UNIVERSITÉ DU QUÉBEC
INSTITUT NATIONAL DE LA RECHERCHE SCIENTIFIQUE
CENTRE INRS-INSTITUT ARMAND-FRAPPIER

ÉTUDE DE LA RADIOSENSIBILISATION VIRALE DANS LES ALIMENTS COMME LA LAITUE ICEBERG APRÈS UTILISATION DE TRAITEMENTS COMBINÉS

Par

Alexandra Gobeil

Mémoire présenté pour l'obtention du grade de *Magister Scientiæ* (M. Sc.)

En sciences de **microbiologie appliquée**

Jury d'évaluation

Examinateur externe	Pr. George Szatmari Université de Montréal
Directrice de recherche	Pre. Monique Lacroix INRS Centre INRS- Institut Armand-Frappier
Examinateur interne et Président du jury	Pr. Philippe Constant INRS Centre INRS- Institut Armand-Frappier

À mes parents qui m'ont tant encouragé et motivé à atteindre mes objectifs.

À ma sœur qui m'a énormément aidé et mentoré de par ses précieux conseils.

À mon cher Philippe, mon meilleur ami, mon compagnon de vie qui a toujours été là pour moi. Je lui dois tout le mérite de ce travail.

Avant-propos

Je tiens premièrement à offrir mes reconnaissances les plus profondes au P^re^ Monique Lacroix pour son dévouement et son aide précieuse tout au long de ma maitrise. Elle a su répondre à tous mes questionnements et me conseiller lors des défis rencontrés.

Sans oublier le support infaillible de mes parents Richard Gobeil et Marie-Josée Paquin et ma sœur D^re^ Sophie Gobeil, à chaque jour de cette longue aventure. Ils ont su m'encourager, m'écouter et me motiver à donner mon maximum dans mes recherches. Ils ont été d'une écoute mémorable. Vous êtes des parents exceptionnels, je vous aime profondément. Sophie, merci pour tes bons conseils, ton expérience fait de toi un mentor sans égal. J'offre ma plus grande gratitude à mon copain Philippe Reddy qui a su m'écouter et me motiver à tous les jours de cette aventure. Il m'a fait grandir et réfléchir tout au long de ma maitrise, ce qui m'a beaucoup aidé à évoluer à travers cette expérience. Il a été d'une écoute irréprochable et d'une gentillesse exemplaire. Je lui en serai à tout jamais reconnaissante, car c'est grâce à lui que je suis qui je suis aujourd'hui. Il est primordial pour moi de valoriser le travail de mes trois stagiaires : Mathilde Gérault, Clémence Bertrand et Flavien Degroisse. Tous trois ont su marquer ma maitrise de par leur débrouillardise, leur travail d'arrache-pied et leur curiosité. Je vous remercie énormément parce que c'est grâce à vous si ce projet à évoluer de cette façon. Sans oublier Stephane Salmieri, Dr. Behnoush Maherani et Dr. Shiv Shankar qui ont su répondre à tous mes questionnements lors de la rédaction de mes protocoles et de la mise en place des manipulations au laboratoire. Ils se sont assuré que j'aie tout ce dont j'ai besoin pour évaluer mes résultats.

Le passage au laboratoire de différents étudiants a su rendre plus qu'exceptionnelles mes journées. Je parle plus particulièrement de mes collègues et amies qui m'ont suivi tout au long de la maitrise, Valérie Robichaud et Johanne Manus. C'était un réel bonheur de les côtoyer chaque jour et de les voir évoluer dans leur maitrise à leur tour. Merci à Jiali Ji et Yosra Ben Fadhel pour leurs conseils et leur écoute. Certains étudiants ne sont restés que pour quelques mois mais ont tout de même su marquer mon expérience, soit Marjorie Fleury, Claire Rioland et Raphaëlle Odic. Un merci particulier à Marwa Khabir qui m'a beaucoup aidé à travers mes difficultés et qui m'a aidé à m'en sortir puis réussir. Toute cette recherche n'aurait pas pu être possible sans l'aide de Nordion et particulièrement de Sébastien Guay qui a irradié tous mes échantillons. Ils ont su m'ajouter à leur horaire chaque fois et ont rendu mes expériences plus paisibles.

Je remercie mes deux meilleures amies Rindra Rakotonarivo et Amandine Chauviat pour leur support et leurs encouragements. Elles ont toujours su me motiver et me faire rire tout en étant toutes deux sur des continents différents du miens.

Table des matières

Résumé

La surface de la laitue iceberg est favorable au développement de pathogènes comme le Norovirus (NoV). Celui-ci est éliminé par la chaleur, inapte sur les produits consommés frais. L'irradiation demeure une technologie intéressante, mais les virus en sont résistants. Le but de ce projet était de mettre au point une méthode combinée afin d'augmenter la radiosensibilisation du NoV pour ainsi réduire la dose d'irradiation nécessaire pour les éliminer ou pour les amener à un niveau propre à la consommation pour l'Homme. Le projet consistait à évaluer les effets d'extraits naturels et d'O_3 combinés à l'irradiation sur la radiosensibilisation virale. Un test *in-situ* a aussi été réalisé afin de voir l'efficacité du traitement sur les propriétés physico-chimiques et la qualité microbienne de la salade. Les objectifs étaient : *1) évaluer les effets d'extraits naturels sur l'élimination virale, 2) déterminer les effets de l'O_3 sur le virus et établir la concentration optimale, 3) déterminer la D_{10} (kGy) en absence et en présence d'antimicrobiens naturels et la radiosensibilisation en présence d'antimicrobiens naturels et 4) évaluer in-situ les traitements combinés sur l'élimination virale, bactérienne et les propriétés physico-chimiques.* Les résultats ont montré que dose optimale de jus de canneberge était de 0.25 %, car la D_{10} était 1.33 pour son activité antimicrobienne et 2.00 pour le contrôle, donc la radiosensibilisation était de 1.5 fois par rapport à l'irradiation seule. La dose optimale pour l'O_3 était de 5 ppm durant 7.5 minutes. La combinaison de traitement optimale (JC-0.25%+ O_3-5 ppm + irradiation-1.5 kGy) a montré une réduction d'environ 2 log $TCID_{50}$ après traitement et entreposage à 4°C. L'étude *in-situ* n'a montré aucun effet négatif sur la texture, le goût et la couleur de la laitue ($p > 0.05$) et ce même durant l'entreposage. L'analyse de la chlorophylle a montré une augmentation non significative de 5.23 à 9.03 µg/mL au cours de l'entreposage 10 jours après le traitement combiné comparativement au contrôle qui a lui-même diminué significativement durant l'entreposage de 9.02 à 3.12 µg/mL, démontrant un bienfait de la combinaison des traitements.

Mots-clés : Norovirus ; Laitue ; Ozonation ; Acides organiques ; Irradiation.

Alexandra Gobeil, étudiante

Professeur Monique Lacroix, directrice de recherche

Abstract

The surface of iceberg lettuce is favorable for the development of pathogens such as Norovirus (NoV). It is eliminated by heat, not possible on products eaten raw. Irradiation remains an interesting technology, but viruses are resistant. The goal of this project was to develop a combined method to increase the radiosensitization of NoV to reduce the dose of irradiation needed to eliminate them or to bring them down to levels which are safe for human consumption. The project evaluated the effects of natural extracts and O_3 combined with irradiation on viral radiosensitization. An *in-situ* test was also conducted to see the effectiveness of the treatment on the physico-chemical properties and microbial quality of the salad. The objectives were: *1) to evaluate the effects of natural extracts on virus elimination, 2) to determine the effects of O_3 on the virus and to establish the optimal concentration, 3) to determine the D_{10} (kGy) in the absence and in the presence natural antimicrobials and radiosensitization in the presence of natural antimicrobials; and 4) in-situ evaluation of combined treatments on viral, bacterial and physicochemical properties.* The results showed that the optimal dose of cranberry juice was 0.25 %, since the D_{10} was 1.33 for its antimicrobial activity and 2.00 for the control, so the radiosensitization was 1.5 times compared to the irradiation alone. The optimal dose for O_3 was 5 ppm for 7.5 minutes. The optimal treatment combination (CJ-0.25% + O_3-5 ppm + irradiation-1.5 kGy) showed a reduction of 2 log $TCID_{50}$ / mL after treatment and storage at 4 ° C. The *in-situ* study showed no negative effect on the texture, taste and color of lettuce ($p > 0.05$) even during storage. Chlorophyll analysis showed a non-significant increase from 5.23 to 9.03 µg / mL during storage 10 days after the combined treatment compared to the control which decreased significantly during storage from 9.02 to 3.12 µg / mL, demonstrating a benefit from the combination of treatments.

Keywords: Norovirus; Lettuce; Ozonation; Organic acids; Irradiation.

Liste des figures

Liste des tableaux

Chapitre 1.

Chapitre 2.

Chapitre 3.

Chapitre 4.

Liste des abréviations

AJ: Apple Juice

AstV: Astrovirus

ATP: Adenosine triphosphate

B-PACs: Blueberry Proanthocyanidins

Cl: Chlorine

ClO_2: Chlorine Dioxide

COD: Chemical Oxygen Demand

CJ: Cranberry Juice

CJ-PACs: Cranberry Juice Proanthocyanidins

CP: Cranberry Polyphenols

$C_2H_4O_3$: Peracetic Acid

EOs: Essential oils

FAO: Food Agriculture Organization

FCV or FCV-F9: Feline Calicivirus (Strain F-9)

GSE: Grape Seed Extract

HAV: Hepatitis-A Virus

HEV: Hepatitis-E Virus

HCV: Hepatitis-C Virus

HHP: High Hydrostatic Pressure

HPH: High-Pressure Homogenization

HSV-1 and HSV-2: Herpes Simplex Virus

H_2O_2: Hydrogen Peroxide

IAEA: United Nations International Atomic Agency

JC: Jus de canneberge

Kbp: Kilo base pair

LAB: Lactic Acid Bacteria

MNV or MNV-1: Murine Norovirus (Strain 1)

NaClO: Sodium Hypochlorite

NoV: Human Norovirus

O_2: Dioxygen

O_3: Ozone

PACs: Proanthocyanidins

PFU/mL: Plaque-forming unit /mL

PJ: Pomegranate juice

PP: Pomegranate Polyphenols

RV: Rotavirus

ssRNA: single-stranded RNA

ssDNA: single-stranded DNA

TCID50/mL: Tissue Culture Infectious Dose 50 / mL

USD: United State Dollars

USDA: United States Department of Agriculture

UV: Ultraviolet light

UVTP: TiO_2 photocatalysis

VLPs: NoV virus-like particles

VSV: Vesicular Stomatitis

WHO: World Health Organization

Chapitre 1 : Introduction générale

1.1 Les virus

La signification latine du mot virus est « *poison* ». Les virus font partie d'un règne encore plus diversifié que le règne des bactéries, des plantes et des animaux tous combinés ensembles. Malgré le fait qu'il n'y ait pas de définitions claires et précises du terme « virus », on peut tout de même le qualifier grâce à ses différentes propriétés communes. Les virus sont acellulaires, ils ne sont donc pas constitués de cellules. Les organismes vivants sont généralement constitués de cellules. Les virus ne sont donc pas vivants selon ce critère d'organisation cellulaire que possèdent les unicellulaires et les pluricellulaires. Ils ne se divisent pas non plus comme les bactéries. Un virus est hôte-dépendant, il a besoin d'un hôte pour se multiplier, souvent une cellule. Il va utiliser ses différentes fonctions et ses composants cellulaires. Ils n'ont pas de machinerie capable de générer de l'ATP (énergie) ni de machinerie de synthèse protéique, les virus utilisent celles de la cellule. D'un autre côté, le virus possède des caractéristiques semblables à celles des organismes vivants. Les virus sont capables d'évoluer dans le temps (mutations) et se multiplient. Ces derniers sont composés majoritairement d'acides nucléiques et de protéines. De plus, leur code génétique est identique à celui des organismes cellulaires (Lemay, 2017). Les virus sont de très petites tailles avec des diamètres d'environ 25-300 nm, ils sont donc invisibles au microscope conventionnel. Parmi les infections alimentaires, les virus deviennent de plus en plus reconnus comme étant responsables. Ces derniers sont majoritairement transmis par la voie fécale-orale.

Tableau 1. Tableau des comparaisons des caractéristiques physiques et métaboliques entre les virus, les bactéries et les cellules animales

Caractéristiques	Virus	Bactéries	Cellules animales
Taille	ηm	1-10 μm	10-100 μm
Production d'ATP (énergie)	Non	Oui, dans la membrane plasmique	Oui, dans la mitochondrie
Reproduction	Parasitisme	Scissiparité	Mitose
Synthèse protéique	Non	Polysome (ribosomes + ARNm)	Polysome (ribosomes + ARNm)

Structure	Capside, ADN ou ARN	Paroi, membrane, cytoplasme, chromosomes, ribosomes	Absence de paroi ou de capside, membrane plasmique

1.1.1 Le génome

Il est possible de retrouver l'acide nucléique des virus sous plusieurs structures, formes et compositions différentes. En effet, le génome viral peut être retrouvé sous forme circulaire, linéaire ou même segmenté. Les virus, contrairement aux cellules, peuvent contenir deux types d'acides nucléiques soit ADN ou ARN. Parmi les virus constitués d'un génome d'acide désoxyribonucléique (ADN), on retrouve les virus ADN double brin (groupe I) et les virus ADN simple brin (groupe II). D'autre part, il existe des virus avec un génome d'acide ribonucléique (ARN). Ces derniers sont classifiés comme étant à double brin (groupe III), à simple brin et à polarité positive (groupe IV) et finalement à simple brin et à polarité négative (groupe V) (Cann, 2005).

1.1.2 La capside

La capside représente une barrière de protection du génome viral. Elle est formée de plusieurs sous-unités protéiques qui englobent l'acide nucléique. Le fait que celle-ci soit forte, flexible et de petite taille implique qu'elle est difficilement brisable par une pression physique. Sa structure est très complexe à cause du faible rendement de production protéique par un seul acide nucléique, ce dernier ne peut que coder que pour une protéine de 15% de son poids à lui-même. Il faudra donc réaliser une capside faite de multiples molécules protéiques pour réaliser l'assemblage de ses sous-unités. La capside hélicoïdale est la version la plus simple, car elle permet d'entourer de façon circulaire plusieurs protéines de formes différentes. Grâce à la cristallographie, il a été démontré que l'empilement de ces structures induisait une hélice plus tôt qu'un cylindre. La capside icosaédrique est une forme solide composée de 20 faces triangulaires arrangées autour du génome de façon sphérique. Il est plus économique pour le virus de préparer un grand nombre de protéines répétées que de préparer moins de protéines mais de plus grandes tailles (Cann, 2005). Avec les années, d'autres morphologies de capside ont été trouvées dont les amphores et les quenouilles (Lemay, 2017).

1.1.3 L'enveloppe

Plusieurs virus sont constitués d'une enveloppe à bicouche lipidique avec des protéines. L'enveloppe n'est toutefois pas essentielle au virus (Lemay, 2017). Le cycle lytique viral permet de libérer plus facilement

les virions de leur hôte cellulaire, mais cela amène plusieurs désavantages. Le fait de tuer chaque fois la cellule diminue la possibilité de produire une infection persistante ou latente. De ce fait, certains virus ont évolué afin de se répliquer sans pour autant avoir à détruire la cellule hôte. Ce type de virus ne va pas affecter la membrane de l'hôte pour le laisser vivant. Les virus modifient leur enveloppe lipidique en faisant la synthèse de plusieurs types de protéines : les protéines de la matrice, glycoprotéines externes et protéines du canal de transport. Les protéines de la matrice servent à lier la nucléocapside à l'enveloppe. Elles ne sont généralement pas glycosylées et très abondantes. Les glycoprotéines externes sont accrochées dans l'enveloppe par leur domaine transmembranaire. Elles représentent les antigènes majeurs des virus enveloppés et permettent un contact avec l'environnement. Les protéines du canal de transport contiennent plusieurs domaines transmembranaires hydrophobes qui forment un canal à travers l'enveloppe. Ces protéines sont très utiles pour régulariser l'environnement interne du virus (Cann, 2005). Le FCV ne possède toutefois pas d'enveloppe lipidique.

1.2 Historique des virus

Les premiers écrits concernant les infections virales étaient en hiéroglyphes originaires de Memphis (Égypte ancienne, 3700 AV. J-C) et concernaient un prêtre démontrant des symptômes liés à la poliomyélite. La variole a été endémique en Chine dans les années 1000 AV. J-C, les chinois ont constaté que les sujets ayant auparavant été infectés étaient protégés lors des infections suivantes. Dès lors et pendant des siècles, des pratiques de variolations (inoculation de pus infecté par le virus sur sujet) étaient effectuées sans jamais savoir si cela allait être fatal ou non. Edward Jenner a été le premier à vacciner des sujets avec du pus de vaches contaminé avec la vaccine (variole de la vache) en 1796. Antony van Leeuwenhoek (1632-1723) a construit le premier microscope et a observé des bactéries. Durant la guerre hispano-américaine (1898) et durant la construction du canal du Panama, beaucoup sont morts infectés par le virus de la fièvre jaune. Walter Reed a ensuite démontré en 1900 que la fièvre jaune était transmissible. Frederick Twort (1915) et Félix d'Herelle (1917) ont remarqué que des virus pouvaient aussi infecter des bactéries, ainsi appelés bactériophages (Cann, 2005).

Tableau 2. Tableau des différentes découvertes, inventions et technologies ainsi que leur inventeur (Adapté de Oldstone, 2009)

Date	Découvreur(s), inventeur(s) et développeur(s)	Découvertes, inventions et technologies
400 AV. J-C	Hippocrate	Père de la médecine, observations épidémiologiques
1775	L. Spallanzani	Première croissance bactérienne en culture
1796	E. Jenner	Application de la vaccine (variole

		des vaches) sur des sujets contre la variole humaine
1835	M. Schleiden, T. Schwann & others	Développement du concept que tous les organismes sont composés de cellules
1857	L. Pasteur and R. Koch	Fondements de la microbiologie
1858	C. Darwin and A. Wallace	Théorie de l'évolution et de la sélection naturelle
1865	G. Mendel	Fondements de la génétique
1868	F. Meischer	Découverte et caractérisation des acides nucléiques
1880s	J. Buist	Découverte des corps élémentaires du virus de la vaccine et de la variole
1883	E. Metchnikoff, J. Bordet, P. Ehrlich	Fondements de l'immunologie
1884	J. Henle, R. Koch, F. Loeffler	Les postulats de Henle-Koch, critères pour relier une cause à un effet
1885	L. Pasteur and E. Roux	Développement d'un vaccin antirabique (rage)
1888	Institut Pasteur	Ouverture de l'Institut Pasteur à Paris
1892	D. Ivanovski	Découverte du virus de la mosaïque du tabac
1898	F. Loeffler and P. Frosch	Découverte du virus causant la maladie pied-main-bouche
1907	P. Ashburn and C. Craig	Découverte du virus de la dengue
1908	V. Ellermann and O. Bang	Découverte du virus de la leucémie aviaire (première découverte de leucémie virale)
1909	K. Landsteiner and E. Popper	Découverte du poliovirus
1910	A. Carrel, H. and M. Maitland, E. Steinhardt. H. Eagle, G. Gey, T. Puck, R. Hamin, J. Enders, T. Weller, F. Robbins et Autres.	Développement de la méthode de culture cellulaire
1917-1919	F. Twort and F. D'Herelle	Découverte des bactériophages
1947-1955	M. Burnet, A. Gottschalk and E. Klenck	Découverte des récepteurs viraux
1948	K. Sandford	Culture de cellules animales

1.3 Le cycle de réplication général des virus

1.3.1 Attachement

La réplication virale compte un total de 7 étapes. Peu importe leur hôte spécifique, tous les virus possèdent ce même processus de réplication général. En premier, il y a l'attachement des virus à leur hôte. Lors de l'attachement, il y a une liaison spécifique entre la protéine d'adhésion virale (anti-récepteur) et un récepteur cellulaire. Les molécules qui constituent le récepteur cellulaire peuvent être à la fois protéique

(glycoprotéines) ou glucidique via le résidu carbohydrate présent soit sur les glycoprotéines ou soit sur les glycolipides. Les rhinovirus humains (HRVs) possèdent une fente connue sous le nom de « canyon » sur la surface de chaque face triangulaire de la capside icosaédrique, formée par les monomères VP1, VP2 et VP3 (Cann, 2005).

1.3.2 Pénétration

L'étape de pénétration est de courte durée après l'attachement du virus à son hôte malgré le fait qu'elle soit énergie-dépendante et qu'elle nécessite une cellule active. Plusieurs mécanismes sont possibles pour la pénétration virale. La première possibilité est la translocation du virus à travers la membrane cytoplasmique. Ce mécanisme n'est toutefois pas encore totalement maitrisé. La seconde est l'endocytose du virus par des vacuoles intracellulaires. Durant ce processus, il y a formation et internalisation de vésicules. La dernière possibilité est la fusion de l'enveloppe virale avec la membrane cellulaire. Ce procédé est dépendant de la présence de protéines de fusion sur le virus (Cann, 2005).

1.3.3 Décapsidation

Après la pénétration, une étape de décapsidation est initialisée, la capside virale est alors complétement ou partiellement enlevée. Dans les endosomes, il y aura une modification du pH dû à leur acidification et cela provoquera la décapsidation (Cann, 2005).

1.3.4 Réplication génomique et expression génique

Dans le cas des virus du groupe I (ADNdb), il existe deux types de réplication. La première est la réplication dépendante de l'hôte cellulaire et se déroule entièrement dans le noyau en utilisant la machinerie cellulaire. La seconde manière est complètement indépendante de la machinerie cellulaire car le virus a évolué de façon à effectuer sa réplication dans le cytoplasme grâce à sa propre machinerie permettant la transcription ainsi que la réplication du génome viral. Pour les virus du groupe II (ADNsb), la réplication se déroule dans le noyau cellulaire. En effet, à l'aide de l'enzyme ADN polymérase, il y aura préparation d'un intermédiaire à double-brin qui servira de matrice pour la synthèse de l'ADNsb. Ensuite, pour les virus du groupe III (ARNdb) qui possèdent un génome segmenté se font transcrire séparément afin de produire des ARNm monocistroniques. Dans le cas des virus du groupe IV (ARNsb+), il existe deux sous-groupes de réplication génomique. Le premier implique des virus possédant des ARNm polycistroniques qui sont traduits en une poly-protéine qui est suite clivée par une protéase pour former des protéines matures. Le deuxième sous-groupe possède une transcription plus ardue, avec deux séances de traduction ou en utilisant des ARNs sous génomiques. Pour les virus du groupe V (ARNsb-), on y compte les virus non segmentés qui sont en premier transcrits par une ARN polymérase ARN-dépendante

pour produire un ARNm monocistronique qui servira de matrice pour la prochaine réplication. Parmi les virus segmentés (*Orthomyxoviridae*), la réplication se déroule dans le noyau de la cellule, avec plusieurs ARNm monocistroniques pour chaque gènes viraux produits par la transcriptase virale à partir du génome viral (Cann, 2005).

1.3.5 Assemblage

L'assemblage permet de produire un virion mature qui sera prêt à quitter la cellule. Le site d'assemblage varie en fonction du site de réplication, il peut être situé dans le cytoplasme ou bien dans le noyau directement. Le déclenchement de cette étape est initié par une hausse de quantité de protéines virales intracellulaires ainsi que de génomes viraux qui atteindront une concentration critique. L'encapsidation du génome viral peut se produire très rapidement durant l'assemblage ou très lentement quand le génome est entouré dans une coque protéique presque complétée (Cann, 2005).

1.3.6 Maturation

L'étape de maturation implique des changements de structure ou de conformation dans la particule virale la rendant ainsi infectieuse. On observera des clivages protéiques par des protéases afin de former un ensemble mature. On observera aussi des condensations des nucléoprotéines du génome viral. Pour certains virus, l'assemblage et la maturation se déroulent ensemble et dans la cellule. Pour d'autres, la maturation arrive après la libération de la particule virale (Cann, 2005).

1.3.7 Relâche du virus

Dans le cas des virus lytiques (surtout pour les virus non enveloppés), la cellule va lyser et relâcher les particules virales. Il en reste une production virale au détriment de la survie cellulaire. Pour les virus enveloppés, ils acquièrent leur membrane lipidique au fur et à mesure qu'ils sortent de la membrane cellulaire ou par l'intermédiaire d'une vésicule intracellulaire. Les protéines virales du virion sont ensuite accrochées à la surface du virus durant son excision. On appelle ce phénomène le « bourgeonnement » (Cann, 2005).

1.4 Le cycle de réplication spécifique des calicivirus

Les principales maladies humaines et animales des virus de la famille des caliciviridaes sont les gastroentérites (Norwalk) et la calicivirose féline, maladie respiratoire chez le chat. La voie de transmission de la gastroentérite humaine est majoritairement par la voie fécale-orale tandis que celle de la calicivirose féline est par la salive, les excrétions du nez et des yeux, l'urine et les excréments. Il s'agit du

plus simple cycle de reproduction virale, par ARNsb+. La première étape est la reconnaissance récepteur et anti-récepteur, l'entrée du virus et ensuite la décapsidation. Dès lors, l'ARN génomique à polarité positive est lu par les ribosomes cellulaires et se fait traduire en protéine polycistronique (Figure 1). Cette dernière se fera cliver par des protéases pour générer plusieurs petites protéines. Ensuite, un brin complémentaire à polarité négative sera formé grâce à l'ARN polymérase-ARN dépendante, ou réplicase. Ce brin servira de matrice pour générer plusieurs copies du génome à polarité positive. Ce génome sera par la suite encapsidé par les protéines de structures de la capside virale générées auparavant.

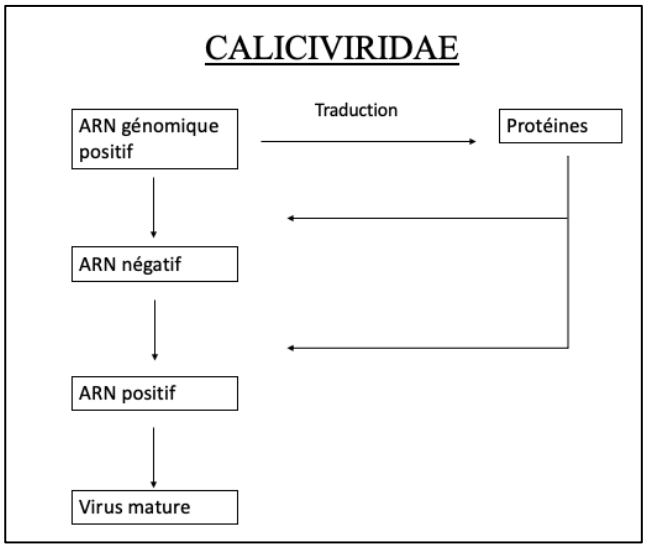

Figure 1. Représentation schématique de la réplication virale d'un virus de la famille des *caliciviridae*

Les virus de la famille des *Caliciviridae* font partie du genre Calicivirus. Les virions possèdent un diamètre compris entre 30-38 nm avec 32 surfaces arrangées en symétrie icosaédrique. Ils sont aussi composés de 180 protéines associées en dimère. La taille du génome de ce virus est de 7.4-7.7 kb en taille. Les virions sont aussi résistants à l'éther, au chloroforme et aux détergents. Dans le genre Calicivirus humain, on retrouve aussi les virus Southampton, Snow Mountain, Hawaii et Taunton. Dans le genre Calicivirus animal, on trouve le Calicivirus félin (FCV), le virus de l'exanthème vésiculaire du porc et la maladie hémorragique virale du lapin (Fields *et al.,* 1996).

1.5 Gastroentérite, pertes économiques associées et analogues du NoV

Parmi les virus entériques humains, on compte les familles suivantes : *Adenoviridae, Astroviridae, Caliciviridae, Parvoviridae, Picornaviridae, Reoviridae, Coronaviridae* et *Hepeviridae* (Adams *et al.,* 2015). Ces virus sont présents en grande quantité dans les diarrhées (jusqu'à10^8-10^{10}/g). Les rotavirus, calicivirus et astrovirus sont les agents majoritairement responsables. Ils ont commencé à être appelé « *Norwalk-Like agents* » après une éclosion de gastroentérite dans une école en Ohio, à Norwalk, en 1968.

Tandis qu'au Royaume-Uni, on les nommait « petits virus ronds » (SRSV). Maintenant, ils sont dans la famille des *Caliciviridae,* du genre NoV. Les génogroupes I, II (95 % des cas) et IV sont plus particulièrement des virus entériques humains. Le temps d'incubation (temps avant l'apparition des symptômes) est de 15-50 heures et ensuite des diarrhées suivies de vomissements persistent en moyenne 24-48 heures. La transmission est faite par voie fécale-orale, incluant la transmission directe de personne-à-personne et la consommation d'eaux ou d'aliments contaminés puis aussi le contact avec des surfaces contaminées. Aux États-Unis, les NoV sont les plus grands responsables des maladies alimentaires et causent plus de 40 à 50 % de toutes les toxi-infections alimentaires (Adams *et al.,* 2015). Chaque année, il se produit environ 19-21 millions de cas de NoV, 400 000 urgences, 56 000-71 000 hospitalisations, 570-800 morts, résultant en une perte économique de plus de 777$ millions USD en coûts de santé aux États-Unis (Hall *et al.,* 2014).

Le NoV et le FCV sont dans la même famille de virus mais possèdent des différences dans leurs propriétés biochimiques (Cannon *et al.,* 2006). Les infections causées par le MNV chez les souris impliquent des symptômes différents que le NoV chez l'humain avec la gastroentérite, mais le MNV reste tout de même un meilleur analogue que le FCV de par sa similarité avec le NoV (Arthur and Gibson, 2015). De plus, le FCV est un analogue moins robuste pour ce qui en est de l'aspect de résistance à l'environnement que le MNV pour le NoV (Cannon *et al.,* 2006). En général, le FCV est plus sensible aux traitements que le MNV ou le virus Tulane (TuV), le TuV cause des fièvres et des diarrhées chez les macaques rhésus (Esseili *et al.,* 2015). Par exemple, le FCV a été démontré comme sensible à un traitement avec du chlore à 200 ppm lorsqu'il était séché sur des surfaces en acier inoxydable alors que le MNV et le TuV sont tous deux demeurés insensibles (Cromeans *et al.,* 2014). Il est possible de déterminer le titre viral entre autres par $TCID_{50}$, mais aussi par « *plaque assay* ». La méthode « *plaque assay* » est quantitative et possède l'avantage de produire un évènement comptable (Darling *et al.,* 1998). La mesure du titre viral est déterminée par le nombre de plaque formée divisée par le volume de l'échantillon original testé. La méthode $TCID_{50}$ est quantitative aussi et est privilégiée lorsque le virus ne peut pas produire de plaques, mais qu'il produit des effets cytopathogènes (CPE) sur les cellules infectées et les puits seront déterminés comme étant positifs ou négatifs en fonction de la présence d'infection (Darling *et al.,* 1998). Il est important d'avoir plusieurs réplicats afin de donner de la précision au résultat (Darling *et al.,* 1998). Il est aussi connu que la méthode « *plaque assay* » est plus sensible que la mesure réalisée avec la méthode $TCID_{50}$ (ATCC, 2014). Une recherche récente a été effectuée sur le MNV et le TuV en utilisant la méthode de « *plaque assay* » pour déterminer le titre viral. Les auteurs ont émis l'hypothèse que comparativement au TuV, le MNV est un meilleur analogue pour le NoV car il est capable de résister à des gammes de pH plus étendues, à du chlore à 2 ppm et à une température de 4 °C durant plus de 30 jours (Hirneisen and Kniel, 2013). Le FCV reste un choix optimal en termes d'efficacité d'infection cellulaire et

d'apparition de CPE, cela prend seulement 1.5 jour pour observer ces effets avec le FCV. Pour les autres virus comme le MNV et la TuV, 2 et 2.5 jours sont nécessaires avant d'observer les CPE (Esseili *et al.,* 2015).

1.6 Contamination des aliments frais par des agents infectieux

Les aliments frais sont très propices à des contaminations par des bactéries, parasites ou virus comme le NoV parce qu'ils ne sont pas cuits et peuvent être contaminés avant ou après la cueillette (Berger *et al.,* 2010 ; Herwaldt *et al.,* 1994). De plus, ils ne sont que très peu ou pas transformés donc sont plus propices aux contaminations (Predmore and Li, 2011). Selon des données, les fruits et légumes frais sont les véhicules majeurs d'infections alimentaires (Heaton *et al.,* 2008). Le NoV serait responsable de 40 % des toxi-infections alimentaires causées par la consommation d'aliments frais entre 1998 et 2005 aux États-Unis (Doyle *et al.,* 2008). Ces éclosions se déroulent majoritairement après contamination des laitues, des fraises, des melons ou des framboises car ils sont tous difficiles à laver à cause de leurs petites cavités (Butot *et al.,* 2009). Plusieurs maladies alimentaires virales associées avec des aliments frais sont causées par des virus entériques tels que le NoV, l'hépatite A (HAV), l'hépatite E (HEV), le rotavirus (RV) et les astrovirus (AstV) (Terio *et al.,* 2017). Actuellement, les moyens pour décontaminer les fruits et légumes frais sont majoritairement l'acide peracétique et l'hypochlorite de sodium, connu sous le nom d'eau de javel. Par contre, plusieurs inconvénients sont notés avec l'eau de javel, dont l'irritation de la peau et du tractus respiratoire. Il y aurait aussi des composés cancérigènes, mutagènes, tératogènes ou toxiques associés à l'eau de javel (Alvaro *et al.,* 2009). Il serait donc intéressant de trouver des alternatives plus propres et qui n'utilisent pas de produits chimiques.

1.7 Les traitements antiviraux utilisés et objectifs

1.7.1 L'irradiation

Les rayons γ interagissent avec les acides nucléiques du virus via la production de radicaux libres. Ces derniers peuvent engendrer des brisures simples-brins, doubles-brins et des dégradations de nucléotides (Feng *et al.,* 2011). La formation de radicaux libres (stress oxydatif) se produit quand une molécule de O_2 se divise en deux pour former des atomes O seuls avec des électrons libres qui seront hautement instables et réactifs. Une réaction en chaine se produit alors car les radicaux libres vont aller tirer des électrons à d'autres molécules et ainsi les rendre en radicaux libres à leur tour. Les dommages causés sont généralement faits sur les protéines, les lipides et les acides nucléiques (Wolff *et al.,* 1986). Le développement de maladies dû aux radicaux libres peut se faire et ainsi causer l'Alzheimer, la maladie de Parkinson et des maladies cardiovasculaires (Götz *et al.,* 1994). Dans le cas du MNV-1, plus la dose

d'irradiation-γ utilisée contre ce virus est élevée et plus l'expression de la protéine majeure de la capside (VP1) est altérée, mais seule cette protéine structurale a été étudier avec SDS-PAGE dans le cas du MNV-1 (Feng *et al.,* 2011) donc il aurait été intéressant d'en étudier d'avantages. L'irradiation-γ a dégradé une majeure partie des protéines VP1, mais il en restait 50% et 30% après une irradiation à 2.8 et 5.6 kGy, respectivement, démontrant ainsi une résistance du MNV-1. De plus, l'irradiation peut briser des liens covalents et non-covalents essentiels pour la structure protéique. Feng *et al.* (2011) a démontré une diminution de 1.7 à 2.4 log PFU/ mL sur des produits frais inoculés avec le virus MNV-1 (tous traités à 5.6 kGy). Le VSV est plus susceptible par l'irradiation que le MNV-1 car une réduction de 3.3 log PFU/ mL a été observée avec ce même traitement. Les auteurs ont attribué ces résultats à la taille du génome en expliquant qu'il existerait une relation inverse entre la dose d'inactivation et la taille du génome viral, en effet le MNV-1 a une taille de 7.4 kb et le VSV de 11 kb, cela expliquerait la sensibilité face aux rayons- γ (Feng *et al.,* 2011). Les auteurs ont aussi ajouté que la taille de la particule virale pourrait aussi influencer la sensibilité à l'irradiation-γ, parce que les particules plus grosses seraient plus propices à être frappé par les rayons γ à cause de leur grande surface.

1.7.2 L'ozonation

L'ozone (O_3) est généré grâce à une grande source d'énergie qui va venir rompre les liens entre de l'O_2, ensuite l'atome d'oxygène (O) seul va aller se lier à une molécule d'O_2 pour ensuite former de l'O_3 (Simek and Clupek, 2002). C'est un procédé plus propre et plus efficace que la chlorination car le seul résidu est de l'O_2. C'est un procédé non thermique et non chimique pouvant être appliqué en état aqueux ou gazeux. L'utilisation de l'O_3 permet de stériliser les surfaces à décontaminer et de former de la glace à partir d'eau (Guzel-Seydim *et al.,* 2004; Kim *et al.,* 2003). L'O_3 est un fort agent oxydant, ce dernier élément cause des dommages à la capside virale et empêche la reproduction virale en empêchant le contact cellulaire (Elvis and Ekta, 2011). Il a été démontré que les virus non enveloppés (comme le NoV) sont plus sensibles à l'O_3 que les virus enveloppés parce qu'ils ont accès aux acides nucléiques plus facilement. Les cellules contiennent des enzymes à leur surface et sont susceptibles à une infection virale (Elvis and Ekta, 2011). Par la suite, une oxydation des composants se déroulera, ce qui mènera à la destruction des cellules infectées et donc au remplacement par des cellules en santé. La réactivité de l'O_3 est causée par l'oxydation, la peroxydation et la génération de radicaux libres, par la suite cela causera une inactivation enzymatique qui mènera à la génération de lésions et donc de morts cellulaires (Elvis and Ekta, 2011). Hirneisen (2011) a étudié l'O_3 dans l'eau à une concentration de 6.25 ppm durant des temps d'exposition de 0.5, 1, 5 et 10 minutes contre le MNV-1 et le FCV-F9 sur des oignons verts et des laitues. Une inactivation plus grande des virus a été observée plus le temps d'exposition au traitement augmentait. Pour ce qui est du FCV, une réduction de 3.08 log $TCID_{50}$/g a été observée après 10 minutes de traitement sur

la laitue et une réduction de 2.02 log $TCID_{50}$/g a été observée sur des oignons verts. Les auteurs ont attribué cette différence de réduction virale à la composition organique des oignons verts et de la laitue car l'O_3 réagit avec les composés organiques complexes des aliments en raison de son potentiel d'oxydation élevé (Hirneisen *et al.,* 2011). Predmore (2015) a étudié les effets de l'ozone 6 % (w/w O_3) dans de l'O_2 durant 0, 10, 20, 30 et 40 minutes. Après 40 minutes, une diminution de 3.3 log PFU/g de MNV-1 a été observée sur des fraises, mais il y avait toujours présence de 2.5 log PFU/g, indiquant ainsi une petite présence de virus sur la laitue même après les traitements.

