to low pH can cause sublethal injury to cell membranes, causing disruption of proton motive force owing to loss of H^+-ATPase.

Chemical composition of individual EO constituents affects the mode of action and antibacterial activity of the plant extracts (Dorman & Deans, 2000). Moreover, interaction between the different

constituents can occur, causing additive and synergistic antimicrobial effects (Espina *et al*., 2011). However, it should be noted that the total antimicrobial activity of an EO cannot be only attributed to its phenolic compounds, but rather to a synergistic effect of all its constituents. Indeed, under low pH conditions, EOs become more hydrophobic and dissolve more easily in the lipids of the bacterial cell membrane, rendering the bacteria more sensitive to the acidic environment created in the presence of organic acids (Tawema *et al.*, 2014).

3.6 Sensory analysis of red peppers and potatoes treated with F2 and F6 formulations

The sensory evaluation of SF red peppers and potatoes without treatment (control), treated with F2 and F6 formulation presented in **Table 2**.

- **Sensorial analysis of peppers**

The odor, color, global appreciation, taste and texture of samples were determined according to 9-points scale hedonic levels, resulting in degrees of appreciation, from 1 (Dislike very much) to 9 (Like very much). Results showed that F2 and F6 coatings did not affect significantly ($p > 0.05$) the sensory properties of peppers. Indeed, the odor, color, global appreciation, taste and texture of control (untreated peppers) and peppers coated with F2 and F6 formulations were not different ($p > 0.05$) between each other. No difference was observed between the color of untreated or treated peppers.

Hedonic data indicated ranges of 6.2-6.9 for odor, 5.8-7.0 for color, 6.0-6.7 for global appreciation, 5.6-6.3 for taste and 5.4-5.6 for texture. These results mean that the trend in appreciation was:

- "Like a little / Like moderately" for odor, color, global appreciation;
- "Like a little" for taste;
- "Indifferent / Like a little" for texture.

In particular, the F2/F6 treatments did not affect the odor and taste of peppers. Therefore, their sensorial attributes could be accepted for further commercialization.

Table 2 – Effect of F2 and F6 coating on the sensory properties of red peppers and potatoes, by 9-points hedonic evaluation.

Food / Coating	Hedonic evaluation of sensory properties[1-3]				
	Odor	**Color**	**Global appreciation**	**Taste**	**Texture**
PEPPERS					
Untreated (control)	6.29 ± 1.38[a]	7.00 ± 1.63[a]	6.11 ± 1.17[a]	6.29 ± 1.38[a]	5.60 ± 1.34[a]
Treated with F2	6.86 ± 1.77[a]	7.00 ± 1.63[a]	6.71 ± 1.38[a]	5.67 ± 1.51[a]	5.40 ± 1.67[a]
Treated with F6	6.22 ± 1.09[a]	5.83 ± 1.72[a]	6.00 ± 1.41[a]	5.57 ± 1.40[a]	5.63 ± 1.30[a]
POTATOES					
Untreated (control)	7.63 ± 1.89[b]	6.17 ± 1.33[a]	7.57 ± 1.27[a]	7.57 ± 1.27[a]	7.50 ± 0.93[a]
Treated with F2	6.78 ± 1.30[ab]	7.29 ± 1.60[a]	7.50 ± 1.31[a]	6.43 ± 1.27[a]	6.40 ± 1.14[a]
Treated with F6	5.86 ± 0.90[a]	6.80 ± 1.30[a]	6.44 ± 1.42[a]	6.71 ± 1.11[a]	7.20 ± 0.84[a]

1 The hedonic evaluation was scaled as follow: 9 = Like very much; 8 = Like a lot; 7 = Like moderately; 6 = Like a little; 5 = Indifferent; 4 = Dislike a little; 3 = Dislike moderately; 2 = Dislike a lot; 1 = Dislike very much.
2 The equality of variances was determined by Levene's test. Data were compared according to Duncan's test for assumed equal variances.
3 For each food product, means with different letters within the same column are significantly different ($p \leq 0.05$).

- **Sensorial analysis of potatoes**

Results showed that F2 and F6 coatings had no significantly effect ($p > 0.05$) on the sensory properties of potatoes, except in odor analysis for which potatoes treated with F6 formulation were significantly affected ($p \leq 0.05$) compared to control. No significant difference ($p > 0.05$) between potatoes treated with F2 formulation and those treated with F6 formulation was observed. Indeed, no significant difference ($p > 0.05$) was observed between the odor of control and F2-treated potatoes (range of 6.8-7.6). Also no significant difference in odor of potatoes treated with F2 formulation and those treated with F6 formulation (range of 5.9-6.8) was observed. However, a significant difference ($p \leq 0.05$) between the odor of control and F6-treated potatoes (7.6 and 5.9, respectively) was felt. Besides the odor; global appreciation, taste, color and texture of control (untreated potatoes) and potatoes treated with F2 and F6 formulations were not different organoleptically ($p > 0.05$). Hedonic data indicated

ranges of 6.2-7.3 for color, 6.4-7.6 for global appreciation, 6.4-7.6 for taste and 6.4-7.5 for texture. These results mean that the trend in appreciation was:

- "Like a little" for the odor of samples treated by F6;
- "Like moderately / Like a lot" for the odor of control and samples treated by F6;
- "Like a little / Like moderately" for the color and texture of all groups;
- "Like a little / Like a lot" for the global appreciation and taste of all groups;

By considering the obtained results, F2 treatment presents an advantageous sensorial attribute compared to F6 treatment on pre-cut/pre-fried potatoes.

4. Conclusion

The *in vitro* inhibition capacity (IC %) of F2 and F6 formulations demonstrated their high antimicrobial potential against pathogenic and non-pathogenic bacteria. In addition, *in vitro* minimum inhibitory concentration (MIC) of F2 and F6 formulations were better against pathogen and non-pathogen bacteria compared to sodium benzoate.

It is interesting to note that although both formulations follow similar profiles, the inhibition rate of F2 formulation is generally higher than F6, possibly attributed to the presence of citral in F2 formulation. As MIC results demonstrated similar MIC values for F2 and F6 formulations against a wide range of bacteria, but a lower MIC values (higher antimicrobial capacity) of F2 against *L. monocytogenes*, non-pathogenic *E.coli* and *S.* Typhimurium.

However, *in situ* results confirmed that both formulations allowed a significant reduction of foodborne pathogenic bacteria in cranberries and red peppers. Otherwise, this study indicated that the differences in sensitivity of the targeted bacteria to the antimicrobial formulations depends strongly on the nature of the food product. It was observed that F2 and F6 formulations are more efficient against *E. coli* and *S.* Typhimurium (Gram-negative bacteria) than *L. monocytogenes* (Gram-positive bacteria) in high-loaded red peppers and cranberry. *Salmonella* was generally the easiest bacterium to eliminate, while the most resistant was *Listeria*. Overall, these antimicrobial formulations were able to significantly reduce or slow down the growth of food pathogens in different RTEs, during storage (as recommended by the manufacturers).

In pre-fried potatoes, F2/F6 formulations presented the same antibacterial activity against *E.coli* non-pathogen and *L. innocua*, however, the higher antibacterial activity was observed in presence of F2 formulation against *S. enterica.* The MAP condition and pre-fried process could have led to increase the bacterial resistance of non-pathogenic bacteria to natural antibacterial formulations. The MAP condition and presence a layer of solid oil on the surface of pre-fried sliced potatoes can decrease the surface contact and the release rate of antibacterial incorporated in emulsified formulation to target product.

Moreover, the sensory analysis of red peppers and potatoes treated with F2 and F6 formulations has shown that both formulations are very acceptable in terms of organoleptic attributes. Indeed, both F2 and F6 formulations could be used to prevent food contamination and to extend the shelf-life of RTE fruits and vegetables, with important bactericidal activity during storage time. Moreover, based on sensorial analysis, it will be potentially meet a high consumers commercial acceptance. In general, F2 presents superior antimicrobial and sensory properties, but the difference between F2 and F6 can be considered as negligible.

The low pH of the formulations induced by organic acid (pH 2.2) in combination with different active molecules presents in EO and citrus extract led to a synergistic antibacterial effect and showed a large spectrum activity, without negative effect on sensorial properties that is applicable to use in RTF industry.

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

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CHAPITRE 4:

DEVELOPPEMENT OF ANTIMICROBIAL METHODS USING GASEOUS OZONE, NATURAL SPRAYING FORMULATIONS AND GAMMA IRRADIATION AND THEIR COMBINATION TO PRESERVE FRESHNESS OF GREEN READY TO EAT PEPPERS

Article en préparation, à soumettre à *Food Control*

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Contributions des auteurs

J'ai réalisé les expériences, décrit et interprété les résultats et rédigé l'article.

Stéphane Salmieri a participé à la planification et au bon déroulement des analyses, et à la rédaction de l'article.

Maherani Behnoush a aidé dans l'interprétation des résultats.

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

Résumé

L'objectif de cette étude est d'évaluer l'effet antibactérien d'une formulation naturelle (coating X1+DF), de l'ozone gazeux et de l'irradiation. Les bactéries qui ont été évaluées sont *Escherichia coli* et *Salmonella enterica* en tant que Gram (-) et *Listeria innocua* en tant que Gram (+).

La formulation (coating X1+DF) a inhibé totalement *E. coli*, a réduit de 1.2 log *S. enterica* après 4 jours de stockage à -20°C dans les poivrons, et 1 log de réduction de *Listeria* a été observé au jour 10.

L'ozone gazeux a été évalué à une concentration de 10 ppm pendant une durée de 5 min et a permis une inhibition totale *d'E. coli* au jour 4 et aussi 1.1 log et 0.6 log de réduction de *L. innocua* a été observé aux jours 4 et 10 respectivement dans les poivrons verts. L'ozone gazeux a réduit aussi le compte de salmonelles de 1.1 et 0.6 log aux jours 4 et 10 respectivement.

La combinaison de l'ozone et de la formulation (coating X1+DF) a réduit rapidement les trois bactéries étudiées. Une réduction de 2 log de E. coli après 24 h a été observée, suivi d'une inhibition totale au jour 4. De plus cette combinaison a réduit de 1.3 et de 1.5 log le taux de *S. enterica* après 24 h et 10 jours.

La vaporisation de la formulation (coating X1+DF) et l'ozone sont des traitements qui ont réduit le compte bactérien, mais la combinaison de ces deux traitements a induit une réduction plus élevée.

Les poivrons irradiés à 0.5 kGy et tous les traitements combinés avec l'irradiation ont montré une inhibition rapide et totale de *E. coli* et de *S. enterica* à partir du jour 0 jusqu'au jour 10 avec 4 log de réduction. De plus, 2.3 log de réduction de *Listeria* a été observé au jour 0 jusqu'au jour 10.

La combinaison de trois traitements ozone, coating X1+DF et l'irradiation a totalement et rapidement inhibé les trois bactéries étudiées. Ce qui veut dire que les trois traitements ont un effet antimicrobien additif pour éliminer les bactéries. Les traitements testés n'ont pas affecté la qualité des poivrons verts (couleur, teneur en chlorophylle, vitamine C).

Abstract

The objectives of this study were to evaluate the antibacterial properties *in situ* of an optimized formulation (coating X1+DF), ozone gas, γ-irradiation and their combination on green peppers quality. The evaluated bacteria were *Escherichia coli* and *Salmonella enterica* as Gram negative bacteria and *Listeria innocua*, as Gram positive bacteria. *In situ* results showed that the coating X1+DF allowed a total inhibition of *E. coli* and 1.2 log reduction of *Salmonella* after 4 days at -20°C on peppers. Also, a 1 log reduction of *Listeria* was observed after 10 days. In addition, ozone at a concentration of 10 ppm during 5 min allowed a total inhibition of *E. coli* after 4 days, as well as a 1.1 log reduction and 0.6 log reduction of *L. innocua and Salmonella* at days 4 and 10 respectively, on peppers.

The combined treatment of ozone and coating allowed a rapid reduction of foodborne pathogens. A reduction by 2 log of *E. coli* after 24 h was observed, followed by a total inhibition at day 4. In addition, 1.3 log and 1.5 log reduction of *Salmonella* was observed after 24 h and 10 days respectively. The combined treatment allowed finally a 1 log reduction of *Listeria* after 10 days. Spray and ozone treatment reduced the bacterial level but combined treatments allowed a higher reduction of bacteria than with each treatment alone.

Irradiated green peppers and all treatment combinations with irradiation showed a total inhibition of *E. coli* and *S. enterica* at day 0 with 4 log reductions until the end of storage. In addition, 2.3 log reduction of *Listeria* was observed at day 0. *Listeria* was the most resistant bacteria however; *E. coli* was the most sensitive one. The combined treatment of ozone, spray and irradiation allowed a total and rapid inhibition of all studied bacteria.Treatments did not affect negatively the quality of green peppers (color, chlorophyll or vitamin C content).

Keywords

Ozone, Gamma irradiation, Natural antimicrobial compound, Antibacterial properties**,** Foodborne pathogenic bacteria.

1. Introduction

Demand for consumption of green peppers has increased recently, especially for use as an ingredient in ready-to-eat (RTE) foodstuffs (Gonzalez-Aguilar *et al.*, 2004; Ketteringham *et al.*, 2006). Also, the consumption of fruits and vegetables generally increased due to the recommendations by nutritionists for eating five fruits and vegetables per day (Kennedy *et al.*, 1996). Foodborne disease affects one on six US residents every year with 128,000 hospitalizations and 3,000 deaths annually (CDC, 2014). In the purpose of reducing the level of microorganisms, most fruits and vegetables are washed with chlorinated water (50-200 ppm). However, the reduction of bacteria obtained by this treatment is < 2 log CFU/g (Beuchat, 1992; Brackett, 1992; Lee *et al.*., 1995; Taormina and Beuchat, 1999). The loss of efficiency of the active sodium hypochlorite (NaOCl) is due to the reaction of nitrogen compounds in foods (Beuchat, 1998). To remove pathogens from the surface of vegetables, water containing NaOCl is generally used to wash produces (Hidaka *et al.*, 1992) but moreover many harmful by-products are produced when food is exposed to NaOCl, for example chloramines and trihalomethanes (Aieta *et al.*, 1984). For this reason, alternative treatments with natural highly antimicrobial compounds (safer than chlorine) are needed for inhibiting human pathogens in fresh produce (Lee *et al.*, 2004).