1.7.3 L'utilisation de jus de canneberge

Le jus de canneberge commercial (JC) est constitué de sucre, d'eau, de sirop de mais, de JC concentré et d'acide citrique, quinique, malique et ascorbique (Ehling and Cole, 2011). Le mécanisme d'action du JC contre les virus alimentaires est encore méconnu mais est pour l'instant attribué aux altérations de la capside virale ainsi que l'acide nucléique. L'inactivation de virus non enveloppés (comme le NoV) est due à la dénaturation de protéines de la capside à la surface du virus et cela est causé par l'acidité des acides organiques trouvés dans le jus de canneberges (Cao, 2013). Su *et al.* (2010) a testé les effets du JC contre le MNV-1, le FCV-F9, le bactériophage MS2 (ssRNA) et le bactériophage φ X-174 (ssDNA) à des temps d'exposition variant de 0 à 60 minutes. Le virus FCV-F9 est celui dont le titre viral a diminué le plus en quantité et ce, le plus rapidement. Une diminution de 5 $\log_{10}$ PFU/mL a été observée après 30 minutes de traitement avec le JC. Su *et al.* (2010) a aussi étudié les effets des proanthocyanidines de canneberges contre ces virus. Les CJ-PAC (0.15 mg/mL and 0.30 mg/mL) ont immédiatement abaissé le titre viral du FCV-F9 jusqu'au seuil de détection limite. Les canneberges contiennent des quantités importantes de flavonoïdes et de composés polyphénoliques qui procurent des bienfaits sur la santé. Les canneberges ont des effets antioxydants et antimicrobiens contre *Helicobacter pylori, Salmonella* et *Escherichia coli*. Le rôle des antioxydants est de neutraliser les dommages causés par les radicaux libres en se faisant tirer un électron par les radicaux libres au lieu qu'ils s'attaquent à nos cellules (Biswas *et al.,* 2013 ; Côté *et al.,* 2011).

1.7.4 Objectifs et hypothèses

La surface de la laitue iceberg est favorable à être contaminée par des pathogènes comme le Norovirus (NoV). Celui-ci est habituellement éliminé par la chaleur créant ainsi un défi sur les produits frais. L'irradiation-γ demeure une technologie intéressante, mais les virus en sont résistants. Le but de ce projet était de mettre au point une méthode de traitements combinés afin d'augmenter la radiosensibilité du FCV-F9 pour ensuite réduire la dose d'irradiation nécessaire pour les éliminer. Ce projet consistait à évaluer les effets d'extraits naturels et ceux de l'O_3 combinés à l'irradiation-γ sur la radiosensibilisation

virale. Un test *in-situ* a aussi été réalisé afin de voir l'efficacité du traitement sur les propriétés physico-chimiques et la qualité microbienne de la salade durant le stockage à 4 °C.

Les objectifs étaient :

1) évaluer les effets d'extraits naturels sur l'élimination virale,

2) déterminer les effets de l'O_3 sur le virus et établir la concentration optimale,

3) déterminer la D_{10} (kGy) en absence et en présence d'antimicrobiens naturels et la radiosensibilisation en présence d'antimicrobiens naturels,

4) évaluer in-situ les traitements combinés sur l'élimination virale, bactérienne et sur les propriétés physico-chimiques.

L'hypothèse de recherche est donc que l'utilisation de l'irradiation en combinaison de traitements permettra une meilleure radiosensibilisation virale et permettra donc l'élimination du FCV sur la laitue iceberg.

1.8 Résultats observés

Dans le chapitre 2 intitulé : Revue de littérature, l'article « Norovirus elimination on the Surface of Fresh Foods » aborde les différents traitements actuels utilisés contre le FCV-F9. Parmi les nombreux traitements, on compte l'utilisation de composés chimiques synthétiques comme le chlore et le dioxyde de chlore. On compte aussi l'utilisation de composés naturels comme les extraits de plante (carvacrol) et les extraits de fruits (CJ). Parmi les traitements non-thermiques, on compte la fermentation, l'ozonation, l'utilisation de High Pressure Homogenizer (HPH) et l'utilisation de rayons UV. Le dernier thème abordé dans la revue de littérature est l'utilisation de traitements combinés, principal sujet de ce mémoire. L'objectif de cet article était de visualiser les traitements actuellement utilisés dans le marché des aliments frais afin de trouver des combinaisons possibles avec l'irradiation-γ.

1.8.1 *In-vitro*

Le chapitre 3 aborde l'élimination virale du FCV-F9 de façon *in-vitro* et s'intitule : « Radiosensitivity increase of FCV-F9 virus using combined treatments with natural antimicrobials and γ-irradiation ». L'objectif de ce papier était de sélectionner des concentrations optimales de BS et de CJ ainsi que la dose d'irradiation optimale contre le FCV-F9 en mesurant leur D_{10} respective. Par la suite, l'hypothèse de recherche était que les combinaisons des extraits naturels avec l'irradiation-γ permettrait de diminuer la dose d'irradiation requise pour éliminer le virus FCV-F9 *in-vitro*. Le jus de canneberge et l'extrait de citron (Biosecur F420B) ont tous deux étés utilisés à différentes concentrations contre le virus FCV-F9 de façon *in-vitro* dans des microtubes contenant la suspension virale, le traitement antiviral ainsi que du DPBS (**Objectif 1**). Le JC contient principalement des acides quiniques, maliques et citriques alors que

l'extrait de citron contient des acides citriques, ascorbiques et lactiques. Utilisés seuls, ils ont démontré que plus leur concentration augmentait et plus une diminution importante du titre viral était observée. Pour ce qui est de l'extrait de citron, des concentrations de BS-0.005%, BS-0.01%, BS-0.025%, BS-0.05% et finalement BS-0.1% ont été utilisées seules contre le FCV-F9. La plus grande réduction de 2.04 log $TCID_{50}$/mL a été observée après l'utilisation du BS-0.1%. De plus, une concentration supérieure de BS-0.50% a été testé seule par la suite et a démontré qu'elle abaissait le titre viral au seuil limite de détection instantanément. La D_{10} a été mesurée en effectuant l'inverse de la pente a =22.02, donc de 0.05 %-BS (**Objectif 3**). Pour ce qui est du JC, la même tendance a été observée, plus la concentration est élevée et plus le titre viral diminue après traitement. Des concentrations de CJ-0.10%, CJ-0.25%, CJ-0.5%, CJ-0.75% et CJ-1.0% ont été testées seules contre le virus FCV-F9. La plus grande réduction de 2.20 log $TCID_{50}$/mL a été observée après l'utilisation de la plus grande concentration de CJ-1.0%. La D_{10} a été mesurée en faisant l'inverse de la pente a =2.34, donc de 0.42 %-CJ (**Objectif 3**). Nous pouvons donc conclure que ces deux mélanges qui contiennent majoritairement des acides organiques et des composés phénolés ont un impact antiviral. Il faudra donc combiner des concentrations optimales d'acides organiques avec des doses d'irradiation afin de voir une synergie entre les traitements. Il faudra faire attention de ne pas utiliser des concentrations trop élevées qui abaisseraient le titre viral trop rapidement et qui empêcheraient de voir une synergie avec l'irradiation-γ. L'irradiation γ seule a aussi été testé seule contre le FCV-F9 *in-vitro* et encore une fois, plus la dose d'exposition était élevée et plus le titre viral diminuait rapidement. Le titre initial était de 5.37 log $TCID_{50}$/mL et après irradiation à 1.25 kGy, une diminution de 1.42 log $TCID_{50}$/mL a été observée (à 3.95 log $TCID_{50}$/mL). Après irradiation à 3.0 kGy, une diminution de 2.11 log $TCID_{50}$/mL a été observée. La dose optimale pour éliminer presque la totalité du virus était de 3 kGy. Le CJ et le BS ont ensuite été combinés avec l'irradiation à des doses variant entre 0 et 1.5 kGy pour toutes les concentrations sauf pour la BS-0.025% où les doses variaient entre 0 et 2.5 kGy et pour le CJ-0.5% avec des doses entre 0 et 2 kGy. Le BS-0.01% a montré la plus grande radiosensibilisation, de 1.28 par rapport au contrôle avec irradiation seule. Avec cette même concentration, la plus grande réduction était observée à 1.50 kGy avec 2.16 log $TCID_{50}$/mL de réduction. Par la suite, le BS-0.025% a montré une radiorésistance de 1.65. La plus grande réduction virale avec le BS-0.025% a été observée à 2.5 kGy avec une réduction de 1.42 log $TCID_{50}$/mL. Ce même phénomène de radiorésistance s'est produit avec le BS-0.1%, avec une radiorésistance de 3.88. La plus petite dose de BS est donc optimale en combinaison avec ces doses d'irradiation. La plus grande réduction avec le BS-0.025% a été observée à 1.5 kGy avec 0.25 log $TCID_{50}$/mL de réduction virale. Les deux concentrations de CJ : CJ-0.1% et CJ-0.5% ont montré une radiosensibilisation respective de 1.50 et radiorésistance de 1.31. Encore une fois, la concentration la plus faible de traitement a été la plus efficace contre le FCV-F9. La plus grande réduction virale observée après le traitement CJ-0.1% était observée à 1.5 kGy, avec 2.01

log $TCID_{50}$/mL de réduction. Finalement, pour le CJ-0.5%, la plus grande réduction était observée à 2.0 kGy, avec 1.04 log $TCID_{50}$/mL de réduction par rapport au titre viral initial. Les extraits naturels comme le JC ont permis de réduire de 8 kGy jusqu'à 5.33 kGy la dose d'irradiation requise afin d'éliminer 6 log $TCID_{50}$/mL du virus et le BS en combinaison avec l'irradiation a permis de réduire de 8 kGy jusqu'à 6.25 kGy la dose nécessaire pour éliminer 6 log $TCID_{50}$/mL du virus FCV-F9.

1.8.2 *In-situ*

Le chapitre 4 aborde le traitement de la laitue iceberg infectée en surface avec le FCV-F9 et présente l'article intitulé : « Radiosensitivity of FCV-F9 on Iceberg lettuce surface after combined treatments with γ-irradiation ». Les hypothèses de recherche de ce papier étaient que l'utilisation de l'O_3, du CJ et de l'irradiation-γ permettrait de diminuer la dose d'irradiation requise pour éliminer le virus à la surface de la laitue tout en gardant les qualités physico-chimiques de la laitue durant l'entreposage à 4 °C. L'activité antibactérienne des traitements a aussi été évaluée et l'hypothèse de recherche associée était que la combinaison de traitements permettrait d'abaisser la flore totale microbienne à la surface de la laitue durant l'entreposage à 4 °C. Les 4 objectifs de ce mémoire ont été abordés dans ce chapitre. Les effets du CJ ont été évalués contre le virus sur la laitue (**Objectif 1**), les effets de l'O_3 contre le FCV-F9 ont aussi été évalués (**Objectif 2**), les D_{10} en absence et en présence d'antimicrobiens naturels et la radiosensibilisation en présence d'antimicrobiens naturels ont été évalués (**Objectif 3**) et dernièrement, les traitements combinés sur l'élimination virale, bactérienne et sur les propriétés physico-chimiques ont été évalués *in-situ* (**Objectif 4**).

Traitement de la laitue iceberg avec O_3, CJ et irradiation sans entreposage à 4 °C

Le modèle alimentaire sélectionné a été la laitue iceberg. En premier, l'irradiation-γ seule a été testée contre le virus FCV-F9 à la surface de la laitue iceberg. Les intervalles de doses d'irradiation contre le virus ont été situées entre 0 et 2.5 kGy. Plus la dose d'irradiation était élevée et plus la réduction du titre viral était grande. Le titre viral inoculé à la surface de la laitue était de 6.31 log $TCID_{50}$/mL. Après des doses d'irradiation de 0.5, 1.0, 1.5, 2.0 et 2.5 kGy contre le virus sur la laitue, des réductions de 0.07, 0.60, 0.64, 1.75 et 1.89 log $TCID_{50}$/mL. Une D_{10} de 1.21 kGy a été observée après l'application de plusieurs doses d'irradiation contre le FCV-F9 à la surface de la laitue sans aucun entreposage à 4 °C. Le traitement d'ozonation à une concentration de 5-ppm a été testée durant plusieurs temps d'exposition variant de 0, 2.5, 5.0, 7.5, 10 et 15 minutes (**Objectif 2**). Une régression linéaire a aussi démontré que plus le temps d'exposition était grand et plus la diminution virale sur la laitue iceberg était grande. Le titre viral est passé de 5.54 $TCID_{50}$/mL initialement jusqu'à 4.99, 4.74, 4.62, 4.57 et 4.41 $TCID_{50}$/mL pour les temps

ascendants respectifs de traitement à l'ozone. Une D_{10} de 14.93-ppm O_3 a été calculée à partir de la pente de la courbe des titres viraux traités à différents temps d'ozonation. Ces résultats ne sont pas effectués à partir de laitue iceberg entreposée à 4 °C durant plusieurs jours, les analyses étaient faites le jour même. Finalement, plusieurs concentrations de CJ ont été testées seules contre le virus FCV-F9 à la surface de la laitue iceberg. Une régression linéaire a été observée suite aux traitements, plus la concentration de CJ augmentait et plus la réduction du titre viral était grande. Le titre viral initial était de 5.70 log $TCID_{50}$/mL et des réductions de 0.58, 0.71, 0.71, 0.83, 0.85 et 0.90 log $TCID_{50}$/mL ont respectivement été observées pour des concentrations de CJ-0.1%, CJ-0.25%, CJ-0.50%, CJ-0.75%, CJ-1.0% et CJ-1.5%. La D_{10} mesurée était de 2.38 %-CJ à partir de la pente de la courbe des différentes concentrations de CJ contre le FCV-F9 à la surface de la laitue iceberg. En combinant le CJ-0.1%, CJ-0.25%, CJ-0.50% et CJ-1.5% avec l'irradiation-γ, cela a permis de mesurer les D_{10} des contrôles et des traitements afin de mesurer les radiosensibilisations après les combinaisons de traitements entre le CJ et l'irradiation. Le CJ-0.1% a donné une D_{10} de 0.95 kGy, le CJ-0.25% de 1.33 kGy, le CJ-0.5% de 1.91 kGy et finalement le CJ-1.5% de 2.08 kGy. La meilleure radiosensibilisation a été observée avec le CJ-0.25%, car la D_{10} du contrôle était de 2.00 kGy donc cela a donné une RS de 1.50. De plus, une réduction de 0.98 log $TCID_{50}$/mL a été observée après un traitement de 1.5 kGy en combinaison avec le CJ-0.25%. Les autres radiosensibilisations étaient de 1.2 pour le CJ-0.1%, 1.09 pour CJ-0.5% et finalement 1.00 pour le CJ-1.5%. La tendance de régression linéaire plus la dose d'irradiation augmentait et plus le titre viral diminuait a été observée avec les 4 concentrations de CJ.

Traitement de la laitue iceberg avec O_3, CJ et irradiation avec entreposage à 4 °C

Les laitues iceberg ont été inoculées avec le FCV-F9 à environ 7.77 log $TCID_{50}$/mL. Ensuite, elles ont été traitées avec : 1) aucun traitement (contrôle), 2) CJ-0.25% seul, 3) O_3 5-ppm durant 7.5 minutes, 4) irradiation-γ à 1.5 kGy et finalement 5) les trois traitements combinés ensembles. Par la suite, elles ont été mises dans des sacs de type Whirlpak et entreposées à 4 °C durant 10 jours pour les tests de virologie, colorimétrie, chlorophylle et de texture et analysées tous les deux jours pour ces paramètres précédemment énumérés (**Objectif 4**). Les feuilles de laitues ont été entreposées jusqu'à 13 jours pour les comptes totaux bactériens et analysées à tous les trois jours. Finalement, les laitues ont été goûtées par une vingtaine de personne aux jours d'entreposage 0, 3 et 5 après les 3 traitements sélectionnés suivants : 1) contrôle, 2) CJ-0.25% seul et 3) les 3 traitements combinés. Pour ce qui est du titre viral après tous les 5 traitements, une réduction de 1.11 log $TCID_{50}$/mL a été observée pour un contrôle sans traitement après 10 jours d'entreposage à 4 °C. Après le traitement contenant du CJ-0.25% contre le FCV-F9 à la surface de la laitue, une diminution de 1.20 log $TCID_{50}$/mL a été observée au jour 8 d'entreposage et une diminution de 0.57 log $TCID_{50}$/mL au jour 10. Cela insinue que le pic d'activité antivirale du CJ-0.25% durant

l'entreposage à 4 °C est situé au jour 8, après quoi l'activité antivirale diminue. Après 10 jours d'entreposage à 4 °C, le titre viral a diminué de 1.49 log $TCID_{50}$/mL après ozonation de la laitue à 5-ppm durant 7.5 minutes. Après l'irradiation-γ à 1.5 kGy seulement, une réduction de 1.63 log $TCID_{50}$/mL a été observée 10 jours après l'entreposage. La meilleure réduction durant le stockage a été observée après le traitement combinant les trois traitements durant les 10 jours d'entreposage à 4 °C, pour une baisse de 2.15 log $TCID_{50}$/mL (99 %). Lors des analyses de l'impact des traitements contre la flore totale initialement présente à la surface de la laitue iceberg (inoculée à 5.5 log CFU/g), l'entreposage a duré 13 jours et les analyses ont été faites tous les trois jours. Le MAPAQ a posé une norme d'acceptabilité de flore totale mésophile sur les aliments frais à 7 log CFU/g. Le groupe contrôle est passé de 5.53 log CFU/g jusqu'à 8.22 log CFU/g au jour 13. La durée de vie de l'aliment sans aucun traitement était de seulement 7 jours. Après avoir traité la laitue avec le CJ-0.25%, les comptes totaux sont passés de 5.49 log CFU/g à 7.55 log CFU/g après 13 jours de stockage. La durée de vie de l'aliment est passée à 10 jours, gagnant 3 jours de plus que l'absence de traitement. Après avoir traité la laitue avec l'ozone 5-ppm durant 7.5 minutes, les comptes totaux mésophiles sont passés de 5.69 log CFU/g jusqu'à 7.34 log CFU/g. La durée de vie de l'aliment est aussi passée à 10 jours après ozonation. Le groupe d'irradiation-γ à 1.5 kGy seul est resté constant à 2.0 log durant toute la durée du stockage, n'atteignant ainsi jamais la limite du MAPAQ et finalement la combinaison de traitement a laissé les comptes totaux à 0 tout au long de l'expérience, signifiant que c'est le meilleur traitement pour le stockage durant 13 jours de la laitue iceberg. Après 13 jours de stockage, la combinaison de traitement était significativement différente de tous les groupes, l'irradiation aussi, le CJ et l'O_3 ensembles et finalement le contrôle était aussi significativement différent de tous les groupes. Visuellement l'aspect des feuilles de laitue après tous les traitements ne montrait pas de différence majeure. Cependant, l'appareil Konica Minolta a été utilisé afin de mesurer les paramètres L*, a* et b* de colorimétrie. Pour le paramètre de clarté et de noirceur de l'aliment, le paramètre L*, après 10 jours de stockage, tous les traitements ont montré une légère hausse de L*, un éclaircissement de la feuille. En ordre de plus grand L* après 13 jours, le premier étant CJ-0.25%, ensuite la combinaison de traitement, le contrôle, l'irradiation et ensuite l'ozonation. Le paramètre a* donne la valeur de vert après 13 jours de stockage à 4 °C. Le groupe contrôle et CJ-0.25% n'ont pas eu de changement significatif. Les groupes ozone et combinaison ont eu une légère diminution du vert alors que le groupe irradiation seule a eu une diminution significative du vert. Pour ce qui est du paramètre b*, celui-ci indique la différence entre le jaune et le bleu. Toutes les feuilles sont devenues un peu plus jaune après 10 jours de stockage sauf le groupe traité avec de l'irradiation. La chlorophylle a été mesurée aux jours 0, 6 et 10 d'entreposage à 4 °C. Une diminution de ce pigment a été observée linéairement pour les groupes contrôles, ozone et irradiation-γ. L'irradiation-γ a donné une diminution très significative de chlorophylle après l'entreposage à partir du jour 6. Le CJ-0.25% a causé une diminution de la chlorophylle lors des jours 0 et 6

d'entreposage mais au jour 10, une augmentation a été observée significativement différente du jour 6. Finalement, une stabilité de la chlorophylle a été observée pour l'entreposage de la laitue traitée avec la combinaison de traitement. Pour ce qui est de la texture, aucune différence significative n'a été observée après tous les traitements durant tous les 10 jours d'entreposage à 4 °C sauf au jour 6 où l'O_3 était significativement différent du contrôle. Finalement, l'analyse sensorielle a été effectuée sur une vingtaine de personne et aucune différence en termes de couleur, d'odeur, de goût et de texture n'a été observée aux jours 0, 3 et 5 d'entreposage des laitues traitées avec 1) contrôle, 2) CJ-0.25% seul et 3) trois traitements combinés sauf pour le contrôle qui a eu des notes inférieures au jour 5 en termes de goût et de couleur. Le traitement favori pour tous les paramètres mesurés était le CJ-0.25% seul au 5ème jour d'entreposage à 4 °C.

Bibliographie

1- Adams, M., Moss, M., and McClure, P. (2015). *Food Microbiology* (4th ed., p. 562). Royal Society of Chemistry.

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

3- Arthur, S. E., and Gibson, K. E. (2015). Physicochemical stability profile of Tulane virus: a human norovirus surrogate. *Journal of Applied Microbiology*, 119 : 868-875.

4- ATCC, (2014). *Q&A, ATCC- Excellence in Research Webinar "ATCC-Influenza Research Materials"*. [online] Available at: https://www.atcc.org/~/media/PDFs/webinars/QA/2014/QA_Webinar_Influenza.ashx [Accessed 16 Nov. 2019]. Virginia, USA.

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

6- Biswas, N., Balac, P., Narlakanti, S. K., Haque, M. D. E., and Hassan, M. D. M. (2013). Identification of phenolic compounds in processed cranberries by HPLC method. *Journal of Nutrition & Food Sciences*, 3.

7- Butot, S., T. Putallaz, R. Amoroso, and G. Sanchez. (2009). Inactivation of enteric viruses in minimally processed berries and herbs. *Applied and Environmental Microbiology,* 75: 4155–4161.

8- Cann, A. J. (2005). Principles of molecular virology. Elsevier Academic Press, 4th Edition. University of Leicester, UK.

9- Cannon, J. L., Papafragkou, E., Park, G. W., Osborne, J., Jaykus, L. A., and Vinjé, J. (2006). Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *Journal of Food Protection*, 69 : 2761-2765.

10- Cao, C. (2013). Chemical Disinfectants for Inactivation of Human Norovirus Surrogates.

11- Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J. F., and Lacroix, M. (2011). Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22: 1413-1418.

12- Cromeans, T., Park, G. W., Costantini, V., Lee, D., Wang, Q., Farkas, T., ... and Vinjé, J. (2014). Comprehensive comparison of cultivable norovirus surrogates in response to different inactivation and disinfection treatments. *Applied and Environmental Microbiology*, 80 : 5743-5751.

13- Darling, A. J., Boose, J. A., and Spaltro, J. (1998). Virus assay methods: accuracy and validation. *Biologicals*, 26 : 105-110.

14- Doyle, M. P., and M. C. Erickson. (2008). Summer meeting 2007—the problems with fresh produce: an overview. *Journal of Applied Microbiology*, 105: 317–330.

15- Ehling, S., and Cole, S. (2011). Analysis of organic acids in fruit juices by liquid chromatography− mass spectrometry: an enhanced tool for authenticity testing. *Journal of Agricultural and Food Chemistry*, 59 : 2229-2234.

16- Elvis, A. M., and Ekta, J. S. (2011). Ozone therapy: A clinical review. *Journal of Natural Science, Biology, and Medicine*, 2 : 66.

17- Esseili, M. A., Saif, L. J., Farkas, T., and Wang, Q. (2015). Feline calicivirus, murine norovirus, porcine sapovirus, and Tulane virus survival on postharvest lettuce. *Applied and Environmental Microbiology*, 81: 5085-5092.

18- Feng, K., Divers, E., Ma, Y., and Li, J. (2011). Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*, 77 : 3507-3517.

19- Fields, B. N., Knipe, D. M., Howley, P. M., Everiss, K. D., and Kung, H. J. (1996). *Fundamental Virology* Philadelphia ePA PA: Lippincott-Raven, pp. 401-402.

20- Götz, M. E., Künig, G., Riederer, P., and Youdim, M. B. (1994). Oxidative stress: free radical production in neural degeneration. *Pharmacology & Therapeutics*, 63 : 37-122.

21- Guzel-Seydim, Z. B., Greene, A. K., and Seydim, A. C. (2004). Use of ozone in the food industry. *LWT-Food Science and Technology*, 37 : 453-460.

22- Hall, A. J., Wikswo, M. E., Pringle, K., Gould, L. H., and Parashar, U. D. (2014). Vital signs: foodborne norovirus outbreaks—United States, 2009–2012. *MMWR. Morbidity and Mortality Weekly Report*, 63: 491.

23- Heaton, J. C., and K. Jones. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *Journal of Applied Microbiology* 104:613–626.

24- Herwaldt, B. L., Lew, J. F., Moe, C. L., Lewis, D. C., Humphrey, C. D., Monroe, S. S., ... and Glass, R. I. (1994). Characterization of a variant strain of Norwalk virus from a food-borne outbreak of gastroenteritis on a cruise ship in Hawaii. *Journal of Clinical Microbiology*, 32 : 861-866.

25- Hirneisen, K. A., Markland, S. M., and Kniel, K. E. (2011). Ozone inactivation of norovirus surrogates on fresh produce. *Journal of Food Protection*, 74: 836-839.

26- Hirneisen, K. A., and Kniel, K. E. (2013). Comparing human norovirus surrogates: murine norovirus and Tulane virus. *Journal of Food Protection*, 76: 139-143.

27- Kim, J. G., Yousef, A. E., and Khadre, M. A. (2003). Ozone and its current and future application in the food industry. *Advances in Food and Nutrition Research*, 45: 167-218.

28- Lemay, G. (2017). *MCB 2994, virologie et pathogenèse virale*. Montréal: Librairie de l'Université de Montréal. Montréal, Canada.

29- Oldstone, M. B. (2009). *Viruses, plagues, and history: past, present and future*. Oxford University Press.

30- Predmore, A., and Li, J. (2011). Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. *Applied and Environmental Microbiology*, 77: 4829-4838.

31- Predmore, A., Sanglay, G., Li, J., and Lee, K. (2015). Control of human norovirus surrogates in fresh foods by gaseous ozone and a proposed mechanism of inactivation. *Food Microbiology*, 50 :118-125.

32- Simek, M., and Clupek, M. (2002). Efficiency of ozone production by pulsed positive corona discharge in synthetic air. *Journal of Physics D: Applied Physics*, 35 : 1171.

33- Su, X., Howell, A. B., and D'Souza, D. H. (2010). Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. *Food Microbiology*, 27: 985-991.

34- Terio, V., Bottaro, M., Pavoni, E., Losio, M. N., Serraino, A., Giacometti, F., ... and Tantillo, G. (2017). Occurrence of hepatitis A and E and norovirus GI and GII in ready-to-eat vegetables in Italy. *International Journal of Food Microbiology*, 249: 61-65.

35- Wolff, S. P., Garner, A., and Dean, R. T. (1986). Free radicals, lipids and protein degradation. *Trends in Biochemical Sciences*, 11 : 27-31.

Le prochain chapitre intitulé : Revue de littérature abordera les différents traitements appliqués à la surface d'aliments afin d'éliminer le FCV-F9, analogue du NoV chez l'humain. C'est un article qui est soumis au journal : Critical Reviews in Food Science and Nutrition. Le chapitre 1 présentait l'introduction générale de ce mémoire alors que le chapitre 2 est un article qui présente les traitements actuels déjà connus et appliqués sur le marché. Grâce au chapitre 2, nous pourrons sélectionner des traitements qui nous semblent intéressants et les combiner avec l'irradiation-γ afin d'éliminer le FCV-F9 *in-vitro* (**chapitre 3**) et *in-situ* (**chapitre 4**).

Chapitre 2: Revue de littérature

Norovirus elimination on the Surface of Fresh Foods

Alexandra Gobeil, Behnoush Maherani, Monique Lacroix*

INRS-Institut Armand-Frappier, Research Laboratories in Sciences, Applied to Food, Canadian Irradiation Centre, 531, Boulevard des Prairies, Laval, Québec, Canada, H7V 1B7.

* Corresponding author: Professor Dr. Monique Lacroix.
E-mail address: monique.lacroix@iaf.inrs.ca
Tel.: 450-687-5010 #4489.
Fax: 450-686-5501.

Contribution des auteurs

Alexandra Gobeil a réalisé la rédaction de l'article.
Behnoush Maherani a participé à la correction de l'article.
Monique Lacroix: Directrice de recherche, a participé à la planification des expériences et aux discussions du sujet de recherche et a aussi procédé à la correction de l'article.
L'article a été soumis au Journal Critical Reviews in Food Science and Nutrition le 29 avril 2019 (BFSN-2019-4175)

Abstract

Fresh foods like fruits, vegetables and shellfish are potential sources for viral infections such as human norovirus (NoV). Chemical treatment like chlorination is a well-known process for food pathogens and virus elimination. However, with the increase of the consumer demands for less toxic treatments, the use of natural antimicrobials like essential oils from spice or plants, fruit extracts, and cold pasteurisation treatments (fermentation, irradiation, ozonation and high pressure) could be considered. The aim of this review is to discuss these technologies and their efficacy to eliminate NoV on the surface of fresh food.

Keywords: Human Norovirus, Inactivation, Feline calicivirus, Murine Norovirus, Fresh Food and Food surfaces.

Abbreviations

AJ: Apple Juice

B-PACs: Blueberry Proanthocyanidins

Cl: Chlorine

ClO_2: Chlorine Dioxide

COD: Chemical Oxygen Demand

CJ: Cranberry Juice

CJ-PACs: Cranberry Juice Proanthocyanidins

CP: Cranberry Polyphenols

$C_2H_4O_3$: Peracetic Acid

EOs: Essential oils

FAO: Food Agriculture Organization

FCV or FCV-F9: Feline Calicivirus (Strain F-9)

GSE: Grape Seed Extract

HAV: Hepatitis-A Virus

HCV: Hepatitis-C Virus

HHP: High Hydrostatic Pressure

HPH: High-Pressure Homogenization

HSV-1 and HSV-2: Herpes Simplex Virus

H_2O_2: Hydrogen Peroxide

IAEA: United Nations International Atomic Agency

Kbp: Kilo base pair

LAB: Lactic Acid Bacteria

MNV or MNV-1: Murine Norovirus (Strain 1)

NaClO: Sodium Hypochlorite

NoV: Human Norovirus

O_2: Dioxygen

O_3: Ozone

PACs: Proanthocyanidins

PFU/mL: Plaque-forming unit /mL

PJ: Pomegranate juice

PP: Pomegranate Polyphenols

ssRNA: single-stranded RNA

ssDNA: single-stranded DNA

TCID50/mL: Tissue Culture Infectious Dose 50 / mL

USD: United State Dollars

USDA: United States Department of Agriculture

UV: Ultraviolet light

UVTP: TiO_2 photocatalysis

VLPs: NoV virus-like particles

VSV: Vesicular Stomatitis

WHO: World Health Organization

ZSO: *Zanthoxylum schinifolium*

1 Introduction

Human norovirus (NoV) is the leading cause of viral gastroenteritis worldwide. Indeed, the annual costs associated with disease are high and include absences from work, hospitalization or death. In fact, according to the United States Department of Agriculture (USDA), food-borne illnesses cost between 10 to 83 billion USD each year (McLinden *et al*., 2014). NoV is ranked among 5 of the costliest pathogens associated with foodborne infections within the United States of America (Scharff, 2012). Fruits, vegetables and shellfish are highly contaminated by viruses such as NoV (Berger *et al*., 2010). They are mainly contaminated before and after harvest in the farm. Indeed, even before harvest, fertilizers and water used for irrigation can contaminate foods (Cheong *et al*., 2009; Mara and Sleigh, 2010; Wei and Kniel, 2010). In addition, it only takes less than 100 infectious particles of NoV to cause the disease (Sair *et al*., 2002).

NoV is a member of the caliciviridae family, containing a positive-sense single-stranded RNA genome (approximately 7.7 kb in size) enclosed in a non-enveloped capsid (Glass *et al*., 2009). This type of virus is highly resistant to environmental conditions and antimicrobials in general (Watanabe *et al*., 1989; Barker *et al*., 2001). NoVs are enteric viruses that are present in faeces and vomit of infected individuals (Rzeżutka and Cook, 2004). Humans are involuntarily infected during their life with NoVs because of the multiple transmission modes. For example, there is direct transmission from person-to-person with contaminated individuals. Plus, there is also transmission through manipulation of surfaces or objects contaminated by NoV (dishes preparation, utensils, handles) and finally the consumption of contaminated foods with NoV such as potable water (Lopman *et al*., 2012). The fact that NoV is able to survive on many environmental surfaces for weeks represents a major concern in finding ways to inactive it (Barker *et al*., 2004).

People who have been exposed to the virus, usually have symptoms from 24 - 48 hours or within the first 12 hours post-consumption (Fretz-Männel *et al*., 2005). The most common symptoms include nausea, vomiting, diarrhea and stomach cramps (Ahmed *et al*., 2014; Patel *et al*., 2008; Ryu *et al*., 2015). Currently, it remains impossible to propagate NoV and to distinguish infectious particles. There are no animal models or cell culture possible for NoV detection. However, there are NoV surrogates such as feline calicivirus (FCV) and murine norovirus (MNV) (Hirneisen *et al*., 2011; Lacombe *et al*., 2017). They represent accurate models since they have the same shape and size as NoV (Wobus *et al*., 2006). Foods such as lettuce are good representative models for research on NoV because of the multipliable leaves and the many inclusions, making it difficult to adequately wash (Fraisse *et al*., 2011).

In fact, there has been an evolution of consumer's demand in correlation with food technologies. Since the 80's, consumers have desired mass-produced, ready-to-eat and individual portions (Cayot, 2007). Moreover, there is increasing need to develop safer and healthier food without synthetic additives. There are many different techniques for food decontamination; two of them are ozonation (Khadre *et al*., 2001; Hirneisen *et al*., 2011) and irradiation (Lacroix and Vigneault, 2007; Holley, 2014). NoV is highly resistant to chemicals such as alcohol and quaternary ammonium compounds (Donaldson *et al*., 2008). It is important to notice that nonenveloped viruses such as NoV are resistant to many antimicrobials under various environmental conditions (Gilling *et al.,* 2014B). This contributes into viral spread of NoV during transmission.

The aim of this review is to discuss on the use of natural extracts to eliminate NoV from food surfaces by using essential oils (EOs), natural plant extracts and also various techniques such as fermentation, irradiation, ozonation and high pressure.

2 Anti-viral Treatments

2.1 Synthetic chemical compounds

Currently, industries use a washing process for ready-to-eat fruits and vegetables that includes detergents, followed by a rinsing step with water (Fraisse *et al.,* 2011). Detergents have been proven effective for their antimicrobial properties. The inactivation of viral infectivity is mainly due to damage of the viral genome. For example, H_2O_2 is an oxidizing agent and produces hydroxyl free radicals (•OH) that target essential cell components like lipids, proteins and DNA (Fraisse *et al.,* 2011). Bleach is probably the most common disinfectant used in food industries, because it acts quickly and efficiently and because of its low cost (Fraisse *et al*., 2011; Seymour and Appleton, 2001). Chlorine Dioxide is known for its antiviral properties such as acting on viral nucleic acids and proteins (Li *et al.,* 2004).

Chlorine (Cl) and Chlorine Dioxide (ClO_2)

Chlorine is an important chemical for treatment of drinking water. It is also found in disinfectants or in detergents and acts as a bleaching agent (Beuchat, 1998). Chlorine Dioxide (ClO_2) and Hydrogen peroxide (H_2O_2) are both oxidizing agents targeting nucleic acids and proteins. For enteric viruses, such as NoV, FCV and MNV, the first target should be the capsid and followed next by the nucleic acid. The gaseous form of these two compounds is more antimicrobial than the aqueous state because of increased diffusibility and the penetration (Montazeri *et al.,* 2017).

Viral FCV suspensions of 20 µL ($2.6x10^6$ TCID) were treated with sodium hypochlorite (NaClO) to determine viral sensitivity to this chemical agent (10 µg/mL) (Urakami *et al*., 2007). The study showed that the sensitivity of viral FCV increased with a reduction in the amount of cellular host debris. They

partially purified the virus from the cellular hosts and demonstrated that FCV infectivity was reduced by more than 4.6 log after a 5 minutes treatment in presence of 300 ng/mL of free chlorine (Urakami *et al*., 2007). Thurston-Enriquez *et al.* (2003) treated FCV in presence of free-chlorine at a concentration of 0.5 mg/L for 0.25 minutes (pH 6.0 and 5 °C) and observed a 4.3 log reduction of FCV in buffered-demand-free (BDF) water.

Duizer *et al.* (2004) studied the effect of 3000 ppm of NaClO on FCV ($2x10^5$ $TCID_{50}$/mL-$1x10^6$ $TCID_{50}$/mL) and obtained a reduction of 5.0 $\log_{10}$ $TCID_{50}$/mL after 10 minutes of treatment at room temperature. Belliot *et al.* (2008) observed a reduction of 4.0 $\log_{10}$ PFU/mL of MNV-1 after a treatment with NaClO at 36.4 mM for 0.5 minute.

Industrial processes used for ready-to-eat vegetables include a prior washing step with tap water containing a disinfectant such as NaClO followed by a rinsing step. Fraisse *et al.* (2011) demonstrated that a washing step with or without ultrasounds followed by another washing step, in presence of disinfectant such as NaClO during 2 minutes at 15 ppm, caused a decrease of 2.9 log units of an FCV population on the surface of fresh lettuce. However, MNV-1 was shown to be a bit more resistant; a 1.4 log reduction was observed with the same treatment. A simple disinfectant-free wash showed a decrease of 0.7 log for FCV and 1.0 log for MNV-1 (Fraisse *et al.,* 2011).

Chlorine dioxide is a yellow/red gas and represents an oxidized form of chlorine. It is also used as a disinfectant and bleaching agent (Du *et al*., 2002). Thurston-Enriquez *et al.* (2005) studied the efficiency of ClO_2 against FCV after 0.75 minutes of treatment with ClO_2 at a concentration of 0.90 mg/L at pH 8.0 at a temperature of 5 °C. A reduction of 3.6 $\log_{10}$ most probable number/mL (MPN/mL) was observed. When a concentration of 0.72 mg/L was used for 0.25 minutes of treatment at pH 8 and at a temperature of 15 °C, a reduction of more than 4.15 $\log_{10}$ MPN/mL was observed.

Peracetic acid ($C_2H_4O_3$) is a biocidal agent and its degradation products (acetic acid and oxygen) are less toxic for the environment (McDonnell and Russell, 1999). The mode of action of this product results in the disruption of chemical bonds that stabilize the cell membrane of microorganisms (Block, 2001). Fraisse *et al.* (2011) demonstrated that a concentration of 100-ppm of $C_2H_4O_3$ can reduce the level of FCV by 3.2 log and MNV-1 by 2.3 log. Their results also showed that a simple disinfectant-free water wash showed a decrease of 0.7 log for FCV and 1.0 log for MNV-1. The washing of fruits and vegetables with chemicals can incur additional costs. For example, sodium-ortho-phenylphenate-hexamine creates dullness of fruits, which then require wax for polishing (Smith, 1962).