According to Thayer (1995), irradiation can be used to reduce pathogenic bacteria on meat. Niemira *et al.* (2003) reported that irradiation also reduces the bacterial level on ready to eat fruits and vegetables. In addition, gamma-irradiation allowed a high reduction of microorganism on red and black peppers compared to pasteurization (Oularbi and Mansouri, 1996). The level of *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* decreased in meat and poultry for doses of irradiation below 3 kGy (Thayer, 1995; Gezgin and Gunes, 2007). Furthermore, the use of 6 kGy for conservation of ground chicken breast allowed a total inhibition of pathogenic bacteria during 28 days under refrigeration (Spoto *et al.*, 2000). According to Thayer and Rajkowski (1999), the shelf life of strawberries, sweet onions, carrots and lettuce was increased when ionizing irradiation was used to reduce microorganism' spoilage. An irradiation dose of 1 kGy allowed a reduction on population of microorganisms and preserved the nutritional value of fresh cut lettuce during 9 days of storage at 4°C (Likui *et al.*, 2004).

In recent years, citrus EOs has attracted great attention due to their strong antimicrobial properties, high yields, aromas and flavours and particularly the presence of flavonoids. Flavonoids are a group of polyphenolic compounds that include flavanones, flavones and their derivatives (polymethoxy flavone aglycones, flavone-O-glycosides, flavone-C-glycosides) (Jing *et al.*, 2015). Flavonoids are a group of pigments contained in plants, fruits, vegetables, flowers, wine, and honey and they are responsible for flower and fruit coloration. Citrus flavonoids have been reported to have many biological activities

such as antioxidant, antimicrobial and anti-inflammatory properties (Tripoli *et al.*, 2007). Citrus flavonoids have a large spectrum of biological activity against a wide range of Gram (-) bacteria (Cormier *et al.*, 2013). Pharmacological studies have also confirmed their anti-inflammatory, anticancer and antiviral properties.

Cranberries are healthy fruits that contribute color, flavor, nutritional value, and functionality. Cranberry juice has several biological activities that have been studied, namely antioxidant, anti-inflammatory effect anti-cancer (Caillet *et al.*, 2011) as well as, prevention of recurrent urinary infection by preventing binding of *Escherichia coli* to urinary mucosa (Howell *et al.*, 2005),. These activities are assigned to pro-anthocyanidins (PACs), also known as condensed tannins which are the most consumed polyphenolic compounds in the human diet. The antimicrobial effect of cranberry juice and of its extracts was investigated against some bacterial strains *E. coli* O157:H7, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* Typhimurium, and *Staphylococcus aureus*. The results indicated that all the bacterial strains, both Gram (+) and Gram (-), were selectively inhibited by the cranberry phenolic compounds (Caillet *et al.*, 2011). A recent study by Harich *et al.* (2016) showed that concentrate cranberry juice reduced significantly the level of *E. coli*, *S.* Typhimurium and *L. monocytogenes* in RTE red peppers and cranberries.

Fermentation is a method of food preservation that has been used for centuries. According to Settanni and Corsetti (2008), lactic acid bacteria are responsible for the production of metabolites during fermentation of milk or other substrates and are known to inhibit pathogenic and spoilage microorganisms. Dussault *et al.* (2011) have also demonstrated the antimicrobial beneficial effect of fermented dextrose containing bacterial metabolites as food additives to reduce the growth rate of the total bacterial flora in vegetables.

Ozone is largely used in the food industry all over the world and is "Generally Recognized As Safe" (GRAS) by FDA and classified as a disinfectant for food industry in the USA (Graham, 1997), especially for the good manufacturing practice (GMP) associated to the treatments of bottled water (FDA, 1995). Xu (1999) reported that ozone is 1.5 times more efficient than chlorine since it allowed a significant reduction but also inhibition of a large number of microorganisms compared to chlorine. Graham (1997) also reported that the efficacy of ozone is 3,000 times faster than chlorine while not producing dangerous by-products after decomposition. In addition, ozone allowed the inhibition of bacteria, molds, yeasts, parasites and viruses, therefore showing a broad spectrum of inhibition and destruction (Kim *et al.*, 1999). The use of ozone in appropriate concentration, during storage in modified atmosphere, allowed a high protection of fruits and vegetables against physical deterioration and pathogenic bacteria (Nadas *et al.*, 2003). Indeed, according to Guzel-Seydim *et al.* (2004), gaseous ozone is not too much toxic when used at low concentration. According to Horvitz and Cantalejo (2012), peppers treated with 0.7 ppm of ozone gas during 1 to 5 min allowed 2.6, 5.8 and 2.3 log CFU/g reduction of mesophilic bacteria, psychrophilic bacteria, and yeasts/molds respectively.

According to Mejia *et al.* (1988), Lee *et al.* (1995) and Markus *et al.* (1999), fresh peppers are an excellent source of vitamins and phenolic compounds. The genotype, maturity and growing conditions can influence the concentration of all the compounds present in peppers. As reported by Lee and Kadar (2000) and Howard *et al.* (2002), vitamin C level depend on cultivar, maturity at harvest, storage conditions and productions practices.

2. Material and methods

2.1. Raw material

Pre-cut green peppers were provided by Bonduelle Americas Inc. (Saint-Denis-sur-Richelieu, QC, Canada) and were stored at -20°C until used. Pre-cut green peppers were used for *in situ* studies.

2.2. Preparation of bacterial cultures

Stock cultures of *E. coli* ATCC 25922, *S. enterica* ATCC 53648 chi 4064 and *L. innocua* NSPQ 3285 were stored at −80°C in Tryptic Soy Broth (TSB) medium (Alpha Biosciences Inc., Baltimore, MD, USA) containing glycerol (10% v/v). Prior to each experiment, stock cultures were grown through two consecutive 24-48 h growth cycles in TSB at 37°C. Working cultures were diluted in peptone water (Alpha Biosciences Inc., Baltimore, MD, USA) to obtain bacterial concentrations of 10^3-10^4 CFU/mL for *in situ* studies.

2.3. Preparation of the natural antimicrobial formulation X1+ DF

The antimicrobial formulation (coating X1+DF) was prepared under sterile conditions, based on a preliminary study demonstrating high inhibiting properties of this formulation when sprayed onto vegetable surfaces. A volume of 0.6 mL of Tween® 80 (emulsifier) was added in 1 L of distilled water. The formulation (coating X1+DF) containing fermented dextrose Prolong 2 (BSA-Québec, Montreal, QC, Canada), citral essential oil (Bio Lonreco Inc., Dorval, QC, Canada), citus extract (Biosecur Lab Inc., Mont St-Hilaire, QC, Canada), and concentrated cranberry juice (Atoka Cranberries Inc., Manseau, QC, Canada) were used at concentrations of 0.075, 0.01, 0.1 and 1% (w/v) respectively.

2.4. Spraying treatment of the formulation (coating X1+DF) on green peppers

Each sample of green peppers (25 g) were placed on a plastic cup and inoculated with 100 µL of bacterial suspension concentrated at 10^6 CFU/mL. Then, all samples were left to dry during 90 min at 4°C in a cold room. The (coating X1+DF) formulation was uniformly sprayed under sterile conditions on each sample using an electrostatic sprayer BSL-DS1 (BSL Technologies Inc., Brossard, QC, Canada), at a distance of 45-60 cm and an outlet air pressure of 25 psi (reservoir pressure of 30 psi).

Samples were sprayed one each side at a concentration of 0.3 mL spray/25 g peppers, and thereafter left to dry for 30 min before storage and microbiological analyses.

2.5. Ozonation of green peppers

Each sample of green peppers (25 g) was placed on a plastic cup and inoculated with 100 µL of bacterial suspension concentrated at 10^6 CFU/mL. Then, all samples were left to dry during 90 min at 4°C in a cold room. Ozonation treatment was performed by using an ozone generator C-10 portable unit equipped with an Ozone Monitor controller and a calibration probe of 10 ppm (Ozone Innovations Inc., Drummondville, QC, Canada). Each sample was deposited onto a stainless steel (SS) perforated plate and transferred into a SS ozone diffusion chamber. Ozonation was carried out at 10 ppm, for 5 min of ozone exposure at a flow of approximately 15 standard cubic feet per hour (SCFH). Thereafter, samples were removed and stored at -20°C before microbiological analyses. The combined treatment consisted in **i)** treating the inoculated peppers with ozone and then **ii**) coating the green peppers with the (coating X1+DF) formulation.

2.6. Microbiological analysis

The antimicrobial capacity of the (coating X1+DF) formulation, ozonation, irradiation and their combined treatment was evaluated *in situ* on green peppers after 0, 1, 4, 7, 10 days of storage, against food pathogens. Samples (25 g) were homogenized for 30 s at 230 rpm in 50 mL of sterile peptone water (0.1% w/v) with a Stomacher® 400-Circulator (Seward Laboratory Systems Inc., Davie, FL, USA). From each homogenate, serial dilutions were prepared, plated onto the surface of appropriate media (Difco, Becton Dickinson, Franklin Lakes, NJ, USA) and incubated for 24-48 h at 37°C before bacteria enumeration. MacConckey agar supplemented with sorbitol, DCLS agar and PALCAM agar were used for the enumeration of *E. coli, S. enterica* and *L. innocua* respectively (detection limit: 10 CFU/g).

2.7. Sensory analysis of green peppers

The color, odor, taste, texture and global appreciation of the green peppers coated with the formulation (coating X1+DF) were evaluated by a panel comprising 20 persons, according to a 9-points hedonic scale test (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). The sensory analysis was carried out 1 day after the treatment. A minimum of 8 consumer panelists was present for each evaluation session. This method was used in order to measure the degree of acceptance or rejection of samples, and eventually to verify that the antimicrobial coating had no significant side-effect ($p > 0.05$) on the organoleptic properties of the peppers.

2.8. Gamma irradiation

Samples of green peppers (25 g) were put into a sterile Deli bag (3 mil nylon/ethylene vinyl acetate/polyethylene; Winpak Division Ltd., St-Leonard, QC, Canada) and sealed using a packaging

machine (model 250 Single Chamber, Sipromac Inc., St-Germain-de-Grantham, QC, Canada). Then gamma irradiation treatment was done at a dose of 0.5 kGy at the Canadian Irradiation Center (CIC) in an underwater UC-15A SS canister (Nordion Int. Inc.) at a dose rate of 16.74 kGy/h.

2.9. Colorimetry

The color of the treated green peppers was measured using a Color reader CR-10 (Konica Minolta, Ramsey, NJ, USA). Measurements of spectral reflectance were performed directly onto the vegetables surface (viewing area = 10 mm × 10 mm). The L*, a*, b* system (CIELAB) was employed; the L* axis represents the lightness from black (L* = 0) to absolute white (L* = 100), the a* axis varies from green (-300) to red (+299), and the b* axis varies from blue (-300) to yellow (+299). To characterize more precisely the color of green peppers, the hue angle (h°) was determined to indicate the color between a* (green color if a* < 0; h° = 180°) and between b* (yellow color if b* > 0;-h° = 90°), the hue angle is measured from the positive a* axis according to the following equations:

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) \qquad \textbf{if a* > 0}$$

$$h^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) + 180^\circ \qquad \textbf{if a* < 0} \qquad [1]$$

The total color difference (ΔE*) between samples and control was also determined by the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad [2]$$

2.10. Determination of chlorophyll content

The determination of chlorophyll content in green peppers was performed according to a procedure of Arnon (1949). A quantity of 10 g of green pepper tissue was extracted in 2 mL of acetone-water 80:20 (v/v), using a mortar and pestle for 2 min. Filtration was followed using a 0.45 µm PTFE filter and the filtrate was transferred to a Falcon tube covered with aluminum foil to prevent the photo-oxidation of chlorophyll. Absorbance was measured at 663 and 645 nm using a Scinco S-3100 UV-visible spectrophotometer (Betatek Inc., Toronto, ON, Canada). The concentration of chlorophyll (a, b, total) was expressed as µg/mL extract and calculated from the following equations:

$$\text{Chlorophyll a } (\mu g/mL) = 12.7\, A_{663} - 2.69\, A_{645} \qquad [3]$$

$$\text{Chlorophyll b } (\mu g/mL) = 22.9\, A_{645} - 4.68\, A_{663} \qquad [4]$$

$$Total\ c\text{hlorophyll } (\mu g/mL) = c\text{hlorophyll a} + c\text{hlorophyll b} = 20.2\, A_{645} - 8.02\, A_{663} \qquad [5]$$

2.11. Determination of vitamin C content

Itis important to note that dehydroascorbic acid exhibit biological activity because it can be converted into ascorbic acid in human body. So, it's crucial to measure both ascorbic and dehydroascorbic acids.

According to Wimalasiri and Wills (1983), Nisperos-Carriedos *et al.* (1992), Howard *et al.* (1994) and Lee *et al.* (1995), ascorbic acid concentration of peppers cultivar was between 46 and 243 mg/100 g. More than 90% of vitamin C in human diet is supplied by fruits and vegetables (Seung and Adel., 2000). Ascorbic acid (AA) is the main biologically active form of vitamin C but also dehydroascorbic acid (DHA) - an oxidation product – that is converted back into ascorbate via the GLU10 glucose transporters into the endoplasmic reticulum of human cells.

The determination of vitamin C (L-ascorbic acid + L-dehydroascorbic acid) content in green peppers was performed by using a VitaFast® Vitamin C P1010 enzymatic test kit (detection limit: 7.8 mg/100 mL; R-Biopharm AG, Darmstadt, Germany). The test was based on a colorimetric method by Boehringer Mannheim GmbH Biochemica (1987).

Sample preparation. A quantity of 10 g of green peppers was added to 50 mL of meta-phosphoric acid 1.5% (pH 3.5). The mixture was homogenized with an Ultra-Turrax T25 disperser (IKA Works Inc) at 15,000 rpm, for 15 min at room temperature. The homogenate was then filtered (0.45 µm) before using directly for the assays.