Industries are now moving towards more natural food-processing pathways, and many new options are being considered, notably the use of natural extracts like plants, spice and fruit extracts. These compounds could be used alone or in combination with cold processes like ozonation, irradiation or high pressure. Fermentation is also considered as an efficient process to eliminate the virus.

Table 1. Recapitulative table of the performance of synthetic chemical compounds against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Free chlorine	300 ng/mL, 5 min	4.6 $\log_{10}$ $TCID_{50}$/mL FCV	Urakami *et al.* (2007)
Free chlorine	0.5 mg/L, 0.25 min at pH 6 and 5 °C	4.3 $\log_{10}$ MPN/mL FCV	Thurston-Enriquez *et al.* (2003)
NaClO	3000 ppm, 10 min at RT	5 $\log_{10}$ $TCID_{50}$/mL FCV	Duizer *et al.* (2004)
NaClO	36.4 mM for 0.5 min	4 $\log_{10}$ PFU/mL MNV-1	Belliot *et al.* (2008)
Washing step (with or without ultrasound) followed by washing with NaClO	2 min at 15 ppm of active chlorine	2.9 $\log_{10}$ $TCID_{50}$/mL FCV 1.4 $\log_{10}$ $TCID_{50}$/mL MNV	Fraisse *et al.* (2011)
ClO_2	0.75 min at 0.90 mg/L at pH 8.0 and at a temperature of 5 °C	3.6 $\log_{10}$ MPN/mL FCV	Thurston-Enriquez *et al.* (2005)
ClO_2	0.25 min at 0.72 mg/L at pH 8.0 and at a temperature of 15 °C	4.15 $\log_{10}$ MPN/mL FCV	Thurston-Enriquez *et al.* (2005)
$C_2H_4O_3$	100 ppm	3.2 $\log_{10}$ $TCID_{50}$/mL FCV 2.3 $\log_{10}$ $TCID_{50}$/mL MNV	Fraisse *et al.* (2011)

MPN: most probable number, RT: room temperature

2.2 Natural compounds used to eliminate virus

2.2.1 Plant extracts

Plant extracts are rich in polyphenols. These compounds include amongst others phenolic acids, flavonoids (flavanones, anthocyanidins) and tannins (Shahidi and Ambigaipalan, 2015). Flavonoid is the most important category and is subdivided into six classes: Flavones, Flavanones, Flavonols, Isoflavones, Anthocyanidins and Flavanols (Manach *et al*., 2004). Flavonoids are widely found in fruits, in vegetables and in red wine. A diet rich in fruits and vegetables is correlated with low incidences of coronary heart disease and cancer (Block *et al*., 1992). Some phenolic compounds present in fruits and vegetables such as flavonoids (apigenin, myricetin, robinetin), isoflavanoids (biochanin A) and lignans (sesamin) showed protective effects against cancer in animal models (Vercauteren and Chèze, 1998).

Carvacrol is a monoterpenic phenol (Ultee *et al.,* 2000). In fact, carvacrol is a powerful bactericidal, antiviral and anti-infectious compound (Nostro and Papalia, 2012). This compound is found in many spices like oregano, savory and thyme (Alinkina *et al*., 2013) and has been recognized as a

natural and economic preservative (Lu and Wu, 2010). It was observed by Gilling *et al.* (2014B) that carvacrol can also inhibit murine norovirus (MNV) by acting on the capsid first and subsequently on the viral RNA. Sanchez *et al.* (2015) tested the efficiency of 0.25, 0.50 and 1% carvacrol against FCV, MNV and Hepatitis A in quantities of 6-7 log Tissue Culture Infectious Dose $_{50}$/mL ($TCID_{50}$, quantity of pathogen agent that will produce pathological change in 50% of cell culture inoculated) during 2 hours at 37 °C. Carvacrol at a concentration of 0.5% (w/w) completely inactivated the two NoVs surrogates (FCV and MNV) and carvacrol at a concentration of 1.0% completely inactivated HAV. Carvacrol was also tested on lettuce and when added to lettuce rinse, the efficiency of carvacrol against MNV virus was dependent on the chemical oxygen demand (COD), which is an indicative measure of the amount of oxygen that is needed for the oxidation of all organic substances in the solution or the amount of oxygen that can be consumed by reactions in a measured solution. A complete elimination of FCV at a concentration of 6-7 log $TCID_{50}$/mL was observed when carvacrol 0.5% (w/w) was added on inoculated lettuce. According to Gilling *et al.* (2014B), the efficiency of carvacrol against MNV-1 can be effective at concentrations < 0.5%. After only 15 minutes, carvacrol (0.25% and 0.5%) decreased the viral titer by 1.03 and 1.28 $\log_{10}$ respectively. After 60 minutes of treatment, a decrease of 1.95 $\log_{10}$ and 3.87 $\log_{10}$ of MNV-1 in presence of carvacrol (0.25% and 0.50%) was respectively obtained.

Antiviral activities of flavones (apigenin, baicalein and luteolin) have been also demonstrated. For example, simultaneous application of apigenin with acyclovir showed antiviral effects against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), Poliovirus type-2 and hepatitis C virus (HCV) (Zakaryan *et al.,* 2017). A research on flavonoids showed that among all 10 flavonoids tested on FCV and MNV, kaempferol exhibited the most antiviral activity at 200 uM reducing FCV titers by 69.76% (Seo *et al.,* 2016). The efficiency of kaempferol was explained by the structure-activity relationship showing that kaempferol worked as a neuraminidase inhibitor.

Su and D'Souza (2013) studied the mechanism of action of flavonoids on NoV surrogates. They tested four flavonoids: myricetin, L-epicatechin, tangeretin and naringenin for their antiviral properties. Their results showed that in presence of myricetin at a concentration of 0.5 mM and 1 mM, FCV-F9 at an initial viral titer of 5 $\log_{10}$ Plaque-forming unit (PFU)/mL, was completely inactivated to undetectable levels after 2 hours of treatment at 37 °C. Tangeretin and naringenin did not reduce the FCV viral titer. The viral titer of FCV-F9 was also decreased by 1.40 $\log_{10}$ PFU/mL when treated with L-epicatechin at a concentration of 0.5 mM. The FCV-F9 (viral titer 7 $\log_{10}$ PFU/mL) after a treatment with myricetin and L-epicatechin at 0.5 mM concentrations, respectively, was decreased by 3.17 and 0.72 $\log_{10}$ PFU/mL and by 1.73 $\log_{10}$ PFU/mL with myricetin at a concentration of 0.25 mM (Su and D'Souza, 2013).

Natural extracts can be used under the form of essential oils and are composed of volatile compounds such as terpenes, terpenoids, aldehydes, esters, phenol-derived aromatic components and

aliphatic components (Bakkali *et al.,* 2008; Oh and Chung, 2014). Steam distillation is one of the most popular methods to extract essential oils from plants (Boutekedjiret *et al.,* 2003). EOs have the potential as natural agents to increase shelf life of foods (Burt and Reinders, 2003). There are more than 3000 EOs that are already recognized (Burt, 2004) and over 300 are on the market and usable. EOs are known for their antibacterial properties against many pathogenic bacteria such as *Campylobacter jejuni, Listeria monocytogenes, Salmonella, Staphylococcus* and *Escherichia coli* O157:H7 (Elizaquivel *et al*., 2013; Ghabraie *et al.,* 2016). Their actions are mainly on the membrane permeability, which results in a loss of metabolites and enzyme denaturation (Oussalah *et al.,* 2006; Boumail *et al*., 2016). So far, the antiviral effects of EOs against human enteric viruses are still mainly unknown (Elizaquivel *et al.,* 2013). On the other hand, antiviral effects have been found with *Zanthoxylum schinifolium* (ZSO) essential oil, which contains oleic acids (composition of 35.36%) and linoleic acids (22.6%) as major fatty acids present in ZSO. ZSO at a concentration of 0.01% reduced by 70% the viral titer of FCV-F9. A direct interaction between NoV surrogates and ZSO components has been proposed (Oh and Chung, 2014). Another research group has attempted to understand the antiviral mechanisms of action of several essential oils against HSV. It was shown that the altered viral multiplication step was just before adsorption or during adsorption itself but not during viral penetration of the cell. This indicates that essential oils may be acting by inhibiting components of virus essential for cell adhesion (Reichlig *et al.,* 2009). According to Gilling *et al.* (2014A) some essential oils like Allspice could act on the viral capsid of MNV to affect its integrity causing the viral genome to be exposed and targeted.

Elizaquivel *et al.* (2013) tested three essential oils including *Origanum compactum*, *Eugenia caryophyllus* and *Zataria multiflora Boiss* against NoV surrogates, FCV and MNV. The essential oils were individually mixed with each virus at titers of 7-8 log $TCID_{50}$/mL and incubated for 2 hours at 4 °C and 37 °C. Results showed that the presence of 2% oregano decreased the FCV titers by more than 3.75 log $TCID_{50}$/mL at 37 °C. A decrease in viral titers of 1.04, 1.17 and 1.62 log $TCID_{50}$/mL of MNV at respective concentrations of 0.5%, 1% and 2% of oregano, was observed. Cloves and zataria followed the same trends as oregano, and maximum viral reduction was achieved with 0.1% zataria at 37 °C to eliminate FCV.

Azizkhani and Tooryan (2016) investigated the antiviral effects of cinnamon (1, 2 and 7%), rosemary (1, 1.5, 2 and 2.5%) and zataria (0.01, 0.04, 0.08 and 0.1%) against FCV and MNV. The essential oils were individually mixed with viruses at titers between 7-8 log $TCID_{50}$/mL and incubated 2 hours at 4 and 37 °C. In samples incubated at 37 °C, cinnamon at a concentration of 3% reduced the FCV titer by 2.38 log $TCID_{50}$/mL. A concentration of 2.5% rosemary reduced the titer by 3.38 log $TCID_{50}$/mL and finally, a concentration of 0.1% of zataria reduced the FCV titer by 4.51 log $TCID_{50}$/mL. For MNV, results showed a reduction of 0.25 log $TCID_{50}$/mL when treated with zataria 0.1% and a reduction of 1.44

log $TCID_{50}$/mL in presence of rosemary (2.5%) at 37 °C (Azizkhani and Tooryan, 2016).

2.2.2 Fruit extracts

Fruit extracts are rich in polyphenols (Block *et al.,* 1992). Polyphenols from cranberries are known for their antioxidant (Côté *et al.,* 2011A; Heinonen, 2007), antibacterial (Côté *et al.,* 2011C), anti-inflammatory (Vorsa *et al.,* 2007), anti-mutagenic (Dixon *et al.,* 2005), anti-viral (Scalbert, 1991) and pharmacological properties (Wu *et al*., 2009). These phytochemical compounds include flavanols and cinnamic acid derivatives like gallic acid. Gallic acid is a phenolic acid that is present in flavonoids (Scalbert, 1991). Tannins are distributed in two groups: proanthocyanidins and hydrolysable tannins (Scalbert, 1991). In fact, tannic acid was found to have more antiviral activity against poliovirus than gallic acid (Konowalchuk and Speirs, 1976). Many plant compounds (simple or complexed) can inactivate enteric viruses (Gilling *et al.,* 2014A). In addition, many organic acids are found in fruit extracts such as malic acid and citric acid that were detected as main organic acids in commercial pomegranate juice (Tezcan *et al.,* 2009). Organic acids have been used to inactivate NoV surrogates and its mechanism of action is not completely understood but can be attributed to alterations of the virus capsid and the nucleic acid (Cao, 2013). It was suggested that the inactivation of non-enveloped viruses (such as NoV) is due to the denaturation of capsid proteins due to acidity of organic acids (Cao, 2013). According to Lipson *et al.* (2010), the anti-viral mechanism of action of cranberry polyphenols or polyphenols originating from cranberries (CP) acts by inhibiting viral penetration in cells. Joshi *et al.* (2016) showed that lingonberry proanthocyanidins (PACs) affect viral penetration and PAC from an African resurrection plant inhibits viral attachment and penetration.

Su *et al*. (2010) tested the effects of cranberry juice (CJ) on MNV-1, FCV-F9, bacteriophage MS2 (ssRNA) and bacteriophage φ X-174 (ssDNA) at exposure times ranging from 0 to 60 minutes at room temperature. Among all these surrogates of food viruses, FCV-F9 was the viral titer that displayed the most rapid and abundant decrease. They observed a 5 $\log_{10}$ PFU/mL reduction after 30 minutes of treatment with CJ (pH 2.7 and 7.0). The total reduction in titer for MNV-1 was 1.90 and 1.66 $\log_{10}$ PFU/mL after 1 hour of treatment with CJ at pH 2.6 and CJ at pH 7.0, respectively (Su *et al*., 2010). Similar results were observed by Côté *et al.* (2011B) where cranberry juice containing water-soluble phenolic compounds inhibited pathogens like *Listeria monocytogenes*.

Su *et al.* (2010) tested the effect of cranberry proanthocyanidins (PAC) on MNV-1, FCV-F9, bacteriophage MS2 (ssRNA) and bacteriophage φ X-174 (ssDNA) at exposure times ranging from 0 to 60 minutes at room temperature. CJ-PAC at both 0.15 mg/mL and 0.30 mg/mL (CJ-PAC concentration) decreased the FCV-F9 titer to an undetectable level immediately. For MNV-1, a reduction of 2.24 and 2.94 $\log_{10}$ PFU/mL at 0.15 mg/mL and 0.20 mg/mL concentration of CJ-PAC was observed respectively,

after one hour of treatment. Blueberry proanthocyanidins (B-PACs) were also shown to be able to decrease viral titers of NoV surrogates (Joshi *et al.,* 2017). The authors assessed the benefits of B-PAC in apple juice (AJ) and in 2% reduced fat milk in simulated gastrointestinal fluids infected with FCV-F9 and MNV-1 and treated at 37 °C for 24 hours. The viruses were inactivated after 15 minutes in AJ at pH 3.6 in the presence of 1, 2 or 5 mg/mL B-PAC. In contrast, B-PAC did not display much virucidal activity in 2% partly-skim milk. In milk, FCV-F9 was reduced by only 0.4 log PFU/mL in presence of 2 mg/mL B-PACs and by 1.09 log PFU/mL in presence of 5 mg/mL of B-PACs after 24 hours. MNV-1 was reduced by 0.81 log PFU/mL in presence of 5 mg/mL of B-PACs after 24 hours. This loss of the antiviral properties against MNV-1 was associated with the presence of carbohydrates, lipids and proteins in the milk matrix and then induced loss of effectiveness.

Antiviral effects of pomegranate polyphenols (PP) were evaluated in presence of 2 mg/mL and 4 mg/mL of PP against FCV-F9, MNV-1 and bacteriophage MS2 at room temperature for up to 1 hour (Su *et al.,* 2011A). Each virus (5 $\log_{10}$ PFU / mL) was mixed with an equivalent volume of PP at 4 or 8 mg/mL and incubated for 0, 10, 20, 30, 45 and 60 minutes at room temperature. A decrease of 4.02, 0.68 and 0.18 $\log_{10}$ PFU/mL for FCV-F9, MNV-1 and MS2, was respectively observed after 20 minutes exposure in the presence of 2 mg/mL of PP (Su *et al*., 2011A). A concentration of 4 mg/mL PP resulted in a decrease of 5.09, 1.14 and 0.19 $\log_{10}$ PFU/mL for FCV-F9, MNV-1 and MS2, respectively after 20 minutes of treatment.

Polyphenolic compounds represent the most studied group of plant phenolics. Pomegranate juice (PJ) contains mainly anthocyanins and anthoxanthins (Aviram and Rosenblat, 2012). Among the most anti-NoV plant polyphenols, anthocyanins, proanthocyanins and catechins are the most characterized. In fact, anthocyanin's glycoside may inhibit MNV-1 during internalization or during an early replication step (Ryu *et al.,* 2015) and anthoxanthins can act as antioxidants (Pate *et al.,* 2017). Su *et al.* (2011A) tested the antiviral effects of PJ at pH 7 against FCV-F9, MNV-1 and bacteriophage MS2 at room temperature for up to 1 hour. Each virus (5 $\log_{10}$ Plaque-Forming Unit / mL, PFU/mL) was mixed with equivalent volumes of PJ and incubated for 0, 10, 20, 30, 45 and 60 minutes at room temperature. In all cases of viral surrogates, viral titers were reduced by more than 50% during only the first 20 minutes post-treatment. A decrease of 3.12, 0.79 and 0.23 $\log_{10}$ PFU/mL in only 20 minutes of exposure with PJ for FCV-F9, MNV-1 and MS2 was observed, respectively.

Subsequently, Su *et al*. (2011B) attempted to evaluate the efficiency of grape seed extract (GSE) against hepatitis A virus (HAV) and against surrogates of NoV (FCV-9, MS2 and MNV-1). High titers and low titers of virus inoculated at 7 $\log_{10}$ PFU/mL (plaque-forming unit/mL) and 5 $\log_{10}$ PFU/mL titer were used, respectively. Equal part of viruses (7 $\log_{10}$ or 5 $\log_{10}$ PFU/mL) and GSE were mixed at different concentrations (0.5, 1 and 2 mg/mL) of GSE and incubated for 2 hours at room temperature. For

FCV-F9 at high titers, reductions of 3.64, 4.10 and 4.61 $\log_{10}$ PFU/mL in presence of three respective concentrations (0.5, 1 and 2 mg/mL) was obtained. For the high titer of MNV-1 (7 $\log_{10}$ PFU/mL), in presence of the three respective concentrations (0.5, 1 and 2 mg/mL), a reduction of 0.82, 1.35 and 1.73 $\log_{10}$ PFU/mL was attained, respectively. MS2 phage, at high titer (7 $\log_{10}$ PFU/mL) was reduced by 1.13, 1.43 and 1.60 $\log_{10}$ PFU/mL as the concentration of GSE increased from 0.5 to 1 and 2 mg/mL, respectively (Su *et al.,* 2011B). For HAV at high titer (7 $\log_{10}$ PFU/mL), the reductions were 1.81, 2.66 and 3.20 $\log_{10}$ PFU/mL in presence of 0.5, 1 and 2 mg/mL of GSE, respectively. At low titer (5 $\log_{10}$ PFU/mL), FCV-F9 was always reduced by 4.98 $\log_{10}$ PFU/mL (Su *et al.,* 2011B).

- **Spice and herbal extracts**

Herbal extracts contain antimicrobial components such as terpenoids, alkaloids and phenolic compounds that are capable of interacting with enzymes or proteins (Mostafa *et al.,* 2018). The antiviral mechanism of herbal extracts (ex: thiophenes, polysaccharides, polyacetylenes and furyl compounds) is due to the inhibition of viral enzymes, viral replication and protein synthesis (Seo *et al.,* 2017). It has been shown that combining herbal extracts with other conditions such as low pH, storage temperature, irradiation, bacteriocins or organic acids causes synergistic antimicrobial activities (Perumalla and Hettiarachchy, 2011).

Aboubakr *et al.* (2016) tested aqueous extracts of flower of clove, fenugreek seeds, garlic and onion bulbs, ginger rhizomes, and jalapeno peppers against FCV. Results showed that non-diluted clove and ginger extracts inactivated 6.0 log $TCID_{50}$ (tissue culture infectious dose 50) and 2.7 log $TCID_{50}$, respectively of the initial viral titer. Eugenol, a phenylpropene (29.5%), was the major component of clove extract and R- (-)-1,2-propanediol (10.7%) was the major component of ginger extract. Eugenol tested alone showed antiviral activities. These findings confirmed that eugenol is the component most likely responsible for viral elimination in cloves.

Extracts of *Camellia sinensis* and *Ficus carica* have also been evaluated. A significant inhibition of FCV (~7 $\log_{10}$ PFU/mL) and MNV (~7 $\log_{10}$ PFU/mL) by 87% and 49% was observed in presence of these respective extracts (Seo and Choi, 2017). In addition, FCV showed 53% inhibition by *Pleuropterus multiflorus* extracts (20 µg/mL) and 50% with *Alnus japonica* extracts (20 µg/mL) (Seo and Choi, 2017). MNV was also inhibited by more than 92% in the presence of 150 µg/mL of *Inonotus obliquus* extracts and 58% in presence of 50 µg/mL of *Crataegus pinnatifida* extracts. In addition, 20 µg/mL of *Coriandrum sativum* extracts reduced MNV titer by about 45% (Seo and Choi, 2017). They attributed the antiviral mechanism of action of bioactive substances to the anti-adhesion of virus on cells, direct virucidal action or immune enhancement (Seo and Choi, 2017).

Table 2. Recapitulative table of the performance of natural compounds against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Carvacrol	0.50% for 2h at 37 °C	Complete inactivation of 6-7 log_{10} $TCID_{50}$/mL FCV and MNV	Sanchez *et al.* (2015)
Carvacrol	1.0% for 2h at 37 °C	Complete inactivation of 6-7 log_{10} $TCID_{50}$/mL HAV	Sanchez *et al.* (2015)
Carvacrol	0.50%, on lettuce	Complete inactivation of 6-7 log_{10} $TCID_{50}$/mL FCV	Sanchez *et al.* (2015)
Carvacrol	0.25%, for 15 min	1.03 log_{10} $TCID_{50}$/mL MNV-1	Gilling *et al.* (2014B)
Carvacrol	0.50%, for 15 min	1.28 log_{10} $TCID_{50}$/mL MNV-1	Gilling *et al.* (2014B)
Carvacrol	0.25%, for 60 min	1.95 log_{10} $TCID_{50}$/mL MNV-1	Gilling *et al.* (2014B)
Carvacrol	0.50%, for 60 min	3.87 $log_{10}TCID_{50}$/mL MNV-1	Gilling *et al.* (2014B)
Kaempferol	200 μM	69.76% reduction of FCV	Seo *et al.* (2016)
Myricetin	0.5 mM and 1 mM, for 2 h at 37 °C (initially 5 log_{10} PFU/mL)	Complete inactivation of 5 log_{10} PFU/mL FCV	Su and D'Souza (2013)
L-epicatechin	0.5 mM, for 2 h at 37 °C (initially 5 log_{10} PFU/mL)	1.40 log_{10} PFU/mL FCV	Su and D'Souza (2013)
Myricetin	0.5 mM, for 2 h at 37 °C (initially 7 log_{10} PFU/mL)	3.17 log_{10} PFU/mL FCV	Su and D'Souza (2013)
L-epicatechin	0.5 mM, for 2 h at 37 °C (initially 7 log_{10} PFU/mL)	0.72 log_{10} PFU/mL FCV	Su and D'Souza (2013)
Myricetin	0.25 mM, for 2 h at 37 °C (initially 7 log_{10} PFU/mL)	1.73 log_{10} PFU/mL FCV	Su and D'Souza (2013)
***Zanthoxylum schinifolium* (ZSO)**	0.01% concentration	70% reduction of FCV-F9	Oh and Chung 2014
Oregano	2%, for 2 h at 37 °C	3.75 log_{10} $TCID_{50}$/mL FCV	Elizaquivel *et al.* (2013)
Oregano	0.50%, for 2 h at 37 °C	1.04 log_{10} $TCID_{50}$/mL MNV	Elizaquivel *et al.* (2013)
Oregano	1.0%, for 2 h at 37 °C	1.17 log_{10} $TCID_{50}$/mL MNV	Elizaquivel *et al.* (2013)
Oregano	2.0%, for 2 h at 37 °C	1.62 log_{10} $TCID_{50}$/mL MNV	Elizaquivel *et al.* (2013)
Cinnamon	3.0%, for 2 h at 37 °C	2.38 log_{10} $TCID_{50}$/mL FCV	Azizkhani and Tooryan (2016)
Rosemary	2.5%, for 2 h at 37 °C	3.38 log_{10} $TCID_{50}$/mL FCV	Azizkhani and Tooryan (2016)

Zataria	0.1%, for 2 h at 37 °C	4.51 $\log_{10}$ $TCID_{50}$/mL FCV	Azizkhani and Tooryan (2016)
Zataria	0.1%, for 2 h at 37 °C	0.25 $\log_{10}$ $TCID_{50}$/mL MNV	Azizkhani and Tooryan (2016)
Rosemary	2.5%, for 2 h at 37 °C	1.44 $\log_{10}$ $TCID_{50}$/mL MNV	Azizkhani and Tooryan (2016)
CJ	30 min at pH 2.7 and 7.0	5 $\log_{10}$ PFU/mL FCV	Su *et al*. (2010)
CJ	1 h at pH 2.6	1.90 $\log_{10}$ PFU/mL MNV	Su *et al*. (2010)
CJ	1 h at pH 7.0	1.66 $\log_{10}$ PFU/mL MNV	Su *et al*. (2010)
CJ-PAC	Immediately, 0.15 mg/mL and 0.30 mg/mL at RT	Complete inactivation FCV	Su *et al*. (2010)
CJ-PAC	0.15 mg/mL, for 1 h at RT	2.24 $\log_{10}$ PFU/mL MNV	Su *et al*. (2010)
CJ-PAC	0.20 mg/mL, for 1 h at RT	2.94 $\log_{10}$ PFU/mL MNV	Su *et al*. (2010)
B-PAC	1, 2 or 5 mg/mL, for 15 min at 37 °C in apple juice at pH 3.6	Complete inactivation of FCV and MNV	Joshi *et al.* 2017
B-PAC	2 mg/mL, for 24 h in 2% reduced-fat milk	0.4 $\log_{10}$ PFU/mL FCV	Joshi *et al.* 2017
B-PAC	5 mg/mL, for 24 h in 2% reduced-fat milk	1.09 $\log_{10}$ PFU/mL FCV	Joshi *et al.* 2017
B-PAC	5 mg/mL, for 24 h in 2% reduced-fat milk	0.81 $\log_{10}$ PFU/mL MNV	Joshi *et al.* 2017
PP	2 mg/mL, for 20 min at RT	4.02, 0.68 and 0.18 $\log_{10}$ PFU/mL for FCV, MNV and MS2 coliphage	Su *et al*. (2011A)
PP	4 mg/mL, for 20 min at RT	5.09, 1.14 and 0.19 $\log_{10}$ PFU/mL for FCV-F9, MNV-1 and MS2 coliphage	Su *et al*. (2011A)
PJ	pH 7.0, for 20 min at RT	3.12, 0.79 and 0.23 $\log_{10}$ PFU/mL for FCV, MNV and MS2 coliphage	Su *et al*. (2011A)
GSE	FCV at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	3.64, 4.10 and 4.61 $\log_{10}$ PFU/mL FCV	Su *et al*. (2011B)
GSE	MNV at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	0.82, 1.35 and 1.73 $\log_{10}$ PFU/mL MNV	Su *et al*. (2011B)
GSE	MS2 at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	1.13, 1.43 and 1.60 $\log_{10}$ PFU/mL MS2	Su *et al*. (2011B)
GSE	HAV at high titer (7 $\log_{10}$ PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	1.81, 2.66 and 3.20 $\log_{10}$ PFU/mL HAV	Su *et al*. (2011B)
GSE	FCV at low titer (5 $\log_{10}$	4.98 $\log_{10}$ PFU/mL	Su *et al*. (2011B)

	PFU/mL) 0.5, 1 and 2 mg/mL for 2 h at RT	FCV for each concentration	
Clove extracts	Pre-treatment of FCV with non-diluted extract at 4 °C, for 24 h	6 $\log_{10}$ $TCID_{50}$/mL FCV	Aboubakr *et al.* (2016)
Ginger extracts	Pre-treatment of FCV with non-diluted extract at 4 °C, for 24 h	2.7 $\log_{10}$ $TCID_{50}$/mL FCV	Aboubakr *et al.* (2016)
***Camellia sinensis* extracts**	100 µg/mL	87% of 7 $\log_{10}$ PFU/mL FCV	Seo and Choi (2017)
***Ficus carica* extracts**	100 µg/mL	49% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)
***Pleuropterus multiflorus* extracts**	20 µg/mL	53% of 7 $\log_{10}$ PFU/mL FCV	Seo and Choi (2017)
***Alnus japonica* extracts**	20 µg/mL	50% of 7 $\log_{10}$ PFU/mL FCV	Seo and Choi (2017)
***Inonotus obliquus* extracts**	150 µg/mL	92% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)
***Crataegus pinnatifida* extracts**	50 µg/mL	58% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)
***Coriandrum sativum* extracts**	20 µg/mL	45% of 7 $\log_{10}$ PFU/mL MNV	Seo and Choi (2017)

RT: room temperature, CJ: Cranberry juice, CJ-PAC: Cranberry juice proanthocyanidins, B-PAC: Blueberry proanthocyanidins, PP: Pomegranate polyphenol, PJ: Pomegranate juice, GSE: Grape seed extract

2.3 Non-thermal treatments

- **Fermentation**

The antimicrobial potential of fermented foods is due to their acidity which is caused by the production of lactic acid and bacteriocins, but there are also important effects caused by high salinity, temperature and ripening (Dortu and Thonart, 2009; Seo *et al*., 2014). Fermentation with lactic acid bacteria (LAB) reduces the risk of foodborne illness by inhibiting the growth of pathogens (Ross *et al*., 2002; Kim *et al*., 2008). Known antimicrobial factors produced by fermentation include acidity, organic acid production, bacteriocins, CO_2, hydrogen peroxide, ethanol, diacetyl production, and low redox potential (Adams and Nicolaides, 1997). Lactic acid fermentation can inactivate and inhibit the growth of pathogenic bacteria (Lee *et al*., 2017). For example, pathogens such as *Salmonella* Typhimurium and *Staphylococcus aureus* have been eliminated with lactic acid fermentation (Nout *et al*., 1989). The antiviral mechanism of action of fermented food is still mainly unknown but can be attributed to high acidity production (Lee *et al.,* 2012). Also, according to Aboubakr *et al.* (2014), *Lactococcus lactis,* a lactic bacteria used in fermentation, possesses an anti-FCV mechanism of action could be attributed to 1) denaturation of capsid proteins due to acid production, 2) trapping viral particles by the membrane peptidoglycans of lactic acid bacteria, 3) production of metabolites that may disturb viral penetration in

cells and 4) competition between bacteria and virus for cell attachment. Fermentation has been shown to be highly effective against NoV on the surface of foods but does not keep the product entirely fresh (Adams and Nicolaides, 1997; Lee *et al*., 2017).

In Korea, it's a common practice to ferment foods such as cabbage, green onions, carrots, cucumbers, etc. (Lee, 1997). Kimchi is a dish made from fermented vegetables and there are many different types such as Dongchimi (Lee *et al*., 2012). It is prepared with sliced radish, 1% green onions, 0.5% garlic, and 0.3% ginger. Salt water (2.5%) is added to the vegetable mix at a ratio of 1: 1.5 (W/V). It was observed that the viral titer of FCV was significantly reduced from 5.69 $\log_{10}$ PFU/g to 1.57 $\log_{10}$ PFU/g after 20 days of fermentation (Lee *et al*., 2012). The viral titer of the MNV was reduced less significantly than FCV, from 5.63 to 3.51 $\log_{10}$ PFU/g after 20 days of fermentation.

Seo *et al.* (2014) evaluated the effects of FCV and MNV-1 survival during oyster fermentation (Seo *et al*., 2014). Lactic acid fermentations in presence of 5% and 10% salt at 18 ° C for 15 days were made. In fermented oysters containing 5% salt, the FCV titer decreased by 3 log and the MNV titer decreased by 1.6 log after 15 days of lactic acid fermentation. It was concluded that the antimicrobial compounds generated during the lactic acid fermentation contributed to the reduction of food viruses (Seo *et al*., 2014).

Lee *et al*. (2017), also evaluated the level of NoV on kimchi stored at 4 °C and 10 °C during lactic acid fermentation (Lee *et al*., 2017). Although, the amount of NoV, originally at 3 $\log_{10}$ genomic copies/200 µL, was reduced by 1.31 $\log_{10}$ genomic copies/reaction, it was detected in all samples tested after 28 days of fermentation (Lee *et al.,* 2017).

Table 3. Recapitulative table of the performance of fermentation against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Fermentation of dongchimi	20 days	4.12 $\log_{10}$ PFU/g FCV	Lee *et al*. (2012)
Fermentation of dongchimi	20 days	2.12 $\log_{10}$ PFU/g MNV	Lee *et al*. (2012)
Fermentation of oysters	5% salt at 18 °C for 15 days	3.0 $\log_{10}$ PFU/g FCV	Seo *et al.* (2014)
Fermentation of oysters	5% salt at 18 °C for 15 days	1.6 $\log_{10}$ PFU/g MNV	Seo *et al.* (2014)
Fermentation of kimchi	4 °C for 28 days	1.31 $\log_{10}$ genomic copies/reaction NoV GII.4	Lee *et al.* (2017)

- **Ozonation**

The effectiveness of ozone (O_3) on viruses can be attributed to the fact that non-enveloped viruses are more sensitive to the presence of O_3. This could facilitate the access of O_3 to nucleic acids. Plus, O_3 is a strong oxidizing agent and disturbs the reproduction cycle of the virus by disrupting the contact with cells (Elvis and Ekta, 2011). O_3 makes the cells susceptible to viral infection and make them susceptible to oxidation and then elimination (Elvis and Ekta, 2011). Ozonation is an O_3 generation process, producing an unstable form of oxygen (highly reactive, making it a powerful oxidizing agent). O_3 is produced by a high source of energy breaking O_2 molecules. Once the bond is broken, these oxygen atoms (O) bind to a molecule of O_2 and then form O_3. This process induces the reactive oxygen molecules specifically damage cellular DNA, usually DNA strand breakage or by chemical modification of bases, which usually leads to cell death (Boumail *et al.,* 2016). Ozonation is a cleaner process than chlorination because O_2 is the only residue at the end of the process and there is no manipulation of chemicals needed (Rice *et al*., 2002). O_3 might also react on cell walls of bacteria by oxidizing major components like proteins and unsaturated lipids (Beuchat, 1992; Komanapalli and Lau, 1998). In addition, chlorination has the disadvantage of forming toxic and carcinogenic chlorinated compounds (Kim *et al*., 2003; Kirk and Mitchell, 1980). There are many advantages using this method. It requires only oxygen, is faster and more efficient than chlorine. It is a non-thermal and non-chemical process and can be applied in aqueous or gaseous state (Rice *et al*., 2002; Kim *et al*., 2003; Khadre *et al*., 2001; Guzel-Seydim *et al*., 2004). Furthermore, it is a safe method because it leaves no residue while it reduces microorganisms in food (Trindade *et al*., 2012).

A study evaluated the effectiveness of ozonated water to inactivate FCV and MNV inoculated respectively at concentrations of 10^7 $TCID_{50}$/mL and 10^6 PFU/mL on green onions and lettuce (Hirneisen *et al*., 2011). The lettuce and green onion samples were submerged in gaseous ozonised water from an O_3 generator (0.9 g of ozone/h at a flow rate of 2.4 L/min) (6.25 ppm). The samples were ozonized for 0.5, 1, 5 and 10 minutes. Viral inactivation was increased as duration of treatment increased. With regard to the inactivation of FCV from surface of lettuce and green onions, regardless of duration of the treatment, the virus inactivation was always greater on the surface of the lettuce compared to other vegetables. For example, after one minute of treatment with 6.25 ppm, they observed a reduction of 1.2 log $TCID_{50}$/mL on surface of treated lettuce compared to a reduction of only 0.5 $TCID_{50}$/mL on surface of green onions (Hirneisen *et al.,* 2011). In the case of MNV on the surface of the same two foods, viral elimination was always more effective on the surface of lettuce, except at the 10-minutes exposure time where there was a higher viral reduction of 3.78 log PFU/g on green onions and only a 3.09 log PFU/g reduction on lettuce (Hirneisen *et al*., 2011). In conclusion, viral inactivation by ozone was lower in green onions in most cases. There is a possibility that this difference in the inactivation is due to the different organic composition of the two items. O_3 reacts with complex organic compounds because of the high oxidation potential (Hirneisen *et al.*, 2011).

Predmore *et al.* (2015) studied the effects of gaseous O_3 on fresh strawberries. Samples were ozonized with 6% (w/w O_3) in oxygen during 0, 10, 20, 30 and 40 minutes. Experiments were done at room temperature at 25°C and gaseous O_3 was pumped into the chamber until 15 psi was reached. After 10 minutes of treatment in presence of gaseous O_3, MNV-1 at a concentration of 10^8 PFU/mL demonstrated a 4.1 log PFU/g reduction in simple liquid virus stock. After 40 minutes of O_3 treatment, the viral titer decreased by 3.3 log PFU/g on the surface of strawberries, but there was still 2.5 log PFU/g remaining, indicating that MNV-1 is partially resistant to O_3.

Table 4. Recapitulative table of the performance of ozonation against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on lettuce for 1 min	1.2 log $TCID_{50}$/mL FCV	Hirneisen *et al.* (2011)
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on green onions for 1 min	0.5 log $TCID_{50}$/mL FCV	Hirneisen *et al.* (2011)
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on green onions for 10 min	3.78 log PFU/g MNV	Hirneisen *et al.* (2011)
Ozonated water	0.9 g of ozone/h at a flow rate of 2.4 L/min at 6.25 ppm on lettuce for 10 min	3.09 log PFU/g MNV	Hirneisen *et al.* (2011)
Gaseous O_3	6%, 15 psi, at 25 °C and RT in liquid stock for 10 min	4.1 log PFU/g MNV	Predmore *et al.* (2015)
Gaseous O_3	6%, 15 psi, at 25 °C and RT on surface of fresh strawberries for 40 min	3.3 log PFU/g MNV	Predmore *et al.* (2015)

- **High Pressure Homogenizer (HPH)**

Homogenization by high-pressure valve is a recent, non-thermal process where a fluid feed is forced through small orifices. High-pressure processes include high hydrostatic pressure (HHP) and high-pressure homogenization (HPH) (D'Souza *et al*., 2009). HPH is a continuous process at low pressure (< 400 MPa) and shorter exposure than HHP (D'Souza *et al*., 2009). The HPH process has proven effective for bacterial, enzymatic and emulsion enhancement (Diels *et al*., 2004; Dybowska, 2005; Picart *et al*., 2006).

Chen *et al.* (2005) demonstrated that FCV at a concentration of ~10^6 PFU/mL was reduced by 1.7 log in culture with an HHP treatment (250 MPa) of 2 minutes (21 °C). Li and Chen (2015) also demonstrated that the same HHP treatment reduced MNV-1, from a viral concentration of 1x 10^7 to 10^8 PFU/ml by only

0.1 log. Therefore, it can therefore be concluded that FCV is more sensitive to this type of treatment than MNV-1 (Li and Chen, 2015).

D'Souza *et al*. (2009) evaluated the efficiency of HPH at different pressures (0, 100, 200, 250 and 300 MPa) against two NoV surrogates; MNV-1 at a concentration of 5.78 log PFU/mL and MS2 coliphage at a concentration of 6.52 log PFU/mL (D'Souza *et al*., 2009) and found that only HPH at pressures of 300 MPa at 75 °C showed virus inactivation by more than 3 log PFU/mL for MS2 with plaque-assay. In addition, at the same pressure, viral titers decrease by more than 0.8 log PFU/mL for MNV-1.

Khadre and Yousef (2002) attempted to compare the anti-viral effects of ozonation and high-pressure treatments on human rotavirus. O_3 (25 µg/mL) decreased viral titers of about approximately 8-9 $\log_{10}$ $TCID_{50}$/mL with a high initial viral titer of ~10^{11} $TCID_{50}$/mL. The high-pressure treatment was shown to be extremely effective against rotavirus. Treatment at 300 MPa for 2 minutes at 25 °C decreased viral titers about 8 $\log_{10}$ $TCID_{50}$/mL. Both treatments appeared to have similar effects on viruses. The mechanism of action of high pressures can be explained by protein denaturation leading to changes in conformation. It might be possible that this denaturation may occur in viral attachment proteins, thereby preventing viral infection and spread (Khadre and Yousef, 2002).

Table 5. Recapitulative table of the performance of high-pressure homogenizer (HPH) and high hydrostatic pressure (HHP) against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
HHP	250 MPa for 2 min at 21 °C	1.7 $\log_{10}$ PFU/mL FCV	Chen *et al.* (2005)
HHP	250 MPa for 2 min at 21 °C From 1x 10^7 to 10^8 PFU/ml	0.1 $\log_{10}$ PFU/mL MNV	Li and Chen (2015)
HPH	300 MPa at 75 °C	3 $\log_{10}$ PFU/mL MS2	D'Souza *et al*. (2009)
HPH	300 MPa at 75 °C	0.8 $\log_{10}$ PFU/mL MNV	D'Souza *et al*. (2009)
HPH	300 MPa for 2 min at 25 °C	8 $\log_{10}$ $TCID_{50}$/mL rotavirus	Khadre and Yousef (2002)

- **Irradiation (γ- rays, electron beam and X- rays)**

Gamma irradiation has been studied extensively for its effectiveness in eliminating pathogens on food. It can lead to the production of oxygen or hydroxyl radicals (Park and Ha, 2017). Gamma rays can interact with nucleic acids because of the DNA modifications and the free radicals (Park and Ha, 2017). Among the different damages on nucleic acids, there are double and single-strand breaks, cross-linkage breaks and nucleotide degradation (Park and Ha, 2017). The expression of the MNV-1 major capsid protein (VP1) gene decreases as the irradiation dose increases. VP1 was the only protein studied in order to seek

alterations after irradiation of MNV-1 during this research (Park and Ha, 2017). Furthermore, gamma irradiation can break covalent and non-covalent bonds (hydrogen bonds, ionic bonds, van der Waals forces and hydrophobic interactions) that are essential for protein structure (Park and Ha, 2017). It has been shown that irradiation applied alone or in combination with other treatments such as using antimicrobial coatings can reduce bacterial contamination in food (Boumail *et al*., 2016; Rahimi *et al*., 2013). It is also a cold process that is usually applied on packaged products (Boumail *et al*., 2016). The maximum dose that can be applied on fresh fruits and vegetables should not exceed 1 kGy (Komolprasert *et al.,* 2008). However, foodborne viruses can only be eliminated by irradiation at doses ranging from 2 to 8 kGy (Monk *et al.,* 1995). Therefore, combining this treatment with antimicrobial coatings or negative air ionization with ozone can decrease the needed dose (Boumail *et al.,* 2016). This technique is generally used to assure food safety, disinfection, extend the shelf life of foods and for delaying the germination of seeds (Farkas and Mohácsi-Farkas, 2011; Lacroix and Ouattara, 2000; Lacroix and Vigneault, 2007).

To date, food irradiation is a public concern, but is considered safe by the World Health Organization (WHO), the United Nations International Atomic Agency (IAEA), and the Food Agriculture Organization (FAO). The use of irradiation in combined treatments has the advantage of reducing the dose required for the elimination of an organism. In the case of resistant organisms such as viruses and Gram (+) bacteria, reducing the required dose to ensure food safety, also helps to better preserve the nutritional and physicochemical quality of food (Lacroix and Ouattara, 2000). Irradiation could be done using electron-beam equipment, an X-ray machine or a source of γ-radiation. However, electron beams are much less penetrating and require higher levels of dose rate (Woo and Sandford, 2002). X-ray irradiation is an electricity-based process and rays are produced by an electron transition phenomenon that is an electron passage to another energy level (Cleland and Stichelbaut, 2013).

Feng *et al.* (2011) have evaluated the effects of γ-irradiation against NoV surrogates such as MNV-1, NoV virus-like particles (VLPs) and vesicular stomatitis virus (VSV). MNV-1 and VLPs are both resistant to gamma irradiation. A reduction of 1.7 to 2.4 log was observed on the surface of fresh products inoculated with MNV-1 and treated at 5.6 kGy. In contrast, VSV appears to be more susceptible to gamma irradiation, a reduction of 3.3 log was observed after the treatment with 5.6 kGy (Feng *et al*., 2011). Authors attributed this sensitivity to γ-irradiation to the genome size. In fact, MNV-1 has a genome of 7.4 kb and VSV has a bigger genome with 11 kb. There are studies showing an inverse relationship between the size of the genome and the inactivation gamma-irradiation dose (Feng *et al*., 2011). Also, there is another hypothesis suggesting that enveloped viruses are more sensitive to gamma irradiation (Feng *et al.,* 2011).

Park and Ha (2017) also showed that gamma irradiation reduced the MNV-1 viral titer in kimchi as the γ-irradiation dose increased. For γ-irradiation doses of 1, 3, 5, 7 and 10 kGy, a respective reduction of

0.34, 0.71, 0.98, 1.45 and 1.76 $\log_{10}$ was observed. In conclusion of this study, γ-irradiation with ≥ 5.75 kGy doses could be effective against NoV contamination in the kimchi preparation industry.

Irradiation treatments in combination with heat processing at temperatures above 55°C reduce considerably the risk of viral contamination on food because of their high heat sensitivity (Farkas, 1989).

Table 6. Recapitulative table of the performance of γ-irradiation against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
γ-irradiation	Fresh produce, 2.8 kGy	1.77 $\log_{10}$ PFU/g for spinach, 1.40 $\log_{10}$ PFU/g for romaine lettuce and 1.31 $\log_{10}$ PFU/g strawberries MNV-1	Feng *et al.* (2011)
γ-irradiation	Fresh produce, 5.6 kGy	1.7 to 2.4 $\log_{10}$ PFU/g in fresh produce MNV-1	Feng *et al.* (2011)
γ-irradiation	Kimchi, 1, 3, 5, 7 and 10 kGy	0.34, 0.71, 0.98, 1.45 and 1.76 $\log_{10}$ PFU/mL MNV-1	Park and Ha (2017)

- **UV irradiation**

Ultraviolet (UV) radiation represents electromagnetic irradiation and has wavelengths of below 450 nm ($\nu \approx 10^{15}$ Hz) and a quantum energy of 3-5 eV (Adams *et al.,* 2016), which is shorter than the wavelengths of visible light at 400-700 nm (Dai *et al.,* 2012). UV-A comprises wavelengths ranging from 320 to 400 nm (Cadet and Douki, 2018), UV-B ranges from 280 to 320 nm (Cadet and Douki, 2018) and UV-C has wavelengths ranging from 200 nm to 280 nm (Dai *et al.,* 2012). The mechanism of action of UV-C against viruses is linked to the attack of the viral nucleic acids (Dai *et al.,* 2012). The most antimicrobial wavelength is between 250-270 nm, because the UV-C spectrum is strongly absorbed by the nucleic acids of the microorganisms (Dai *et al.*, 2012). The UV-C type rays have a low wavelength (200-280 nm) and are therefore less energetic and are longer than x-rays who have wavelengths < 100 nm (Dai *et al.*, 2012). UV-C is normally used for the sterilization of surfaces and transparent objects (Silindir and Ozer, 2009). Among the mutations observed after UV treatment, UV-A rays genotoxic actions involve oxidative processes by the production of reactive oxygen species (ROS) after exciting photosensitizers. The ROS damage major components of cells such as proteins and lipids (Wondrak *et al.,* 2006). UV-B rays are able to create cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4 PPs) and these modifications in DNA strongly affect the functioning of the cell and can induce its death (Cadet and

Douki, 2018; Lankinen *et al.,* 1996). UV-C rays create cyclobutane pyrimidine and pyrimidine pyrimidone dimers, blocking the elongation of nucleic acid transcripts (Vreeswijk *et al.,* 1994). UV-radiation at 253.7 nm wavelength (UV-C) was tested against FCV and a 3-log reduction of viral titer was achieved with a fluence of 120 J/m^2 (De Roda Husman *et al.,* 2004). It was shown that the first target of UV-radiation is the capsid of the virus using capsid epitope binding experiments (Nuanualsuwan and Cliver, 2003).

Table 7. Recapitulative table of the performance of UV-irradiation against virus

Treatment	Conditions of treatment (pH, concentration, temperature and duration)	Reduction of viral titer	Author
UV-C	UV-C at 253.7 nm and 120 J/m^2	3 $\log_{10}$ $TCID_{50}$/mL FCV	De Roda Husman *et al.* (2004)

2.4 Combined treatments

Combining irradiation and EOs could decrease the amount of radiation required to reduce food pathogens, while weeping all nutrients, flavours and physico-chemical aspects of the fresh products (Ndoti-Nembe *et al.*, 2015).

- **UV Irradiation combined with free Chlorine/Monochloramine**

Shang *et al*. (2007) investigated the inactivation of MS2 coliphage with different types of combined treatments. Among the treatments, they tested sequential ultraviolet (UV-C) light/free chlorine exposure, sequential UV-C light/monochloramine exposure, simultaneous UV-C light/free chlorine exposure, and simultaneous UV-C light/monochloramine exposure using either low-pressure (LP) at 254 nm (UV-C) or medium-pressure (MP) ranging from 220 nm (UV-C) to 580nm UV lamps. All tests were conducted at pH 7 and 20 ± 2°C. Sequential exposure to UV-C or simultaneous exposition (UV-C dose at 51 mJ /cm^2) in the primary disinfection stage increased MS2 inactivation during the second disinfection stage by monochloramine about 4 to 5 times and with chlorine, by 1.5–2.7 times, compared to the rates by free chlorine.

- **UV-assisted TiO_2 photocatalysis combined with high hydrostatic pressure**

Kim *et al.* (2017) tested the effects of single and combined UV-C (254 nm) assisted TiO_2 photocatalysis (UVTP) and HHP against MNV-1. UVTP (4.5 mW/cm^2) for 10 minutes decreased the MNV-1 titer by 2.9 $\log_{10}$ post-treatment. The use of HHP at 500 MPa during 5 minutes at room temperature reduced the MNV-1 titer by 3.5 $\log_{10}$. When UVTP was followed by HHP, the titer was

decreased by 5.5 $\log_{10}$ (under detection limit). The study showed a synergistic effect between those 2 treatments (Kim *et al*., 2017).

- **UV-C light in combination with hydrogen peroxide (H_2O_2)**

Xie *et al.* (2008) tested the combination of UV-C and hydrogen peroxide at a concentration of 2% vol/vol (H_2O_2) against MS2 coliphage with an initial concentration of 7.63 log PFU/mL on the surface of iceberg lettuce. UV-C light treatment alone for 60 seconds reduced the viral titer by 1.31 log PFU/mL. H_2O_2 treatment alone for 60 seconds reduced the viral titer by 3.19 log PFU/mL and finally, the treatment combining UV-C light with H_2O_2 for 60 seconds reduced the viral titer by more than 3.54 log PFU/mL. The study showed that the combination of UV-C and H_2O_2 against MS2 coliphage could represent an alternative to hypochlorite-based washes on fresh food products (Xie *et al.,* 2008).

3 Conclusion

Many chemical treatments, such as peracetic acids and chlorine compounds have been shown to be effective in the elimination of many different types of viruses. On the other hand, as consumers are increasingly looking for greener and less toxic alternatives for the environment and for health, researchers are turning to natural and non-thermal physical treatments. EOs such as: oregano, clove, zataria, cinnamon, rosemary, tea tree, Japanese alder, hasuo and fig tree were shown to be effective against NoV surrogates. Fruit extracts, including their polyphenols, like pomegranate, cranberry and grape seed showed high efficiency in viral elimination. Finally, non-thermal physical treatments like fermentations, ozonation and high-pressure treatments have also been shown to display noticeable antiviral properties. Furthermore, high-pressure treatments and ozonation seems to be effective against enteric viruses. Combined treatments against NoV are very recent and seem to be very promising. Combined treatments of irradiation or ozonation with the utilization of natural antimicrobials such as EOs alone or in combination could bring benefits to the food industry and could decrease the required dose to eliminate viruses while maintaining nutritional and organoleptic quality of food products. The hope of finding effective treatments might be in the path of combining treatments.

4 Acknowledgements

This work was supported by an operating grant from the Natural Sciences Engineering Research Council of Canada (discovery program no:RGPIN: 2017-05947).

5 References

1. Aboubakr, H. A., El-Banna, A. A., Youssef, M. M., Al-Sohaimy, S. A., and Goyal, S. M. (2014). Antiviral effects of Lactococcus lactis on feline calicivirus, a human norovirus surrogate. *Food and Environmental Virology*, 6 : 282-289.
2. Aboubakr, H. A., Nauertz, A., Luong, N. T., Agrawal, S., El-Sohaimy, S. A., Youssef, M. M., and Goyal, S.M. (2016). In vitro antiviral activity of clove and ginger aqueous extracts against feline calicivirus, a surrogate for human norovirus. *Journal of Food Protection*, 79 :1001-1012.
3. Adams, M. R., Moss, M. O., and McClure, P. (2016). Food Microbiology. Royal Society of Chemistry, 4th edition. Cambridge, UK.
4. Adams, M. R., and Nicolaides, L. (1997). Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control*, 8: 227-239.
5. Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ... and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, 14: 725-730.
6. Alinkina, E. S., Misharina, T. A., and Fatkullina, L. D. (2013). Antiradical properties of oregano, thyme, and savory essential oils. *Applied Biochemistry and Microbiology*, 49 : 73-78.
7. Aviram, M., and Rosenblat, M. (2012). Pomegranate protection against cardiovascular diseases. *Evidence-Based Complementary and Alternative Medicine*, Volume 2012 : 20 p.
8. Azizkhani, M., and Tooryan, F. (2016). Effect of Cinnamon, Rosemary and Zataria Essential Oils on Food-borne Gastroenteritis Viruses. *Journal of Mazandaran University of Medical Sciences*, 26: 8-15.
9. Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chemical Toxicology*, 46 : 446-475.
10. Barker, J., Stevens, D., and Bloomfield, S. F. (2001). Spread and prevention of some common viral infections in community facilities and domestic homes. *Journal of Applied Microbiology*, 91: 7-21.
11. Barker, J., Vipond, I. B., and Bloomfield, S. F. (2004). Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. *Journal of Hospital Infection*, 58 : 42-49.
12. Belliot, G., Lavaux, A., Souihel, D., Agnello, D., and Pothier, P. (2008). Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. *Applied and Environmental Microbiology*, 74 : 3315-3318.
13. Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., and Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology,* 12: 2385–2397.

14. Beuchat, L. R. (1992). Surface disinfection of raw produce. *Dairy, Food and Environmental Sanitation*, 12: 6-9.
15. Beuchat, L. R. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. *Journal of Food Protection*, 61: 1305–1311.
16. Block, S. S. (Ed.). (2001). *Disinfection, Sterilization, and Preservation*. Lippincott Williams & Wilkins.
17. Block, G., Patterson, B., and Subar, A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer*, 18 : 1-29.
18. Boumail, A., Salmieri, S., and Lacroix, M. (2016). Combined effect of antimicrobial coatings, gamma radiation and negative air ionization with ozone on Listeria innocua, Escherichia coli and mesophilic bacteria on ready-to-eat cauliflower florets. *Postharvest Biology and Technology*, 118 : 134-140.
19. Boutekedjiret, C., Bentahar, F., Belabbes, R., and Bessiere, J. M. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. *Flavour and Fragrance Journal*, 18 : 481-484.
20. Burt, S. A., and Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against Escherichia coli O157: H7. *Letters in Applied Microbiology*, 36: 162-167.
21. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94: 223-253.
22. Cadet, J., and Douki, T. (2018). Formation of UV-induced DNA damage contributing to skin cancer development. *Photochemical & Photobiological Sciences*, 17 :1816-1841.
23. Cao, C. (2013). Chemical Disinfectants for Inactivation of Human Norovirus Surrogates.
24. Cayot, N. (2007). Sensory quality of traditional foods. *Food Chemistry*, 102: 445-453.
25. Chen, H., Hoover, D. G., and Kingsley, D. H. (2005). Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection*, 68: 2389-2394.
26. Cheong, S., Lee, C., Song, S. W., Choi, W. C., Lee, C. H. and Kim, S. J. (2009). Enteric viruses in raw vegetables and groundwater used for irrigation in South Korea. *Applied and Environmental Microbiology*, 75: 7745-7751.
27. Cleland, M. R., and Stichelbaut, F. (2013). Radiation processing with high-energy X-rays. *Radiation Physics and Chemistry*, 84: 91-99.
28. Côté, J., Caillet, S., Doyon, G., Dussault, D., Salmieri, S., Lorenzo, G., ... and Lacroix, M. (2011A). Effects of juice processing on cranberry antioxidant properties. *Food Research International*, 44: 2907-2914.

29. Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J. F., and Lacroix, M. (2011B). Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22: 1413-1418.
30. Côté, J., Caillet, S., Dussault, D., Sylvain, J. F., and Lacroix, M. (2011C). Effect of juice processing on cranberry antibacterial properties. *Food Research International*, 44: 2922-2929.
31. Dai, T., Vrahas, M. S., Murray, C. K., and Hamblin, M. R. (2012). Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? *Expert Review of Anti-Infective Therapy*, 10 : 185-195.
32. De Roda Husman, A. M., Bijkerk, P., Lodder, W., Van Den Berg, H., Pribil, W., Cabaj, A., ... and Duizer, E. (2004). Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (gamma) radiation. *Applied and Environmental Microbiology*, 70 : 5089-5093.
33. Diels, A. M., Callewaert, L., Wuytack, E. Y., Masschalck, B., and Michiels, C. W. (2004). Moderate Temperatures Affect Escherichia coli Inactivation by High-Pressure Homogenization Only through Fluid Viscosity. *Biotechnology Progress*, 20 : 1512-1517.
34. Dixon, R. A., Xie, D. Y., and Sharma, S. B. (2005). Proanthocyanidins–a final frontier in flavonoid research? *New Phytologist*, 165: 9-28.
35. Donaldson, E. F., Lindesmith, L. C., Lobue, A. D., and Baric, R. S. (2008). Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. *Immunological Reviews*, 225 : 190-211.
36. Dortu, C., and Thonart, P. (2009). Les bactériocines des bactéries lactiques: caractéristiques et intérêts pour la bioconservation des produits alimentaires/Bacteriocins from lactic acid bacteria: interest for food products biopreservation. *Biotechnologie, Agronomie, Société et Environnement*, 13: 143.
37. D'Souza, D. H., Su, X., Roach, A., and Harte, F. (2009). High-pressure homogenization for the inactivation of human enteric virus surrogates. *Journal of Food Protection*, 72: 2418-2422.
38. Du, J., Han, Y., and Linton, R. H. (2002). Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces. *Food Microbiology*, 19: 481-490.
39. Duizer, E., Bijkerk, P., Rockx, B., De Groot, A., Twisk, F., and Koopmans, M. (2004). Inactivation of caliciviruses. *Applied and Environmental Microbiology*, 70: 4538-4543.
40. Dybowska, B. E. (2005). Emulsion reproducibility in two-stage homogenizer Niro Soavi type Panda. *Milchwissenschaft*, 60 : 367-370.
41. Elizaquível, P., Azizkhani, M., Aznar, R., and Sánchez, G. (2013). The effect of essential oils on norovirus surrogates. *Food Control*, 32: 275-278.
42. Elvis, A. M., and Ekta, J. S. (2011). Ozone therapy: A clinical review. *Journal of Natural Science, Biology, and Medicine*, 2 : 66.

43. Farkas, J. (1989). Microbiological safety of irradiated foods. *International Journal of Food Microbiology*, 9: 1-15.
44. Farkas, J., and Mohácsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science & Technology*, 22 : 121-126.
45. Feng, K., Divers, E., Ma, Y., and Li, J. (2011). Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*, 77: 3507-3517.
46. Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., and Perelle, S. (2011). Comparison of chlorine and peroxyacetic-based disinfectant to inactivate Feline calicivirus, Murine norovirus and Hepatitis A virus on lettuce. *International Journal of Food Microbiology*, 151: 98-104.
47. Fretz-Männel, R., Baumgartner, A., Lüthi, Schmid, H., Schorr, D., Svoboda, P., Witschi, A. (2005). Norovirus- Caractéristiques biologiques, épidémiologie, tableau clinique, prévention Recommandations pour la gestion des épidémies. Édition Office fédéral de la santé publique. Berne, Suisse.
48. Ghabraie, M., Vu, K. D., Tata, L., Salmieri, S., and Lacroix, M. (2016). Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. *LWT-Food Science and Technology*, 66 : 332-339.
49. Gilling, D. H., Kitajima, M., Torrey, J. R., and Bright, K. R. (2014A). Mechanisms of antiviral action of plant antimicrobials against murine norovirus. *Applied and Environmental Microbiology*, 80 : 4898-4910.
50. Gilling, D. H., Kitajima, M., Torrey, J. R., and Bright, K. R. (2014B). Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *Journal of Applied Microbiology*, 116: 1149-1163.
51. Glass, R. I., Parashar, U. D., and Estes, M. K. (2009). Norovirus gastroenteritis. *New England Journal of Medicine*, 361: 1776-1785.
52. Guzel-Seydim, Z. B., Greene, A. K., and Seydim, A. C. (2004). Use of ozone in the food industry. *LWT-Food Science and Technology*, 37: 453-460.
53. Heinonen, M. (2007). Antioxidant activity and antimicrobial effect of berry phenolics–a Finnish perspective. *Molecular Nutrition & Food Research*, 51: 684-691.
54. Hirneisen, K. A., Markland, S. M., and Kniel, K. E. (2011). Ozone inactivation of norovirus surrogates on fresh produce. *Journal of Food Protection*, 74: 836-839.
55. Holley, R. A. (2014) Food irradiation adds cost but makes sense. Canadian Food Insights 2:28-30.

56. Joshi, S. S., Howell, A. B., and D'Souza, D. H. (2016). Reduction of enteric viruses by blueberry juice and blueberry proanthocyanidins. *Food and Environmental Virology*, 8 : 235-243.
57. Joshi, S., Howell, A. B., and D'Souza, D. H. (2017). Blueberry proanthocyanidins against human norovirus surrogates in model foods and under simulated gastric conditions. *Food Microbiology*, 63: 263-267.
58. Khadre, M. A., Yousef, A. E., and Kim, J. G. (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science*, 66 : 1242-1252.
59. Khadre, M. A., and Yousef, A. E. (2002). Susceptibility of human rotavirus to ozone, high pressure, and pulsed electric field. *Journal of Food Protection*, 65 : 1441-1446.
60. Kim, J. G., Yousef, A. E., and Khadre, M. A. (2003). Ozone and its current and future application in the food industry. *Advances in Food and Nutrition Research*, 45: 167-218.
61. Kim, S. H., Shahbaz, H. M., Park, D., Chun, S., Lee, W., Oh, J. W., … and Park, J. (2017). A combined treatment of UV-assisted TiO2 photocatalysis and high hydrostatic pressure to inactivate internalized murine norovirus. *Innovative Food Science & Emerging Technologies*, 39 : 188-196.
62. Kim, Y. S., Zheng, Z. B., and Shin, D. H. (2008). Growth inhibitory effects of kimchi (Korean traditional fermented vegetable product) against Bacillus cereus, Listeria monocytogenes, and Staphylococcus aureus. *Journal of Food Protection*, 71 : 325-332.
63. Kirk, J. R., and Mitchell, S. K. (1980). Risks and benefits associated with chlorine in the food industry. *Water Chlorination. Environ-Chlorination of Organic Compounds,* 273 : 218-225.
64. Komanapalli, I. R., and Lau, B. H. S. (1998). Inactivation of bacteriophage k, Escherichia coli, and Candida albicans by ozone. *Applied Microbiology and Biotechnology*, 49 : 766-769.
65. Komolprasert, V., Bailey, A., and Machuga, E. (2008). Irradiation of Food Packaging Materials: Regulatory Report. FDA. *Center for Food Safety and Applied Nutrition.*
66. Konowalchuk, J., and Speirs, J. I. (1976). Antiviral activity of fruit extracts. *Journal of Food Science*, 41 : 1013-1017.
67. Lacombe, A., Niemira, B. A., Gurtler, J. B., Sites, J., Boyd, G., Kingsley, D. H., and Chen, H. (2017). Nonthermal inactivation of norovirus surrogates on blueberries using atmospheric cold plasma. *Food Microbiology*, 63 : 1-5.
68. Lacroix, M., and Ouattara, B. (2000). Combined industrial processes with irradiation to assure innocuity and preservation of food products—a review. *Food Research International*, 33 : 719-724.
69. Lacroix, M., and Vigneault, C. (2007). Irradiation treatment for increasing fruit and vegetable quality. *Stewart Postharvest Review*, 3: 1-8.

70. Lankinen, M. H., Vilpo, L. M., and Vilpo, J. A. (1996). UV-and γ-irradiation-induced DNA single-strand breaks and their repair in human blood granulocytes and lymphocytes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 352: 31-38.
71. Lee, C. H. (1997). Lactic acid fermented foods and their benefits in Asia. *Food Control*, 8 : 259-269.
72. Lee, M. H., Yoo, S. H., Ha, S. D., and Choi, C. (2012). Inactivation of feline calicivirus and murine norovirus during Dongchimi fermentation. *Food Microbiology*, 31: 210-214.
73. Lee, J. H., min Lee, H., Kim, S. H., Eun, J. B., and Ha, J. H. (2017). Recovery of intact human norovirus from cabbage Kimchi stored at 4° C and 10° C during fermentation. *LWT-Food Science and Technology*, 78: 258-264.
74. Li, J. W., Xin, Z. T., Wang, X. W., Zheng, J. L., and Chao, F. H. (2004). Mechanisms of inactivation of hepatitis A virus in water by chlorine dioxide. *Water Research*, 38 : 1514-1519.
75. Li, X., and Chen, H. (2015). Evaluation of the porcine gastric mucin binding assay for high-pressure-inactivation studies using murine norovirus and tulane virus. *Applied and Environmental Microbiology*, 81: 515-521.
76. Lipson, S. M., Gordon, R. E., Karthikeyan, L., Singh, M., Burdowski, A., Roy, M., and Stotzky, G. (2010). Cranberry and Grape Juice Drinks Affect Infectivity, Integrity, and Pathology of Enteric Viruses in an Animal Model. In *ACS symposium series* (Vol. 1035, pp. 177-195). Oxford University Press.
77. Lopman, B., Gastanaduy, P., Park, G. W., Hall, A. J., Parashar, U. D. and Vinjé, J. (2012). Environmental transmission of norovirus gastroenteritis. *Current Opinion in Virology*, 2 : 96-102.
78. Lu, Y., and Wu, C. (2010). Reduction of Salmonella enterica contamination on grape tomatoes by washing with thyme oil, thymol, and carvacrol as compared with chlorine treatment. *Journal of Food Protection*, 73: 2270-2275.
79. Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79 : 727-747.
80. Mara, D., and Sleigh, A. (2010). Estimation of norovirus infection risks to consumers of wastewater-irrigated food crops eaten raw. *Journal of Water and Health*, 8: 39-43.
81. McDonnell, G., and Russell, A. D. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews*, 12: 147-179.
82. McLinden, T., Sargeant, J. M., Thomas, M. K., Papadopoulos, A., and Fazil, A. (2014). Component costs of foodborne illness: a scoping review. *BMC Public Health*, 14: 509.
83. Monk, J. D., Beuchat, L. R., and Doyle, M. P. (1995). Irradiation inactivation of food-borne microorganisms. *Journal of Food Protection*, 58 : 197-208.

84. Montazeri, N., Manuel, C., Moorman, E., Khatiwada, J. R., Williams, L. L., and Jaykus, L. A. (2017). Virucidal Activity of Fogged Chlorine Dioxide-and Hydrogen Peroxide-Based Disinfectants against Human Norovirus and Its Surrogate, Feline Calicivirus, on Hard-to-Reach Surfaces. *Frontiers in Microbiology*, 8 : 1031.
85. Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., and Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, 25 : 361-366.
86. Ndoti-Nembe, A., Vu, K. D., Han, J., Doucet, N., and Lacroix, M. (2015). Antimicrobial Effects of Nisin, Essential Oil, and γ-Irradiation Treatments against High Load of Salmonella typhimurium on Mini-carrots. *Journal of Food Science*, 80 : 1544-1548.
87. Nostro, A., and Papalia, T. (2012). Antimicrobial activity of carvacrol: current progress and future prospectives. *Recent patents on anti-infective drug discovery*, 7 : 28-35.
88. Nout, M. R., Rombouts, F. M., and Havelaar, A. (1989). Effect of accelerated natural lactic fermentation of infant good ingredients on some pathogenic microorganisms. *International Journal of Food Microbiology*, 8: 351-361.
89. Nuanualsuwan, S., and Cliver, D. O. (2003). Capsid functions of inactivated human picornaviruses and feline calicivirus. *Applied and Environmental Microbiology*, 69 : 350-357.
90. Oh, M., and Chung, M. S. (2014). Effects of oils and essential oils from seeds of Zanthoxylum schinifolium against foodborne viral surrogates. *Evidence-Based Complementary and Alternative Medicine*, Volume 2014.
91. Oussalah, M., Caillet, S., and Lacroix, M. (2006). Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157: H7 and Listeria monocytogenes. *Journal of Food Protection*, 69 : 1046-1055.
92. Park, S. Y., and Ha, S. D. (2017). Application of gamma radiation for the reduction of norovirus and the quality stability in optimally ripened cabbage kimchi. *Food Research International*, 100 : 277-281.
93. Pate, K. M., Rogers, M., Reed, J. W., van der Munnik, N., Vance, S. Z., and Moss, M. A. (2017). Anthoxanthin Polyphenols Attenuate Aβ Oligomer-induced Neuronal Responses Associated with Alzheimer's Disease. *CNS Neuroscience & Therapeutics*, 23 : 135-144.
94. Patel, M. M., Widdowson, M. A., Glass, R. I., Akazawa, K., Vinjé, J., and Parashar, U. D. (2008). Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerging Infectious Diseases*, 14: 1224.
95. Perumalla, A. V. S., and Hettiarachchy, N. S. (2011). Green tea and grape seed extracts—Potential applications in food safety and quality. *Food Research International*, 44 : 827-839.

96. Picart, L., Thiebaud, M., René, M., Guiraud, J. P., Cheftel, J. C., and Dumay, E. (2006). Effects of high pressure homogenisation of raw bovine milk on alkaline phosphatase and microbial inactivation. A comparison with continuous short-time thermal treatments. *Journal of Dairy Research*, 73: 454-463.
97. Predmore, A., Sanglay, G., Li, J., and Lee, K. (2015). Control of human norovirus surrogates in fresh foods by gaseous ozone and a proposed mechanism of inactivation. *Food Microbiology*, 50 : 118-125.
98. Rahimi, E., Faghihi, R., Baradaran-Ghahfarokhi, M.,Alavaian-Ghavinini, A., Baradaran-Ghahfarokhi, H. R., Siavashpour, S., … and Rafie, F. (2013). Effects of gamma irradiation on microbial load and quality characteristics of veal. *Advanced Biomedical Research*, Vol 2.
99. Reichling, J., Schnitzler, P., Suschke, U., and Saller, R. (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–an overview. *Complementary Medicine Research*, 16 : 79-90.
100. Rice, R. G., Graham, D. M., and Lowe, M. T. (2002). Recent ozone applications in food processing and sanitation. *Food Safety Magazine*, 8:10-17.
101. Ross, R. P., Morgan, S., and Hill, C. (2002). Preservation and fermentation: past, present and future. *International Journal of Food Microbiology*, 79: 3-16.
102. Ryu, S., You, H. J., Kim, Y. W., Lee, A., Ko, G. P., Lee, S. J. and Song, M. J. (2015). Inactivation of norovirus and surrogates by natural phytochemicals and bioactive substances. *Molecular Nutrition & Food Research*, 59: 65-74.
103. Rzeżutka, A., and Cook, N. (2004). Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews*, 28 : 441-453.
104. Sair, A. I., D'Souza, D. H., Moe, C. L., and Jaykus, L. A. (2002). Improved detection of human enteric viruses in foods by RT-PCR. *Journal of Virological Methods*, 100: 57-69.
105. Sánchez, C., Aznar, R., and Sánchez, G. (2015). The effect of carvacrol on enteric viruses. *International Journal of Food Microbiology*, 192: 72-76.
106. Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, 30 : 3875-3883.
107. Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection*, 75:123-131.
108. Seo, D. J. and C. Choi (2017). Inhibition of Murine Norovirus and Feline Calicivirus by Edible Herbal Extracts. *Food Environmental Virology,* 9: 35-44.
109. Seo, D. J., Jeon, S. B., Oh, H., Lee, B. H., Lee, S. Y., Oh, S. H., ... and Choi, C. (2016). Comparison of the antiviral activity of flavonoids against murine norovirus and feline calicivirus. *Food Control*, 60 : 25-30.

110. Seo, D. J., Lee, M. H., Seo, J., Ha, S. D., and Choi, C. (2014). Inactivation of murine norovirus and feline calicivirus during oyster fermentation. *Food Microbiology*, 44 : 81-86.
111. Seo, D. J., Lee, M., Jeon, S. B., Park, H., Jeong, S., Lee, B. H., and Choi, C. (2017). Antiviral activity of herbal extracts against the hepatitis A virus. *Food Control*, 72 : 9-13.
112. Seymour, I. J., and Appleton, H. (2001). Foodborne viruses and fresh produce. *Journal of Applied Microbiology*, 91: 759-773.
113. Shahidi, F., and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, 18 : 820-897.
114. Shang, C., Cheung, L. M., and Liu, W. (2007). MS2 coliphage inactivation with UV irradiation and free chlorine/monochloramine. *Environmental Engineering Science*, 24 : 1321-1332.
115. Silindir, M. and Özer, A. Y. (2009). Sterilization methods and the comparison of E-beam sterilization with gamma radiation sterilization. *Fabad Journal of Pharmacology* Sciences, 34: 43.
116. Smith, W. L. (1962). Chemical treatments to reduce postharvest spoilage of fruits and vegetables. *The Botanical Review*, 28 : 411-445.
117. Su, X., Howell, A. B., and D'Souza, D. H. (2010). Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. *Food Microbiology,* 27: 985-991.
118. Su, X., Sangster, M. Y., and D'Souza, D. H. (2011A). Time-dependent effects of pomegranate juice and pomegranate polyphenols on foodborne viral reduction. *Foodborne Pathogens and Disease*, 8: 1177-1183.
119. Su, X., and D'Souza, D. H. (2011B). Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology*, 77: 3982-3987.
120. Su, X., and D'Souza, D. H. (2013). Naturally occurring flavonoids against human norovirus surrogates. *Food and environmental virology*, 5: 97-102.
121. Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., and Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, 115 : 873-877.
122. Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., and Gerba, C. P. (2003). Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Applied and Environmental Microbiology*, 69 : 3979-3985.
123. Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., and Gerba, C. P.(2005). Inactivation of enteric adenovirus and feline calicivirus by ozone. *Water Research*, 39 : 3650-3656.

124. Trindade, M. A., Kushida, M. M., Montes Villanueva, N. D., Pereira, D. U. D. S., and de Oliveira, C. E. L. (2012). Comparison of ozone and chlorine in low concentrations as sanitizing agents of chicken carcasses in the water immersion chiller. *Journal of Food Protection*, 75 : 1139-1143.
125. Ultee, A., Slump, R. A.., Steging, G., and Smid, E. J. (2000). Antimicrobial activity of carvacrol toward Bacillus cereus on rice. *Journal of Food Protection*, 63: 620–4.
126. Urakami, H., Ikarashi, K., Okamoto, K., Abe, Y., Ikarashi, T., Kono, T., and Tanaka, N. (2007). Chlorine sensitivity of feline calicivirus, a norovirus surrogate. *Applied and Environmental Microbiology*, 73: 5679-5682.
127. Vercauteren, J., and Chèze, C. (1998). *Polyphenols 96: 18th International Conference on Polyphenols, Bordeaux (France), July 15-18, 1996* (No. 87). Editions Quae.
128. Vorsa, N., Vvedenskaya, I. O., Huang, M. T., and Rosen, R. T. (2007). *U.S. Patent No. 7,270,837*. Washington, DC: U.S. Patent and Trademark Office.
129. Vreeswijk, M. P., Van Hoffen, A., Westland, B. E., Vrieling, H., Van Zeeland, A. A., and Mullenders, L. H. (1994). Analysis of repair of cyclobutane pyrimidine dimers and pyrimidine 6-4 pyrimidone photoproducts in transcriptionally active and inactive genes in Chinese hamster cells. *Journal of Biological Chemistry*, 269 : 31858-31863.
130. Watanabe, Y., Miyata, H., and Sato, H. (1989). Inactivation of laboratory animal RNA-viruses by physicochemical treatment. *Experimental Animals*, 38: 305-311.
131. Wei, J., and Kniel, K. E. (2010). Pre-harvest viral contamination of crops originating from fecal matter. *Food and Environmental Virology*, 2: 195-206.
132. Wobus, C. E., Thackray, L. B., and Virgin, H. W. (2006). Murine norovirus: a model system to study norovirus biology and pathogenesis. *Journal of Virology,* 80: 5104-5112.
133. Wondrak GT, Jacobson MK, and Jacobson EL. (2006). Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochemical and Photobiological Sciences,* 5:215-237.
134. Woo, L., and Sandford, C. L. (2002). Comparison of electron beam irradiation with gamma processing for medical packaging materials. *Radiation Physics and Chemistry*, 63 : 845-850.
135. Wu, V. C., Qiu, X., Benildo, G., Lin, C. S., and Pan, Y. (2009). Application of cranberry concentrate (Vaccinium macrocarpon) to control Escherichia coli O157: H7 in ground beef and its antimicrobial mechanism related to the downregulated slp, hdeA and cfa. *Food Microbiology*, 26: 32-38.
136. Xie, Y., Hajdok, C., Mittal, G. S., and Warriner, K. (2008). Inactivation of MS2 F (+) coliphage on lettuce by a combination of UV light and hydrogen peroxide. *Journal of Food Protection*, 71 : 903-907.

137. Zakaryan, H., Arabyan, E., Oo, A., and Zandi, K. (2017). Flavonoids: promising natural compounds against viral infections. *Archives of Virology*, 162 : 2539-2551.