Determination of L-(+)-ascorbic acid (AA) and dehydroascorbic acid (DHA). Two assays were performed to determine the content of AA and DHA, based on **i)** indirect quantification by ascorbate oxidase (AAO; from *Cucurbita* sp.) to differentiate them from other reducing substances and **ii)** indirect quantification by dithiothreitol (DDT) for the specific determination of AA and (AA+DHA).

Principle of the determination of AA content. AA (which is present in the sample extract as reductant ascorbate) and some other reducing substances reduce the tetrazolium salt MTT [3(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide] into MTT-formazan, in the presence of the electron carrier PMS (5-methylphenazinium methyl sulphate) at pH 3.5. The sum of all the reducing substances is then measured in the sample, namely corresponding to the sample determination (without AAO). For the specific determination of AA content, the AA fraction of the sample is oxidized into DHA by AAO in the presence of oxygen. The DHA formed does not react with MTT/PMS and then, the remaining reductants reduce the MTT into formazan, namely corresponding to the sample blank determination (with AAO). The formazan formed in the assays is proportional to the sum of reductants and was measured at 578 nm using a microplate reader BioTek ELx800 (Winooski, VT, USA). Hence, the AA specific content was calculated based on the absorbance difference of the sample assay minus that of the sample blank assay, according to the equation:

$$\Delta A_{AA} = \Delta A_{Sample,\,AA} - \Delta A_{Sample\ blank,\,AA} \quad [6]$$

Results were expressed based on an ascorbic acid standard curve.

Principle of the determination of (AA+DHA) content. The determination of the (AA+DHA) content was performed similarly to the AA content, but the DHA fraction initially present in the sample was reduced by DTT into AA, before the reaction with MTT. Hence, the (AA+DHA) specific content was calculated based on the absorbance difference of the sample assay minus that of the sample blank assay, according to the equation:

$$\Delta A_{AA+DHA} = \Delta A_{Sample,\ AA+DHA} - \Delta A_{Sample\ blank,\ AA+DHA} \quad [7]$$

The difference between the total (AA+DHA) (**Eq. 7**) and free AA (**Eq. 6**) resulted in the DHA content. **Table 1** summarizes the combined tests of indirect quantification of (AA and AA+DHA).

Table 1 Combined assays of indirect quantification (intermediate selective reactions) for the specific determination of AA and DHA.

Intermediate selective reactions	**For the determination of AA**		**For the determination of AA+DHA**	
	Sample blank	**Sample**	**Sample blank**	**Sample**
DTT-reaction	No	No	Yes	Yes
AAO-reaction	Yes	No	Yes	No

Calculations. Based on the standard curve, the weighed amount of solid sample and the dilution factor F, the concentration of ascorbic acid (free AA or total AA+DHA) was calculated as follows:

$$\text{Ascorbic acid } (mg/100\ mL) = \frac{(\Delta A - y\text{–}intercept) \times F}{\text{slope} \times \text{weighed sample (g/mL)}} \quad [8]$$

2.12. Statistical analysis

All measurements were performed in triplicate (n = 3). Analysis of variance (ANOVA), Duncan's multiple range test (for equal variances) and Student *t* test (for independent samples) were performed for statistical analysis (PASW Statistics 18; IBM Corporation, Somers, NY, 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. Inhibiting properties of the coating (X1+DF) formulation, ozonation, irradiation and their combined treatment

3.1.1. Inhibiting properties against *E. coli*

The antimicrobial properties of different treatments were evaluated *in situ* against *E. coli* in green

peppers. Results show that green peppers without treatment (control) allowed a high microbial level of *E. coli* during 10 days of storage (4 - 2.1 log CFU/g) this decrease is due to freezing (-20°C). Samples treated with the natural formulation using an electrostatic spray showed a rapid reduction of *E. coli* level after day 1 (0.8 log reduction), followed by a decrease to the limit of the detectable level. No growth was observed after day 4. Samples treated with ozone showed a 1.2 log reduction after 24 h followed by a total inhibition at days 4, 7 and 10. Combined treatments allowed a 2.1 log reduction after 24 h followed by a total inhibition at days 4, 7 and 10. Bermudez-Aguirre and Barbosa-Cánovas (2013) reported that the treatment of tomatoes using ozone gas at 5 ppm during 3 to 15 min reduced by 2.2 log the concentration of *E. coli.*

Irradiated peppers at 0.5 kGy and all combined treatments with irradiations allowed a total inhibition of *E.coli* from day 0 (4 log CFU/g reduction) until the end of storage. According to Jo *et al.* (2005) 2 kGy eliminate *E. coli* from imitation crab leg and surimigel after 24 h of storage. In addition, Wu (2008) reported that injury of microorganisms can occur during gamma irradiation. Furthermore, Prakash *et al.*, (2000) reported also that 1 kGy eliminated *E. coli* from diced celery.

Table 2 Antibacterial activity of sprayed (coating X1+DF) formulation, ozonation (10 ppm; 5 min), irradiation (0.5 kGy) and their combination against *E. coli* inoculated on green peppers, for 10 days of storage at -20°C.

Treatment \ Day	0 (Log CFU/mL)	1 (Log CFU/mL)	4 (Log CFU/mL)	7 (Log CFU/mL)	10 (Log CFU/mL)
Control	$4.0 \pm 0^{c\,E}$	$3.6 \pm 0.05^{e\,D}$	$3.2 \pm 0.15^{b\,C}$	$2.3 \pm 0.12^{b\,B}$	$2.1 \pm 0.12^{b\,A}$
Coating (X1+DF)	$4.0 \pm 0^{c\,C}$	$3.2 \pm 0.2^{d\,B}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$
Ozonation	$4.0 \pm 0^{c\,C}$	$2.8 \pm 0.03^{c\,B}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$
Combined: Ozonation+Coating	$3.7 \pm 0.05^{b\,C}$	$1.5 \pm 0^{b\,B}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$
Irradiation	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$
Combined: irradiation+ Coating	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$
Combined: coating+ ozone +Irradiation	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$	$ND^{a\,A}$

ND: Not detectable (< 10 CFU/g)
[1] Means followed by the same lowercase letter in a same column are not significantly different ($p > 0.05$).
[2] Means followed by the same uppercase letter in a same row are not significantly different ($p > 0.05$).

3.1.2. Inhibiting properties against *S. enterica*

The antimicrobial activity of different treatments was evaluated against *Salmonella enterica* and results are presented in Table 3. Peppers without treatment (control) allowed growth of *Salmonella* during the storage at -20°C (between 3.2 and 3.6 log CFU/g). Peppers treated with (coating X1+DF) allowed a 0.7 log reduction after 24 h followed by a 1.2 log reduction at day 4, then 1 and 0.9 log reduction was observed at days 7 and 10 respectively. Ozone treatment allowed a 1 log reduction after 24 h followed by a 1.2 log reduction at day 7 then 0.7 log reduction at day 10. Peppers treated with

combined treatments allowed 1.3 log reduction after 24 h then 1.4, 1.6 and 1.5 log reduction at days 4,7 and 10 respectively. Das *et al.* (2006), reported that the treatment of tomatoes with ozone at a concentration between 2,500 and 9,500 ppm during 5 to 20 min reduced by 7 log CFU/g *Salmonella* level.

Irradiated green peppers and all combination with irradiation allowed a total inhibition of *Salmonella enterica* from day 0 (with 3.6 log CFU/g reduction) until the end of storage, as with *E. coli*. Previous work had shown that fresh cut lettuce irradiated at 1 kGy decreased microbial spoilage and improved the quality of the products. (Likui Zhang *et al.*, 2004). According to Ahn *et al.* (2005) irradiation at 1 kGy or above (2 or 3) can be used to enhance the microbial safety of minimally process Chinese cabbage without affecting the quality.

Table 3 Antibacterial activity of sprayed (coating X1+DF) formulation, ozonation (10 ppm; 5 min), irradiation and their combination against *S. enterica* inoculated on green peppers, for 10 days of storage at -20°C.

Treatment \ Day	0 (Log CFU/mL)	1 (Log CFU/mL)	4 (Log CFU/mL)	7 (Log CFU/mL)	10 (Log CFU/mL)
Control	3.6 ± 0.03 $^{d\,C}$	3.4 ± 0.06 $^{e\,B}$	3.5 ± 0.1 $^{d\,BC}$	3.2 ± 0.15 $^{d\,A}$	3.5 ± 0.02 $^{e\,B}$
Coating (X1+DF)	3.4 ± 0.02 ^{bD}	2.7 ± 0.06 $^{d\,C}$	2.3 ± 0.16 $^{c\,B}$	2.2 ± 0.05 $^{c\,A}$	2.6 ± 0.05 $^{c\,C}$
Ozonation	3.5 ± 0.02 $^{c\,E}$	2.5 ± 0.02 $^{c\,C}$	2.4 ± 0.05 $^{c\,B}$	2.22 ± 0.05 $^{c\,A}$	2.8 ± 0.05 $^{d\,D}$
Combined Ozone + Coating	3.47 ± 0.04 $^{b\,C}$	2.1 ± 0.12 $^{b\,B}$	2.0 ± 0.17 $^{b\,B}$	1.6 ± 0.17 $^{b\,A}$	2.0 ± 0.1 $^{b\,B}$
Irradiation	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}
Combined Irradiation+ Coating	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}
Combined: Coating+ ozone +Irradiation	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}

ND: Not detectable (< 10 CFU/g).

[1] Means followed by the same lowercase letter in a same column are not significantly different ($p > 0.05$).

[2] Means followed by the same uppercase letter in a same row are not significantly different ($p > 0.05$).

3.1.3. Inhibiting properties against *L. innocua*

The antibacterial activity of different antibacterial treatments was evaluated against *Listeria* and results are presented in Table 4. Results showed that peppers without treatment (control) allowed a

growth of a high-level of *Listeria* during storage at -20°C (3.95 and 3.5 log CFU/g). Coating (X1+DF) treatment allowed a little reduction of 0.5 log after 4 days, followed by a decrease of 0.8 log and 1 log at days 7 and 10 respectively. Ozone treatment showed a similar effect to spray treatment during the 4 first days. With a reduction of 0.5 log at day 4, then 0.3 and 0.4 log reduction at days 7 and 10, this treatment did not reduce significantly *Listeria innocua*, due to the high level of *Listeria* and the resistance of this bacteria. Combined treatment allowed a 0.7 log reduction after 4 days followed by 0.8 log reduction at day 7 then 1 log reduction was observed at day 10.

It was reported that the efficiency of ozone on the reduction of microbial count in fresh produce depend on the dose, exposition time and initial microbial concentration (Han, 2002; Das *et al.*, 2006). Samples treated with irradiation alone allowed a decrease of 2.2 log CFU/g at day 0 then 2.3 log reduction was observed until the end of storage. The combined treatment of irradiation at 0.5 kGy and spray allowed a 2.38 log reduction at day 0 followed by 2.23, 2.23 and 2.63 log CFU/g at days 4, 7 and 10 respectively. The combined treatment of ozone, spray and irradiation inhibited totally the growth of *Listeria* from day 0 until the end of storage.

Table 4 Antibacterial activity of sprayed (coating X1+DF) formulation, ozonation (10 ppm; 5 min), irradiation 0.5 kGy and their combination against *L. innocua* inoculated on green peppers, for 10 days of storage at -20°C.

Treatment \ Day	0 (Log CFU/mL)	1 (Log CFU/mL)	4 (Log CFU/mL)	7 (Log CFU/mL)	10 (Log CFU/mL)
Control	3.9 ± 0.04 $^{c, C}$	3.94 ± 0.01 $^{c, C}$	3.64 ± 0.02 $^{e, B}$	3.5 ± 0.06 $^{f, A}$	3.9 ± 0.05 ^{f,C}
Coating (X1+Df)	3.91 ± 0.07 $^{c, D}$	3.91 ± 0.07 $^{c D}$	3.2 ± 0.02 $^{d C}$	2.7 ± 0.19 $^{d A}$	2.9 ± 0.09 $^{d, B}$
Ozonation	3.94 ± 0.01 $^{d C}$	3.94 ± 0.01 $^{c C}$	3.16 ± 0.13 $^{d A}$	3.2 ± 0.09 e A	3.5 ± 0.02 $^{e B}$
Combined Coating+ozone	3.7 ± 0.12 $^{c B}$	3.7 ± 0.12 $^{c B}$	3.0 ± 0.19 $^{c A}$	2.7 ± 0.07 $^{d A}$	2.9 ± 0.06 $^{d A}$
Irradiated at 0.5 kGy	1.7± 0.17 $^{b A}$	1.63 ± 0.27 $^{b A}$	1.59 ± 0.1 $^{b A}$	1.6 ± 0.1 $^{c A}$	1.6 ± 0.1 $^{c A}$
Combined irradiation + coating	1.57 ±0.17 $^{b AB}$	1.67± 0.17 $^{b B}$	1.47 $^{b AB}$	1.27 ± 0.17 $^{b A}$	1.27 ± 0.17 $^{b A}$
Combined: coating+ ozone +Irradiation	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}	ND ^{aA}

ND: Not detectable (< 10 CFU/g).
[1] Means followed by the same lowercase letter in a same column are not significantly different ($p > 0.05$).
[2] Means followed by the same uppercase letter in a same row are not significantly different ($p > 0.05$).

3.2. Sensory analysis of green peppers

The sensory evaluation of green peppers coated with the coating (X1+DF) formulation is presented in **Table 5**. Results showed that the X1+DF antimicrobial coating did not affect significantly ($p > 0.05$) any of the sensory properties of peppers. Hedonic data indicate ranges of 6.5-7.9 for color, 5.8-6.4 for odor, 6.1-6.4 for taste, 6.4-6.5 for texture and 6.7-6.8 for global appreciation. These results mean that the trend in appreciation was Like moderately/Like very much for color; Like slightly for odor, taste and texture; Like moderately for global appreciation**.**

In particular, the coating (X1+DF) formulation did not affect the color, odor and taste of peppers. Therefore, the sensorial attributes of this preservative formulation could be accepted for further commercialization.

Table 5 Sensory analysis of sprayed (coating X1+DF) formulation, ozonation on green peppers.

Treatment	Hedonic evaluation [1-3]				
	Color	**Odor**	**Taste**	**Texture**	**Global appreciation**
Control (untreated peppers)	6.5 ± 1.2[a]	6.5 ± 1.2[a]	6.4 ± 1.2[a]	6.4 ± 1.2[a]	6.7 ± 1.1[a]
Peppers coated with COATING	7.9 ± 1.0[a]	5.8 ± 1.5[a]	6.1 ± 1.4[a]	6.5 ± 1.2[a]	6.8 ± 1.2[a]

[1] The hedonic evaluation was scaled as follow: 9 = Like extremely; 8 = Like very much; 7 = Like moderately; 6 = Like slightly; 5 = Neither like nor dislike; 4 = Dislike slightly; 3 = Dislike moderately; 2 = Dislike very much; 1 = Dislike extremely.
[2] The equality of variances was determined by Levene's test. Data were compared according to Student *t* test.
[3] In each column, means followed by the same letter are not significantly different ($p > 0.05$).

3.3 Colorimetry of green peppers

Results of colorimetric measurements of green peppers are presented in **Table 6**.

3.3.1. Analysis of the lightness (L*)

At Days 0 and 1, no significant difference of L* ($p > 0.05$) is observed between control and all treatments. At Day 3, a significant decrease of L* ($p \leq 0.05$) was noted after ozonation and the combination of all treatments (coating+ozone+irradiation) (slight darkening from 35.5 to 33.5%). At Day 7, a significant increase of L* ($p \leq 0.05$) was obtained after the combined treatment (coating+ozone) (slight whitening from 33.9 to 35.3%) and a significant decrease ($p \leq 0.05$) was measured after irradiation (darkening from 33.9 to 32.2%). However, these variations are not so important at a macroscopic level (no visual change) and can be attributed to slight variations of whiteness/darkness in peppers lots, therefore suggesting a relative stability of lightness in green peppers throughout storage. Regarding the evolution of L* for each treatment in function of time storage, results show that L* was stable in all samples (values ranging from 32.2 to 35.8%) except in samples treated by coating where a significant increase ($p \leq 0.05$) was observed at Day 1 (from 32.6 to 34.4%). Here again, this whitening variation can be assumed to be due to slight variations of lightness in peppers lots, therefore suggesting a relative stability of lightness in green peppers throughout storage.

3.3.1. Analysis of the a* parameter (green area)

At Day 0, no significant change ($p > 0.05$) was observed between control and treatment excepted for the treatment (ozone+irradiation) after which a significant increase of a* ($p \leq 0.05$) was measured

from -5.8 to -4.0, as a resulting slight reduction of green color. Thereafter, no significant change ($p > 0.05$) was observed after all treatments until Day 7, suggesting a high stability of green color in peppers over storage. Regarding the evolution of a* for each treatment, results show a stability of a* ($p > 0.05$) in all samples (values ranging from -5.9 to -4.0) except in samples treated by coating and by (coating+ozone) after which a significant increase of a* ($p \leq 0.05$) was detected over time, assessing a slight decrease of green color from -6.5 to -5.2, from -6.6 to -5.4 and from -5.4 to -5.1 respectively. However, these variations are not sufficient at a macroscopic visual level and can be related to intrinsic green changes in peppers lots.

3.3.2. Analysis of the b* parameter (yellow area)

The b* parameter tended to be more stable at the detection level of the analyses compared to the L* and a* parameter. Indeed, at Day 0, no significant change ($p > 0.05$) was observed between the samples with values ranging at 13.3-18.2. At Day 1, no significant difference ($p > 0.05$) was noted in-between treatments except significant increases ($p \leq 0.05$) between control samples (13.3), and samples treated by ozone (16.1), (ozone+irradiation) (17.6) and (coating+ozone+irradiation) (15.6), suggesting a slight increase of yellowness in peppers, commonly due to the ozone treatment. However, these variations could also be related to the slight whitening (increase of L*) of peppers at Day 0 after ozonation, were not sufficient to involve visible changes and could be neglected. Thereafter, no significant change ($p > 0.05$) was noted within the groups until Day 7, with a stable b* value ranging from 14.8 to 16.2. This stability of the b* parameter is supposed to generate constant values of hue (h°) as a more suitable parameter to quantify the color properties in vegetables. Regarding the evolution of b* for each treatment, results show a stability of b* values ($p > 0.05$) in all sample groups except for samples treated with the combination (coating+ozone) for which a significant increase of b* ($p \leq 0.05$) occurred at Day 3 (from 14.4 at Day 1 to 16.2 at Day 7). This increase of yellowness parameter could be associated with the increase of L* after treatment by ozonation and combined treatment (coating+ozone), but these changes are not susceptible to produce visual macroscopic modifications.

3.3.3. Analysis of the hue angle (h°)

The hue (h°) is a color parameter mostly used to define the variation of color implying a* and b* axes altogether in the CIELAB color space as an angle on the a*-b* plane calculated using Equation 1, indicating in this case a variation in the yellow-green quarter (90-180°) of the color system. At Day 0, a significant decrease of h° ($p \leq 0.05$) was observed after the treatment (ozone+irradiation) from 110.5 to 102.3°. Morover, at Day 1, a significant decrease was still observed after the treatment (ozone+irradiation) (from 113.0 to 106.0°), but also after coating treatment (109.7°) and ozone treatment (108.9°). These changes of h° angle can be neglected at a visual level as the differences are lower than 10°. Thereafter, no significant change ($p > 0.05$) was observed at Days 3 and 7 between all

samples. This relative high stability of h° throughout 1 week of storage is a quality parameter to indicate that all treatments applied on green peppers are suitable by considering the visual properties of vegetables. Regarding the evolution of h° for each treatment, results show no significant changes ($p > 0.05$) except for the combined treatments (coating+ozone) and (ozone+irradiation) but this difference was attenuated by no transitory difference ($p > 0.05$) of h° at Day 1 with the other days of storage. Moreover, the respective variations of hue from 114.5 to 108.4° and from 102.3 to 107.3° during storage corresponds to a very slight green-yellow color change that is not noticeable visually.

3.3.4. Analysis of the total color difference (ΔE)

The total change of color (ΔE) of green peppers throughout storage was determined in order to evaluate the impact of the treatments in comparison to control samples ($\Delta E = 0$) at each day of analysis. However, the lower accuracy when calculating the index ΔE is due to the fact that it computes the difference in the 3 axes of the CIELAB system. No significant change ($p > 0.05$) was observed at days 0, 1, 3 and 7 for all treatments. Regarding the evolution of ΔE for each treatment, results show no significant variation of ΔE ($p > 0.05$) between Day 0 and Day 7, which demonstrates no color variation when comparing all the treatments. Furthermore, such low variations of the ΔE index do not implicate any perceptible visual changes of color between samples. Therefore, from these experiments, it can be postulated that $\Delta E < 5$ do not indicate noticeable visual changes in green peppers and that the different treatments had no noticeable visual impact on the lightness, the hue and the total color difference of green peppers.

Table 6 Effects of different antimicrobial treatments on the color change of green peppers.

Treatment	Days	Color parameters[1-2]				
		L*	a*	b*	h°	ΔE
Control	**0**	32.6 ± 1.3 a, AB	-5.8 ± 0.3 a,AB	15.6 ± 2.3 a, AB	110.5 ± 2.9 a, BC	NA[3]
	1	33.6 ± 2.0 a, A	-5.6 ± 0.2 a, A	13.3 ± 0.3 a, A	113.0 ± 1.0 a, B	NA
	3	35.5 ± 0.5 a, B	-5.8 ± 0.6 a, A	15.5 ± 1.0 a, AB	110.7 ± 2.8 a, A	NA
	7	33.9 ± 0.5 a, BC	-5.5 ± 0.2 a, A	15.7 ± 0.8 a, A	109.5 ± 1.1 a, A	NA
Coating	**0**	32.6 ± 0.3 a, AB	-6.5 ± 0.4 a, A	16.9 ± 2.2 a, AB	111.3 ± 3.3 a, BC	2.3 ± 1.2 a, A
	1	34.4 ± 1.5 b, A	-5.4 ± 0.4 b, A	15.2 ± 2.5 ab, ABC	109.7 ± 1.8 a, A	2.5 ± 1.4 a, AB
	3	34.8 ± 0.7 b, B	-5.2 ± 0.1 b, A	16.0 ± 0.9 a, AB	108.0 ± 0.9 a, A	1.4 ± 0.3 a, AB
	7	34.6 ± 1.0 b,CD	-5.2 ± 0.1 b, A	16.0 ± 0.9 a, A	108.0 ± 0.9 a, A	1.3 ± 0.6 a, A
Ozonation	**0**	34.6 ± 0.4 ab, AB	-5.3 ± 0.3 a, B	15.5 ± 2.2 a, AB	109.1 ± 3.1 a, B	2.7 ± 0.7 ab, A
	1	35.0 ± 1.5 b, A	-5.5 ± 0.6 a, A	16.1 ± 1.0 b, CD	108.9 ± 2.7 a, A	3.3 ± 1.4 b, AB
	3	33.4 ± 0.6 ab, A	-5.5 ± 0.8 a, A	14.9 ± 0.7 a, AB	110.4 ± 3.6 a, A	2.4 ± 0.6 ab, B
	7	33.2 ± 0.7 a, AB	-5.5 ± 0.8 a, A	14.9 ± 0.7 a, A	110.4 ± 3.6 a, A	1.3 ± 0.8 a, A
Combined Coating+ ozone	**0**	34.2 ± 0.6 a, AB	-6.6 ± 0.5 a, A	14.4 ± 0.6 a, A	114.5 ± 2.5 b, C	2.3 ± 1.2 a, A
	1	33.9 ± 2.4 a, A	-5.2 ± 0.8 b, A	13.8 ± 0.3 ab, AB	110.5 ± 3.2 ab,AB	2.0 ± 1.2 a, A
	3	35.4 ± 0.9 a, B	-5.4 ± 0.3 b, A	15.7 ± 0.3 b, AB	108.8 ± 1.1 a, A	1.0 ± 0.2 a, A
	7	35.3 ± 1.0 a, D	-5.4 ± 0.3 b, A	16.2 ± 0.9 b, A	108.4 ± 1.4 a, A	1.8 ± 0.9 a, A
Irradiated	**0**	32.4 ± 0.9 a, A	-5.9± 0.11 a, AB	15.5 ± 2.3 a, AB	111± 2.9 a, BC	3.45 ± 1.2b A
	1	33.5 ± 0.65 a, A	-5.7 ± 0.4 a, A	14.2 ± 0.9 a, ABC	111.9 ± 2.5 a, B	1.2 ± 0.7 aA
	3	35.5 ±0.5 b, B	-5.8 ± 0.55 a, A	15.5 ± 1 a, AB	110.7 ± 2.8 a, A	1 ± 0.2a A
	7	32.2±0.7 a, A	-5.7 ± 0.1 a, A	15 ± 0.6 a, A	110.8 ± 1 a, A	1.9 ± 0.6 aA
Ozone+ irradiation	**0**	34.9 ± 0.65 a, B	-4 ±0.8 b, C	18.2 ± 0.3 a, B	102.3± 2.2 a, A	2.8 ± 1.4 ab,A
	1	35.8 ± 0.4 a, A	-5 ± 0.6 a, A	17.6± 0.7 a, D	106 ± 2.4 ab, A	4.8 ± 0.8 b B
	3	34.9±0.5 a, B	-5 ±0.05 a, A	17 ± 1.7 a, B	106.4 ± 1.4ab,A	2 ± 1.4 a, AB
	7	34.7±0.6 a, CD	-4.9± 0.15 ab, A	15.9 ± 2.7 a, A	107.3 ± 3 b, A	2.4 ± 0.9 a, A
Coating+ irradiation	**0**	34.9±1.8 a, B	-5.4 ± 0.4 a, B	13.3± 2 a, A	112.2± 2.9 a, BC	1.9 ± 0.75a, A
	1	35.1±0.2 a, A	-5 ± 1.1 a, A	14.7± 0.7 a, ABC	108.8 ± 3.6 a, AB	2.4 ± 0.2a, AB
	3	35.2 ± 0.3 a, B	-5 ±1 a, A	15.5 ±0.9 a, AB	108.2 ± 4 a, A	1.4 ± 0.4 a, AB
	7	34.8 ±0.8 a, CD	-5.1 ± 1 a, A	15.5 ± 0.9 a, A	108.2 ± 4.1 a, A	1.6 ± 0.5 a, A
Coating+ ozone +irradiation	**0**	32.4 ± 0.9 a, A	-5.3 ± 0.55 a, B	15.4 ± 1.4 a, AB	108.9± 1.9 a, B	2.7 ± 1.4 A
	1	34.3 ± 1.5 b, A	-5.2 ± 0.17 a, A	15.6± 1 a, BCD	108.5 ± 1.7 a, AB	2.6 ± 1.2 a, AB
	3	33.7± 0.46 ab, A	-5.1 ±0.6 a, A	14.8 ± 1.5 a, A	109 ± 2.2 a, A	2.4 ± 0.6 a, B
	7	33.5 ±0.4 ab, BC	-5.1 ± 0.6 a,A	14.8 ±1.5 a, A	109 ± 2.2 a, A	1.6 ± 0.7 a, A

[1] For each treatment, means followed by the same lowercase letter from Day 0 to Day 7, are not significantly different ($p > 0.05$)

[2] For each day of analysis, means followed by the same uppercase letter for the comparison of treatments, are not significantly different ($p > 0.05$).