Le chapitre 2 intitulé : Revue de littérature, l'article « Norovirus elimination on the Surface of Fresh Foods » abordait les différents traitements actuels utilisés contre le FCV-F9. L'utilisation de composés chimiques synthétiques comme le chlore et le dioxyde de chlore a été développée. On comptait aussi l'utilisation de composés naturels comme les extraits de plante (carvacrol) et les extraits de fruits (CJ). Les traitements non-thermiques comme la fermentation, l'ozonation, l'utilisation de High Pressure Homogenizer (HPH) et l'utilisation de rayons UV ont été abordés. Finalement, le dernier thème abordé dans la revue de littérature a été l'utilisation de traitements combinés. L'objectif de cet article était de visualiser les traitements actuellement utilisés dans le marché des aliments frais afin de trouver des combinaisons possibles avec l'irradiation-γ. Le prochain chapitre (**Chapitre 3**) aborde l'application de traitements comme le jus de canneberge et l'extrait citrus utilisés seuls ou en combinaison avec l'irradiation-γ contre le virus FCV-F9 *in-vitro*.

Chapitre 3: Radiosensitivity increase of FCV-F9 virus using combined treatments with natural antimicrobials and γ-irradiation

Alexandra Gobeil, Shiv Shankar, Monique Lacroix*

INRS-Armand-Frappier, Health and Biotechnology Centre, Research Laboratories in Sciences, Applied to Food, Nutraceutical Institute and Functional Foods, Canadian Irradiation Centre, 531, Boulevard des Prairies, Laval, Québec, Canada, H7V 1B7.

* Corresponding author: Professor Monique Lacroix.

Tel.: 450-687-5010 #4489.

Fax: 450-686-5501; E-mail address: monique.lacroix@iaf.inrs.ca

Contribution des auteurs

Alexandra Gobeil a réalisé les manipulations et la rédaction de l'article.

Shiv Shankar a participé à la correction de l'article.

Monique Lacroix: Directrice de recherche, a participé à la planification des expériences et aux discussions des résultats et a aussi procédé à la correction de l'article.

L'article est soumis dans : Journal of Applied Microbiology (JAM-2019-1719) le 25-09-2019.

Abstract

Aims: Evaluate the possible synergistic effect of cranberry juice (CJ) and commercial citrus extract against FCV-F9 viral titer *in-vitro* in combination with γ-irradiation in order to determinate the D_{10} values and radiosensitivity increase.

Methods and Results: Virus samples were treated with a formulation containing a mixture of citrus extract (BS) or cranberry juice (CJ). Results showed a D_{10} of 0.05 %-BS, 0.42 %-CJ and 1.34 kGy for the virus treated with the BS, the CJ and the irradiation alone, respectively. Concentrations needed to reduce 6 log $TCID_{50}$ mL^{-1} of viral titer were BS-0.3%, CJ-2.52% and 8.04 kGy. Irradiation combined with BS-0.01% against FCV-F9 virus showed a D_{10} value of 0.74 kGy and a viral radiosensitization of 1.28, whereas γ-irradiation combined with CJ-0.1% showed a D_{10} of 0.72 kGy and a viral radiosensitization of 1.50.

Conclusion: These findings indicate that CJ and BS could be used as antiviral agents alone or in combination with γ-irradiation and the use of CJ and BS could be helpful in prevention of NoV outbreaks.

Significance and Impact of Study: CJ and BS could be used in hurdle approaches in combined treatment with γ-irradiation to assure food safety without detrimental effect on nutritional value and maintain low processing cost.

Keywords: Viruses, Food safety, Antimicrobials, Non-thermal processes, Post Harvest.

1 Introduction

Fresh fruits and vegetables such as iceberg lettuce are potential sources for viral infections like human norovirus (NoV). In 2010, the Centers for Disease Control and Prevention (CDC) estimated approximately 76 million new cases of foodborne diseases in the USA (Scharff, 2010). There are more than 325 000 hospitalizations and 5000 deaths by foodborne diseases (Scharff, 2010). NoV are among the top 10 infectious agents mainly responsible for foodborne diseases (Mattison *et al.,* 2007). They also represent 65 % of the nonbacterial gastroenteritis in the United States (Mattison *et al.,* 2007). Among foodborne viruses, the most common are hepatitis A and Norwalk-like gastroenteritis virus, NoV was estimated to cause 5.46 million infections per year with 14663 hospitalizations and 149 deaths representing annual costs of 2896 million US dollars (USD) per year (Cliver, 1997; Scharff, 2012). NoVs are non-enveloped viruses and are part of the *Caliciviridae* family. NoVs are stable and resistant to environmental degradation and to chemical inactivation processes (Cheesbrough *et al.,* 2000; Kuusi *et al.,* 2002; Widdowson *et al.,* 2004). NoVs are excreted in the feces and vomitus of the infected person (Cannon *et al.,* 2006). The main modes of transmission of NoVs are directly from person to person, consumption of contaminated food, and vomitus (Fankhauser *et al.,* 2002). Personal hygiene and effective food safety measures, especially for ready-to-eat foods such as salad bars, deli meats, fresh produce, and shellfish are important for the control of NoVs infection (Cannon *et al.,* 2006). However, waterborne outbreaks have been reported but do not represent a large fraction of all diseases (Lopman *et al.,* 2012).

The safety of food products can be enhanced by appropriate implementation of effective control and preventive strategies. Owing to the consumers' preference of natural compounds over artificial chemicals, natural compounds that possesses antimicrobial properties without causing any undesirable sensory changes of the product, toxicity and adverse health effects are in growing demand. Among the fruit extracts, cranberry extracts attracted much attention as they are known to exhibit a range of antibacterial, antiviral, and pharmacological properties (Côté *et al.,* 2011; Zhao *et al.,* 2018; Kim *et al.,* 2019). CJ reportedly helps prevent and reduce the recurrence of urinary tract infections (Liu *et al.,* 2008; Pinzón-Arango *et al.,* 2009; Maki *et al.,* 2016, 2018). CJ is rich in polyphenols (Côté *et al.,* 2010) and organic acids like quinic, malic, and citric acid (Coppola *et al.,* 1978). Previous studies have demonstrated *in-vitro* (Côté *et al.,* 2011) and *in-situ*, good capacity to inhibit *E. coli* and *Listeria* (Lacroix and Ouattara, 2000). In addition, other natural antimicrobials such as citrus extracts are full of organic acids and are qualified as non-genetically modified organism (OGM), clean label and generally recognized as safe (GRAS) product. Citrus extracts contain organic acids including citric acid, ascorbic acid, and lactic acid (Methot *et al.,* 2017). These acids are responsible for the acidity of the product and also aid in the stabilization and protection of anthocyanins (Caillet *et al.,* 2011). Suzuki *et al.* (2005) demonstrated that ethyl acetate layer of *Citrus unshiu* peel extract could decrease viral HCV; the authors attributed these

results to the antioxidant and anti-tumor properties of the extract. Also, it has been showed that polyphenol compounds present in green tea can inhibit HCV. Epigallocatechin-3-gallate (EGCG) has been identified as an inhibitor of HCV entry in the cell. Authors demonstrated that EGCG acts by blocking viral attachment to target cells (Ciesek *et al.,* 2011).

Also, γ-irradiation is a non-thermal process used to assure food safety and to enhance the shelf-life of food (Lacroix and Ouattara, 2000). γ-irradiation acts directly on microbial DNA and can inhibit bacterial growth at doses range from 1-10 kGy (Severino *et al.,* 2015).Viruses are more resistant to inactivation by ionizing radiation than bacteria, parasites, or fungi because of their small size and their type of nucleic acid ($_{ss}$RNA, $_{ds}$RNA, $_{ss}$DNA, $_{ds}$DNA), but it has been showed that foodborne viruses can be inactivated by γ-irradiation at doses from 2.7 to 3.0 kGy (Bidawid *et al.,* 2000). However, the doses of irradiation required to eliminate viruses can affect the physico-chemical quality of fresh vegetables like lettuce (Diehl, 1992).^{60}Co is mostly used for the emission of γ-rays (Bidawid *et al.,* 2000). According to Feng *et al.* (2011), it is suggested that enveloped viruses (like VSV virus) are more sensitive to γ-irradiation than non-enveloped virus. NoV are non-enveloped viruses and possesses a highly stable capsid that protects their genetic material.

Generally, NoV surrogates such as Feline Calicivirus (FCV) and Murine Norovirus (MNV) represent the best substitute for inactivation studies, because there is no animal or cell culture available for NoV propagation. Both FCV and MNV have the same size and shape as the human NoV (Hirneisen *et al.,* 2011). NoV are not enveloped viruses and possess round viral particles with a diameter of 27 to 40 nm (Buckow *et al.,* 2008). According to Cannon *et al.* (2006), NoV have a low infectious dose of 10-100 virus particles for infection.

There are reports available on the antimicrobial and antiviral activities using natural antimicrobial compounds or heat treatment (Su *et al.,* 2010; Gibson and Schwab, 2011). Heat treatments cannot be used for products like fresh fruits and vegetables and the reductions found with natural antimicrobials are more effective at high concentrations (Gilling *et al.,* 2014).

The present study aims to evaluate the possible synergistic effect of CJ and commercial citrus extract against NoV (FCV-F9 virus) viral titer *in-vitro* used in combination with γ-irradiation.

2 Materials and methods

2.1 Cellular Culture of CRFK cells

CRFK cells (ATCC-CCL94) were used as hosts for the virus and were maintained in Dulbecco's minimum essential medium (DMEM: Fisher Scientific, Ottawa, Ontario, Canada) supplemented with 10 % fetal bovine serum (FBS, Wisent, Saint-Jean-Baptiste, Québec, Canada) and 1 % Streptomycin-Penicillin antibiotic (Gibco, Fisher Scientific, Ottawa, Ontario, Canada). The medium was added to a 75

cm^2 culture flasks and incubated at 37 °C, in a humidified incubator containing a 5 % CO_2 atmosphere. Cells were subcultured every 2-3 days.

2.2 Preparation of FCV-F9 viral stocks

CRFK cells (ATCC-CCL94) were grown to 90 % confluency in 25 cm^2 culture flasks (Sarstedt, Montréal, Québec, Canada), then the culture medium (DMEM+ 10 % FBS) was aspirated out and the monolayer of cells adhered to the bottom of the flask was washed twice with Dulbecco's Phosphate-Buffered Saline (Ph 7.0) (DPBS) (HyClone, GE Healthcare Life Sciences, Utah, USA). Thereafter, a 200 µL aliquot of viral inoculum (FCV-F9 virus (ATCC VR-651)) was added to each flask and incubated at 37 °C under 5 % CO_2 atmosphere for 90 min to allow viral adsorption. Subsequently, 7 mL of medium (DMEM+ 2 % FBS + 1 % strep-pen) was added to each flask and incubated for 16-18 h at 37 °C in 5 % CO_2 incubator that resulted in virus-induced destruction of nearly 90 % of the monolayer. Each virus-infected flask was first frozen at -80 °C for 5 min and then thawed at 37 °C for 5 min and this cycle was repeated twice. The content was centrifuged at 1500 x g for 15 min to remove cell debris and the supernatant containing viruses at ~ 5 log $TCID_{50}$/mL was dispensed into 1 mL aliquot and stored at -80 °C.

2.3 Preparation of CJ and BS formulation

Cranberry Juice Concentrate (CJ) 12100-5/ °BRIX (ATOKA, Manseau, Québec, Canada) was prepared in distilled water. Quantities of 0.1g, 0.25g, 0.50g, 0.75g and 1.0 g of CJ were respectively added in 99.90 mL (CJ-0.1%), 99.75 mL (CJ-0.25%), 99.50 mL (CJ-0.5%), 99.25 mL (CJ-0.75%) and 99.0 mL (CJ-1%) of distillated water, respectively. Citrus Extract Biosecur F420B (BS) (Biosecur, Mont Saint-Hilaire, Québec, Canada) was also prepared in distilled water. Quantities of 0.005g (BS-0.005%), 0.01g (BS-0.01%), 0.025g (BS-0.025%), 0.05 (BS-0.05%) and 0.1g (BS-0.10%) were respectively added to 99.995 mL, 99.99 mL, 99.975 mL, 99.95 mL and 99.90 mL of distillated water, respectively.

2.4 Treatment of virus

Thereafter, 100 µL of various concentrations of CJ or BS was mixed with 90 µL DPBS and 10 µL virus stock in a 1.5 mL microtube. This mix was incubated for 2 h in an agitator (Forma Scientific Model 435 Orbital Shaker Incubator) at 37 °C, 150 RPM (0.31 x g) in order to reduce viral titer.

2.5 γ-irradiation treatment

Viral samples (5-5.75 log $TCID_{50}$ mL^{-1}) of FCV-F9 virus were treated at doses from 0 to 3 kGy in order to determine the D_{10} value (kGy). Irradiation was also applied on pre-treated virus samples with BS-0.01%, BS-0.025%, BS-0.10% treated with doses ranging from 0 to 1.5 kGy for BS-0.01% and BS-0.10% and doses ranging from 0 to 2.5 kGy for BS-0.025%. A treatment was also done with CJ-0.1% and CJ-0.5% on 5-5.75 log $TCID_{50}$ mL^{-1} against FCV-F9 virus and treated at doses ranging from 0 to 1.5 kGy in order to reduce viral titer and calculate the D_{10} values. The irradiation treatments were done at the Canadian Irradiation Center, in a UC-15 A (SS canister) underwater calibrator (Nordion Inc., Kanata, Ontario, Canada) equipped with a ^{60}Co source and having a dose rate of 9.5 kGy h^{-1}.

Relative radiation sensitivity was determined using the following equation:

Relative radiation sensitivity = (radiation D_{10} of control sample)/ (radiation D_{10} of sample treated in presence of antimicrobial compound)

The D_{10} value (kGy) is defined as the radiation dose required reducing 90 % of the population (reduction by 1 - log $TCID_{50}$/mL) of viable feline calicivirus infecting the CRFK cell. To calculate D_{10} value (kGy), viral titer counts (log $TCID_{50}$/mL) were plotted against radiation doses, and the reciprocal of the slope of the trendline was extracted from the plot.

2.6 Infection of CRFK cells in 96-well plate

The above treated viral particles were serially diluted from 10^2 to 10^9 log $TCID_{50}$ mL^{-1} using Phosphate-buffered saline (PBS) in a 96 well plate in order to determine viral titer by $TCID_{50}$ method. Infection of CRFK cells by FCV-F9 virus was performed after 90 % confluency was reached. Confluency is the degree of separation that exists between adherent cells and is measured by visual inspection under microscopy. For this, the CRFK cells were grown to the 90 % confluency, then the medium was discarded followed by washing twice with DPBS. Then the CRFK cells were trypsinized by adding 1 mL of trypsin-EDTA (0.25%, pH 7.2-8.0) (Fisher Scientific, Ottawa, Ontario, Canada) and the cells were seed in a new 96 well plates (flat bottom for adherent cell culture) with the density of 10^5 cells per well in 100 µl DMEM (10 % FBS, 1 % Pen/Strep), and incubated overnight at 37 °C under 5 % CO_2. The cells reached confluency of 80 – 90 % after overnight incubation, then the media was removed, cells were washed with PBS buffer twice and 20 µL of diluted treated virus particles were added to the corresponding wells. The cells without virus particles were taken as control. The microplates were incubated for 30-60 min at 37 °C and a quantity of 150 uL of DMEM + 2 % FBS was then added to the microplate and incubated for 2 days (5 % CO_2, 37 °C). Cytopathic effects were determined by visual inspection under the optical microscope

in order to measure the viral titer of FCV-F9 virus. The limit of detection of this method is 3.20 log $TCID_{50}$ mL^{-1}.

2.7 Analysis by Tissue Culture Infectious Dose 50 ($TCID_{50}$)

The viral titer was measured with the endpoint dilution assay with Tissue Culture Infective Dose 50 ($TCID_{50}$) in order to evaluate the FCV-F9 titer. This method of endpoint dilution quantifies the quantity of virus needed to infect 50 % of the cellular culture or to produce cytopathic effects in 50 % of cellular culture. To calculate viral titer ($TCID_{50}$ mL^{-1}) after infection (Hirneisen *et al.,* 2011), the Spearman-Karber method was used (Brié *et al.,* 2017).

2.8 Statistical analysis

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

3 Results

3.1 Effect of CJ and BS on CRFK cells and FCV-F9 virus titer

Results showed that CJ-0.1%, CJ-0.5% and CJ-1% and BS-0.01%, BS-0.025%, BS-0.1% and BS-0.5% did not show any signs of cytotoxicity against CRFK cells used in the present study. This showed that CJ and BS at the concentrations used for viral titration had no obvious effect on the viability of these cell lines and would hamper our ability to use these cell lines to determine antiviral effects (Su *et al.,* 2010). However, the FCV-F9 virus titer ($TCID_{50}$ mL^{-1}) was strongly affected by treatment with BS. A decrease of the viral titer with an increase of BS concentration was observed (Fig.1A). A treatment of the virus with BS-0.005% decreased the viral titer from 5.49 log $TCID_{50}$ mL^{-1} to 5.32 log $TCID_{50}$ mL^{-1}. A treatment of BS-0.01% did not significantly affect the viral titer, the same reduction from 5.49 log $TCID_{50}$ mL^{-1} to 5.32 log $TCID_{50}$ mL^{-1} was observed. A treatment with BS-0.025% decreased the viral titer from 5.49 log $TCID_{50}$ mL^{-1} to 4.62 log $TCID_{50}$ mL^{-1}. The two last concentrations of BS, BS-0.05% and BS-0.1%, induced a significant ($p \leq 0.05$) decrease of viral titer to 3.66 and 3.45 log $TCID_{50}$ mL^{-1}, respectively (Table 1). The value of the D_{10} after treatment with different concentrations of BS measured was 0.05 %-BS.

Similar to BS, the treatment with CJ also exhibited a strong reduction in FCV-F9 virus titer at various concentrations (Fig. 1B). A linear negative regression was also observed. The initial virus titer was about 5.57 log $TCID_{50}$ mL^{-1}, which decreased to 5.49 (0.08 log $TCID_{50}$ mL^{-1} reduction), 5.45 (0.12 log $TCID_{50}$ mL^{-1} reduction), and 4.24 (1.33 log $TCID_{50}$ mL^{-1} reduction) log $TCID_{50}$ mL^{-1} when CJ at the concentration of CJ-0.10%, CJ-0.25%, and CJ-0.5% was used, respectively. The treatments with CJ-0.75% and CJ-1% reduced the FCV-F9 virus titer by 1.54 log (4.03 log $TCID_{50}$ mL^{-1}) and 2.2 log (3.37 log $TCID_{50}$ mL^{-1}) $TCID_{50}$ mL^{-1}, respectively (Table 1). The D_{10} calculated when FCV-F9 was treated with CJ was 0.42 %-CJ.

3.2 Effect of γ-irradiation on FCV-F9 virus titer

γ-irradiation treatment also induced the diminution of virus titer of FCV-F9 (Fig. 1C). γ-irradiation also exhibited a negative linear regression on viral titer (Table 1). As the dose of γ-irradiation increased, a concomitant decrease in viral titer was observed. The initial viral titer was 5.37 log $TCID_{50}$ mL^{-1}, which was reduced to 5.08 (0.29 log reduction), 5.07 (0.30 log reduction), 4.57 (0.80 log reduction), 4.52 (0.85 log reduction), 3.95 (1.42 log reduction), 3.82 (1.55 log reduction), 3.82 (1.55 log reduction), 3.73 (1.64 log reduction), and 3.26 (2.11 log reduction) log $TCID_{50}$ mL^{-1} after γ-irradiation treatment at doses of 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0 and 3.0 kGy, respectively. The D_{10} calculated when FCV-F9 was treated with irradiation was 1.34 kGy.

Table 1. Recovery of viral titers after different treatments used alone (γ-irradiation, CJ or BS)

Treatment	Viral titer	Reduction	Treatment	Viral titer	Reduction
BS (%)	**(log $TCID_{50}$ mL^{-1})**	**(Log)**	**Irradiation (kGy)**	**(log $TCID_{50}$ mL^{-1})**	**(Log)**
0	5.49^{abc}	0	**0**	5.37^{abc}	0
0.005	5.32^{abc}	0.17	**0.25**	5.08^{abcdf}	0.29
0.01	5.32^{abc}	0.17	**0.50**	5.07^{abcd}	0.30
0.025	4.62^{bc}	0.87	**0.75**	4.57^{bcdefg}	0.80
0.05	3.66^{d}	1.83	**1.0**	4.52^{de}	0.85
0.10	3.45^{d}	2.04	**1.25**	3.95^{bdfgh}	1.42
CJ (%)	**(log $TCID_{50}$ mL^{-1})**	**(Log)**	**1.50**	3.82^{dfghi}	1.55
0	5.57^{ab}	0	**1.75**	3.82^{bdfgh}	1.55

0.10	5.49[ab]	0.08	**2.0**	3.73 [fghi]	1.64
0.25	5.45[ab]	0.12	**3.0**	3.26 [gi]	2.11
0.50	4.24 [abc]	1.33			
0.75	4.03 [bc]	1.54			
1.0	3.37 [bc]	2.20			

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

In Fig. 1A, the effects of various concentrations of BS on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -22.02 and a constant "b" of 5.34. The standard deviation of the regression is 4.29 and the coefficient of determination (R^2) is 0.87. In Fig. 1B, the effects of various concentrations of CJ on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -2.34 and a constant "b" of 5.71. The standard deviation of the regression is 0.27 and the R^2 is 0.95. In Fig. 1C, the effects of various doses of γ-irradiation on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.75 and a constant "b" of 5.21. The standard deviation of the regression is 0.08 and the R^2 is 0.92. In analyzing these results, the steepest slope is the one with the BS used alone, which implies that the treatment used alone that is the most efficient is BS because it reduces the viral titer the most strongly. BS is also the treatment showing the highest standard deviation, meaning that the values are more spread around their mean. All three of R^2 are really high, with values that are more than 0.87, meaning that the majority of the values fits the regression.

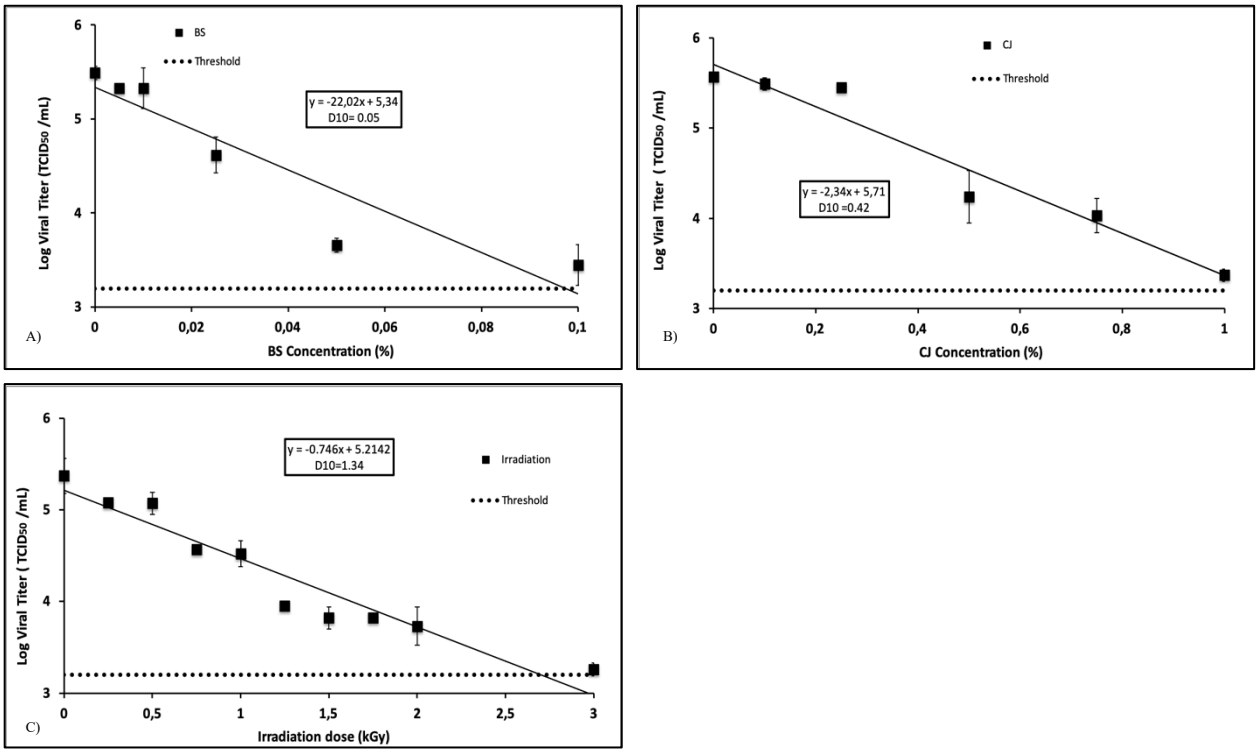

Fig. 1 (A) Viral titer reduction ($TCID_{50}$ mL^{-1}) of FCV-F9 after treatment with BS at different concentrations. (B) Viral titer reduction ($TCID_{50}$ mL^{-1}) of FCV-F9 after treatment with CJ at different concentrations. (C) Viral titer reduction ($TCID_{50}$ mL^{-1}) of FCV-F9 after treatment with γ-irradiation at different doses.

3.3 Effect of combined treatment of γ-irradiation and BS on virus titer

A combined effect of γ-irradiation doses in the range of 0-3 kGy and BS concentrations at BS-0.01%, BS-0.025% and BS-0.1% was also tested, and the results are presented in Fig. 2. The results showed a slope of treatment regression and a more accentuated slope was found when the lowest concentration of BS was used. As shown in Fig. 2A, the D_{10} values of the samples treated with BS-0.01% and the D_{10} of the control were 0.74 and 0.95 kGy, respectively, meaning a relative radiosensitivity (RS) of 1.28. When this previous BS concentration (BS-0.01%) was treated in combination with γ-irradiation doses of 0.25 to 1.50 kGy, the viral titers decreased from 5.36 log to 3.20 log $TCID_{50}$ mL^{-1}. The relative RS values are presented in table 4 for further comparison. The higher the concentration of BS was, lower the relative RS was, exhibiting an inverse relation between the concentration of BS and efficacy of combined treatment. Fig. 2B illustrated the D_{10} values of the combined treatment consisting of γ-irradiation with BS-0.025% curve in comparaison with the control curve, 1.75 kGy and 1.06 kGy, respectively, showing a relative radioresistance (RR) of 1.65. The viral titers decreased from 4.62 log to

3.20 $TCID_{50}$ mL^{-1}, when samples were treated with BS-0.025% in combination with a γ-irradiation dose range from 0 to 2.5 kGy. In presence of BS-0.1%, the D_{10} values found was 5.55 kGy as compared to 1.43 kGy for the control, showing a relative RR of 3.88 (Fig. 2C). When BS-0.1% was combined with γ-irradiation doses of 0 to 1.5 kGy, the viral titers decreased from 3.45 log to 3.20 $TCID_{50}$ mL^{-1}. The reductions in viral titers were not found to be significant when BS-0.1% was used in combination at these γ-irradiation doses (Table 2).

Table 2. Recovery of viral titers after different concentration of BS in combination with γ-irradiation

BS-0.01%	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**	**Control**	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**
Irradiation (kGy)			**Irradiation (kGy)**		
0	5.36^{a}	0	**0**	5.20	0
0.25	4.87^{b}	0.49	**0.25**	5.20	0
0.50	4.32^{c}	1.04	**0.50**	5.07	0.13
0.75	4.24^{c}	1.12	**0.75**	4.70	0.50
1.0	4.16^{c}	1.20	**1.0**	4.45	0.75
1.25	3.45^{d}	1.91	**1.25**	4.07	1.13
1.50	3.20^{d}	2.16	**1.50**	3.70	1.50
BS-0.025%	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**	**Control**	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**
Irradiation (kGy)			**Irradiation (kGy)**		
0	4.62^{abc}	0	**0**	5.57	0
0.50	4.16^{abcde}	0.46	**0.50**	5.20	0.37
1.0	3.62^{abcd}	1.0	**1.0**	4.57	1.0

1.5	3.37^{bcde}	1.25	**1.5**	3.95	1.62
2.0	3.24^{bde}	1.38	**2.0**	3.45	2.12
2.5	3.20^{abcde}	1.42	**2.5**	3.45	2.12

BS-0.1%	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**	**Control**	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**
Irradiation (kGy)			**Irradiation (kGy)**		
0	3.45^{a}	0	**0**	5.57	0
0.25	3.45^{a}	0	**0.25**	5.20	0.37
0.50	3.45^{a}	0	**0.50**	5.09	0.48
0.75	3.37^{a}	0.08	**0.75**	4.57	1.0
1.0	3.32^{a}	0.13	**1.0**	4.57	1.0
1.50	3.20^{a}	0.25	**1.50**	4.57	1.0

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

In Fig. 2A, the effects of BS-0.01% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -1.35 and a constant "b" of 5.24. The standard deviation of the regression is 0.13 and the R^2 is 0.95. In Fig. 2B, the effects of BS-0.025% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.57 and a constant "b" of 4.41. The standard deviation of the regression is 0.09 and the R^2 is 0.89. In Fig. 2C, the effects of BS-0.1% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.18 and a constant "b" of 3.49. The standard deviation of the regression is 0.03 and the R^2 is 0.91. According to our results, the steepest slope is the treatment with BS-0.01%, meaning that this treatment in combination with γ-irradiation is the most efficient for increasing the radiosensitization of the virus. All three standard deviation are really low (0.13,0.09 and 0.03 for BS-0.01%, BS-0.025% and BS-0.1%, respectively). The R^2 of all three treatments are above 0.89, meaning that the majority of the values fits the regression.

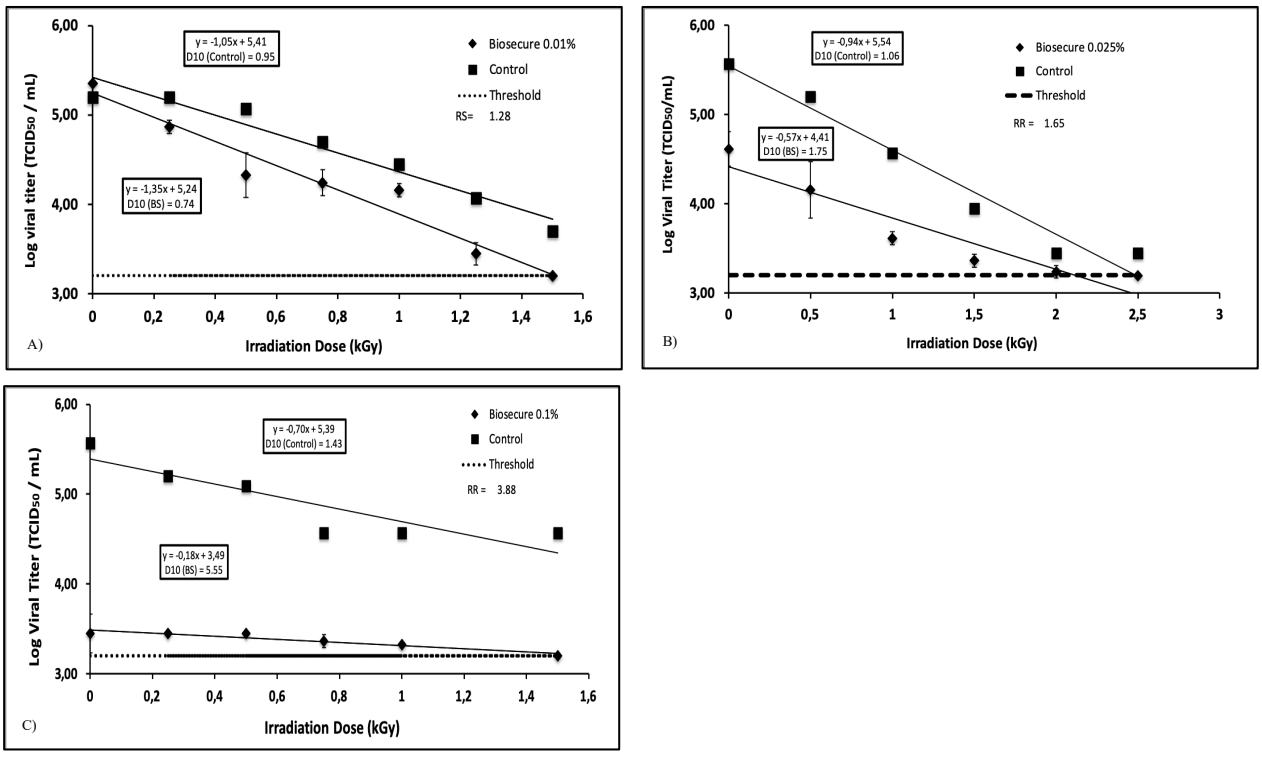

Fig. 2 Viral titer reduction ($TCID_{50}$ mL^{-1}) of FCV-F9 after treatment with γ-irradiation at various doses in combination with BS at (A) BS-0.01%, (B) BS-0.025%, and (C) BS-0.1%.

3.4 Effect of combined treatment of γ-irradiation and CJ on viral titer

The results of the effect of combined treatment of γ-irradiation at doses between 0 kGy and 3 kGy and CJ at A) CJ-0.1% and B) CJ-0.5% are shown in Fig. 3. The slope of treatment regression was found to be more accentuated when the lowest concentration of CJ was used. In Fig. 3A with CJ-0.1%, the D_{10} values of the treatment and of the control were 0.72 kGy and 1.08 kGy, respectively, exhibiting a relative RR of 1.50. The viral titer reduced from 5.75 log $TCID_{50}$ mL^{-1} to 3.74 log $TCID_{50}$ mL^{-1} when samples were treated with CJ-0.1% in combination with γ-irradiation at doses of 0 to 1.5 kGy (Table 3). The relative RS values are regrouped in Table 4. As the concentration of CJ increased, RS was found to decrease proportionally, which shows an inverse relation between the concentration of CJ and efficacy of combined treatment. In Fig. 3B, when the virus was treated with CJ-0.5%, the D_{10} values of the treatment and the control were 1.52 kGy and 1.16 kGy respectively, showing a relative RR of 1.31. The viral titer reduced from 4.24 log $TCID_{50}$ mL^{-1} to 3.20 log $TCID_{50}$ mL^{-1} for CJ-0.1% in combination with γ-irradiation doses.

Table 3. Recovery of viral titers after different concentration of CJ in combination with γ-irradiation

CJ-0.10%	Viral titer (log $TCID_{50}$ mL^{-1})	Reduction (log)	Control	Viral titer (log $TCID_{50}$ mL^{-1})	Reduction (log)
Irradiation (kGy)			**Irradiation (kGy)**		
0	5.75^{a}	0	**0**	5.20	0
0.25	5.57^{a}	0.18	**0.25**	5.20	0
0.50	4.99^{b}	0.76	**0.50**	5.20	0
0.75	4.66^{bc}	1.09	**0.75**	4.82	0.38
1.0	4.32^{cd}	1.43	**1.0**	4.57	0.63
1.25	4.07^{cd}	1.68	**1.25**	4.32	0.88
1.50	3.74^{d}	2.01	**1.50**	3.82	1.38
CJ-0.50%	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**	**Control**	**Viral titer (log $TCID_{50}$ mL^{-1})**	**Reduction (log)**
Irradiation (kGy)			**Irradiation (kGy)**		
0	4.24^{abc}	0	**0**	5.20	0
0.25	4.32^{ab}	-0.08	**0.25**	5.07	0.13
0.50	4.32^{ab}	-0.08	**0.50**	4.95	0.25
0.75	4.20^{ab}	0.04	**0.75**	4.57	0.63
1.25	3.57^{abc}	0.67	**1.25**	3.95	1.25
1.50	3.28^{ac}	0.96	**1.50**	3.82	1.38
2.0	3.20^{abc}	1.04	**2.0**	3.70	1.50

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

In Fig. 3A, the effects of CJ-0.1% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -1.39 and a constant "b" of 5.77. The standard deviation of the regression is 0.07 and the R^2 is 0.99. In Fig. 3B, the effects of CJ-0.5% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.66 and a constant "b" of 4.47. The standard deviation of the regression is 0.11 and the R^2 is 0.88. According to our results, the steepest slope is the treatment with CJ-0.1%, meaning that this treatment in combination with γ-irradiation is the most efficient for increasing the radiosensitization of the virus. Both standard deviations are really low,

meaning that the values are not much spread around the mean. The R^2 of both treatments are above 0.88, meaning that the majority of the values fits the regression.

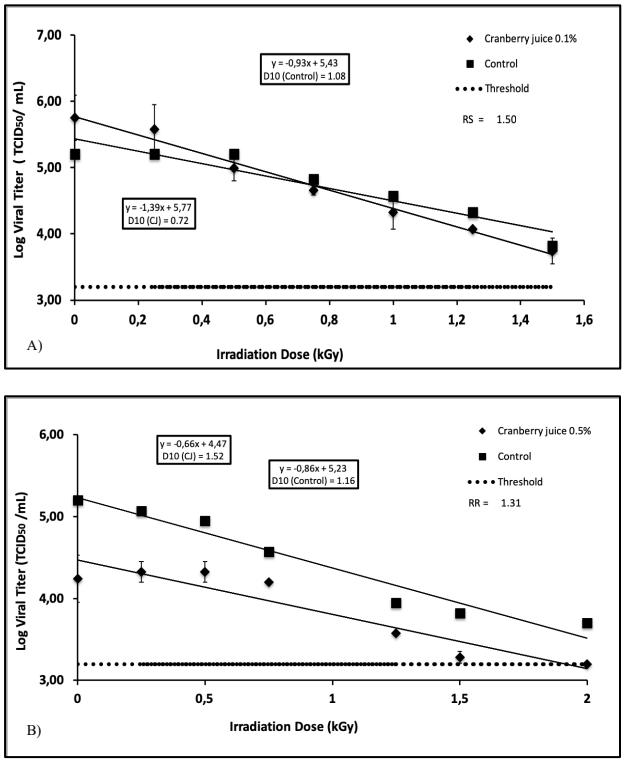

Fig. 3 Viral titer reduction ($TCID_{50}$ mL^{-1}) of FCV-F9 after treatment with γ-irradiation at various doses in combination with CJ at (A) CJ-0.1% and (B) CJ-0.5%.

Table 4. Relative Radiosensitivity or Radioresistance (D_{10} control/D_{10} treatment) of FCV-F9 after treatment with BS and CJ

Treatment	D_{10} treatment (kGy)	Relative RS or RR
BS (%)		
0.01 (C)	0.95	RS 1.00
0.01 (BS)	0.74	RS 1.28
0.025 (C)	1.06	RS 1.00
0.025 (BS)	1.75	RR 1.65
0.10 (C)	1.43	RS 1.00
0.10 (BS)	5.55	RR 3.88
CJ (%)		
0.1 (C)	1.08	RS 1.00

0.1 (CJ)	0.72	RS 1.50
0.5 (C)	1.16	RS 1.00
0.5 (CJ)	1.52	RR 1.31

RR: Radioresistance. RS: Radiosensitization. BS: Biosecur in combination with γ-irradiation. CJ: Cranberry juice in combination with γ-irradiation. C: Control with irradiation only.