3.4. Determination of chlorophyll content

The effect of the treatments on the chlorophyll content in green peppers is presented in **Table 7**. Results are expressed in content of chlorophyll-a (Ch_a), chlorophyll-b (Ch_b) and total chlorophyll ($Ch_{total} = Ch_a + Ch_b$). Ch_b differs from Ch_a in one aldehyde group bound to the porphyrin ring, and is consequently more soluble than Ch_a in polar solvents. Although Ch_a is recognized as the main pigments which convert light energy into chemical energy by photosynthesis, Ch_b are important accessory pigments as they contribute indirectly in photosynthesis by transferring the light they absorb to Ch_a. (Sciencing 2017). The experimental results show relative stability in chlorophyll content during storage. The slight differences due to the treatments could be associated with intrinsic variations of chlorophyll content in some peppers lots rather than oxidation generated by the coating components, irradiation or ozonation process

3.4.1. Analysis of Ch_a

At day 1, a significant decrease ($p \leq 0.05$) of Ch_a is observed after ozone and irradiation treatments (decrease from 3.48 to 3.32 and 3.30 µg/mL respectively). However, this decrease is not so important at a biological level (slight decrease of 0.16-0.18 µg/mL), possibly due to the intrinsic variation of chlorophyll content in green peppers lots. At days 3 and 7, coating and combined treatment (coating+ozone) allowed a significant decrease ($p \leq 0.05$) of Ch_a content from 3.60 to 3.38 µg/mL at day 3, and from 3.50 to 3.31 µg/mL at day 7. The other treatments did not affect the Ch_a content over time compared to control samples. Regarding the evolution of Ch_a content for each treatment in function of time storage, control samples, samples treated by ozone and those treated by (coating (X1+DF)+ozone+irradiation) showed a stable value of Ch_a (3.48- 3.60 µg/mL in control and 3.32-3.44 µg/mL after ozone). On the other hand, peppers treated by (coating X1+DF), and combined treatment (coating+ozone) were analyzed with a significant decreasing Ch_a content during storage (similar reductions from 3.41 to 3.31 µg/mL). In particular, peppers treated by irradiation showed a significant increase ($p \leq 0.05$) of Ch_a from 3.30 to 3.40 between days 1 and 7. Here again, these decreases are not so important at a biological level and can be attributed to slight variations in peppers lots.

3.4.2. Analysis of Ch_b

At day 1, only the ozone treatment increased significantly ($p \leq 0.05$) the Ch_b content from 6.33 to 6.60 µg/mL whereas all other treatments did not affect Ch_b values ($p > 0.05$). At day 3, the Ch_b content decreased significantly ($p \leq 0.05$) in peppers treated by all treatments except ozone process, with decreases from 6.67 to a range of 6.26-6.26 µg/mL. Contrarily to day 1, the ozone treatment did not affect ($p > 0.05$) the Ch_b content of peppers at day 3, with a value similar to control samples (6.67 µg/mL). At day 7, the Ch_b content was affected ($p \leq 0.05$) by all treatments except irradiation, with decreases ranging from 6.50 to 6.13-6.30 µg/mL. Here again, as observed for Ch_a measurements, a tendency of irradiation process to maintain a stable Ch_b content was noted throughout storage.

Regarding the evolution of Ch_b content for each treatment from day 1 to day 7, results show that Ch_b content was stable in control samples (6.33-6.50 µg/mL), samples treated by coating (X1+DF) (6.13-6.18 µg/mL) and (coating+ozone) (6.13-6.18 µg/mL). On the other hand, Ch_b values decreased in peppers treated by ozone (from 6.60 to 6.26 µg/mL) and (coating+ozone+irradiation) processes (from 6.38 to 6.15 µg/mL). Similarly to Ch_a results, peppers treated by irradiation showed a significant increase ($p \leq 0.05$) of Ch_b from 6.12 to 3.30 between days 1 and 7, suggesting a higher protective effect of chlorophyll content during storage.

3.4.3. Analysis of Ch_{total}

As a subsequent result of Ch_a and Ch_b analyses, no significant effect of the treatments ($p > 0.05$) was observed at days 1 except for samples treated by irradiation for which Ch_{total} was measured at a significantly lower value ($p \leq 0.05$) of 9.43 µg/mL compared to all other samples that display a range of 9.59-9.92 µg/mL. At day 3, the treatments of coating, (coating+ozone), irradiation and (coating+ozone+irradiation) generated a significant decrease ($p \leq 0.05$) of Ch_{total} with values ranging from 9.64 to 9.73 µg/mL compared to 10.27 µg/mL in control samples. In comparison, the ozone treatment did not affect significantly ($p > 0.05$) the Ch_{total} content in peppers with a value of 10.27 µg/mL similar to control samples. At day 7, the coating (X1+DF), (coating+ozone) and (coating+ozone+irradiation) treatments led to a significant decrease of Ch_{total} from 10.01 µg/mL in control samples to 9.43-9.47 µg/mL. In contrast, the ozone and irradiation treatments did not affect adversely ($p > 0.05$) the Ch_{total} content in peppers with respective values of 9.71 and 9.79 µg/mL. Regarding the evolution of Ch_{total} for each treatment during time, the coating and (coating+ozone) treatments induced a significant decrease ($p \leq 0.05$) of Ch_{total} throughout storage (down to 9.43 and 9.79 µg/mL) whereas Ch_{total} was stable in control samples and peppers treated by ozone and (coating+ozone+irradiation), with values ranging at 9.37-10.27 µg/mL. Moreover, as observed for Ch_a and Ch_b measurements, the Ch_{total} content of peppers after irradiation increased significantly ($p \leq 0.05$) over storage from 9.43 to 9.71 µg/mL. Overall, based on the evolution of Ch_a, Ch_b and Ch_{total}, these results suggest that some statistical differences were noted in chlorophyll content of green peppers after coating and combined treatments. But very slight (non-detrimental) decreases were determined at a biological level. Hence, it can be considered that the different treatments did not affect adversely the chlorophyll content in green peppers, which is in accordance with colorimetric results. Otherwise, ozonation and irradiation processes did not generally affect the chlorophyll content throughout storage, suggesting these technologies can be used in combination with antimicrobial sprayed coating without affecting the quality of green peppers based on their green pigmentation and photosystem units.

Table 7 Effects of different antimicrobial treatments on the chlorophyll content of green peppers.

Treatment	Day	Ch_a (µg/mL)	Ch_b (µg/mL)	Ch_{total} (µg/mL)
Control	1	3.48 ± 0.01 [a, C]	6.33 ± 0.06 [a, A]	9.81 ± 0.07 [a, B]
	3	3.60 ± 0.22 [a, B]	6.67 ± 0.41 [a, B]	10.27 ± 0.64 [a, B]
	7	3.50 ± 0.14 [a, BC]	6.50 ± 0.29 [a, B]	10.01 ± 0.43 [a, B]
Coating (X1+DF)	1	3.41 ± 0.02 [b, BC]	6.18 ± 0.04 [ab, A]	9.59 ± 0.06 [b, AB]
	3	3.38 ± 0.01 [b , A]	6.26 ± 0.01 [b, A]	9.64 ± 0.01 [b, A]
	7	3.31 ± 0.03 [a , A]	6.13 ± 0.07 [a, A]	9.43 ± 0.10 [a, A]
Ozonation	1	3.32 ± 0.09 [a , AB]	6.60 ± 0.36 [c, B]	9.92 ± 0.36 [ab, B]
	3	3.60 ± 0.04 [b , B]	6.67 ± 0.08 [b, B]	10.27 ± 0.12 [b, B]
	7	3.53 ± 0.18 [ab , C]	6.26 ± 0.01 [a, A]	9.79 ± 0.18 [a, AB]
Combined Coating+ozone	1	3.41 ± 0.02 [b , BC]	6.18 ± 0.04 [ab, A]	9.59 ± 0.06 [b, AB]
	3	3.38 ± 0.01 [b A]	6.26 ± 0.01 [b, A]	9.64 ± 0.01 [b, A]
	7	3.31 ± 0.15 [a, A]	6.13 ± 0.07 [a, A]	9.43 ± 0.10 [a, A]
Irradiation	1	3.3 ± 0.03 [a A]	6.12 ± 0.06 [a A]	9.43 ± 0.1[a A]
	3	3.45 ± 0.01 [c AB]	6.27 ± 0.04 [b A]	9.73 ± 0.048 [b A]
	7	3.4 ± 0.01[b AB]	6.3 ± 0.01 [b AB]	9.71 ± 0.02 [b AB]
Combination: ozone + irradiation + coating	1	3.44 ± 0.07 [a C]	6.38 ± 0.15 [b A]	9.82 ± 0.22 [a B]
	3	3.44 ± 0.05 [a AB]	6.23 ± 0.08 [ab A]	9.67 ± 0.14 [a A]
	7	3.32 ± 0.03 [a AB]	6.15 ± 0.07 [a A]	9.47 ± 0.11 [a A]

[1] For each treatment, means followed by the same lowercase letter from Day 1 to Day 7, are not significantly different ($p > 0.05$)

[2] For each day of analysis, means followed by the same uppercase letter for the comparison of treatments are not significantly different ($p > 0.05$).

Ch_b = chlorophyll b; Ch_a = chlorophyll a; Ch_{total} = total chlorophyll.

3.5. Determination of vitamin C content

The effect of the treatments on the vitamin C content in green peppers is presented in **Table 8**. Results are expressed in content of free ascorbic acid (AA), dehydroascorbic acid (DHA) and total ascorbic acid (AA+DHA), in mg/100 g extract. DHA always occurs in food in conjunction with AA. The simultaneous determination of DHA and AA is therefore always necessary. The accuracy of the

determination of DHA depends on its relation to AA (the weight ratio of AA/DHA should be < 5:1). The differences related to the treatments could be associated with intrinsic variations of vitamin C content in some peppers lots rather than oxidation of AA into DHA generated by the coating components or ozonation process. However, it was observed that the combined treatments in presence of ozone and/or irradiation induced a higher sensitivity of vitamin C than the other treatments, as discussed below.

3.5.1. Analysis of AA (free ascorbic acid)

At day 1, the AA content is significantly higher ($p \leq 0.05$) for peppers treated by coating and the combined treatments (coating+ozone), (ozone+irradiation) and (coating+irradiation) (60.1, 56.2, 53.9 and 49.5 mg/100 g respectively) compared to control, ozonation and irradiation (45.8, 46.6 and 44.3 mg/100 g respectively). These higher values are most probably due to the presence of ascorbic acid in the coating formulation, especially the content of ascorbic acid in the Foodgard F440 citrus extract. Otherwise, samples treated by irradiation and (coating+ozone+irradiation) induced a significant decrease ($p \leq 0.05$) of AA content to 41.0 mg/100g. At day 14, a lower significant AA content ($p \leq 0.05$) was observed in peppers after all treatments except coating process, with values ranging at 14.1-15.9 mg/100 g, compared to 17.5 mg/100 g for control samples and 19.2 mg/100 g after coating process. These observations suppose that a higher proportion of AA was oxidized during storage, taking into account the impact of ozonation, irradiation and the additional content of vitamin C contained in the antimicrobial sprayed coating. Furthermore, at day 14, the higher AA content is found in control samples and samples treated by coating, confirming that ozone and irradiation promoted the oxidation of AA after combined treatments. Regarding the evolution of AA content for each treatment in function of time storage, significant reductions of AA content ($p \leq 0.05$) are systematically measured for each treatment between day 1 and day 14. Although low final values of 14.1-19.2 mg/100 g are obtained in all sample groups at day 14, the AA reductions are composed of two groups of magnitude: **i)** higher magnitude for samples treated with coating, (coating+ozone), irradiation, (ozone+irradiation) and (coating+irradiation) which have a decrease of 67.8-73.4% of AA; **ii)** lower magnitude for control samples and samples treated with ozone and (coating+ozone+irradiation) which have a decrease of 61.8-65.9% of AA. Otherwise, as observed in previous results of color and chlorophyll analyses, these magnitudes can also be considered as inherent to the oxidation process in green peppers after 14 days of storage. Hence, these differences could be neglected at a global biological level. As a result, it is clearly evidenced that after 14 days, the AA content in green peppers was maintained at an acceptable concentration notwithstanding the kind of treatment.

3.5.2. Analysis of AA+DHA (total ascorbic acid)

The quantification of AA+DHA was carried out similarly to that of AA, but in presence of a reducing agent (DTT) to promote the reverse conversion of DHA into AA. At day 1, proportionally to the AA content, the comparison of the AA+DHA content in function of the treatment followed the same pattern. Significantly higher AA+DHA contents ($p \leq 0.05$) were observed in samples treated by coating and (coating+ozone) (65.4 and 79.0 mg/100 g respectively) compared to control samples (55.5 mg/100 g), due to the presence of ascorbic acid in the coating formulation. Oppositely, a significant decrease ($p \leq 0.05$) was observed in samples treated by (coating+ozone+irradiation) (46.2 mg/100 g) compared to control, probably due to the influence of combined ozone and irradiation to affect the vitamin C in green peppers. Other treatments such as ozone, irradiation, (ozone+irradiation) and (coating+irradiation) did not affect ($p > 0.05$) the AA+DHA content of peppers with values ranging from 49.9 to 56.5 mg/100 g respectively. At day 14,a similar trend is observed in AA analysis, a higher AA+DHA content ($p \leq 0.05$) was measured in peppers treated by coating (58.3 mg/100 g) compared to control samples (47.6 mg/100 g). On the other hand, a significant decrease of AA+DHA content ($p \leq 0.05$) was observed in peppers treated by the combinations (coating+irradiation) and (coating+ozone+irradiation) with respective values of 30.8 and 37.0 mg/100g, whereas no significant difference ($p > 0.05$) was noted between control samples and samples treated by ozone, (coating+ozone), irradiation and (ozone+irradiation) altogether at a range of 40.6-48.1 mg/100 g. Overall, an important aspect of these results is that these variations of AA+DHA are masked by the additional ascorbic acid contained in the coating formulation. Indeed, regarding the evolution of AA+DHA content for each treatment between day 1 and day 14, significant reductions ($p \leq 0.05$) are evidenced for all samples except control samples and samples treated by ozone (due to a low initial AA+DHA content at day 1). Additionally, typical percentages of reduction of AA+DHA were calculated in **Table 8**. Especially, higher percentages were determined after all the combined treatments of (coating+ozone) (39.2%), (ozone+irradiation) (27.0%), (coating+irradiation) (44.8%) and (coating+ozone+irradiation) (20%) compared to control samples and samples treated by single treatments (range of 10.9-14.3% reduction). Therefore, as mentioned for AA analysis, these reductions suggest that ozone and irradiation might contribute to a higher loss of total vitamin when combined with the coating process. Overall, despite these variations, it is important to mention that after 14 days, the AA+DHA (total vitamin C) content in green peppers was maintained at very acceptable concentrations at a biological level, notwithstanding the additional value of vitamins from the coating formulation.

3.5.3. Analysis of DHA (dehydroascorbic acid)

The quantification of DHA in peppers was established by calculating the difference between

AA+DHA and AA measurements. Therefore, it was expected that the evolution of DHA between samples and throughout storage would be inversely proportional to AA measurements. At day 1, the DHA content is significantly higher ($p \leq 0.05$) for peppers treated with the combination (coating+ozone) (22.9 mg/100 g) compared to other samples (4.7-9.7 mg/100 g). This higher value can be due to the additional ascorbic acid in the coating formulation and a higher oxidized part after ozonation (conversion of AA into DHA). In counterpart, all the other treatments did not affect ($p > 0.05$) the DHA content compared to control samples, at a range of 4.7-6.3 mg/100 g, and no significant difference ($p > 0.05$) was observed between each other. At day 14, a higher DHA content is found in peppers treated by coating (39.1 mg/100 g), not significantly different ($p > 0.05$) from samples treated by (coating+ozone) (33.1 mg/100 g), but significantly different ($p \leq 0.05$) from all other samples. Here again, these higher DHA concentrations can be explained by higher initial AA contents related to the addition of the coating that contains ascorbic acid. Regarding the evolution of DHA content for each treatment, significant increases ($p \leq 0.05$) of DHA are systematically reported in all samples, therefore indicating a significant oxidation of initial AA into DHA after 14 days of storage. However, as the DHA level was measured at an acceptable range between untreated and treated samples (16.5-39.1 mg/100 g), it can be postulated that the degree of oxidation of vitamin C in peppers was not affected by treatments after 14 days of storage. This evaluation of DHA allowed determining the level of oxidation of AA, but the oxidized form DHA is as biologically effective as AA in the mechanism of action of total vitamin C.

Finally, these analyses showed some statistical differences in vitamin C content after coating treatment and mostly after combined treatments implying ozone and/or irradiation, mainly due to the presence of ascorbic acid in the coating formulation, as a major part of vitamin oxidized by ozone and irradiation. However, the range of vitamin C was measured in all samples at very satisfying concentrations ranging from 30.9 to 58.3 mg/100 g peppers, therefore confirming that the different treatments did not adversely affect the vitamin C content at a biological level.

Table 8 Concentrations (mg/100 g) of L-(+)-ascorbic acid (vitamin C) decomposed into free ascorbic acid (AA), dehydroascorbic acid (DHA) and total ascorbic acid (AA+DHA) in green peppers extracts as a function of treatments and storage time.

Treatment	Days	Vitamin C content (mg/100 g)[1-3]			Percentage of reduction (AA+DHA) (%)[4]
		AA	DHA	AA+DHA	
Control	**1**	45.84 ± 1.68 ^{b}	9.65 ± 2.34 ^{a}	55.50 ± 4.01 ^{b}	14.3
	14	17.49 ± 1.19 ^{c*}	30.07 ± 5.54 ^{cd*}	47.57 ± 6.74 ^{cd}	
Coating (X1+DF)	**1**	60.10 ± 3.2 ^{e}	5.34 ± 0.61 ^{a}	65.44 ± 2.6 ^{c}	10.9
	14	19.22 ± 1.11 ^{d*}	39.11 ± 1.83 ^{e*}	58.33 ± 1.13 ^{e*}	
Ozone	**1**	46.58 ± 0.46 ^{bc}	5.05 ± 0.46 ^{a}	51.63 ± 0.80 ^{ab}	11.2
	14	15.88 ± 0.37 ^{b*}	29.95 ± 4.30 ^{cd*}	45.83 ± 4.17 ^{cd}	
Coating + Ozone	**1**	56.16 ± 2.66 ^{d}	22.87 ± 7.88 ^{b}	79.04 ± 10.14 ^{d}	39.2
	14	14.96 ± 0.93 ^{ab*}	33.11 ± 2.00 ^{de*}	48.06 ± 1.19 ^{d*}	
Irradiation	**1**	44.35 ± 1 ^{ab}	5.56 ± 1.05 ^{a}	49.9 ± 1.3 ^{ab}	11.1
	14	14.14 ± 0.4 ^{a*}	30.2 ± 1.5 ^{cd*}	44.34 ± 1.78 ^{cd*}	
Ozone + Irradiation	**1**	49.55 ±1^{c}	6.31 ± 1.4 ^{a}	55.9 ± 2.2 ^{b}	27
	14	14.76 ± 0.92 ^{ab*}	25.8 ± 3.7 ^{bc*}	40.6 ± 4^{bc*}	
Coating + Irradiation	**1**	53.86 ± 2.26 ^{d}	4.75 ± 2 ^{a}	56.46 ± 1.22 ^{b}	44.8
	14	14.39 ± 1.3 ^{ab*}	16.46 ± 4 ^{a*}	30.85 ± 4.2 ^{a*}	
Coating + Ozone + Irradiation	**1**	41 ± 1.44 ^{a}	5.19 ± 1 ^{a}	46.21 ± 2.31 ^{a}	20
	14	14.89 ± 0.42 ^{ab*}	22.15 ± 3.11 ^{ab*}	37 ± 3.5 ^{ab*}	

[1] For each day of analysis, means followed by the same lowercase letter, for the comparison of treatments, are not significantly different ($p > 0.05$).

[2] For each treatment, means followed by an asterisk (*), from Day 0 to Day 14, are significantly different ($p \leq 0.05$).

[2] AA=Ascorbic Acid, DHA=Dehydroascorbic acid.

[3] Percentage of reduction of total vitamin C (AA+DHA) determined between Day 1 and Day 14.

4. Conclusion

In this work, different antimicrobial treatments have been tested against three target bacteria. Our results suggested a high antimicrobial effect of all tested treatments: coating, ozone and irradiation). Irradiation at 0.5 kGy was the most effective treatment to reduce all studied microorganisms. The combination of ozone and coating reduces significantly all studied bacteria. It is important to note that the combination of (ozone, coating and irradiation) inhibits quickly and effectively *L. innocua*, *E. coli* and *S. enterica*.

The results of colorimetry suggested that all treatment did not affect the color parameters (L*, a*, b*,

hue and ΔE) of green peppers. In addition, chlorophyll results showed that the use of ozonation and coating alone or in combination did not affect negatively the chlorophyll content. The effect of the coating, ozonation, irradiation, and combined treatment on the chlorophyll content of the peppers has shown no detrimental decrease of total chlorophyll at a biological level of 9.4-10.0 µg/mL of extract.

The presence of vitamin C in Foodgard can sometimes help to counterbalance the loss of vitamin C during the storage and the treatments. But overall, similar trends of vitamin C content were determined within all groups (control and treated samples) at very acceptable concentrations, ensuring a possible application of such treatments during storage.

The evaluation of organoleptic attributes of coating revealed no difference compared to control samples.

This development of antimicrobial treatments can be used in the food industry with the purpose to reduce and inhibit foodborne pathogens and to ensure food safety.

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CHAPITRE 5 : DISCUSSION GÉNÉRALE

Cette étude avait pour objectifs de mettre en place différentes méthodes pour la réduction et l'inactivation des bactéries pathogènes qui risquent de contaminer les fruits et légumes prêts à manger. Pour ce faire, on peut subdiviser notre étude en cinq parties :

Essentiellement, une première partie consacrée à l'étude des composés naturels bioactifs qui ont la capacité de réduire les bactéries pathogènes tels que les huiles essentielles, les acides organiques, le jus de canneberge et le dextrose fermenté. Une deuxième partie, pour l'évaluation de l'ozone gazeux sur les légumes incluant les pré-tests faits afin de sélectionner la concentration la plus efficace et le temps le plus adéquat pour réduire la concentration des bactéries étudiées. Une troisième partie dédiée à l'évaluation de l'irradiation gamma sur les légumes prêts à manger. Une quatrième partie pour voir les effets synergiques des combinaisons entre les traitements cités précédemment (ozone, formulation antimicrobienne, irradiation). Une cinquième partie qui consiste à faire des analyses de qualité des aliments testés et vérifier si les traitements ont affecté leurs attributs sensoriels.

1. Les formulations antimicrobiennes naturelles

Les résultats *in vitro* ont montré que la capacité inhibitrice du jus de canneberge était la plus élevée par rapport aux autres traitements (acides citrique, quinique, malique). Le jus de canneberge neutralisé a montré une capacité d'inhibition plus faible que celle du jus à un pH de 2.4. En effet, pour la même formulation, la diminution du pH augmente l'activité antimicrobienne. De plus, Almajano *et al.* (2007) ont observé les mêmes résultats en présence de l'acide caféique dans une émulsion d'huile dans l'eau. Le pH et les changements dans l'état d'ionisation et la proportion de molécules non dissociées de l'acide caféique à différentes valeurs de pH modifient l'activité antimicrobienne. En effet, l'activité antimicrobienne des acides faibles dépend du pH en raison de l'influence de ce paramètre sur la proportion de molécules d'acide non dissociées dans la solution. Coté *et al.* (2011) ont démontré que le pH faible n'est pas responsable tout seul de l'effet antimicrobien, mais aussi la présence d'autres composés peut être lié à l'activité antimicrobienne. Parmi ces composés, Coté *et al.* (2011) ont cité : les flavanols, flavan-3-ols, anthocyanins, tannins et les acides phénoliques dérivés. Ces composés bioactifs ont beaucoup d'effets bénéfiques sur la santé, ils jouent le rôle d'agents antioxydants, antimicrobiens et anticancéreux. Il est important de mentionner que les résultats obtenus ont montré un ordre de résistance des bactéries au jus de canneberges était comme suit : *L. monocytogenes* > *S.* Typhimurium > *E. coli.* Les composés antimicrobiens présents dans le jus de canneberges peuvent endommager la paroi cellulaire et induire ainsi la lyse cellulaire, ce qui provoque la perte des constituants cellulaires (Wu *et al.*, 2008).

Les résultats de la détermination des concentrations inhibitrices ont montré que le jus de canneberges avait les plus faibles CMI (c'est-à-dire un effet antimicrobien plus efficace) de 3133 contre les trois bactéries étudiées par rapport aux autres acides (quinique, citrique, malique). Le jus de canneberge a été plus efficace que le benzoate de sodium contre les trois bactéries.

Nos résultats sur l'effet synergique entre le faible pH (causé par les acides organiques) et la présence des composés phénoliques sont en accord avec ceux d'Alnoman *et al.* (2015).

Le jus de canneberge a réduit de 1 et de 1.8 log UFC/g le niveau de *L. monocytogenes* aux jours 1 et 7 respectivement dans les poivrons rouges à 4°C. D'après Wu *et al.* (2008), *L. monocytogenes* est la bactérie la plus résistante en la comparant à *E. coli* et à *S.* Typhimurium. Seltmann et Holst (2002) ont rapporté que les peptidoglycanes sont responsables de l'augmentation de la rigidité des cellules bactériennes. En effet, *L. monocytogenes* n'était pas facilement détruite par la formation de lésions dans la paroi cellulaire (Wu *et al.*, 2008).

Une réduction de 2.4 log UFC/g de *E. coli* a été observée après 7 jours à 4°C, il a été observé que l'effet antimicrobien du jus augmente avec le temps. Wu *et al.* (2009) ont mentionné aussi que le temps et la concentration ont un effet synergique sur la réduction d'*E. coli* O157:H7 en présence du concentré de canneberges. Coté *et al.* (2011) ont aussi trouvé que *E. coli* a été affectée par la présence de fractions de jus de canneberges neutralisé riche en anthocyanines.

Le jus de canneberge a réduit de 1.2 log UFC/g le niveau de *S.* Typhimurium au jour 1 suivi d'une inhibition totale après 7 jours à 4°C. L'activité antimicrobienne du jus de canneberge est due à la présence de composés bioactifs tels que les anthocyanines, non anthocyanines, polyphénols (acides benzoïque, caféique, etc.) en combinaison avec le bas pH qui est dû à la présence d'acides organiques. D'après Wu *et al.* (2009) l'exposition de *S.* Typhimurium au concentré de canneberges à une concentration de 5 µL/mL a induit des dommages morphologiques, perte de la structure de la paroi cellulaire et la présence de débris d'ADN dans l'espace extracellulaire.

Le jus de canneberge était largement plus efficace que l'hypochlorite de sodium. De plus, l'utilisation de ce dernier produit chimique peut induire la formation de sous-produits dangereux pour la santé (chloramines et trichloramines) et aussi peut altérer le goût des fruits et légumes. En effet, l'utilisation du chlore pour le traitement des fruits et de végétaux peut induire des saveurs désagréables et altérer le goût (Hasenberg *et al.*, 2008).

Les formulations F2 et F6 contenant un mélange de composés actifs mis au point précédemment dans notre laboratoire ont été testées sur deux légumes prêts à manger (poivrons rouges découpés, pommes de terre découpées et pré-frites) et un fruit (canneberges).

Les résultats de la détermination de CMI ont montré que F2 et F6 ont induit de faibles CMI (fortes capacités antibactériennes). F2 était plus efficace que F6 avec une CMI de 1 700 ppm contre

L. monocytogenes et *S.* Typhimurium. F2, F6 ont agit comme des antimicrobiens efficaces contre les bactéries dans un intervalle de 1 700 à 3 500 ppm.

L. monocytogenes a été faiblement affectée par F2 et F6 par rapport à *E. coli*. En effet F2 et F6 ont réduit seulement 90% la population de *L. monocytogenes*. Aydin *et al.* (2005) ont expliqué que la cible principale du carvacrole est la membrane bactérienne suivie de l'interaction avec l'ADN. De plus, d'après Farber et Peterkin (1991), *L. monocytogenes* peut s'adapter aux faibles températures en produisant des phospholipides avec des acides gras courts. Suivi d'un changement dans l'expression de la membrane lipidique avec une déstabilisation des ribosomes. La formulation F2 était plus éfficace que F6 et a réduit de plus de 99% la concentration d'*E. coli* dans les poivrons rouges. En fait, Burt *et al.* (2007) a rapporté que l'origan appliqué à une concentration de 0.3, 0.5, 0.8, ou à 1 mM était responsable de l'augmentation de la heat shock protéine HSP60 et responsable aussi de l'inhibition de la synthèse du flagelle de *E. coli* O157:H7. Guarda *et al.* (2011) ont aussi rapporté que le thymol et la carvacrol ont désintégré la membrane externe chez les bactéries Gram (-) et cela est due à la libération des lipopolysaccharides et à l'augmentation de la perméabilité à l'adénosine triphosphate (ATP) dans la membrane cytoplasmique. Ce qui a modifié la perméabilité passive de la cellule et par la suite a détruit la bactérie. Les enrobages F2 et F6 ont montré un effet bactéricide élevé contre *S.* Typhimurium avec une réduction de plus de 99% dans les poivrons rouges.