4 Discussion

One of the important aims of the study of antimicrobial agents derived from plants and natural extracts is to find natural products for preventing viral infections. Humans have been consuming a variety of plant extracts as traditional medicine, natural therapies, and phytochemicals for centuries. The advantages of plant extracts in the prevention and treatment of infectious diseases include cost-effectiveness and safety, as compared to synthetic chemical antimicrobials and disinfectants. However, studies on the effectiveness of plant extracts against NoV are limited and focused on specific phytochemical compounds such as tannins and flavonoids from berries and other fruits (Zhang *et al.,* 2012; D'Souza, 2014). In the present study, the antiviral effect of CJ and BS alone or in combination with γ-irradiation in FCV-F9 using CRFK cells was investigated. An FCV-F9 model was used due to the difficulty of cultivating human NoV in the laboratory. It is well documented that cranberries and BS possess antibacterial and antiviral properties (Su *et al.,* 2010; Methot *et al.,* 2017). Overall, the antiviral activity of CJ and BS was found to be concentration dependent and increased concentrations were more effective against virus, which is similar to previously reported results for organic acids (Su *et al.,* 2010; Joshi *et al.,* 2019).

In the present study, a reduction of 2.04 log $TCID_{50}$ mL^{-1} of FCV-F9 viral titer was observed at a concentration of BS-0.1%, that might be due to the presence of citric acid, ascorbic acids and lactic organic acids (Table 1). The D_{10} value calculated from the slope was 0.05% -BS (Fig. 1A). It has been shown that vitamin C (ascorbic acid) possesses antiviral activities against RNA and DNA viruses (Jariwalla and Harakeh, 1996). Ascorbate can inactivate a wide spectrum of viruses *in-vitro* such as herpes simplex, vaccinia, foot-and-mouth disease, rabies, tobacco mosaic, and bacterial viruses (Jungeblut, 1935). Citric acid at pH 2.5 and at an active concentration of 2.5 % is also efficient against FCV at a short-contact time (1 min) (Whitehead and McCue, 2010). Lactic acid is very efficient against FCV virus (Lee *et al.,* 2012). In fact, during the fermentation of Dongchimi, lactic acid bacteria (LAB) and acidity increases (Lee *et al.,* 2012). It was also showed that the LAB might be responsible for the denaturation of capsid proteins, which can reduce attachment from the virus to the host (Aboubakr *et al.,* 2014). Macinga *et al.* (2008) tested the effect of a new ethanol-based sanitizer containing a synergistic blend of

polyquaternium polymer (PQ-37) and organic acid (citric acid). These results showed a reduction greater than 3 $\log_{10}$ PFU mL^{-1} after a 30 seconds exposure of MNV-1 and FCV-F9. PQ-37 and citric acid together maximizes the activity against mammalian non-enveloped viruses because of the high acidity.

Also, a reduction of 2.20 log $TCID_{50}$ mL^{-1} of initial FCV-F9 viral titer was achieved when the virus was treated with CJ-1% (Table 1). The D_{10} value calculated from the slope is 0.42 %-CJ (Fig. 1B). Su *et al.* (2010) tested the effects of CJ on FCV-F9 at exposure times ranging from 0 to 60 min at room temperature. A viral titer reduction of 5 $\log_{10}$ PFU mL^{-1} after 30 min of treatment with CJ (pH 2.7 and 7.0) was observed. By comparing these results with this present study, it is shown that the authors showed a higher inactivation of FCV using a different viral titration method where the cells are overlaid with DMEM and agarose. It was proposed that the CP inhibit viral penetration to the cells (Lipson *et al.,* 2010). The mechanisms of action of organic acids or polyphenols against NoV surrogates are not yet understood but some hypothesis proposed that it could be attributed to the alteration of the viral capsid and also the nucleic acid (Cao, 2013). NoV is a non-enveloped virus and it was also suggested that due to the acidic nature of organic acids, they denature the proteins of viral capsid, resulting in the inactivation of virus (Cao, 2013). Bioactive compounds such as gallic acid, epicatechin, tannins, and proanthocyanidins found in berry fruits are known to be effective antimicrobial agents (Bahadoran *et al.,* 2013; Khurana *et al.,* 2013; Li *et al.,* 2013). Polyphenols from fruits such as pomegranate, blueberry, and other plant extracts have been shown to possess antiviral properties including against NoV surrogates (Su *et al.,* 2010; Oh *et al.,* 2012). However, the phenolic composition and amounts vary between the various fruit types, where cranberries mainly contain A-type proanthocyanidins. Also, Seo and Choi (2017) showed that pre-treatment with fisetin (50 and 100 μM), EGCG (100 μM), quercetin (100 μM), daidzein (50 μM), and ECG (150 μM), five flavonoids, significantly reduced MNV titer by 50.00 ± 7.14 to 60.67 ± 9.26 %. Authors attributed the antiviral effects of flavonoids to upregulation of expression of antiviral cytokines (IFN-α, IFN-λ, and TNF-α) and interferon-stimulating genes (Mx and ZAP).

γ-rays can interact with nucleic acids by the production of free radicals, oxygen and hydroxyl radicals (Park and Ha, 2017). Foodborne viruses can be eliminated by irradiation at doses ranging from 2 to 8 kGy (Monk *et al.,* 1995). In this present study, the highest viral titer reduction of 2.11 log $TCID_{50}$ mL^{-1} was achieved after treatment with a 3.0 kGy γ-irradiation dose (Table 1). The D_{10} value calculated from the slope was 1.34 kGy (Fig. 1C).

γ-irradiation has been shown to inactivate MNV-1 by altering virion geometry, by damaging the capsid protein and genomic RNA and might also disrupt the primary, secondary, tertiary, and quaternary structures of viral proteins (Feng *et al.,* 2011). Kang *et al.* (2016) showed that γ-irradiation at a dose > 7 kGy was effective in reducing MNV-1 titers by more than 1 $\log_{10}$ PFU mL^{-1} in half-dried seafood products during the cold storage at 10 °C. Also, it was shown that ultrafast laser irradiation at 60 femtosecond (fs)

pulses at a repetition rate of 80 Megahertz (MHz) induces partial unfolding of viral proteins of the capsid by disrupting hydrogen bonds and/or hydrophobic interactions, leading to aggregation of closely associated viral proteins and inactivation of the virus (Tsen *et al.,* 2012). A reduction of more than 5 log PFU mL^{-1} of murine cytomegalovirus (MCMV) was observed and no significant change in virion structure of capsid and membranes was observed.

The combinations of CJ or BS with γ-irradiation showed synergy against FCV-F9. The lower the concentration of BS (Fig. 2) or CJ (Fig. 3) was, higher the relative RS value was, exhibiting an inverse relation between the concentration of BS or CJ and efficacy of combined treatment. Relative RS can be observed after combining quercetin to radiotherapy against DLD-1 human colon tumor fragment. Quercetin is one of the main components in flavonoids which have been characterized to have the functions of enhancing immune function, anti-oxidation, anti-viral, anti-inflammatory and anti-tumoral. The *in-vitro* relative RS of the tumor after X-rays irradiation with quercetin in DLD1 cells line was 1.87 kGy (Lin *et al.,* 2012).

It is possible that the relative RR observed with higher concentrations of natural extracts is explained by presence of polyphenols in BS and CJ that both act as antioxidants. Assemand *et al.* (2003) have demonstrated that the irradiation of caeruloplasmin, a protein with good antioxidant properties, did not affect its structure and biological properties when sterilized by γ-irradiation in presence of tyrosine. Tyrosine can act as an antioxidant by forming bi-tyrosine by oxidation, which could protect the caeruloplasmin protein. The viral capsid is composed of proteins and it is possible that the proteins may have been protected by the high quantities of antioxidants in the natural extracts. Relative RR may also be caused by the chemical components of the substrate medium. For example, the presence of alcohols, carbohydrates and proteins in food could increase the tolerance of microorganism (Lawrence and Block, 1968). Scavengers of reactive oxygen species (ROS) may react with the free radicals produced by γ-irradiation and could protect the radiation damage to the virus-infected cell normally attacked by the free radicals (Thornley, 1963). The presence of antioxidants in meat such as carnosine can decrease the antimicrobial efficacy of irradiation because they neutralize the free radicals before they attacked the DNA of the microorganism (Sommers and Fan, 2002). It has also been showed that high quantities of protein in the medium when treated with γ-irradiation of FCV-F9 virus induced RR of virus (de Roda Husman *et al.,* 2004). Yeasts are also radioresistant because of their production of organic acids like lactic acid, acetic acid and alcohols that act as scavengers to protect yeasts from free radicals produced by γ-irradiation (Aquino, 2011). Other studies showed the effects of hydrostatic pressure, ultraviolet (UV) light, and γ-irradiation on FCV-F9 as a human NoV surrogate and around 3 log reductions were reported for all the three processing methods (Husman *et al.,* 2004; Sharma *et al.,* 2008). Jung *et al.* (2009) revealed that the presence of organic substances (protein, sugar, and fat) and irradiation temperature were the important

factors for the inactivation of viruses by γ-irradiation, but pH and salt had a little effect on the effectiveness of γ-irradiation. It was shown that cold temperature (-20 °C) increased RR of poliovirus, a surrogate of NoV. Also, the efficiency of γ-irradiation is inhibited by organic substances such as protein, sugar and fat as they act as scavengers (Jung *et al.,* 2009).

The present study helps to provide a better understanding on the effective optimum concentration levels of CJ and BS in combination with γ-irradiation to inactivate the target viruses for use as viral elimination. At respective concentration of BS-0.01% and CJ-0.1%, a relative RS of more than 1.28 and 1.50 was respectively observed (Table 4). Natural extracts in combination with γ-irradiation has permitted to reduce the needed irradiation dose to eliminate 6 log $TCID_{50}$ mL^{-1} of virus from 8 kGy to 5.33 kGy for CJ and from 8 kGy to 6.25 kGy for BS. The reported results provide useful data for designing new chemical and physical treatments to reduce or eliminate viral contamination.

The present study demonstrates the effect of combined cold processes like the addition of natural extracts and γ-irradiation treatment on the growth of FCV-F9. It was shown that BS used alone is more efficient than CJ used alone against FCV-F9 virus *in-vitro*. Furthermore, the viral titer reduction was highest with the higher concentrations of CJ (CJ-1%) or BS (BS-0.10%) used alone. However, when CJ and BS were used in combination with γ-irradiation, the maximum viral titer reduction was obtained with the lowest concentrations of CJ (CJ-0.1%) and BS (BS-0.01%) and showed a viral radiosensitization of 1.50 and 1.28, respectively. This finding indicates that CJ and BS could be used as antiviral agents alone or in combination with γ-irradiation. The results show promise for the utilization of CJ and BS in the prevention of NoV outbreaks. Potential applications include use for viral reduction in ready-to-eat foods, such as dressings for deli meats, salads, or for use in juices or juice-cocktail combinations. CJ and BS could also be used in Hurdle technology along with other processing technologies such as high pressure or low heat to maintain low processing cost. However, further studies need to be undertaken with food models, as well as sensory evaluations on treated products, before any claims can be made or to be used or recommended for prevention and control. Future work is currently being undertaken *in-situ* using CJ, BS in combination with γ-irradiation on lettuce as a food model to determine antiviral effects.

5 Acknowledgments

This research was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). The authors wish to thank Atoka Cranberries Inc. (Manseau, QC, Canada) for providing CJ. The authors are also grateful to Nordion International for the irradiation operations. Finally, the authors are thankful for the help from Dr. Suresh Pillai (Director of the National Center for Electron Beam Research at Texas A&M University) for the protocol development of virus

titration. The assistance of the International Atomic Energy Agency (IAEA) is also acknowledged. This research is a part of the CRP on radiation inactivation of biohazards (code F23033). A.G was a fellowship recipient of NSERC and the Foundation Armand-Frappier.

Conflict of interest

No conflict of interest declared.

6 References

1. Aboubakr, H. A., El-Banna, A. A., Youssef, M. M., Al-Sohaimy, S. A., and Goyal, S. M. (2014). Antiviral effects of Lactococcus lactis on feline calicivirus, a human norovirus surrogate. *Food and Environmental Virol*ogy, 6 : 282-289.
2. Aquino, S. (2011). Gamma radiation against toxigenic fungi in food, medicinal and aromatic herbs. *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, Mendez-Vilas, A. (Ed.)*, 272-281.
3. Assemand, E., Lacroix, M., and Mateescu, M. A. (2003). Caeruloplasmin sterilized by γ-irradiation in the presence of l-tyrosine maintains structural and catalytic characteristics. *Biotechnology and Applied Biochemistry*, 38: 143-149.
4. Bahadoran, Z., Mirmiran, P., and Azizi, F. (2013). Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *Journal of Diabetes and Metabolic Disorders,* 12: 43.
5. Bidawid, S., Farber, J. M., and Sattar, S. A. (2000). Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. *International Journal of Food Microbiology*, 57 : 91-97.
6. Brié, A., Razafimahefa, R., Loutreul, J., Robert, A., Gantzer, C., Boudaud, N., and Bertrand, I. (2017). The effect of heat and free chlorine treatments on the surface properties of murine norovirus. *Food and Environmental Virology*, 9 :149-158.
7. Buckow, R., Isbarn, S., Knorr, D., Heinz, V., and Lehmacher, A. (2008). Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Applied and Environmental Microbiology*, 74 :1030-1038.
8. Caillet, S., Côté, J., Doyon, G., Sylvain, J. F., and Lacroix, M. (2011). Antioxidant and antiradical properties of cranberry juice and extracts. *Food Research International*, 44: 1408-1413.
9. Cannon, J. L., Papafragkou, E., Park, G. W., Osborne, J., Jaykus, L. A., and Vinjé, J. (2006). Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *Journal of Food Protection*, 69 : 2761-2765.

10. Cao, C. (2013). Chemical Disinfectants for Inactivation of Human Norovirus Surrogates.
11. Cheesbrough, J. S., Green, J., Gallimore, C. I., Wright, P. A., and Brown, D. W. G. (2000). Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiology & Infection*, 125 : 93-98.
12. Ciesek, S., von Hahn, T., Colpitts, C. C., Schang, L. M., Friesland, M., Steinmann, J., ... and Pietschmann, T. (2011). The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry. *Hepatology*, 54: 1947-1955.
13. Cliver, D. O. (1997). Virus transmission via food. *World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales*, 50 :90-101.
14. Coppola, E. D., Conrad, E. C., and Cotter, R. (1978). High pressure liquid chromatographic determination of major organic acids in cranberry juice. *Journal Association of Official Analatylical Chemists*, 61 : 1490-1492.
15. Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J.-F., and Lacroix, M. (2011). Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22: 1413-1418.
16. Côté, J., Caillet, S., Doyon, G., Sylvain, J. F., and Lacroix, M. (2010). Bioactive compounds in cranberries and their biological properties. *Critical Reviews in Food Science and Nutrition*, 50: 666-679.
17. de Roda Husman, A. M., Bijkerk, P., Lodder, W., Van Den Berg, H., Pribil, W., Cabaj, A., ... and Duizer, E. (2004). Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (gamma) radiation. *Applied and Environmental Microbiology*, 70 :5089-5093.
18. Diehl, J. F. (1992). Food irradiation: is it an alternative to chemical preservatives? *Food Additives & Contaminants*, 9 : 409-416.
19. D'Souza, D.H. (2014). Phytocompounds for the control of human enteric viruses. *Current Opinions in Virology*, 4: 44–49.
20. Fankhauser, R. L., Monroe, S. S., Noel, J. S., Humphrey, C. D., Bresee, J. S., Parashar, U. D., ... and Glass, R. I. (2002). Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. *Journal of Infectious Diseases*, 186 : 1-7.
21. Feng, K., Divers, E., Ma, Y., and Li, J. (2011). Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*, 77: 3507-3517.
22. Gibson, K. E., and Schwab, K. J. (2011). Thermal inactivation of human norovirus surrogates. *Food and Environmental Virology*, 3 : 74.

23. Gilling, D. H., Kitajima, M., Torrey, J. R., and Bright, K. R. (2014). Mechanisms of antiviral action of plant antimicrobials against murine norovirus. *Applied and Environmental Microbiology*, 80 : 4898-4910.
24. Hirneisen, K. A., Markland, S. M., and Kniel, K. E. (2011). Ozone inactivation of norovirus surrogates on fresh produce. *Journal of Food Protection*, 74: 836-839.
25. Husman, A.M.D., Bijkerk, P., Lodder, W., van den Berg, H., Pribil, W., Cabaj, A., Gehringer, P., Sommer, R., and Duizer, E. (2004). Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (Gamma) radiation. *Applied Environmental Microbiology,* 70: 5089-5093.
26. Jariwalla, R. J., and Harakeh, S. (1996). Antiviral and immunomodulatory activities of ascorbic acid. *Subcellular Biochemistry*, 215-231.
27. Joshi, S.S., Howell, A.B., and D'Souza, D.H. (2019). Antiviral effects of blueberry proanthocyanidins against Aichi virus. *Food Microbiol*ogy, 82: 202-208.
28. Jung, P. M., Park, J. S., Park, J. G., Park, J. N., Han, I. J., Song, B. S., ... and Chung, Y. J. (2009). Radiation sensitivity of poliovirus, a model for norovirus, inoculated in oyster (Crassostrea gigas) and culture broth under different conditions. *Radiation Physics and Chemistry*, 78 : 597-599.
29. Jungeblut, C. W. (1935). Inactivation of poliomyelitis virus in vitro by crystalline vitamin C (ascorbic acid). *The Journal of Experimental Medicine*, 62 : 517-521.
30. Kang, S., Park, S. Y., and Ha, S. D. (2016). Application of gamma irradiation for the reduction of norovirus in traditional Korean half-dried seafood products during storage. *LWT Food Science and Technology*, 65 : 739-745.
31. Khurana, S., Venkataraman, K., Hollingsworth, A., Piche, M., and Tai, T. C. (2013). Polyphenols: benefits to the cardiovascular system in health and in aging. *Nutrients*, 5: 3779–3827.
32. Kim, H.W., Chung, D.H., Kim, S.A., and Rhee, M.S. (2019). Synergistic cranberry juice combinations with natural-borne antimicrobials for the eradication of uropathogenic Escherichia coli biofilm within a short time. *Letters in Applied Microbiology,* 68: 321-328.
33. Kuusi, M., Nuorti, J. P., Maunula, L., Minh, N. T., Ratia, M., Karlsson, J., and Von Bonsdorff, C. H. (2002). A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. *Epidemiology and. Infection*, 129 : 133-138.
34. Lacroix, M., and Ouattara, B. (2000). Combined industrial processes with irradiation to assure innocuity and preservation of food products—a review. *Food Research International*, 33 : 719-724.

35. Lawrence, C. A., Block, S. S. (1968). *Disinfection, sterilization, and preservation: [edited by] Carl A. Lawrence [and] Seymour S. Block.* Philadelphia: Lea and Febiger.
36. Lee, M. H., Yoo, S. H., Ha, S. D., and Choi, C. (2012). Inactivation of feline calicivirus and murine norovirus during Dongchimi fermentation. *Food Microbiology*, 31 : 210-214.
37. Li, D., Baert, L., and Uyttendaele, M. (2013). Inactivation of food-borne viruses using natural biochemical substances. *Food Microbiology,* 35*:* 1–9.
38. Lin, C., Yu, Y., Zhao, H. G., Yang, A., Yan, H., and Cui, Y. (2012). Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiotherapy and Oncology*, 104 : 395-400.
39. Lipson, S. M., Gordon, R. E., Kathikeyan, L., Singh, M., Burdowski, A., Roy, M., and Stotzky, G. (2010). Cranberry and Grape Juice Drinks Affect Infectivity, Integrity, and Pathology of Enteric Viruses in an Animal Model. In *ACS symposium series* (Vol. 1035, pp. 177-195). Oxford University Press.
40. Liu, Y., Gallardo-Moreno, A. M., Pinzon-Arango, P. A., Reynolds, Y., Rodriguez, G., and Camesano, T. A. (2008). Cranberry changes the physicochemical surface properties of E. coli and adhesion with uroepithelial cells. *Colloids and Surfaces B : Biointerfaces*, 65 : 35-42.
41. Lopman, B., Gastanaduy, P., Park, G. W., Hall, A. J., Parashar, U. D., and Vinjé, J. (2012). Environmental transmission of norovirus gastroenteritis. *Current Opinions in Virology*, 2: 96-102.
42. Macinga, D. R., Sattar, S. A., Jaykus, L. A., and Arbogast, J. W. (2008). Improved inactivation of nonenveloped enteric viruses and their surrogates by a novel alcohol-based hand sanitizer. *Applied and Environmental Microbiology*, 74 : 5047-5052.
43. Maki, K.C., Kaspar, K.L., Khoo, C., Derrig, L.H., Schild, A.L., and Gupta, K. (2016) Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. The *American Journal of Clinical Nutrition*, 103: 1434-1442.
44. Maki, K.C., Nieman, K.M., Schild, A.L., Kaspar, K.L., and Khoo, C. (2018). The effect of cranberry juice consumption on the recurrence of urinary tract infection: relationship to baseline risk factors. The *Journal of American College of Nutrition*, 37: 121-126.
45. Mattison, K., Karthikeyan, K., Abebe, M., Malik, N., Sattar, S. A., Farber, J. M., and Bidawid, S. (2007). Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *Journal of Food Protection*, 70 : 500-503.
46. Methot, Y., Essler-Gravesen, D., and Talbot, J. (2017). Citrus-based antimicrobial composition. US Patent 2017/0079281 A1.

47. Monk, J. D., Beuchat, L. R., and Doyle, M. P. (1995). Irradiation inactivation of food-borne microorganisms. *Journal of Food Protection*, 58 : 197-208.
48. Oh, M., Bae, S. Y., Lee, J. H., Cho, K. J., Kim, K. H., and Chung, M. S. (2012). Antiviral effects of black raspberry (*Rubus coreanus*) juice on foodborne viral surrogates. *Foodborne Pathogens and Disease,* 9: 915–921.
49. Park, S. Y., and Ha, S. D. (2017). Application of gamma radiation for the reduction of norovirus and the quality stability in optimally ripened cabbage kimchi. *Food Research International*, 100 : 277-281.
50. Pinzón-Arango, P. A., Liu, Y., and Camesano, T. A. (2009). Role of cranberry on bacterial adhesion forces and implications for Escherichia coli–uroepithelial cell attachment. *Journal of Medicinal Food*, 12 : 259-270.
51. Scharff, R. L. (2010). Health-related costs from foodborne illness in the United States. Produce Safety Project, Georgetown University, Washington, DC.
52. Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection*, 75: 123-131.
53. Seo, D. J., and Choi, C. (2017). Inhibitory mechanism of five natural flavonoids against murine norovirus. *Phytomedicine*, 30 : 59-66.
54. Severino, R., Ferrari, G., Vu, K. D., Donsì, F., Salmieri, S., and Lacroix, M. (2015). Antimicrobial effects of modified chitosan-based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against Escherichia coli O157: H7 and Salmonella Typhimurium on green beans. *Food Control*, 50 : 215-222.
55. Sharma, M., Shearer, A.E.H., Hoover, D.G., Liu, M.N., Solomon, M.B., Kniel, K.E. (2008). Comparison of hydrostatic and hydrodynamic pressure to inactivate foodborne viruses. *Innovative Food Science and Emerging Technologies*, 9: 418-422.
56. Sommers, C. H., and Fan, X. (2002). Antioxidant power, lipid oxidation, color, and viability of Listeria monocytogenes in beef bologna treated with gamma radiation and containing various levels of glucose. *Journal of Food Protection*, 65 : 1750-1755.
57. Su, X., Howell, A. B., and D'Souza, D. H. (2010). Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. *Food Microbiology*, 27: 985-991.
58. Suzuki, M., Sasaki, K., Yoshizaki, F., Fujisawa, M., Oguchi, K., and Cyong, J. C. (2005). Anti-hepatitis C virus effect of citrus unshiu peel and its active ingredient nobiletin. *The American Journal of Chinese Medicine,* 33: 87-94.

59. Thornley, M. J. (1963). Radiation resistance among bacteria. *Journal of Applied Bacteriology (England)*, *26*.
60. Tsen, S. W. D., Chapa, T., Beatty, W., Tsen, K. T., Yu, D., and Achilefu, S. (2012). Inactivation of enveloped virus by laser-driven protein aggregation. *Journal of Biomedical Optics*, 17: 128002.
61. Whitehead, K., and McCue, K. A. (2010). Virucidal efficacy of disinfectant actives against feline calicivirus, a surrogate for norovirus, in a short contact time. *The American Journal of Infection Control,* 38: 26-30.
62. Widdowson, M. A., Cramer, E. H., Hadley, L., Bresee, J. S., Beard, R. S., Bulens, S. N., ... and Mintz, E. (2004). Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United States, 2002. *Journal of Infectious Diseases*, 190 : 27-36.
63. Zhang, X. F., Dai, Y. C., Zhong, W., Tan, M., Lv, Z. P., Zhou, Y. C., and Jiang, X. (2012). Tannic acid inhibited norovirus binding to HBGA receptors, a study of 50 Chinese medicinal herbs. *Bioorganic & Medicinal Chemistry*, 20 :1616-1623.
64. Zhao, S., Liu, H., and Gu, L. (2018). American cranberries and health benefits–an evolving story of 25 years. *Journal of the Science of Food Agriculture,* 2018.

Le chapitre 3 abordait l'élimination virale du FCV-F9 de façon *in-vitro* et s'intitule : « Radiosensitivity increase of FCV-F9 virus using combined treatments with natural antimicrobials and γ-irradiation ». L'objectif de ce papier était de sélectionner des concentrations optimales de BS et de CJ ainsi que la dose d'irradiation optimale contre le FCV-F9 en mesurant leur D_{10} respective. L'hypothèse de recherche était que les combinaisons des extraits naturels avec l'irradiation-γ permettrait de diminuer la dose d'irradiation requise pour éliminer le virus FCV-F9 *in-vitro*. Nous avons donc sélectionné le jus de canneberge afin de poursuivre nos recherches de traitements efficaces contre le FCV-F9 à la surface de laitue iceberg. Nous avons ensuite testé dans le chapitre 4 intitulé : « Radiosensitivity of FCV-F9 on Iceberg lettuce surface after combined treatments with γ-irradiation » l'ozonation, l'utilisation de jus de canneberge et l'irradiation-γ de façon seule ou en combinaison afin de trouver la combinaison optimale de traitement. Le CJ-0.25%, l'O_3 à 5 ppm durant 7.5 minutes et l'irradiation à 1.5 kGy étaient les conditions les plus optimales de traitements.

Chapitre 4: Radiosensitivity of FCV-F9 on Iceberg lettuce surface after combined treatments with γ-irradiation

Alexandra Gobeil, Shiv Shankar, Monique Lacroix*

INRS, Armand-Frappier, Health and Biotechnology Centre, Research Laboratories in Sciences, Applied to Food, Canadian Irradiation Centre, 531, Boulevard des Prairies, Laval, Québec, Canada, H7V 1B7.

* Corresponding author: Professor Monique Lacroix.
Tel.: 450-687-5010 #4489.
Fax: 450-686-5501; E-mail address: monique.lacroix@iaf.inrs.ca

Contribution des auteurs

Alexandra Gobeil a réalisé les manipulations et la rédaction de l'article.
Shiv Shankar a participé à la correction de l'article.
Monique Lacroix: Directrice de recherche, a participé à la planification des expériences et aux discussions des résultats et a aussi procédé à la correction de l'article.

L'article est soumis dans le Journal of Food Protection (JFP-19-464) le 25-09-2019.

Abstract

The surface of iceberg lettuce (*Lactuca sativa* L.) is favorable to the survival of pathogens like bacteria, parasites, and viruses such as Norovirus. The present study investigates the antiviral properties of cranberry juice (CJ), ozone (O_3) and gamma irradiation (γ-irradiation) alone or in combination against FCV-F9 virus present on the surface of iceberg lettuce. The lettuce leaves were inoculated with viral suspensions at titers of ~ 6 log $TCID_{50}$/mL and treated with CJ, O_3, and γ-irradiation alone and in combination during storage at 4°C. The D_{10} values of 1.21 kGy, 2.23 %-CJ, and 14.93 ppm-O_3 were obtained when samples were treated with various doses of irradiation, CJ, and O_3, respectively. Relative radiosensitization (RS) of FCV-F9 virus on lettuce was 1.20, 1.50, 1.09, and 1.00 after combined treatments with CJ-0.1%, CJ-0.25%, CJ-0.50%, and CJ-1.50%, respectively. Optimum doses of treatments were found to be 5 ppm for 7.5 min for O_3, CJ-0.25%, and γ-irradiation at 1.5 kGy when each treatment was executed alone. The combination of the three treatments showed the highest reduction of 2.15 log $TCID_{50}$/mL from initial inoculated viral load on lettuce (~ 7 log $TCID_{50}$/mL) during 10 days of storage at 4 °C. Also, the antibacterial properties of treatments and physico-chemical quality of lettuce were investigated during 13 days of storage at 4 °C. The treatment of lettuce alone with γ-irradiation (1.5 kGy) reduced by 3 log CFU/g the total flora, however, the combination of CJ (0.25%) with irradiation (1.5 kGy) reduced ~ 5 log CFU/g after 13 days of storage at 4 °C. The texture and color of the treated lettuce with the combined treatment changed slightly during storage, while chlorophyll increased by 3.81 μg/mL after 10 days of storage at 4 °C. Significant differences in taste and color were observed in lettuce without treatments after 5 days of storage, whereas no difference was observed after treatments with CJ-0.25% or with combined treatments.

Keywords: Human Norovirus (NoV), Feline calicivirus (FCV), Lettuce, Organic acids, Irradiation, Ozone.

Highlights

- Combination of γ-irradiation, CJ and O_3 is more efficient against FCV than alone treatment.
- Irradiation alone and combined treatments exhibited potent antibacterial activity.
- Combined treatment did not change the physico chemical and sensorial properties of lettuce.
- Chlorophyll pigment increased in iceberg lettuce after combination of treatments.

1 INTRODUCTION

Leafy green vegetables like iceberg lettuce (*Lactuca sativa* L.) are known for transmission of human pathogens such as bacteria, viruses, and parasites and for causing major foodborne diseases world-wide (Aw *et al.,* 2016; Mescle *et al.,* 1988). Human Noroviruses (NoV) are the cause of 50 % of foodborne disease outbreaks in the United States (Widdowson *et al.,* 2004) and cause 26 % of hospitalizations and 11 % of all deaths linked to foodborne diseases (Kniel *et al.,* 2017). NoV are transported by fecal-oral route and can occur before and after harvest by cross-contamination (Kniel *et al.,* 2017; Leuenberger *et al.,* 2007; Su and D'Souza, 2013).

Since there is no actual cellular model for NoV, MNV-1 and FCV-F9 surrogates are used because of their same size and shape of NoV. CRFK and RAW264.7 cell lines were used to propagate FCV-F9 and MNV-1 viruses, respectively (Su and D'Souza, 2013). Since NoV is inactivated by heat treatment during cooking at more than 145 °F (CDC, 2019; Kniel *et al.,* 2017), the use of cold pasteurisation in combined treatments could be an alternative method to assure food safety. For example, ozonation (Elvis and Ekta, 2011; Hirneisen *et al.,* 2011), fermentation (Lee *et al.,* 2012), γ-irradiation (Feng *et al.,* 2011), and edible coatings using natural antimicrobials like fruit extracts (Su and D'Souza, 2011) could be used.

The use of combined treatments could be more effective by reducing the time of the treatment or the treatment level (Yuk *et al.,* 2006). The maximum irradiation dose permitted for lettuce is between 1-4 kGy depending of the country (Adams *et al.,* 2016, Fan *et al.,* 2012B) but the technology is not yet accepted in Canada for fresh lettuce (CFIA, 2019). Several studies on the quality of vegetables such as lettuce mentioned that the visual quality is maintained up to a dose of 1 kGy (Fan and Sokorai, 2008). Fan *et al.* (2012A) found that fresh-cut lettuce stored under modified atmosphere packages (MAP) after an irradiation treatment at 1 or 2 kGy did not get deteriorated in appearance and texture.

Cranberry juice (CJ) contains organic acids; the most predominant are quinic, malic, shikimic and citric acids, which show strong antimicrobial activity (Côté *et al.,* 2011; Jensen *et al.,* 2017). These organic acids have pKas around 4.5 and transform into the protonated form at low pH and cross the cell membrane to destroy microbial cells (Kniel *et al.,* 2017). CJ has also antiviral activities and this property is mainly due to the decrease in pH (Akbas and Ölmez, 2007).

Ozone (O_3) is also an effective disinfectant agent that has a large antimicrobial spectrum against bacteria, viruses, as well as bacterial and fungal spores (Khadre *et al.,* 2001). In addition, it can be applied in aqueous or gaseous states, leaves no residues and only requires air for its production (Agriculture-Manitoba, 2019). O_3 damages the proteins and peptidoglycan molecules present on the viral capsid (Wysok *et al.,* 2006).

Irradiation treatment can also inhibit food viruses but requires doses in excess of 10 kGy to eliminate HAV (Bidawid *et al.,* 2000). The use of combined treatments allows for a reduction of treatment

levels, while increasing viral sensitivity and permits a better preservation of the physico-chemical and nutritional quality of the food (Gibriel *et al.,* 2013).

Therefore, the aim of this research is to verify the possible effectiveness of CJ and O_3 in combination with γ-irradiation to eliminate the viral load on the surface of iceberg lettuce.

2 MATERIALS AND METHODS

2.1 Culture of CRFK cells.

CRFK cells (ATCC-CCL94) were used as cellular host for FCV-F9 virus and were maintained in Dulbecco's minimum essential medium (DMEM: Fisher Scientific, Ottawa, Ontario, Canada) supplemented with 10 % fetal bovine serum (FBS, Wisent, Saint-Jean-Baptiste, Québec, Canada) and 1 % Streptomycin-Penicillin antibiotic (Gibco, Fisher Scientific, Ottawa, Ontario, Canada). The medium was added to a 75 cm^2 culture flasks and incubated at 37 °C, in a humidified incubator containing 5 % CO_2. Cells were subcultured every 2-3 days.

2.2 Preparation of viral pool.

CRFK cells were grown to 90 % confluency in 25 cm^2 culture flasks (Sarstedt, Montréal, Québec, Canada), the culture medium (DMEM + 10 % FBS) was then removed, and the monolayer of cells was washed twice with Dulbecco's Phosphate-Buffered Saline (DPBS) (pH 7.0-7.2). A 200 µL aliquot of viral inoculum (FCV-F9 virus (ATCC VR-651)) was then added to each flask and incubated at 37 °C under 5 % CO_2 for 90 min to allow viral adsorption. Then, 7 mL of medium (DMEM + 2 % FBS + 1 % strep-pen) was added to each flask and incubated for 16-18 h at 37 °C and 5 % CO_2 which resulted in virus-induced destruction of nearly 90 % of the monolayer verified by microscopy. Each virus-infected flask was first frozen at -80 °C for 5 min and then thawed at 37 °C for 5 min followed by centrifugation at 1500 x g for 15 min to remove cell debris. The supernatant containing the virus at ~ 6 log $TCID_{50}$/mL at was dispensed into 1 mL aliquots and stored at -80 °C.

2.3 Antimicrobial formulation preparation.

Cranberry juice concentrate (CJ) 12100-5/°BRIX (ATOKA, Manseau, QC, Canada) was prepared in distilled water. Respective concentrations of CJ were prepared by adding 0.1, 0.25, 0.5, 0.75, 1, 1.5 and 2 g of CJ in 99.9 (CJ-0.1%), 99.75 (CJ-0.25%), 99.5 (CJ-0.5%), 99.25 (CJ-0.75%), 99 (CJ-1.0%), 98.5 (CJ-1.5%) and 98 (CJ-2.0%) mL of distilled water. The CJ solutions were produced next to a Bunsen burner and also tested for possible bacterial contamination.

2.4 Raw material and spraying treatment with the formulation.

Iceberg lettuce was purchased from a local store IGA (Montréal, IGA extra Supermarché Pierre Leduc Inc.). Each lettuce sample (5 g) was washed with 200 ppm bleach water (La Parisienne, Montréal, Québec, Canada) and dried for 60 min under UV-light in a level 2 biosafety cabinet. The samples were then placed

on a sterile aluminum sheet and inoculated with 250 µL of viral suspension at titers of ~ 6 log $TCID_{50}$/mL. All samples were left to dry for 45-60 min under sterile conditions. The formulation containing CJ was uniformly sprayed on lettuce in order to cover the surface of the leaf using an Air Spray Gun (MasterCraft), at a distance of 30 cm and an outlet air pressure of 30 psi. Samples were left to dry for 1 h and stored overnight at 4 °C before microbiological analysis.

2.5 Ozonation treatment.

The lettuce samples (5 g) were inoculated with virus as above. Then the samples were transferred on stainless steel (SS) perforated plate and put into a SS O_3 diffusion chamber. Ozonation treatment at 5 ppm was performed using an ozone generator (Ozone Innovations Inc., Drummondville, Qc, Canada) at 15 standard cubic feet/h (SCFH) for 0, 2.5, 5, 7.5, 10, and 15 min. Samples were stored overnight at 4 °C before microbiological analysis.

2.6 γ-irradiation treatment.

Lettuce samples were inoculated with ~ 6 log $TCID_{50}$/mL of FCV-F9 virus and irradiated with γ-irradiation doses from 0 to 2.5 kGy to determine D_{10} values. Irradiation was also applied from 0 to 1.5 kGy on pre-treated lettuce with CJ-0.1%, CJ-0.25%, CJ-0.5%, and CJ-1.5% against FCV-F9 virus. Another treatment was performed with a combination of CJ-0.25%, O_3 at 5 ppm for 7.5 min, and γ-irradiation at 1.5 kGy to evaluate the total flora and physico-chemical parameters of lettuce. γ-irradiation was carried out at the Canadian Irradiation Center, in a UC-15 A underwater calibrator (Nordion Inc., Kanata, Ontario, Canada) equipped with a ^{60}Co source and having a dose rate of 9.5 kGy/h. The D_{10} is defined as the radiation dose required for reducing 90 % population (reduction by 1 log $TCID_{50}$/mL) of viable feline calicivirus in the CRFK cell line.

To calculate the D_{10} value, viral titer counts (log $TCID_{50}$ / mL) were plotted against radiation doses, and the reciprocal of the slope of the trend line was extracted from the plot. Relative radiation sensitivity was determined using the following equation:

Relative radiation sensitivity = (D_{10} of control sample) / (D_{10} of sample treated in presence of antimicrobial compound).

2.7 Viral analysis and infection of CRFK cells.

Iceberg lettuce samples (5 ± 0.5 g) were homogenized for 2 min at 230 rpm in 24.75 mL of sterile peptone water (0.1 % w/v) with a stomacher 400-Circulator (Seward Laboratory Systems Inc., Davie, FL, USA). The homogenate was 10-fold serially diluted in Phosphate-buffered saline (PBS) using a 96 well plate with a round bottom. Infection of CRFK cells by FCV-F9 virus was made after 90 % confluency was reached. The cells were washed twice with DPBS. The confluent CRFK cells were trypsinized with 1 mL of trypsin (concentration 1X, pH 7.2-8.0) (Fisher Scientific, Ottawa, Ontario, Canada) in 75 cm^2 flasks.