Les résultats *in situ* obtenus ont montré que les formulations F2 et F6 étaient efficaces pour réduire les pathogènes étudiés sur les canneberges.

De plus, les deux formulations F2 et F6 ont inhibés totalellement *E. coli* au jour 4. Le niveau de *S.* Typhimurium a été réduit et une inhibition totale a été observée après le jour 14, indiquant un comportement similaire à celui de *E. coli* et *L. monocytogenes*. Finalement, on peut conclure que les traitements F2, F6 ont agit en tant qu'antibactériens efficaces pour réduire les trois pathogènes étudiées sur les poivrons rouges.

Nos résultats obtenus sur les pommes de terre découpées et pré-frites conditionnées sous MAP (pour éviter le brunissement) ont montré que les formulations F2 et F6 avaient un effet bactéricide contre *E. coli* et *Salmonella* avec 0.5 log UFC/g de réduction pour *E. coli* et 1 log UFC/g de *Salmonella.* Pas d'inhibition *de L. innocua* sur les pommes de terre. En effet, d'après Canillac et Mourey (2004) et Ismaiel *et al.* (1990), l'inhibition de l'effet antimicrobien du carvacrol sur le développement microbien est peut-être due à la présence de gras ou de protéines dans l'aliment. L'effet de F2 et F6 a été réduit car les pommes de terre étaient grasses et imbibées d'huile de friture ce qui pourrait avoir crée une barrière protectrice entre la bactérie et l'agent antibactérien.

Nous constatons ainsi que les formulations F2 et F6 étaient plus efficaces contre Gram (-) *E. coli* et *S.* Typhimurium dans les poivrons rouges que contre les Gram (+) *L. monocytogenes.* La formulation F2 peut être employée comme antimicrobien efficace contre les trois pathogènes étudiés dans les poivrons

rouges. De plus, notre étude est en accord avec celle de Tawema *et al.* (2014) ou *L. monocytogenes* était la bactérie la plus résistante aux différents traitements vaporisés avec les antimicrobiens naturels. Nos résultats confirment l'hypothèse de la libération contrôlée des composés bioactifs incorporés dans l'émulsion. Il est important de noter que l'activité antimicrobienne des huiles essentielles est attribuée aux composés phénoliques, et aussi à l'effet synergique entre les composés comme les térpénoides, aldéhydes etc. (Tawema *et al.*, 2014; 2016).

La combinaison synergique entre les acides organiques et les huiles essentielles a été déjà démontrée par plusieurs études (Aksit *et al.*, 2006 ; Friedly *et al.*, 2009), en réduisant le pH et en augmentant l'hydrophobicité des huiles essentielles pour affecter la membrane bactérienne.

De plus, les résultats des analyses sensorielles ont montré que les formulations F2 et F6 sont très acceptables sur le plan organoleptique et sensoriel. Ces deux enrobages n'ont pas affecté négativement les propriétés organoleptiques des poivrons rouges. F2 a été préféré par rapport à F6 dans les pommes de terre.

Les huiles essentielles d'agrumes sont connues pour leur activité antimicrobienne élevée. Elles sont une source importante d'arômes et particulièrement de flavonoïdes qui sont des composés polyphénoliques incluant les flavanones, les flavones et leurs dérivés.

Les résultats *in vitro* de la capacité d'inhibition de l'extrait d'agrumes (Foodgard F410b) et celle du benzoate de sodium (conservateur synthétique) contre les bactéries Gram (-) et Gram (+) ont montré que l'extrait d'agrumes était largement plus efficace que le benzoate de sodium (qui était efficace à des concentrations supérieures aux limites autorisés par la FDA). On peut conclure que l'extrait d'agrumes était plus efficace que le benzoate contre tous les pathogènes. Néanmoins, Alnoman *et al.* (2015) ont rapporté que le sorbate de potassium et le benzoate de sodium n'ont pas pu inhiber le développement de spores de *C. perfringens* dans le poulet et il est vraisemblable qu'il en serait de même pour l'extrait de citrus que nous n'avons pas testé contre des spores.

Les résultats des concentrations minimales inhibitrices ont montré aussi des résultats similaires à ceux des résultats de capacité d'inhibition. L'extrait d'agrumes était 20 à 50 fois plus efficace que le benzoate. D'après ces résultats, l'extrait d'agrumes pourrait être utilisé comme conservateur naturel avec une activité antimicrobienne importante pour éliminer les Gram (+) et les Gram (-). En effet, cet extrait naturel contient des bioflavonoïdes comme ingrédients actifs bénéficiant de fonctionnalités antimicrobiennes et préservatives. Cosentino *et al.* (1999) ont rapporté que les composés phénoliques qui sont présents dans les extraits de citrus ont montré une activité antimicrobienne contre les bactéries Gram (-) et Gram (+). Cet extrait était plus efficace contre les bactéries Gram (+) que contre les Gram (-) et cela peut être expliqué par la présence d'une membrane externe chez les bactéries Gram (-) qui réduit l'accessibilité de l'extrait de citrus à la structure cellulaire interne de la bactérie. Belletti *et al.*

(2004), Helander *et al.* (1998) et Puuponen-Pimia *et al.* (2004) ont attribué l'activité antimicrobienne à la présence de phénols, d'acides phénoliques, de quinones, de saponines, de flavonoïdes, de tannins, de coumarine, de terpénoides et d'alcaloïdes.

Les résultats *in situ* montrent que l'incorporation de l'extrait d'agrumes à une concentration de 0.2% dans les garnitures de fraises (pH = 3.21, a_w = 0.935) préalablement inoculées avec *L. monocytogenes* a réduit de 1.3 log UFC/g le niveau de *L. monocytogenes* au jour 1 à 4°C, suivi d'une inhibition totale au jour 7. Ces résultats suggèrent une activité antimicrobienne rapide de l'extrait d'agrumes. Par contre, le benzoate a réduit de 1.3 log UFC/g le niveau de *L. monocytogenes* et l'a inhibé totalement au jour 4 ce qui est encore plus rapide.

L'extrait d'agrumes a permis une inhibition totale de *S. aureus* (4 log de réduction) après 4 jours de stockage dans les garnitures de fraises. Cet antimicrobien naturel était plus rapide et plus efficace que le benzoate de sodium employé à une concentration de 1% où 2 log UFC/g de réduction de *S. aureus* seulement ont été observées au jour 4, suivi d'une inhibition totale au jour 21.

L'incorporation du Foodgard (extrait d'agrumes) dans les garnitures de fraise contaminées avec *B. cereus* a réduit le niveau bactérien à 2.5 log UFC/g au jour 1, suivi de 1 log UFC/g de réduction au jour 7. Une inhibition totale a toutefois été observée au jour 14 (réduction de 4 log UFC/g). Le benzoate de sodium a induit 0.9 log UFC/g de réduction après une semaine de stockage à 4°C dans les garnitures de fraises ensuite une inhibition totale a été observée au jour 14. L'extrait d'agrumes a exhibé une activité antibactérienne élevée et a permis une réduction significative de 4 log UFC/g au jour 14. L'extrait d'agrumes a montré une inhibition similaire au benzoate de sodium ou encore plus efficace que le benzoate contre Gram (+). De plus, l'extrait d'agrumes a réduit la concentration d'*E. coli* sous les limites de détection au jour 4 et a inhibé totalement le développement de *S.* Typhimurium au jour 4 (4 log de réduction). Le benzoate a quant à lui montré une inhibition totale de *S.* Typhimurium après le jour 7. Nos résultats sont en accord avec ceux de Chaisawadi *et al.* (2003) qui a rapporté que les huiles essentielles extraites de la pelure d'agrumes ont montrées une activité antimicrobienne contre *B. cereus*, *S. aureus* et *S.* Typhimurium. En effet, l'activité des huiles essentielles dépend de leur composition. Les composés volatils majeurs de l'huile essentielle d'agrumes étaient les monoterpènes hydrocarbonés qui ont un effet destructif sur les bactéries. Plusieurs études ont montré que les composés phénoliques présent dans les extraits d'agrumes peuvent affecter l'activité enzymatique, causent la dénaturation des protéines, modifient la perméabilité cellulaire, induisent l'augmentation de la perméabilité de la membrane cellulaire provoquant la perte des constituants cellulaires et aussi diminuent la concentration d'ATP et le pH intracellulaire.

Il est important de noter que l'activité antimicrobienne totale de l'extrait d'agrumes (Foodgard) ne peut pas seulement être attribuée aux composés phénoliques, mais aussi à l'effet synergique entre ces

constituants (flavonoïdes, acides phénoliques, flavanones) et aussi la composition des garnitures de fraises où on trouve une présence importante des anthocyanines et des flavan3-ols, de la catéchine ou des procyanidines (Aaby *et al.*, 2012).

La couleur, la texture, l'odeur, la saveur et l'appréciation globale des garnitures de fraises ont été déterminées selon un test hédonique de 9 chiffres. L'incorporation de l'extrait d'agrumes dans les garnitures de fraises n'a pas affecté significativement la couleur, la texture, la saveur, l'odeur ni l'appréciation globale des garnitures de fraises. Aucune différence significative n'a été observée entre les différents traitements ($p > 0.05$). Foodgard a permis une acceptation sensorielle avec une bonne appréciation de la qualité globale par rapport au benzoate.

La formulation mise au point à base de jus de canneberge, d'huiles essentielles, de dextrose fermenté et de l'extrait d'agrumes a été efficace pour réduire les trois bactéries étudiées. En effet, une inhibition totale d'*E. coli* au jour 4 (3 log UFC/g de réduction)a été observée dans les poivrons verts. De plus, cette formulation a réduit significativement le niveau de *S. enterica* (1.2 log UFC/g) Aussi, une réduction de 1 log UFC/g de *L. innocua* dans les poivrons verts a été constaté au jour 10. Ainsi, l'ordre de sensibilité à la formulation x1+df est le suivant *E. coli* > *S.* Typhimurium > *L. innocua.*

2. Ozone gazeux

Ce traitement a réduit de 1.2 log UFC/g le niveau de *E. coli* dans les poivrons verts après 24h, suivi d'une inhibition totale à partir du jour 4 et jusqu'à la fin du stockage à -20°C. Bermudez-Aguirre *et* et Barbosa-Cánovas (2013) ont rapporté que le traitement des tomates à l'ozone gazeux à une concentration de 5 ppm pendant 3 à 15 min a réduit de 2.2 log UFC/g la concentration *d'E. coli*.

De plus, ce traitement de 10 ppm pendant 5 min a aussi réduit de 1 log UFC/g le niveau de *S. enterica* dans les poivrons verts après 24 h, suivi d'une réduction de 1.2 et de 0.7 log UFC/g aux jours 7 et 10 respectivement.

L'ozone a montré un effet similaire à celui de la formulation X1+DF durant les 4 premiers jours avec 0.5 log UFC/g de réduction de *L. innocua,* dans les poivrons verts, suivi d'une réduction de 0.3 et 0.4 log aux jours 7 et 10 de stockage à -20°C. Ce traitement n'a pas réduit suffisament le niveau de *Listeria innocua* et cela est dû à la concentration initiale qui est élevée (4 log UFC/g) et aussi à la résistance de *Listeria* à ce traitement. L'efficacité du traitement d'ozone sur la réduction microbienne dans les produits frais dépend de la dose, du temps d'exposition et de la concentration microbienne initiale (Han *et al.*, 2002; Das *et al.*, 2006).

3. L'irradiation

L'irradiation des poivrons verts à une dose de 0.5 kGy a permis une inhibition totale de *E. coli* (4 log UFC/g de réduction) au jour 0 jusqu'à la fin du stockage. D'après Jo *et al.* (2005) une dose de 2 kGy a éliminé *E. coli* dans les imitations de pattes de crabes après 24 h de stockage. Aussi Prakash

et al. (2000) ont rapporté que 1 kGy a éliminé *E. coli* dans les céleris coupés en dés. De plus, Wu *et al.* (2008) ont rapporté qu'au cours de l'irradiation gamma, des lésions des microorganismes peuvent se produire.

Le traitement des poivrons verts à une dose de 0.5 kGy a éliminé totalement *Salmonella* au jour 0 et jusqu'à la fin du stockage. L'irradiation des laitues découpées à une dose de 1kGy a réduit le niveau des microorganismes d'altération et a amélioré sa qualité (Likui *et al.*, 2004). D'après Ahn *et al.* (2005), l'irradiation à 1 kGy ou plus (2 ou 3 kGy) peut être utilisée pour augmenter la sécurité sanitaire des choux chinois sans affecter la qualité.

Après le traitement des poivrons verts à 0.5 kGy, *L. innocua* a été réduit de 2.2 log UFC/g au jour 0. Cependant, pas d'inhibition totale n'a été observée de *L. innocua* durant le stockage.

4. Combinaison de traitements

La combinaison de l'ozone gazeux (10 ppm – 5 min) et de la formulation X1+DF a réduit de 2.1 log le niveau de *E. coli* dans les poivrons verts au jour 1, suivi d'une inhibition totale à partir du jour 4.

De plus, une réduction de 1.3 log de *S. enterica* a été observée au jour 1 dans les poivrons verts suivi d'une réduction de 1.5, 1.6, et de 1.5 log UFC/g aux jours 4, 7 et 10 respectivement.

L. innocua était la bactérie la moins affectée par cette combinaison par rapport aux deux autres bactéries testées. En effet, le niveau de *L. innocua* a été réduit de 0.7 log UFC/g au jour 4 dans les poivrons verts suivi de 0.8 de 1 log UFC/g aux jours 7 et 10. Ainsi, l'ordre de sensibilité des bactéries au traitement combiné (ozone + Coating) est le suivant : *E. coli* > *S. enterica* > *L. innocua.*

Le traitement combiné de l`irradiation à 0.5 kGy et de la formulation X1+DF a inhibé totalement *E. coli* et *S. enterica* dans les poivrons verts du jour 0 jusqu'au jour 10. Ces résultats montrent que l'irradiation et la formulation antimicrobienne ont exercés un effet antimicrobien additif et ont permis une inhibition rapide et immédiate des deux bactéries. Ce traitement combiné a aussi réduit de 2.4 log UFC/g le niveau de *L. innocua* au jour 0 suivi d'une réduction de 2.2, 2.2, et de 2.63 log UFC/g aux jours 4, 7 et 10 de stockage à -20°C. Ici aussi, il est clair que les deux traitements antimicrobiens ont augmenté la réduction du niveau de *Listeria*. Tawema *et al.* (2016) ont démontré que l'application de faibles doses d'UV-C ou d'irradiation gamma suivies de l'application de faibles quantités d'agents antimicrobiens ont réduit les pathogènes d'origine alimentaire et augmenté la durée de vie des choux-fleurs. Il a été démontré aussi que l'irradiation des végétaux en présence de composés actifs naturels peut réduire les doses létales de bactéries pathogènes (Takala *et al.*, 2011).

La combinaison des trois traitements mise au point (irradiation à 0.5 kGy + ozone gazeux à 10 ppm – 5 min + formulation antimicrobienne naturelle) a induit une inhibition totale des trois bactéries étudiées (*E. coli, S. enterica, L. innocua*) dans les poivrons verts (avec 4 log UFC/g de réduction) à

partir du jour 0 et jusqu'à la fin du stockage. Cette combinaison a manifesté un pouvoir antimicrobien élevé et rapide. Dans une autre étude, certains composés bioactifs et huiles essentielles ont augmenté la radiosensibilité d'*E. coli* dans la viande (Turgis *et al.*, 2008). En effet, Turgis *et al.* (2008) ont trouvé que la sensibilité d'*E. coli* aux radiations a augmenté dans la viande hachée de 1 à 3.57 logs de réduction dépendant de l'huile essentielle et de ses composés majeurs. Par exemple, en présence de l'huile essentielle de cannelle de Chine ou d'origan Espagnol, la dose minimale de radiation gamma a été réduite de 1.2 à 0.35 kGy (Turgis *et al.*, 2008).

5. Analyses de la qualité des poivrons verts découpés en cubes

La couleur

La mesure des paramètres de la couleur (**L*, a*, b*, teinte, ΔE**) a été réalisée afin de voir l'effet des traitements sur la couleur. Les résultats obtenus ont montré qu'il n'y a pas de différence significative entre les traitements ($p > 0.05$). En effet, les poivrons verts découpés en cubes n'ont pas été affectés négativement par les différents traitements antimicrobiens.

Les résultats de mesures du paramètre de clarté **L*** (qui est un paramètre variant de 0 correspondant au noir à 100 correspondant au blanc) ont montré que les échantillons de poivrons non traités et traités à l'ozone, à l'irradiation, à la formulation (coating X1+DF), et aux différentes combinaisons étaient stables, les différents traitements antimicrobiens n'ont pas affectés négativement le paramètre de clarté L*.

Les résultats de mesures du paramètre a* (qui nous indiquent la variation de la couleur du vert (-300) au rouge (+299) indiquent que l'application des traitements antimicrobiens n'a pas affecté négativement ce paramètre. Certaines différences très faibles étaient dues aux paramètres intrinsèques des poivrons verts découpés.

Le paramètre **b*** représente la variation de la couleur du bleu (-300) au jaune (+299), était un paramètre plus stable que **a*** et **L***. Les résultats obtenus montrent que les traitements antimicrobiens n'ont pas affecté négativement le paramètre **b***, avec des valeurs comprises entre 13.3 et 18.

Le paramètre de couleur, la **teinte**, souvent utilisée pour déterminer la variation de couleur impliquant les axes a* et b* ensembles dans l'espace des couleurs CIELAB indiquant la variation de l'angle du jaune au vert (90-180°). Les traitements antimicrobiens appliqués sur les poivrons verts découpés ont faiblement affecté la teinte. En effet, une variation de la valeur de la teinte a été observée de 114.5 à 108.4° après l'application des différents traitements durant le stockage à -20°C, ce qui correspond à un léger changement de couleur du vert au jaune qui n'est pas perceptible à l'œil nu.

Le paramètre **ΔE**, correspondant au changement total de couleur, a été déterminé dans le but d'évaluer l'impact des traitements antimicrobiens en comparaison par rapport au témoin. Des variations très faibles ont été observées n'indiquant aucun changement de couleur notable visuellement.

La teneur en chlorophylle

La mesure de la teneur en chlorophylle a montré que les différents traitements antimicrobiens n'ont pas affectés négativement la qualité des poivrons verts.

Les résultats obtenus de la teneur en chlorophylle (a) ont montré une stabilité de cette teneur durant le stockage à -20°C. En effet, une légère diminution a été observée après le traitement à l'ozone et à l'irradiation, mais cette diminution n'est pas importante à un niveau biologique probablement dû aux variations intrinsèques de la teneur en chlorophylle dans les poivrons verts (de 3.3 à 3.6 µg/mL).

Les résultats de la teneur en chlorophylle (b) ont montré une légère variabilité dans les valeurs, mais ces variations ne sont pas importantes. Les différents traitements antimicrobiens n'ont pas affecté négativement la teneur en Ch (b) (6.3 à 6.67 µg/mL).

De faibles variations de la teneur en chlorophylle totale ont été observées mais elles ne sont pas importantes, en effet, elles ne sont pas dues à l'effet des traitements antimicrobiens, mais aux propriétés intrinsèques des poivrons verts découpés. Les changements constatés sont négligeables et et ceci est vrai autant pour la chlorophylle (a) que (b).

La teneur en vitamine C

Les valeurs élevées d'acide ascorbique obtenues pour la formulation (coating X1+DF) et le traitement combiné coating/ozone est due à la présence additionnelle d'acide ascorbique dans la formulation (coating X1+DF) qui provient majoritairement du Foodgard F440 extrait d'agrumes.

Au jour 14, la teneur en acide ascorbique a diminué pour tous les échantillons, cela est dû à la transformation de l'acide ascorbique en acide déhydroascorbique par oxydation. Les valeurs obtenues pour les échantillons poivrons témoins et les échantillons traités à la formulation (coating X1+DF) présentaient des valeurs plus élevées (de 17.5 et 19.2 mg/100 g pour le témoin et le coating X1+DF respectivement) que les autres échantillons de poivrons.

Un faible pourcentage de réduction de vitamine C totale a été observé au jour 14 pour les échantillons de poivrons verts non traités et ceux traités à la formulation (coating X1+DF), ozone, irradiation, et la combinaison (coating/ozone/irradiation) ayant les pourcentages de réduction suivants : 14.3, 10.9, 11.2, 11.1 et 20% respectivement.

Les réductions les plus importantes étaient observées pour les échantillons traités aux combinaisons (ozone+coating), (coating+irradiation) et (ozone+irradiation) ayant les valeurs de (39.2, 44.8, 27%). La faible réduction est due à la présence additionnelle de vitamine C dans les échantillons de poivrons

traités avec la formulation. En effet, le coating contient un taux de vitamine C provenant de l'extrait d'agrumes Biosecur F440 et qui a donné des valeurs plus élevées que pour les autres échantillons au jour 1.

Après 14 jours de stockage à -20°C, une augmentation de la teneur en acide déhydroascoribique et une diminution de la teneur en acide L-ascorbique ont été observées. Sans modification importante de la teneur totale en vitamine C pour les traitements suivant : ozone, coating, irradiation, control, ozone + irradiation. La combinaison de tous les traitements a induit une faible réduction de 9.21 mg/100 g de la teneur en vitamine C totale (probablement due à l'effet synergique entre les traitements et la congélation). Il est important de mentionner que l'acide déhydroascorbique exerce une activité biologique importante parce qu'il peut être converti en acide ascorbique dans le corps humain.

Qualité sensorielle

La formulation X1+ DF vaporisée électrostatiquement sur les poivrons n'a pas affectée négativement le goût, la couleur, l'odeur ou la texture et l'appréciation globale des poivrons verts.

Finalement, les traitements mis au point ont montré une importante capacité antimicrobienne, et pourraient être appliqué en industrie alimentaire afin d'éliminer les bactéries indésirables et garantir un produit sain et de qualité pour le consommateur. Ceci diminuera les risques de maladies d'origines alimentaires.

CONCLUSION

Nous avons dans cette étude travaillé sur trois pathogènes : *Escherichia coli* O157:H7, *Salmonella* Typhimurium et *Listeria monocytogenes.* Ainsi, il nous a été possible de mettre au point deux formulations appelées F2 et F6. Ces solutions antimicrobiennes ont induit une réduction significative *d'E. coli*, *S.* Typhimurium et de *L. monocytogenes* dans les poivrons rouges et les canneberges. De plus, ces deux formulations ont permis une réduction *d'E. coli*, *Listeria innocua* et de *S. enterica* dans les pommes de terre pré-frites et conditionnées sous atmosphère modifiée. Les deux formulations utilisées n'ont pas affecté la qualité sensorielle des poivrons rouges et des pommes de terre.

L'extrait d'agrumes Foodgard incorporé dans les garnitures de fraise à une concentration de 0.2% était plus efficace que le benzoate de sodium à une concentration de 1% contre les bactéries Gram (+), Gram (-), les levures et moisissures. En effet, Foodgard a totalement inhibé *E. coli*, *S.* Typhimurium et *L. monocytogenes*. De plus, cet extrait d'agrumes a réduit de 3.5 log UFC/g le niveau de *B. cereus* et de 4 log UFC/g *S. aureus* dans les garnitures de fraise. Cet antimicrobien naturel n'a pas affecté négativement la qualité sensorielle des garnitures de fraises. Il pourrait ainsi jouer le rôle de conservateur naturel dans les produits sucrés et acides.

Probablement en grande partie grâce à son contenu en composés phénoliques, le jus de canneberge a réduit significativement les trois pathogènes alimentaires (*E. coli*, *S.* Typhimurium et *L. monocytogenes*). Le jus de canneberge a assuré une réduction de 2.5, 1.8 et 5 log UFC/g d'*E. coli*, *L. monocytogenes* et de *S.* Typhimurium respectivement au jour 7 dans les poivrons rouges. Une inhibition totale de *L. monocytogenes* dans les canneberges et une réduction de *S.* Typhimurium de 3 log CFU/g ont aussi été observées. Les composés antimicrobiens présents dans le jus de canneberge peuvent être utilisés afin de formuler d'autres antimicrobiens plus efficaces à base de produits naturels.

La formulation mise au point X1+ DF vaporisée électrostatiquement, contenant un mélange d'huiles essentielles, de jus de canneberges et de dextrose fermenté a aussi induit des effets antibactériens importants en inhibant totalement *E. coli* et en réduisant de 1.2 log UFC/g de *S. enterica*. De plus, X1+ DF a réduit de 1 log UFC/g *Listeria innocua* au jour 10. La qualité sensorielle des poivrons verts n'a pas été affectée par cette formulation. Avec l'utilisation du vaporisateur électrostatique, il a été possible de réduire le volume de formulation vaporisée de 1.2 à 0.3 mL car ce système permet une vaporisation homogène sur toute la surface des fruits et légumes testés et aussi permettant à la formulation de bien adhérer sur la surface vaporisée.

Le traitement à l'ozone gazeux a aussi permis de réduire la charge bactérienne. La concentration, le temps d'exposition, le type d'aliments, le type de bactéries et la température ambiante, tous ces

paramètres ont été pris en considération lors de l'utilisation de l'ozone gazeux. Nos résultats montrent que l'ozone gazeux appliqué à une concentration de 10 ppm pendant 5 min a réduit totalement *E. coli* au jour 4. Une réduction de 1.1 et de 0.6 log UFC/g de *Listeria innocua* a été observée aux jours 4 et 10. Il faut noter que l'application de l'ozone doit se faire sans affecter la qualité du produit alimentaire (couleur, teneur en chlorophylle, vitamine C).

La combinaison des deux traitements antimicrobiens (ozone et coating) a assuré une réduction plus rapide et plus importante de bactéries. En effet, 2 log UFC/g de réduction de *E. coli* ont été observés après 24 h, suivi d'une inhibition totale au jour 4. De plus 1.3 et 1.5 log UFC/g de réduction de *Salmonella* ont été observés aux jours 0 et 10. Aussi, ce traitement combiné a réduit de 1 log UFC/g de *Listeria innocua* au jour 0.

L'irradiation a aussi été étudiée dans ce travail de recherche, seule et en combinaison de traitements. Dans notre recherche, le traitement des poivrons verts découpés en cubes à 0.5 kGy d'irradiation ou en combinant l'irradiation au spray ou à l'ozone a réduit totalement *E. coli* et *Salmonella.* De plus, la combinaison des trois traitements antimicrobiens (ozone, coating et l'irradiation à 0.5 kGy) a totalement inhibé toutes les bactéries étudiées. Ces traitements n'ont pas affecté négativement la qualité des poivrons verts (couleur, teneur en chlorophylle et en vitamines).

Les formulations et les méthodes de préservation et d'élimination de bactéries ont été améliorées dans ce mémoire. Étant donné que le nombre de maladies d'origines alimentaires est en augmentation, des solutions telles que présentées dans ce mémoire pourront être considérées afin de remédier à cette problématique. Les traitements mis au point ont éliminé les bactéries étudiées. Cela permettra de prévenir les intoxications, infections et toxi-infections dues aux bactéries pathogènes.

Les traitements antimicrobiens mis au point au laboratoire seuls ou en combinaisons, pourront être transférés en industrie afin de les appliquer lors de la transformation des aliments prêts à manger. Ainsi, le procédé de combinaisons des traitements permettra une bonne maîtrise de la qualité microbiologique et nutritionnelle des aliments prêts à manger.

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