Cells were seeded in 96 well plates (flat bottom for adherent cell culture) with a density of 10^5 cells per well in 100 µl of DMEM + 10 % FBS + 1 % Pen/Strep and then incubated overnight in 5 % CO_2 at 37 °C. Cells reached a confluency of 80- 90 % after overnight incubation. Once viral dilutions were completed, 20 µL of each dilution was added into the corresponding wells in the microplate containing cells. The medium was removed before addition of diluted viral samples. The last 2 columns were kept as controls without virus. Microplates with flat bottom were incubated for 30-60 min at 37 °C. A quantity of 150 µL of DMEM + 2% FBS was then added to the microplate. The microplate was incubated for 48 h in 5 % CO_2 at 37 °C. Cytopathic effects were determined by visual inspection under the optical microscope.

2.8 Analysis by $TCID_{50}$.

The viral titer was measured with the endpoint dilution assay with Tissue Culture Infective Dose 50 ($TCID_{50}$) in order to evaluate the FCV-F9 titer. This method quantifies the quantity of virus needed to produce cytopathic effects in 50 % of cellular culture. The Spearman-Karber method was used (Brié *et al.,* 2017) to calculate viral titer ($TCID_{50}$/mL) after infection (Hirneisen *et al.,* 2011).

2.9 Preparation of bacterial suspension.

A quantity of 25 g iceberg lettuce was immersed in 75 mL of peptone water (0.1 % w/v) and homogenized for 2 min using a Stomacher. Subsequently, 1 mL of the resulting mixture was incubated in 9 mL of Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, MI, USA) at 37 °C for 24 h. Total mesophilic flora (TMF) were stored at −80 °C in TSB in the presence of 10 % (v/v) glycerol. Before each experiment, bacteria were propagated through 2 consecutive cycles of 24 h in TSB at 37 °C. The cultivated cultures were centrifuged at 5,000 x g for 15 min and the pellets were washed twice in peptone water (0.1 % w/v) to obtain working cultures containing ~ 10^8 CFU (colony forming unit)/g.

2.10 Effects of bioactive coating, ozonation, and γ-irradiation on total flora.

Iceberg lettuce samples (25±1 g) were washed with 200 ppm of chlorine water and then dried on an aluminum sheet under a biological hood under UV light. Samples were inoculated with 1 mL of TMF at ~10^5 CFU/g and dried for 1 h. Treatments of O_3 (Ozone Innovations Inc., Drummondville, QC, Canada) at 5 ppm for 7.5 min, uniform spraying of CJ-0.25%, and γ-irradiation at 1.5 kGy were made alone or in combination. Samples were stored at 4°C in Whirlpak bags sealed under normal atmosphere. Five groups were tested (1) no treatment, (2) CJ-0.25%, (3) γ-irradiation at 1.5 kGy, (4) O_3 at 5 ppm for 7.5 min and (5) combination of the three treatments. Microbiological analysis was done on day 0, 1, 4, 7, 10, and 13. On analysis day, samples (25±1 g) were homogenized for 2 min at 230 rpm in 225 mL of sterile peptone water (0.1 % w/v) with a stomacher. A quantity of 100 µL of the homogenate and all serial dilutions 1:10 in peptone water were inoculated on TSA (Tryptic Soy Agar) for bacterial enumeration of TMF. Inoculated plates were incubated at 37 °C for 24 h.

2.11 Color, chlorophyll and texture measurements.

Color determination was carried out on 5 preselected locations of 5 cm diameter (on the extremity and on the center) on the surface of each lettuce sample using a Minolta Colorimeter Color reader CR10 (Konica Minolta Sensing, Inc, Mahwah, NJ, USA). L* (lightness, + = lighter, - = darker), a* (+ = redder, - = greener), b* (+ = yellower, - = bluer) and H*, hue angle° (difference in hue) were quantified in each sample. Chlorophyll content was determined at 1 preselected location on the surface of each sample according to Lichtenthaler and Welburn (Lichtenthaler and Wellburn, 1983). A quantity of 2.0 g of sample was combined with 2 mL acetone 80 % and blended using a mortar and pestle. This homogenate was then centrifuged at 5,000 RPM for 5 min at 4 °C. The blending step was repeated until the homogenate became transparent and all supernatants were pooled together and made the volume to 10 mL with acetone 80 %. Absorbance was taken at 663 and 646 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). Texture analysis was carried out on the samples during the storage using a Stevens LFRA TA-1000 Texturometer (Texture Technology Corp., Scarsdale, NY, USA). Each sample was measured on 3 different parts of the leaf. The analysis was conducted with TA7 probe of 2 mm diameter. The distance through the sample was set at 10 mm. Color, chlorophyll and texture analysis was measured during storage on day 0, 2, 4, 6, 8, and 10 after all treatments.

2.12 Sensory evaluations.

Sensory evaluation was carried out using a hedonic test with a nine points scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) with three samples (combination of irradiated at 1.5 kGy, sprayed with CJ-0.25% and ozonated at 5ppm; sprayed with CJ-0.25% alone; and a control without any treatment) by 15 panelists during storage at days 1, 3, 5. For each panelist, 3 pieces of lettuce (~ 5 cm × 5 cm) were served to evaluate for the texture, the smell, the appearance, and the flavor.

2.13 Statistical analysis.

Each experiment was done in triplicate (n=3) and for each replicate, 3 samples were analyzed. Analysis of variance (ANOVA), Tukey HSD's test for equal variances and Tamhane's T2 test for unequal variances were made for statistical analysis with SPSS 18.0 software (SPSS Inc., USA). Differences in means were significant when confidence interval was less than 5 % ($p \leq 0.05$).

3 RESULTS AND DISCUSSION

3.1 Effects of γ-irradiation, CJ, and O_3 against FCV-F9 virus.

γ-Irradiation at doses ranging from 0 to 2.5 kGy were used against FCV-F9 inoculated on iceberg lettuce and results are presented in Figure 1A). The lettuce was inoculated with an initial viral load of FCV-F9 at 6.31 ± 0.05 log $TCID_{50}$/mL and after irradiation at 0.5, 1.0, 1.5, 2.0 and 2.5 kGy, viral titers were reduced to 6.24 ± 0.0, 5.71 ± 0.05, 5.67 ± 0.05, 4.56 ± 0.07, and 4.42 ± 0.02 log $TCID_{50}$/mL, respectively, showing a 1.89 log $TCID_{50}$/mL reduction after a treatment of 2.5 kGy. A previous study on

kimchi showed that γ-irradiation was effective on a NoV surrogate as the dose increased and a dose of 10 kGy was necessary to reduce the NoV surrogate by 1.76 log PFU/mL (Park and Ha, 2017). Feng *et al.* (2011) tested the effect of γ-irradiation at 5.6 kGy against vesicular stomatitis virus (VSV) and MNV-1 inoculated on fresh food and found that VSV was more susceptible than MNV-1 most likely due to the size of its genome. γ-irradiation generates oxygen and hydroxyl radicals that damage biological structures of microorganisms and nucleic acids of viruses (Park and Ha, 2017).

In Figure 1B), various concentrations of CJ (from 0.1% to 1.5%) were used against FCV-F9 on iceberg lettuce leaf. The initial virus titer present on lettuce was 5.70 log $TCID_{50}$/mL which was reduced to 5.12, 4.99, 4.99, 4.87, 4.85 to 4.80 log $TCID_{50}$/mL with CJ-0.1%, CJ-0.25%, CJ-0.50%, CJ-0.75%, CJ-1.0%, and CJ-1.5%, respectively. NoV is normally stable under acidic conditions including those found in the human stomach (Leuenberger *et al.,* 2007). Organic acids in small concentrations, in combination with nisin, can be effective against flavor flaws in meat products (Thurston-Enriquez *et al.,* 2005). Su *et al.* (2010) showed that CJ at pH of 2.6 and pH of 7.0 decreased the FCV-F9 viral titer from 5 $\log_{10}$ PFU/mL to undetectable levels within 30 and 10 min, respectively.

Figure 1C) shows an exposition time from 0 to 15 min of O_3 at 5 ppm was sufficient to reduce the FCV viral titer on iceberg lettuce. The initial viral load was 5.54 log $TCID_{50}$/mL on lettuce and after a treatment with O_3 at 5 ppm for 2.5, 5.0, 7.5, 10 and 15 min, the viral titer was reduced to 4.99, 4.74, 4.62, 4.57, and 4.41 log $TCID_{50}$/mL, respectively. A reduction of 90 % of the initial viral load was observed on the surface of iceberg lettuce after 15 min of contact with 5 ppm O_3. Some authors found that FCV is inactivated by 4.28 log with 1 mg/L of deionized sterile ozonated water for 15 sec, at pH 7 and 5 °C (Thurston-Enriquez *et al.,* 2005). NoV has also been reduced by more than 3 log after an O_3 treatment in water during 10 sec, at pH 7 and 5 °C (Shin and Sobsey, 2003). Hirneisen *et al*. (2011) showed that a 5-10 min exposition with O_3 6.25 ppm (0.9 g of O_3/h) was needed to decrease viral titers of FCV on lettuce and green onions. The authors attributed this to the organic composition of the vegetable because O_3 reacts with the complex organic compounds in foods due to the high oxidation potential (Hirneisen *et al.,* 2011).

In Fig. 1A, the effects of various γ-irradiation doses on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.83 and a constant "b" of 6.52. The standard deviation of the regression is 0.13 and the coefficient of determination (R^2) is 0.91. The p-value calculated is 0.003, meaning that the regression is significant ($p \leq 0.05$). In Fig. 1B, the effects of various concentrations of CJ on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.42 and a constant "b" of 5.29. The standard deviation of the regression is 0.17 and the R^2 is 0.54. The p-value calculated is on the threshold of statistic of =0.059, meaning that the regression is not significant ($p > 0.05$) but is close to be significant. In Fig. 1C, the effects of various O_3 exposition time on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope

of -0.07 and a constant "b" of 5.26. The standard deviation of the regression is 0.02 and the R^2 is 0.80. The p-value calculated is 0.02, meaning that the regression is significant ($p \leq 0.05$). Despite the fact that the slope of treatment with CJ is not significant of very little, the treatment with the steepest slope is with the γ-irradiation alone. The highest standard deviation is observed with CJ treatment (0.17) and showed the lowest R^2 (0.54). Overall, the best treatment to use alone against FCV-F9 on lettuce is the γ-irradiation.

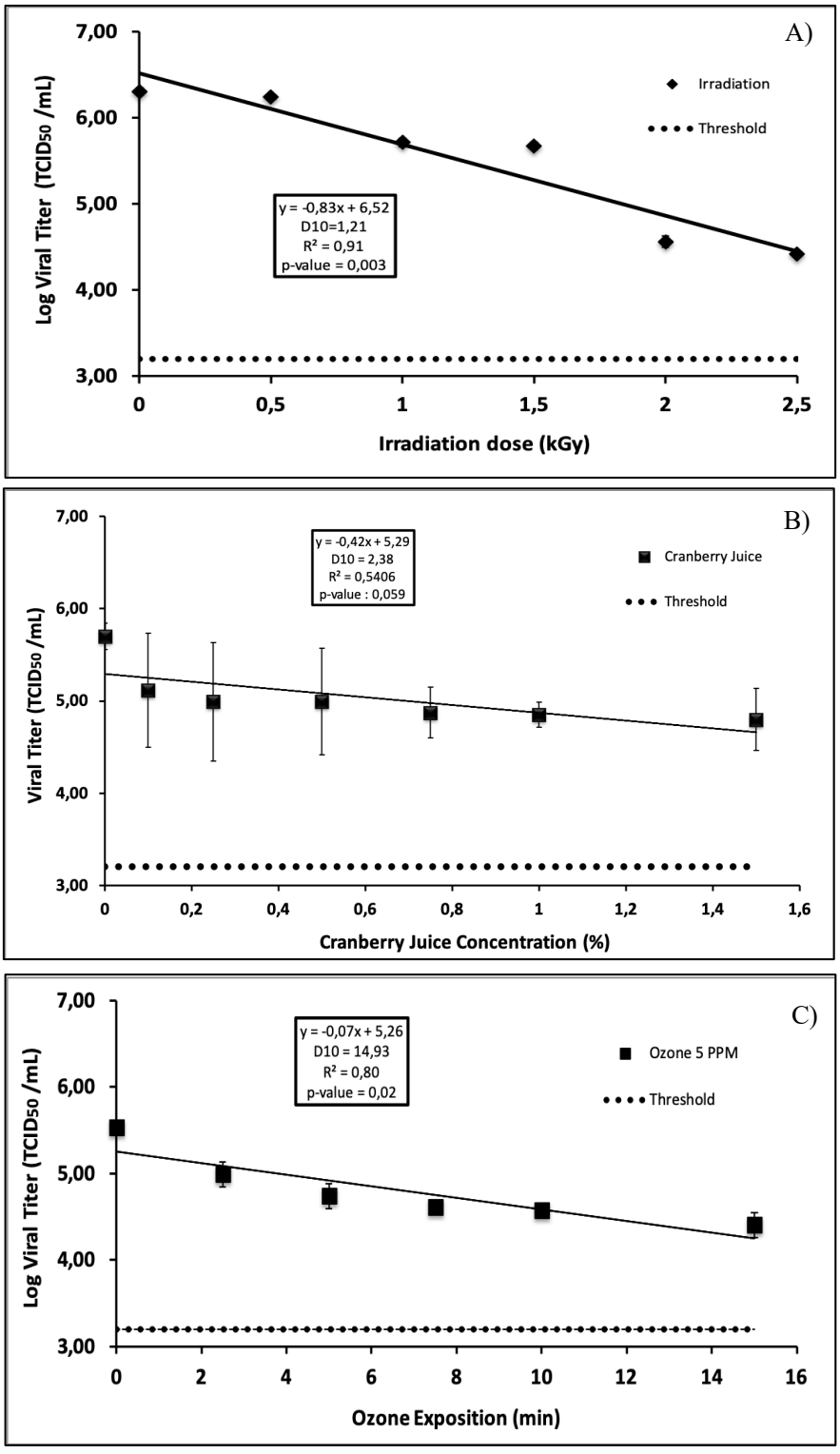

Fig. 1. Individual effects of (A) γ-irradiation, (B) CJ and (C) O_3 on the viral titer of FCV-F9 at different doses on iceberg lettuce at Day 0 of storage

3.2 Combined effects of γ-irradiation and CJ against FCV-F9 virus.

Figure 2A) shows the effect of combined treatments of CJ-0.10 with γ-irradiation doses of 0, 0.25, 0.50, 0.75, 1.0 and 1.5 kGy on virus. The initial viral load was 6.16 log $TCID_{50}$/mL which reduced by 0.71, 0.84, 0.96, 1.21 and 1.67, respectively, as the irradiation doses increased. The D_{10} values (kGy) for combined treatment with CJ-0.10% and control were 0.95 and 1.14 kGy respectively. Relative radiosensitization (RS) is 1.2 for the combined treatment using γ-irradiation with CJ-0.10% showing that the used of combined treatment of irradiation with CJ-0.10% concentration is better than the irradiation treatment without CJ.

Figure 2B) shows the effect of combined treatments of CJ-0.25% in combination with γ-irradiation. The initial viral load on lettuce was 6.05 log $TCID_{50}$/mL and the reductions from 0.02 to 0.98 log $TCID_{50}$/mL for doses ranging from 0.25 to 1.5 kGy were observed. A higher reduction was observed at 1.5 kGy in presence of CJ-0.1%. The D_{10} values (kGy) of samples treated with CJ-0.25% and control (irradiation without CJ) were 1.33 and 2.0, respectively, showing a relative RS of 1.5. By extrapolation, γ-irradiation alone at 4 kGy would be needed to eliminate the FCV-F9 virus on lettuce but when irradiation was done in combination in presence of CJ-0.25%, an irradiation dose of only 2.66 kGy would be needed to eliminate the virus.

Figure 2C) shows the effect of combined treatments of CJ-0.50% with the same γ-irradiation doses as above. It was demonstrated that there was a significant difference between 0 kGy and 1.5 kGy ($p \leq 0.05$). Initial viral load on lettuce was 5.95 log $TCID_{50}$/mL, and after irradiation at 0.25 and 1.5 kGy, virus titer reductions were from 0.08 to 0.71 log $TCID_{50}$/mL, respectively. The D_{10} values (kGy) of samples irradiated in presence of CJ-0.5% and of sample without CJ were 1.91 and 2.08 respectively, showing a relative RS of 1.09 when the irradiation treatment was done in presence of CJ.

Figure 2D) illustrates the effect of combined treatment of CJ-1.5% with the irradiation doses as above. The initial viral load was 5.80 log $TCID_{50}$/mL and reductions of 0.02 to 0.60 log $TCID_{50}$/mL were observed as the irradiation doses increased. The viral titer decreased slowly after treatment with CJ-1.5%. The D_{10} values (kGy) of samples irradiated in presence of CJ-1.5% and without CJ (control) were 2.08 and 2.09, respectively, showing a relative RS of 1, meaning no significant difference ($p > 0.05$) against FCV-F9 virus when CJ-1.5% is added before irradiation treatment. The highest RS is observed when combining CJ-0.25% and γ-irradiation. The phenomenon is a synergy between two factors (γ-irradiation and CJ), where a low concentration of CJ is better for viral RS, but CJ-0.1% was not as effective as CJ-0.25%. There are not many studies combining organic acids or even no study using CJ and γ-irradiation in combination against NoV on the food surface. Organic acids and γ-irradiation combined were more effective against microbial counts and coliforms during storage of pork meat (Kim *et al.,* 2004). Previous

research has shown an interaction between ascorbic acid and γ-irradiation on total coliforms and aerobic plate counts (APCs) on beef patties (Giroux *et al.,* 2001).

In Fig. 2A, the effects of various γ-irradiation doses combined with CJ-0.1% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -1.05 and a constant "b" of 6.27. The standard deviation of the regression is 0.15 and the R^2 is 0.92. The p-value calculated is 0.002, meaning that the regression is significant ($p \leq 0.05$). In Fig. 2B, the effects of various γ-irradiation doses combined with CJ-0.25% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.75 and a constant "b" of 6.25. The standard deviation of the regression is 0.15 and the R^2 is 0.87. The p-value calculated is 0.007, meaning that the regression is significant ($p \leq 0.05$). In Fig. 2C, the effects of various γ-irradiation doses combined with CJ-0.50% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.52 and a constant "b" of 6.30. The standard deviation of the regression is 0.04 and the R^2 is 0.97. The p-value calculated is 0.0002, meaning that the regression is highly significant ($p \leq 0.05$). In Fig. 2D, the effects of various γ-irradiation doses combined with CJ-1.50% on FCV-F9 viral titer are shown. According to statistical analyzes, the data showed a regression slope of -0.48 and a constant "b" of 6.29. The standard deviation of the regression is 0.05 and the R^2 is 0.96. The p-value calculated is 0.0007, meaning that the regression is highly significant ($p \leq 0.05$). In correlation with our results, the most efficient combination of CJ with γ-irradiation is CJ-0.1% because of the steepest slope of -1.05. All four of R^2 are high, 0.92, 0.87, 0.97 and 0.96 for CJ-0.1%, CJ-0.25%, CJ-0.50% and CJ-1.5%, respectively. The respective standard deviations are 0.15, 0.15, 0.04 and 0.05 (for ascending CJ concentrations), meaning that the treatment with values that are not much spread around the mean is CJ-0.5%. All four treatments show p-values that are really significant ($p \leq 0.05$).

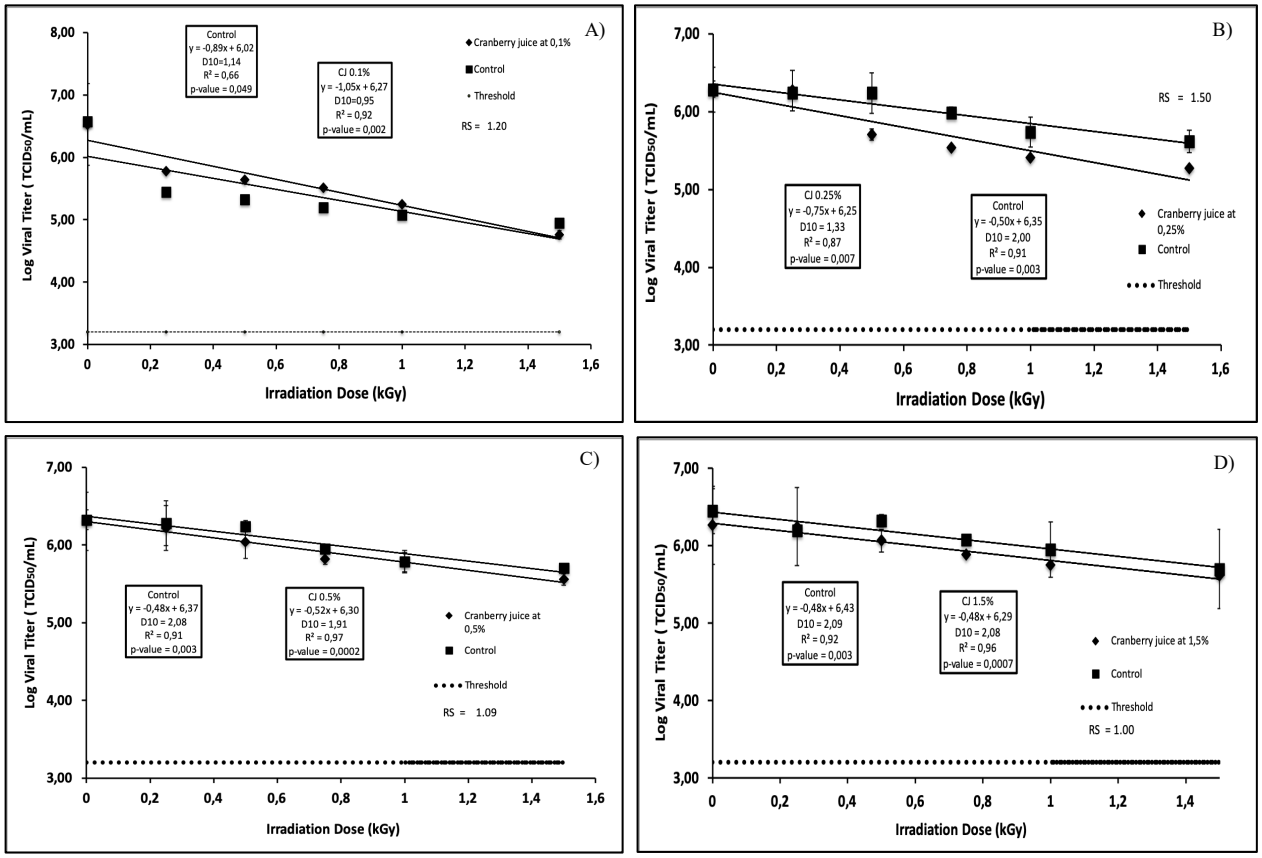

Fig. 2. Combined effects of various concentrations of CJ (A) CJ-0.10%, (B) CJ-0.25%, (C) CJ-0.50% and (D) CJ-1.5% with γ-irradiation at doses of 0, 0.25, 0.50, 0.75, 1.0 and 1.5 kGy on viral titer of FCV-F9 on iceberg lettuce at day 0

3.3 Effect of treatments on FCV-F9 viral titer on lettuce during storage at 4°C for 10 days.

The iceberg lettuce was treated with CJ-0.25%, O_3 at 5ppm for 7.5 min, γ-irradiation (1.5 kGy), and the effect of the combined treatment was evaluated after inoculation of FCV virus (~ 7 log $TCID_{50}$/mL). After 48 h of incubation at 37 °C and 5 % CO_2, visual inspection of 96 well plates were carried out to verify the presence of cytopathic effects such as rounding and aggregation of cells in order to measure viral titer.

At day 0, the control sample exhibited a viral titer of 7.77 log $TCID_{50}$/mL (Fig. 3), and after treatment with CJ-0.25%, O_3 at 5 ppm for 7.5 min, or by γ-irradiation (1.5 kGy), the viral titer was reduced by 1, 0.96 and 1.22 log $TCID_{50}$/mL, respectively. However, the combination of the three treatments showed a viral titer reduction of ~ 0.91 log $TCID_{50}$/mL.

After 10 days of storage at 4 °C, the combination of the three treatments showed the highest reduction (2.15 log $TCID_{50}$/mL reduction) of viral titer inoculated on lettuce. Bidawid *et al.* (2000)

reported an instant reduction of viral titer of Hepatitis A Virus (HAV) on lettuce, when treated with γ-irradiation at different doses (1, 2, 3 and 10 kGy) (Bidawid *et al.,* 2000). After inoculation with 6.90 ± 0.04 log PFU/mL, the level of HAV virus titer was 6.90, 6.20, 5.60, and 3.36 log PFU/mL, respectively.

In the present study, the lettuce treatment with CJ-0.25% was efficient against FCV virus on day 8 showing a reduction of 1.20 log $TCID_{50}$/mL, however, the viral titer increased further after 8 days and reached 7.2 log $TCID_{50}$/mL on day 10, CJ-0.25% was not as much efficient anymore on day 10 post-treatment. Konowalchuk and Speirs (1975) showed that among tested vegetables (celery, lettuce, and radishes *etc*.), enteric viruses such as Coxsackievirus type B5, poliovirus type 1 (Sabin), echovirus type 7, reovirus type 1, and adenovirus type 7a could survive longer on lettuce. The authors attributed this virus survival to the moisture of the food. Celery was also shown to be a good survival medium for enteric virus survival because of its level of moisture. According to the author's hypothesis, it is possible that CJ obviously containing a high level of moisture, made lettuce more hydrated and increased the survival of the virus over time (Konowalchuk and Speirs, 1975).

Ozonation at 5 ppm for 7.5 min showed a reduction of 1.49 log $TCID_{50}$/mL after 10 days of storage at 4 °C. The reduction of the virus titer during storage time was almost constant. Katzenelson *et al*. (1974) reported a kinetic of effects caused by O_3 against poliovirus-1 at concentrations of O_3 from 0.3 to 1.5 mg/L at 4 °C (Katzenelson *et al.,* 1974). They observed two stages of reduction of poliovirus; the first step took less than 8 sec with viral inactivation of 99.5 % and the second step lasted from 1 to 5 min, where viral counts remained stable. At an O_3 concentration of 1.5 mg/L, there was an increase of inactivation rate during the second stage (Katzenelson *et al.,* 1974). Park and Ha (2017) reported the increase in the shelf life of kimchi by γ-irradiation due to the generation of oxygen and hydroxyl radicals by γ-irradiation. Irradiation of kimchi treated with 1 to 10 kGy induced a reduction of 0.34 to 1.76 log PFU/mL in MNV-1 titers (Park and Ha, 2017).

The control group reduced FCV viral titer by 1.11 log $TCID_{50}$/mL after 10 days of storage at 4 °C. Viral titer reduction can be seen in Table 1, and statistically ($p \leq 0.05$) different groups are also shown. A previous study by Kurdziel *et al. (*2001*)* showed more than 90 % natural decrease (without treatments) of viral titer of poliovirus (enterovirus) on lettuce and white cabbage after 12 days of storage at 4 °C (Kurdziel *et al.,* 2001). These results are in agreement with the observations in the present study, where a natural decrease of 90 % of viral titer is observed after 10 days of storage at 4 °C (Fig 3).

Table 1 shows the reduction of viral titer in log $TCID_{50}$/mL after all treatments during 10 days of storage at 4 °C. The viral titer in all samples on day 0 before treatments was 7.77 log $TCID_{50}$/mL.

In control samples, the viral titer was reduced to 6.66 log $TCID_{50}$/mL on day 10, which represents a decrease of 1.11 log $TCID_{50}$/mL. After every treatment, the viral titers were instantly reduced on day 0, and the final reductions were compared on day 0 before treatments and day 10 after storage at 4 °C.

After ozonation on day 0, the viral titer was reduced from 7.77 to 6.81 log $TCID_{50}$/mL and on day 10 to 6.28 log $TCID_{50}$/mL which represent a reduction of 1.49 log $TCID_{50}$/mL.

After treatment with CJ-0.25%, there was an instant reduction of 0.99 log $TCID_{50}$/mL on day 0, however, on day 10, it increased to 7.20 log $TCID_{50}$/mL which represents a 0.57 log $TCID_{50}$/mL reduction of initial inoculated virus. We are aware that an increase of the viral titer on such a day of storage is unexpectable since viruses don't multiplicate on food, however the viral titer observed on day 10 is not higher than the viral quantity inoculated on lettuce on day 0 before treatments (7.77 log $TCID_{50}$/mL). This means that CJ stopped being effective from day 8. A similar study was conducted on celery and spinach irrigated with water containing polio virus and stored for 76 days at 4 °C after harvest, and the virus levels varied greatly over time (Ward and Irving, 1987). The authors hypothesized that the amounts of vegetables in the bags were too variable (0.5-3.0 kg), leading to variation in the amounts of virus. In our case, quantities were standardized so this hypothesis is not feasible. A similar trend with variations in viral titer during storage of green onions, lettuce, white cabbage and raspberries was observed. The authors attributed these variations to the sampling size and the complexity of the surface on food (Kurdziel *et al.,* 2001). Another study evaluated the viral titer of polio virus in cranberry sauce and orange juice after storage at 4 °C for 168 h. In cranberry sauce, between 48 h and 96 h of storage at 4 °C, the viral titer went from 2.0 x 10^4 to 2.7 x10^4 PFU/mL, respectively. Also, in orange juice, between 96 h and 168 h of storage at 4 °C, the viral titer went from 5.0 x10^4 to 5.7 x10^4 PFU/mL, respectively (Heidelbaugh and Giron, 1969). The authors attributed the variation in the viral titer to the change in the adherence of the viral particle to the food and the change in the ability to form plaques under the conditions studied. The authors also suggested the need for a better method for separation of virus particles from foods (Heidelbaugh and Giron, 1969).

It is difficult to say whether an increase in viral titer after treatments consists of a possible biological phenomenon or an artefact. These variations have been observed before (Heidelbaugh and Giron, 1969, Kurdziel *et al.,* 2001, Ward and Irving, 1987) and these increases were justified by the amount of food studied in a bag, the smoothness of the food, the size of the sampling, the complexity of the food, the viral adherence on food, and even the ability of the virus to make plaques. As long as we do not have absolutely ideal methods to collect all the viruses on food and good methods of detection and confirmation of the viral presence (Heidelbaugh and Giron, 1969), this possible biological phenomenon of increase in viral titer can be attributed to handling errors. To increase the rigor of the results and to validate the possibly observable biological phenomenon, it would be necessary to increase the number of replicates. The methods of determination of the viral titer ($TCID_{50}$, *plaque assay*) must be carried out meticulously because if not, they are not reliable (Darling *et al.,* 1998).

Irradiation caused instant reduction of 1.22 log $TCID_{50}$/mL and after 10 days of storage, it decreased to 6.14 log $TCID_{50}$/mL representing a reduction of 1.63 log $TCID_{50}$/mL.

The most efficient treatment is the combination of the three treatments after 10 days of storage with a reduction of 2.15 log $TCID_{50}$/mL from the inoculated virus.

In the MAPAQ report of 2013-2014, several files transferred were related to gastroenteritis of viral origin for which the identification of the virus was not confirmed by stool samples, this proportion of food-related infection was therefore called "undetermined virus" because they couldn't confirm the virus implicated. In the MAPAQ thermoguide (MAPAQ, 2006), there are no viral standards associated with fresh vegetables currently. Level 2 viruses are now in the category "unacceptable microbiological quality with high risk to human health" and are their presence is difficult to detect and confirm. Considerable viral titers for public health as high as those studied in this research will probably never occur in actual foods (Heidelbaugh and Giron, 1969). A study was conducted in Denmark on 191 calicivirus-associated episodes between 2005 and 2011 (Franck *et al.,* 2014). This revealed that in 27% of cases, the contaminations occurred during the production of frozen berries, lettuce and oysters. In another 29% of the cases, the contaminations took place with food of self-serve buffets. In the majority of other cases (34%), the contaminations occurred with food handlers during the preparation, and in 64% of these previous cases, the food handler was asymptomatic. A research on transfer from fecally-contaminated fingers to foods showed that a 3 log inactivation (>1000 viral particles) of these agents is required to reduce the contamination of food by infected food handlers (Koopmans and Duizer, 2004). The reductions observed after 10 days of storage at 4 °C of lettuce treated with combined treatments (2.15 log $TCID_{50}$/mL) were close to the suggested reductions (3 log) from the previous research.

Table 1. Diminution of viral titer (log $TCID_{50}$/mL) after treatments with O_3 at 5-ppm, CJ-0.25% and γ-irradiation 1.5 kGy or the combination after storage at 4°C for 10 days

Treatment	Day 0 (Before treatment)	Day 0 (After treatment)	Day 10 (After treatment)	Diminution (Log $TCID_{50}$/ mL)
Control	$7.77\pm0.21^{a, A}$	$7.77\pm0.21^{a, A}$	$6.66\pm0.28^{b, BC}$	1.11
O_3 5-ppm, 7.5 min	$7.77\pm0.21^{a, A}$	$6.81\pm0.47^{b, B}$	$6.28\pm0.43^{b, AB}$	1.49
CJ-0.25%	$7.77\pm0.21^{a, A}$	$6.78\pm0.14^{b, B}$	$7.20\pm0.37^{b, C}$	0.57
Irradiation 1.5 kGy	$7.77\pm0.21^{a, A}$	$6.55\pm0.20^{b, B}$	$6.14\pm0.28^{b, AB}$	1.63

Combination of O_3, CJ-0.25%, & γ- irradiation (1.5 kGy)	7.77±0.21$^{a, A}$	6.86±0.26$^{b, B}$	5.61±0.31$^{c, A}$	2.15

For each treatment, means followed by the same lowercase letter from Day 0 to Day 10, are not significantly different ($p > 0.05$). For each day of analysis, means followed by the same uppercase letter for the comparison of treatments are not significantly different ($p > 0.05$).

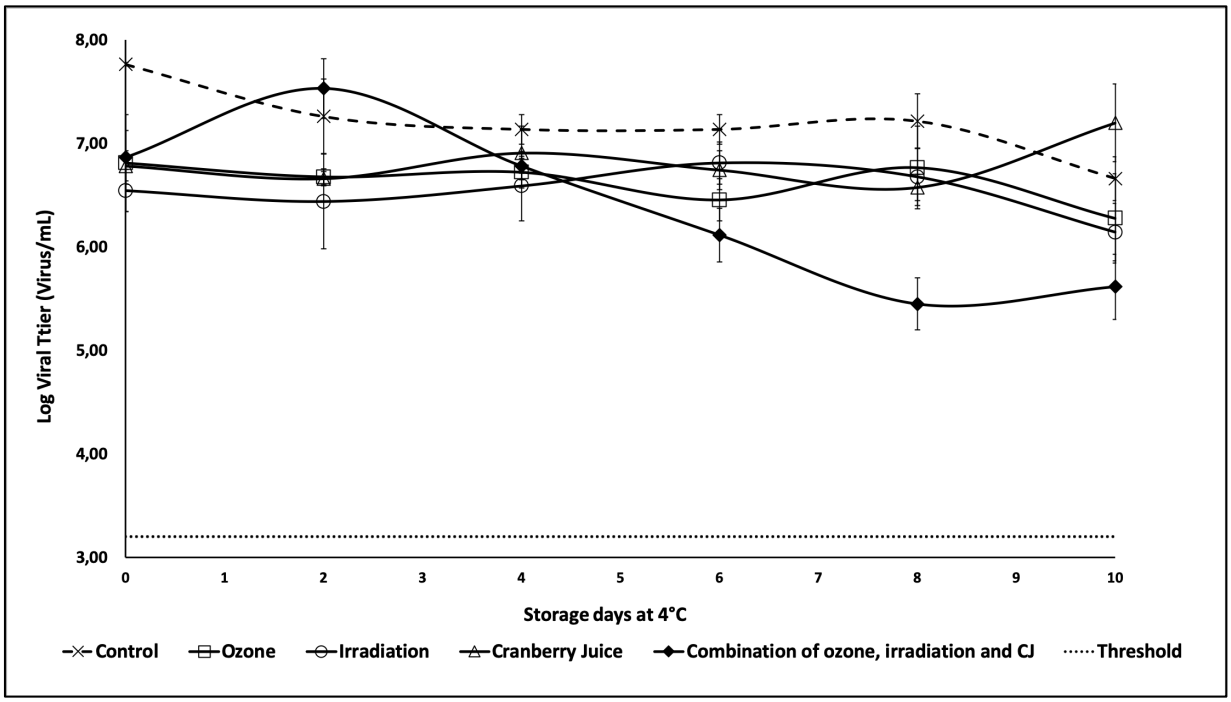

Fig. 3. Effects of CJ-0.25% in combination with γ-irradiation at 1.5 kGy and ozonation at 5ppm on viral (in log $TCID_{50}$/mL) shelf-life of iceberg lettuce during storage at 4 °C for 10 days

3.4 Effect of treatments on total mesophilic flora of iceberg lettuce during storage at 4°C.

Figure 4 shows the effects of treatments on the total flora inoculated (~ 5.5 log CFU/g) on the lettuce leaves. Total microflora was inhibited by the γ-irradiation and combination of the three treatments (CJ-0.25%, O_3 at 5ppm for 7.5 min, and γ-irradiation (1.5 kGy) during the entire storage of 13 days at 4 °C. The results are in agreement with the finding of Monk *et al.* (1995), where they reported that the combination of γ-irradiation at doses of 1.0 or 3.0 kGy in presence of 0.19 % sodium benzoate (salt of benzoic acid found in cranberries) extended the shelf life of Dover sole stored at 5.6-6.7 °C (Monk *et al.,* 1995). They also stated that the aerobic microbial populations of 10^6 CFU/g were attained in 6 and 10 days after irradiation treatment when they were only irradiated with 1.0 and 3.0 kGy, respectively. However,

with the combination of γ-irradiation at doses of 1 or 3 kGy in presence of sodium benzoate treatment increased the shelf life to 9 and 19 days, respectively. In the present study, the lettuce was treated with CJ-0.25% and O_3 at 5ppm for 7.5 min before irradiation (1.5 kGy), and results showed that these combined treatments helped to increase the shelf-life of lettuce during storage.

After γ-irradiation alone at 1.5 kGy, the total flora present on lettuce stayed stable at 2 log CFU/g during the entire storage period. Both, irradiation treatment alone and the combination of treatments maintained the shelf-life of lettuce longer than experiment duration (13 days). The Ministry of Agriculture, Fisheries and Food of Quebec (MAPAQ, 2006) has established a standard for fresh vegetables such as lettuce in terms of the amount of total mesophilic bacteria negatively affect the quality of food at 7 log CFU/g (MAPAQ, 2006). It has been reported that γ-irradiation alone at 1.0 kGy increased the shelf-life of fresh pork after storage at 25 °C (Monk *et al.,* 1995). This irradiation dose reduced mesophilic bacterial populations by 2 log CFU/g. The bacteria grown in unirradiated pork rose to unacceptable levels after 2 days of storage, however, it took more than 10 days for pork samples irradiated at 1.0 kGy to reach these levels (Monk *et al.,* 1995).

A control sample without any treatment showed a significant increase in total flora count from 5.5 log CFU/g to 7.44 log CFU/g during the first 7 days of storage and finally to 8.22 log CFU/g on day 13 ($p \leq 0.05$). Without any treatment, the shelf-life of iceberg lettuce is 7 days and with the three combined treatments, the shelf-life of lettuce increased to more than 13 days. In the MAPAQ thermoguide document, it is mentioned that lettuce should be kept for 7 days at 4°C, which is in confirmation with the results we found (MAPAQ, 2006).

After treatment with CJ-0.25%, the shelf-life of lettuce was extended to 10 days, where total flora growth increased to 7.37 log CFU/g and 7.55 log CFU/g on day 10 and 13, respectively. The addition of CJ as a natural antimicrobial containing phenolic compounds and organic acids represents a good alternative to reduce food poisoning and economic losses due to microbiological contaminations, and to extend the shelf-life of processed food. Cranberries have been found to be active against pathogens like *Helicobacter pylori, Salmonella,* and *Campylobacter.* Bacterial growth inhibition properties of cranberries are related to the low pH of cranberries and the presence of phenolic compounds (Côté *et al.,* 2011). Akbas and Olmez (2007) showed that dipping in citric or lactic acid resulted in lower mesophilic and psychrotrophic counts than those of other dipping treatments during 12 days of storage of fresh-cut iceberg lettuce (Akbas and Ölmez, 2007). The authors attributed this antimicrobial property to the reduction in pH, the ratio of the undissociated fraction of the acids, chain length, cell physiology, and metabolism.

After using O_3 at 5ppm for 7.5 min, the shelf-life was also extended from 7 to 10 days at 4 °C and the growth of total flora increased from 5.69 log CFU/g to 7.12 and 7.34 log CFU/g on day 10 and 13,

respectively. Some studies showed that O_3 at 4 mg/L for 2 min reduced populations of mesophilic and psychrotrophic organisms by ~ 3.0 log CFU/g after 12 days of storage. They proposed that the antimicrobial properties of O_3 are attributed to the oxidative changes in numerous cellular constituents (proteins, unsaturated lipids and peptidoglycans) (Akbas and Ölmez, 2007). According to Yuk *et al.* (2006) dipping lettuce in water containing O_3 (5 ppm) for 10 min reduced the aerobic bacteria by more than 1.5 log CFU/g (Koseki *et al.,* 2001). O_3 inactivates bacteria due to oxidative changes to cellular components such as proteins, unsaturated lipids and respiratory enzymes, peptidoglycans in cell envelopes and enzymes (Yuk *et al.,* 2006).

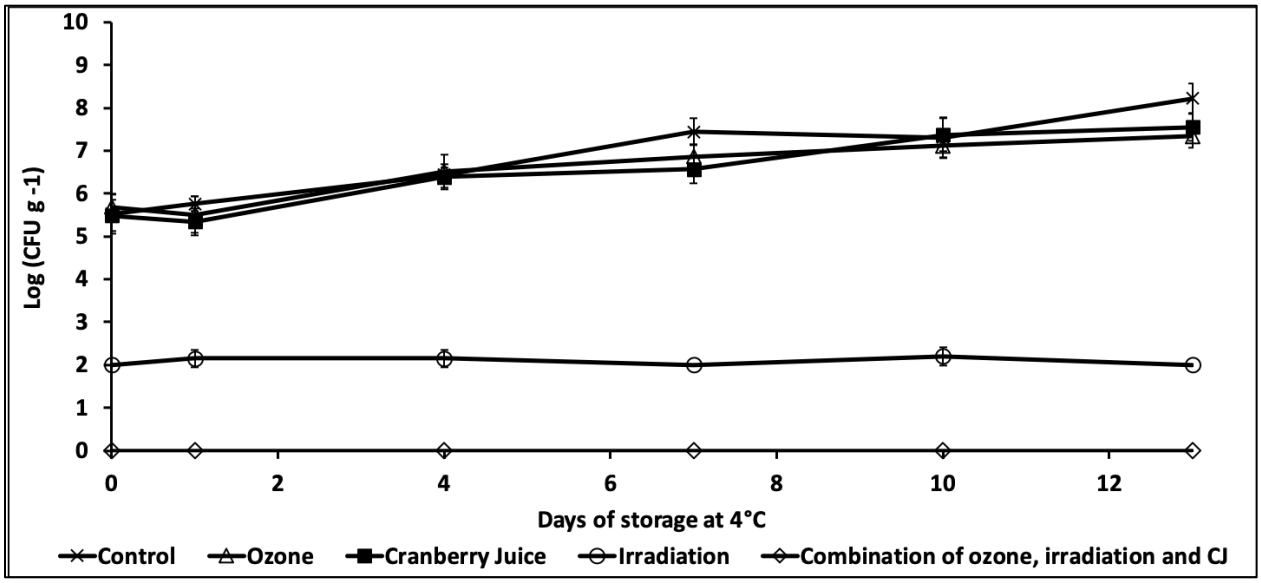

Fig. 4. Effects of CJ-0.25% in combination with γ-irradiation at 1.5 kGy and ozonation at 5ppm on bacterial (in log CFU/g) shelf-life of iceberg lettuce during storage at 4 °C for 10 days

3.5 Effects of treatments on color properties of iceberg lettuce during storage.

After treating iceberg lettuce and storing them at 4 °C, color parameters were recorded, every two days. Figure 5 shows the graphs of the variation of specific parameters: L* (variation in darkness and brightness, positive values are desirable), a* (variation between red and green, negative values are desirable), b* (variation in yellow and blue, positive values are desirable), and H* (defines the Hue angle).

Figure 5A shows the variation of L* during storage which did not change significantly during storage ($p > 0.5$). After 10 days of storage the L* values changed from 53.02 to 54.13 on control samples. For samples treated with O_3 at 5ppm for 7.5 min the L* values changed from 46.62 to 48.59. For samples treated with γ-irradiation (1.5 kGy), the L* values changed from 50.82 to 52.58. When the 3 treatments

were done in combination the L* values changed from 58.18 to 60.03. The sample treated with O_3 exhibited that the lettuce leaves were darker, whereas combination of the three treatments showed higher L* values which indicate that the leaves were lighter. Significant changes of L* value was observed after 10 days of storage after treatment with CJ-0.25% ($p \leq 0.05$). The lettuce leaf seemed lighter (high L* value) after storage when treated with CJ-0.25%.

The a* values shown in Figure 5B specify the effects of treatments after 10 days of storage at 4 °C. After storage at 4 °C, the lettuce leaf treated with O_3 showed a significant difference ($p \leq 0.05$) in a* value of the leaf (a* from -6.94 to -5.74), which appeared less green even if the a* value remained stable during the entire storage period. The a* value of samples treated with γ-irradiation showed a significant difference ($p \leq 0.05$) after 10 days of storage (a* from -5.3 to -2.97) but values fluctuated during entire storage. In samples treated with CJ-0.25% and the use of the 3 combined treatment samples, no significant change was observed (a* value from -5.28 to -5.86 for CJ-0.25% and from -5.32 to -4.63 for the combined treatments).

The b* value (Figure 5C) specifies the difference in blue and in yellow. The general pattern of almost all treatments observed is that the lettuce leaf initially has a low b* value and then, on day 2, the degree of yellow (B* value) increases, on day 4 return to a low b* value and then the value increases with time until day 10 of storage at 4 °C. The pattern is slightly different for the control and the O_3 treated samples, both of which remained stable during entire storage, with no significant changes ($p > 0.05$). Both samples showed average values of b* (from 22.10 to 23.03 for control and from 21.81 to 22.71 for O_3) during the entire storage time. After 10 days of storage, the γ-irradiated samples did not show any significantly change of b* values (from 21.06 on day 0 to 19.7 on day 10) which indicates that the lettuce was less yellow. For the combination of the three treatments and for samples treated with CJ-0.25%, there was a significant difference after 10 days of storage ($p \leq 0.05$). The b* values of the 3 combined treatments increased from 24.9 to 29.5 on day 10 and samples treated with CJ-0.25% showed an increase of the b* values from 23.5 to 27.9 on day 10 showing that the lettuce leaves were becoming more yellow during storage, both of these treatments exhibited highest b* values during the entire storage.

The H* value (Fig. 5D) shows the difference in hue, showing the difference/similarity from pure red, blue and green. Green is generally between °60 and °180. In control samples, the H* value decreased from 103.3 on day 0 to 101.2 on day 10 which shows that the lettuce leaves became slightly yellow. O_3 treated samples did not show any significant difference in H* values between day 0 (104.5) and day 10 (103.4). However, the γ-irradiated samples showed the increased yellowness of the samples where H* value decreased significantly ($p \leq 0.05$) from 103.5 (day 0) to 98.7 (day 10). The combination of the 3 treatments showed a similar pattern as the γ-irradiation treated samples where the H* value decreased

from 102.5 to 98.3 ($p \leq 0.05$). Samples treated with CJ-0.25% showed a small but significant decrease in H* value ($p \leq 0.05$) from 103.3 to 101.3.

Baur *et al*. (2004) showed that the lettuce samples treated with O_3 at 1 mg/L for 120 sec exhibited an increase in L* and a* values, but a decrease in b* and H* values (Baur *et al.,* 2004).

Prakash *et al.* (2000) showed an increase in a* values (loss of green) and b* values (increasing of yellow), but a decrease in L* values in the irradiated sample after storage during 22 days at 4 °C (Prakash *et al.,* 2000). The authors attributed this to the chlorophyll breakdown that would increase the a* value. This correlates with our research, where we observed a decrease of chlorophyll over time of storage after γ-irradiation treatment. The authors also mentioned that phenolic oxidation and bacterial spoilage can cause darkening of the leaves over time.

Akbas and Ölmez (2007) showed the effect of dipping in organic acid on storage quality of fresh-cut iceberg lettuce (Akbas and Ölmez, 2007). Dipping the lettuce in citric (5 g/L) and lactic (5 mL/L) acid solutions did not change the L*, a*, b* values of lettuce just after dipping. However, after 8 days of storage, significant reductions in b* values were observed, whereas, after 12 days of storage, L* and b* values decreased while a* value increased. Bolin and Huxsoll (1991) also did not observe an increase in a* value.

Sagong *et al*. (2011) reported that a combination of organic acids (malic acid, lactic acid, and citric acid) and ultrasound (5 L at 40 kHz) did not significantly change lettuce quality and L*, a* and b* during more than 7 days of storage at 4°C ($p > 0.05$) (Sagong *et al.,* 2011).

Bolin and Huxsoll (1991) showed that storage temperature influenced the colorimetry parameters of lettuce (Bolin and Huxsoll, 1991). They showed that storage at 10 °C darkened the lettuce samples compared to lettuce stored at 2 °C. In addition, a loss in green pigment (increasing a* value) was observed in samples stored at a higher temperature (10 °C). Green color disappeared (when a* value become 0) on day 11, 20 and 38 during storage of the same products stored at 10, 5, and 2 °C, respectively. In this current study, we stored lettuce at refrigerator temperature (4 °C) explaining why a* values increased, and chlorophyll dropped. Figure 6 shows the pictures of lettuce leaves on day 0 after treatments and on day 10 after treatments.

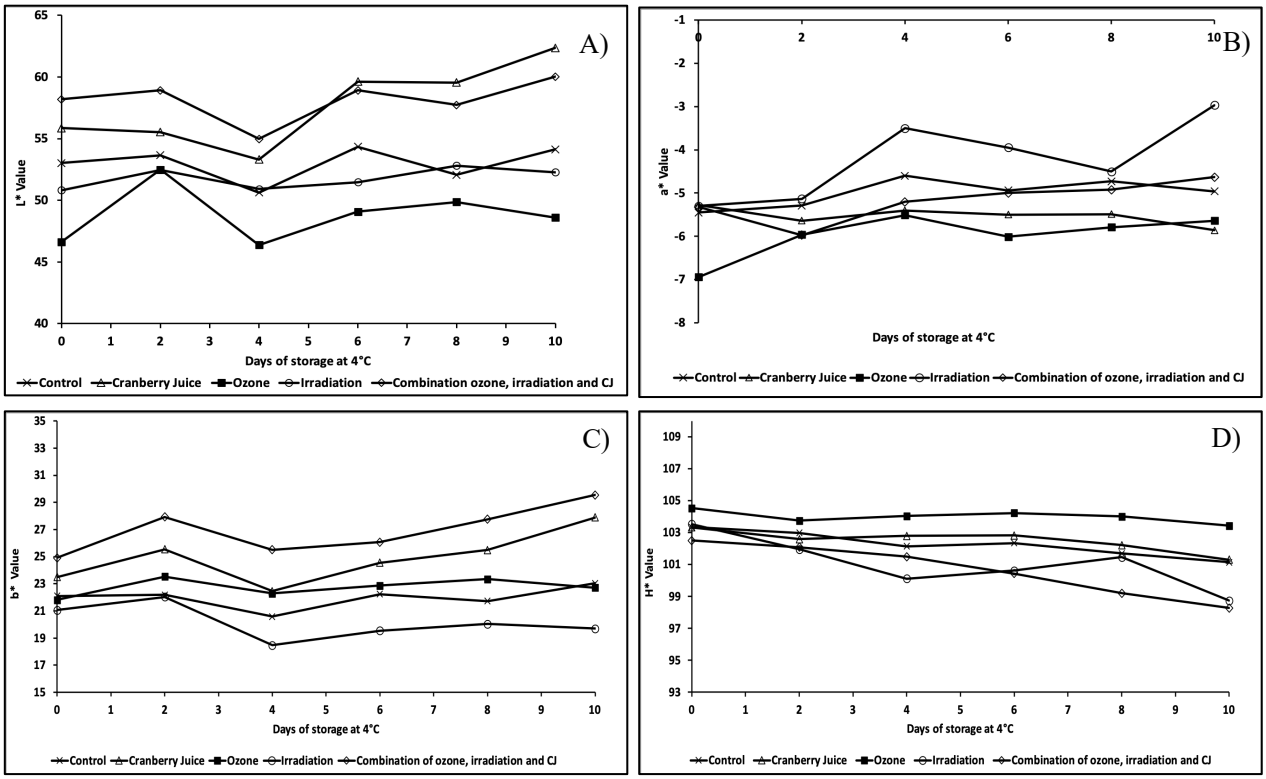

Fig. 5. Effect of CJ coating of CJ-0.25% in combination with O_3 at 5-ppm or γ-irradiation at 1.5 kGy on the color parameters (A) L*, (B) a*, (C) b* and (D) H* of the CIELab color scale of iceberg lettuce samples during storage at 4 °C for 10 days

On Figure 6 are shown the physical differences of the aspect of the lettuce leaves between day 0 and day 10 post-treatment. Overall, the lettuce leaves seemed brighter on day 10 post-treatment. In comparison with Figure 5A, the only treatment that has shown a significant brightening (higher L*) on day 10 is CJ-0.25% used alone. A loss of the green color (negative a* value) should be observed on day 10 after treatment with O_3 only and γ -irradiation only but is not predominant on Figure 6, the only area where a significant loss in green is noticeable is on top of the leaf. A significant increase in yellow (positive b* value) should be observed 10 days after combined treatments and the use of CJ-0.25% alone. On Figure 6, the combination of treatments on lettuce has shown the more gain of yellow after 10 days of storage. The H* value shows the difference in hue, showing the difference/similarity from pure red, blue and green. Green is generally between °60 and °180. On Figure 5D, all treatments induced a decrease in H* value, meaning that the lettuce leaves were more yellow, this is in correlation with Figure 6.

Fig. 6. Visual appearance of iceberg lettuce with various treatments during storage at 4 °C on day 0 (on top) and 10 (on the bottom).

3.6 Effects of treatments and storage time on chlorophyll pigment of iceberg lettuce.

Analysis was made every 2 days for 10 days and results of day 0, 6 and 10 are shown in Table 2. It is important to mention that in this study, chlorophyll analysis is complementary to colorimetry. The colorimetry was an overview of the lettuce leaf and the chlorophyll takes into account the upper part of the leaf during sample. Chlorophyll is the green pigment that captures light energy and transforms it into chemical energy by photosynthesis (Rabinowitch, 1965). A previous study has shown the different parameters that consumers are paying attention to when buying a lettuce. The authors recommended to the grocery merchants to refrigerate lettuce at 4 °C in order to keep the visual appearance and to retain more chlorophyll, because consumers will prefer a brighter green lettuce than a yellowish one (Nam and Kwon, 1997). After grinding 2 g of the piece of lettuce untreated (control) in acetone 80 % on day 0, 9.02 µg/mL of total chlorophyll was detected.

On day 6 and 10, in control samples, the chlorophyll content decreased significantly to 7.91 µg/mL and 3.12 µg/mL, respectively, which represents a reduction of 5.90 µg/mL (65%). Studies showed that storage temperature (4 °C or 10 °C) of *Valeriana* salad caused significant reduction in chlorophyll. After 8 days of storage, a 22 % decrease of the initial amount of chlorophyll was observed on lettuce stored at 4 °C while a 35 % reduction was observed during storage at 10 °C. In our study, after 6 days of storage at 4 °C, the total chlorophyll was reduced by more than 12 % which is correlated with previous studies where chlorophyll naturally decreased during storage at 4 °C (Ferrante and Maggiore, 2007). The total chlorophyll content of leafy vegetables starts declining a few days after harvest and this decrease is

temperature-dependent (Ferrante and Maggiore, 2007). Production of ethylene during storage of lettuce can increase the chlorophyllase activity causing the natural decrease of chlorophyll during storage at 4 °C (Agüero *et al.,* 2008).

The same tendency of chlorophyll reduction was observed after treating the lettuce leaf with O_3 at 5 ppm for 7.5 min. On day 0 after treatment, the chlorophyll was at 10.04 µg/mL which is significantly different from the 3 combined treatments on this day 0 of analysis. On day 6 and 10, it was reduced to 7.56 µg/mL and 3.37 µg/mL, respectively. A research on fresh-cut lettuce showed no significant differences in chlorophyll a or chlorophyll b comparing a control unwashed, a control washed with distilled water and also after treatment of fresh-cut lettuce with O_3 at 12.0 ± 0.5 mg/L during water wash (Karaca and Velioglu, 2014). In fact, the authors found 16.4, 16.1 and 16.4 g/kg dry matter of chlorophyll-a after no washing, washing with distilled water and O_3 wash respectively. For chlorophyll b, they found 7.9, 7.7, 7.9 g/kg dry matter, respectively (Karaca and Velioglu, 2014).

In our study, after CJ (0.25%) treatment of lettuce, total chlorophyll was reduced from 7.60 µg/mL to 4.35 µg/mL on day 6 and increased to 8.38 µg/mL on day 10. Since the chlorophyll content increased after treatments, it is normal that the a* value did not change significantly. Chlorophyll breakdown in the cells would increase a* values (Bolin and Huxsoll, 1991). Some speculations of the increase in chlorophyll observed previously will be explained later in this paragraph.

In the irradiated sample, the total chlorophyll on day 0 was 13.27 µg/mL which significantly reduced to 3.06 µg/mL and 2.53 µg/mL after 6 and 10 days of storage, respectively. Bolin and Huxsoll (1991) proposed that the decrease in green pigmentation would probably result from loss of chlorophyll pigment during storage of lettuce (Bolin and Huxsoll, 1991).

After combining the 3 treatments, on day 0, 5.23 µg/mL of total chlorophyll was observed that increased to 5.64 and to 9.03 µg/mL on days 6 and 10, respectively. Each day of analysis for the combined treatments are not significantly different ($p > 0.05$). Lemoine *et al.* (2008) reported that the combination of irradiation with UV-C light (8 kJ/m^2) and then heating (45 °C, 3 h in air oven) decreased chlorophyll content in control and in treated samples during storage at 20 °C of broccoli florets (Lemoine *et al.,* 2008). According to Agüero *et al.* (2008) the location of chlorophyll sampling on lettuce leaves provides different variations in chlorophyll levels (Agüero *et al.,* 2008). If the sample is taken at the bottom or on the middle of the leaf, a variation of chlorophyll will be observed. On top of the leaf, there is a lot of chlorophyll initially due to easier access to light and O_2. More chlorophyll variation will be found on the top of the leaf will be observed during storage at 4 °C. The authors noted an increase in chlorophyll in the outer zone of the leaf without any treatment between days 5 and 8 and between days 16 to 28. They proposed that the presence of environmental factors such as humidity, acidity, temperature, light, oxygen, ethylene is responsible for the loss of chlorophyll (Agüero *et al.,* 2008). By speculating with the results

shown in this research, it is possible that water movements and water losses may have concentrated chlorophyll in the top of the leaf after our treatment with CJ-0.25% and thus demonstrated an increase in pigment during storage at 4 °C.

Table 2. Evaluation of chlorophyll content (µg/mL) after treatments with O_3 at 5 ppm, CJ-0.25% and γ-irradiation at 1.5 kGy or the combination after storage at 4°C for 10 days

Treatment	Day	Chl. A (ug/mL)	Chl. B (ug/mL)	Chl. Total (ug/mL)
Control	0	$5.80\pm1.19^{ab, B}$	$3.22\pm0.66^{bc, B}$	$9.02\pm1.46^{abc, B}$
	6	$5.21\pm0.69^{b, B}$	$2.70\pm0.48^{b, B}$	$7.91\pm1.12^{b, B}$
	10	$2.59\pm0.31^{a, A}$	$0.53\pm0.34^{a, A}$	$3.12\pm0.62^{a, A}$
O_3 at 5 ppm, 7.5 min	0	$6.89\pm1.48^{ab, B}$	$3.15\pm0.18^{bc, C}$	$10.04\pm1.67^{bc, B}$
	6	$5.26\pm1.32^{b, AB}$	$2.30\pm0.51^{ab, B}$	$7.56\pm1.78^{ab, B}$
	10	$2.49\pm0.52^{a, A}$	$0.88\pm0.16^{a, A}$	$3.37\pm0.66^{a, A}$
CJ-0.25%	0	$5.12\pm1.15^{a, AB}$	$2.48\pm0.63^{ab, A}$	$7.60\pm1.74^{ab, AB}$
	6	$2.82\pm0.64^{ab, A}$	$1.53\pm0.74^{ab, A}$	$4.35\pm1.37^{ab, A}$
	10	$5.81\pm0.69^{b, B}$	$2.57\pm0.49^{b, A}$	$8.38\pm0.97^{b, B}$
γ-irradiation at 1.5 kGy	0	$9.17\pm1.78^{b, B}$	$4.10\pm0.58^{c, B}$	$13.27\pm2.35^{c, B}$
	6	$2.18\pm0.39^{a, A}$	$0.88\pm0.13^{a, A}$	$3.06\pm0.52^{a, A}$
	10	$1.57\pm0.46^{a, A}$	$0.96\pm0.14^{a, A}$	$2.53\pm0.59^{a, A}$
Combination of O_3, CJ-0.25% and γ-irradiation 1.5 kGy	0	$3.68\pm0.94^{a, A}$	$1.55\pm0.59^{a, A}$	$5.23\pm1.34^{a, A}$
	6	$3.83\pm1.42^{ab, A}$	$1.81\pm1.0^{ab, AB}$	$5.64\pm2.39^{ab, A}$
	10	$5.73\pm1.58^{b, A}$	$3.30\pm0.19^{b, B}$	$9.03\pm1.52^{b, A}$

For each treatment, means followed by the same lowercase letter (a, b, c) for the comparison of days of analysis (0, 6, 10), are not significantly different ($p > 0.05$). For each day of analysis, means followed by the same uppercase letter (A, B, C) for the comparison of treatments are not significantly different ($p > 0.05$). *Chl.b = chlorophyll b; Chl.a = chlorophyll a; Ch. Total = total chlorophyll.

3.7 Effects of treatments on texture of iceberg lettuce.

The texture of lettuce leaves was analyzed every two days for 10 days (Table 3). Results showed that after the measuring texture of control samples without treatment, it was found to be 1.43, 0.80 and 1.13 N/mm for day 0, 6, and 10, respectively which is not significantly different ($p > 0.05$). In another

study, storage temperatures of 2, 5, and 10 °C did not affect the texture of the shredded lettuce (Bolin *et al.,* 1977). Similarly, in our study the texture of lettuce without treatment stored at 4 °C did not change significantly.

After O_3 at 5 ppm treatment, 7.5 min, the texture was 1.54, then 1.43 and 1.14 N/mm for day 0, 6 and 10 respectively, with no significant difference ($p > 0.05$). Baur *et al.* (2004) performed a similar experiment on shredded iceberg lettuce where they used ozonated water containing 1 mg/L O_3 on lettuce and stored at 4 °C for 7 days (Baur *et al.,* 2004). They found that the crispness of all samples dropped during storage (Baur *et al.,* 2004) but no significant changes were observed in the texture of samples washed with O_3. Fan *et al.* (2003) also did not find significant changes in measurement of firmness of iceberg lettuce after 14 days of storage at 3 °C; these results are in agreement with this current study (Fan *et al.,* 2003).

After CJ-0.25% treatment, the texture on day 0 was 1.12 N/mm and it increased to 1.31 N/mm on day 6 and remained at 1.31 N/mm on day 10, without a significant difference ($p > 0.05$). Another report showed that there was no significant change in texture observed in samples of lettuce dipped in lactic acid at 5g/L (pH 2.3) and citric acid at 5 mL/L (pH 2.4) (Akbas and Ölmez, 2007). The authors mentioned that firmness could decrease by loss of cell turgor due to water loss during storage.

Irradiation at 1.5 kGy resulted in a texture of 1.44 N/mm on day 0, which decreased to 1.00 on day 6 and then increased to 1.28 N/mm on day 10 after irradiation treatment, representing a non-significant change ($p > 0.05$). Prakash *et al. (*2000*)* demonstrated that irradiation of romaine lettuce at doses of 0.15 kGy and 0.35 kGy and storage for 21 days reduced the firmness of food during storage (Prakash *et al.,* 2000).

After combining the three treatments, the texture of the lettuce did not change significantly during storage ($p > 0.05$). The texture values of lettuce on day 0, 6 and 10 were 1.11, 1.15 and 1.07 N/mm, respectively. Overall there is no difference in texture applied by the treatments compared to the control without treatments during entire storage at 4°C. Baur *et al.* (2004) found no significant changes in texture after 9 days of storage of shredded iceberg lettuce after washing with water, chlorine-water and ozonated water (Baur *et al.,* 2004). Also, Sagong *et al.* (2011) showed that the combination of organic acids and ultrasound did not significantly change lettuce texture during more than 7 days of storage at 4°C ($p > 0.05$) (Sagong *et al.,* 2011).

Table 3. Evaluation of texture (N/mm) after treatments with O_3 at 5 ppm, CJ-0.25% and γ-irradiation at 1.5 kGy or the combination after storage at 4°C for 10 days

Treatment	Day	Texture (N/mm)

Control	0	1.43±0.44[a, A]
	6	0.80±0.22 [a, A]
	10	1.13±0.04 [a, A]
O_3 at 5 ppm, 7.5 min	0	1.54±0.43[a, A]
	6	1.43±0.33[b, A]
	10	1.14±0.06 [a, A]
CJ-0.25%	0	1.12±0.13[a, A]
	6	1.31±0.33 [ab, A]
	10	1.31±0.10 [a, A]
γ-irradiation at 1.5 kGy	0	1.44±0.22[a, A]
	6	1.00±0.12 [ab, A]
	10	1.28±0.50 [a, A]
Combination of O_3, CJ-0.25%, and γ- irradiation 1.5 kGy	0	1.11±0.16[a, A]
	6	1.15±0.32 [ab, A]
	10	1.07±0.20 [a, A]

For each treatment, means followed by the same lowercase letter (a, b, c) for the comparison of days of analysis (0, 6, 10), are not significantly different ($p > 0.05$). For each day of analysis, means followed by the same uppercase letter (A, B, C) for the comparison of treatments are not significantly different ($p > 0.05$).

3.8 Effects of treatments on sensory analysis of lettuce with storage at 4 °C for 5 days.

Sensory analysis was performed on lettuce on three groups: (1) control, (2) CJ-0.25% treated samples and (3) combination of O_3 at 5ppm, 7.5 min, CJ-0.25%, and γ-irradiation at 1.5 kGy. The color, the smell, the texture, and the taste parameters at day 0, 3 and 5 of storage at 4°C were recorded in Table 4.

Results showed that control's color changes significantly from day 0 to day 5 from 7.87, 7.47 and 6.27 for day 0, 3 and 5, respectively. There was a change in the color of lettuce without treatment observed by panelists. The analysis of smell on control was not significantly different for day 0, 3 and 5 with values of 7.67, 7.73 and 7.33, respectively. The texture of iceberg lettuce did not significantly change over the storage of lettuce at 4 °C, from 8.20 to 8.27 and finally to 8.20 for day 0, 3 and 5, respectively. The taste of lettuce without treatment significantly changed ($p \leq 0.05$) from day 0 to day 3 and 5 with values 8.40, 8.0 and 7.07, respectively. The panelists found that the taste was not fresh after 5 days of storage of iceberg lettuce at 4 °C.

The CJ-0.25% treated samples were also tested for sensory analysis during storage at 4 °C after treatment. Results showed that color did not significantly change throughout the storage with values of 7.60, 7.87 and 7.47 on days 0, 3 and 5, respectively. The smell of lettuce after CJ-0.25% treatment did not significantly change during storage but an increase in the evaluation was observed. The level of appreciation on day 0, was 7.47, on day 3 it was 7.67 and finally, on day 5, it was 8.00. The texture of lettuce after CJ-0.25% treatment did not significantly change during storage and a level of appreciation of 8.00, 7.80 and 8.27 were observed at day 0, 3 and 5, respectively. The taste did not significantly change, and a level of appreciation of 7.67, 7.60 and 8.07 on days 0, 3 and 5, was observed, respectively. On day 5, the highest level of appreciation was observed for CJ-0.25% treated samples on all parameters (color, smell, taste, and texture).

The combination of the three treatments was tested for sensory analysis during 5 days of storage. The results showed that the color did not significantly change, and values of 7.07, 6.93 and 6.27 were observed on day 0, 3 and 5, respectively. The smell of lettuce did not change during storage and the values were 8.00, 7.80 and 7.60 after day 0, 3 and 5, respectively. The texture decreased slightly over time, but it was not significant ($p > 0.05$). The level of appreciation was 7.53, 7.27 and 7.20 after day 0, 3 and 5, respectively. Finally, the taste did not change significantly during storage at 4°C after the 3 combined treatment and the level of appreciation were 7.07, 6.53 and 7.20 after day 0, 3 and 5 of storage, respectively.

Samples treated with the 3 combined treatments after 5 days of storage were better than control for smell and taste parameters and had the same color grade as the control. No significant changes ($p > 0.05$) were observed in color, taste, texture and taste parameters between the different treatments (groups) for the same day of analysis. Zhang *et al.* (2006) showed that γ-irradiation at 1.5 kGy slightly affected the color of lettuce after 8 days of storage at 4°C (Zhang *et al.,* 2006). In general, lettuce leaves irradiated at doses of 0 and 0.5 kGy were the least appreciated and the lettuce leaves irradiated at 1.0 kGy had the optimal effects on the sensory quality of lettuce (Zhang *et al.,* 2006). Another study tested the effects of an organic acid like Peroxyacetic acid (PAA) at two concentrations of 80 mg/L and 250 mg/L on iceberg lettuce. None of the treatments affected the sensory quality of iceberg lettuce when compared with water washing samples (Vandekinderen *et al.,* 2009). Acetic acid seems to be an optimal choice, considering it increases shelf life and adds an extra flavor in food. A study on marinated mussels evaluated the antiviral effects of acetic acid-based marinade against HAV, NoV and FCV. A reduction from 10.3 log $TCID_{50}$/mL of FCV in a PBS-based solution to 7.8 log $TCID_{50}$/mL was observed, and a reduction from 10.3 log $TCID_{50}$/mL of FCV in an acetic acid marinade to < 1.6 log $TCID_{50}$/mL was observed after 1 week of marination (Hewitt and Greening, 2004) showing the efficiency of acetic acid.

Table 4. Sensory analysis after treatments with combination of O_3 at 5 ppm, CJ-0.25% and γ-irradiation at 1.5 kGy, CJ-0.25% alone and control after storage at 4°C for 10 days

Parameter	Day 0	Day 3	Day 5
Color			
Control	$7.87 \pm 1.36^{a, B}$	$7.47 \pm 1.68^{a, AB}$	$6.27 \pm 1.67^{a, A}$
CJ-0.25%	$7.60 \pm 1.40^{a, A}$	$7.87 \pm 1.68^{a, A}$	$7.47 \pm 1.46^{a, A}$
O_3, γ- irradiation and CJ-0.25%	$7.07 \pm 1.49^{a, A}$	$6.93 \pm 1.39^{a, A}$	$6.27 \pm 1.87^{a, A}$
Smell			
Control	$7.67 \pm 1.11^{a, A}$	$7.73 \pm 1.10^{a, A}$	$7.33 \pm 1.72^{a, A}$
CJ-0.25%	$7.47 \pm 1.55^{a, A}$	$7.67 \pm 1.54^{a, A}$	$8.00 \pm 1.00^{a, A}$
O_3, γ- irradiation and CJ-0.25%	$8.00 \pm 0.85^{a, A}$	$7.80 \pm 1.15^{a, A}$	$7.60 \pm 1.68^{a, A}$
Texture			
Control	$8.20 \pm 1.26^{a, A}$	$8.27 \pm 0.88^{a, A}$	$8.20 \pm 1.15^{a, A}$
CJ-0.25%	$8.00 \pm 1.00^{a, A}$	$7.80 \pm 1.32^{a, A}$	$8.27 \pm 1.28^{a, A}$
O_3, γ- irradiation and CJ-0.25%	$7.53 \pm 1.30^{a, A}$	$7.27 \pm 1.33^{a, A}$	$7.20 \pm 1.47^{a, A}$
Taste			
Control	$8.40 \pm 0.83^{a, B}$	$8.0 \pm 1.00^{a, AB}$	$7.07 \pm 1.33^{a, A}$
CJ-0.25%	$7.67 \pm 1.05^{a, A}$	$7.60 \pm 1.30^{a, A}$	$8.07 \pm 1.22^{a, A}$
O_3, γ -irradiation and CJ-0.25%	$7.07 \pm 2.12^{a, A}$	$6.53 \pm 1.41^{a, A}$	$7.20 \pm 1.52^{a, A}$

For each day of analysis, means followed by the same lowercase letter (a, b, c) for the comparison of treatments (control, CJ-0.25% and combination) are not significantly different ($p > 0.05$). For each treatment, means followed by the same uppercase (A, B, C) letter for the comparison of days of analysis (0-3-5), are not significantly different ($p > 0.05$).

In conclusion, it was shown that the combination of the 3 treatments was the most effective against viruses during 10 days of storage at 4 °C, against total flora for more than 13 days of storage at 4 °C, without affecting the overall quality of lettuce. Irradiation alone at 1.5 kGy was also effective against total flora of lettuce, reducing it to 2 log CFU/g. An irradiation dose of more than 7 kGy would be needed to completely inhibit FCV virus. The combination of the 3 treatments reduced the viral titer by 2.15 log $TCID_{50}$/mL whereas, irradiation alone at 1.5 kGy reduced it by 1.63 log $TCID_{50}$/mL after 10 days of storage, representing a gain of efficiency of more than 25%. There was no significant change in texture after treatments during 10 days of storage at 4 °C. After combining the 3 treatments, the quantity of chlorophyll pigment remained stable after 10 days of storage.

4 ACKNOWLEDGMENTS

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), discovery program. A.G. is a recipient of NSERC and Armand-Frappier Foundation fellowship. The authors wish to thank Atoka Cranberries Inc. (Manseau, QC, Canada) for providing CJ. The authors are grateful to Nordion Int. for the irradiation operations. The authors are thankful for the help from Dr. Suresh Pillai (Director of the National Center for Electron Beam Research at Texas A&M University) for the protocol development for virus titration. The assistance of International Atomic Energy Agency (IAEA) is also acknowledged. This research is a part of the CRP on radiation inactivation of biohazards (code F23033).

5 REFERENCES

1. Agriculture - Province of Manitoba. (2019). Ozonation in Food Applications. Available at : https://www.gov.mb.ca/agriculture/food-safety/at-the-food-processor/ozonation.html. Accessed 17 March 2019.
2. Agüero, M. V., Barg, M. V., Yommi, A., Camelo, A., and Roura, S. I. (2008). Postharvest changes in water status and chlorophyll content of lettuce (Lactuca sativa L.) and their relationship with overall visual quality. *Journal of Food Science*, 73: S47-S55.
3. Adams, M., Moss, M. and McClure, P. (ed. 4th). (2016). Food Microbiology. 4th edition. Royal Society of Chemistry. Cambridge, United Kingdom.
4. Akbas, M. Y., and Ölmez, H. (2007). Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. *Journal of the Science of Food and Agriculture*, 87:2609-2616.
5. Aw, T. G., Wengert, S., and Rose, J. B. (2016). Metagenomic analysis of viruses associated with field-grown and retail lettuce identifies human and animal viruses. *International Journal of Food Microbiology*, 223:50-56.
6. Baur, S., Klaiber, R., Hammes, W. P., and Carle, R. (2004). Sensory and microbiological quality of shredded, packaged iceberg lettuce as affected by pre-washing procedures with chlorinated and ozonated water. *Innovative Food Science & Emerging Technologies*, 5:45-55.
7. Bidawid, S., Farber, J. M., and Sattar, S. A. (2000). Rapid concentration and detection of hepatitis A virus from lettuce and strawberries. *Journal of Virological Methods*, 88*:*175-185.
8. Bolin, H. R., and Huxsoll, C. C. (1991). Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *Journal of Food Science*, 56:60-62.
9. Bolin, H. R., Stafford, A. E., King Jr, A. D., and Huxsoll, C. C. (1977). Factors affecting the storage stability of shredded lettuce. *Journal of Food Science*, 42:1319-1321.

10. Brié, A., Razafimahefa, R., Loutreul, J., Robert, A., Gantzer, C., Boudaud, N., and Bertrand, I. (2017). The effect of heat and free chlorine treatments on the surface properties of murine norovirus. *Food and Environmental Virology*, 9:149-158.
11. Canadian Food Inspection Agency. (2019). *Irradiated foods*. Available at: http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/labelling/for-industry/irradiated-foods/eng/1334594151161/1334596074872#tc_fi. Accessed 17 Mar. 2019.
12. Centers for Disease Control and Prevention (CDC). (2019). Available at: https://www.cdc.gov/norovirus/preventing-infection.html. Accessed 9 May 2019.
13. Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J. F., and Lacroix, M. (2011). Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22:1413-1418.
14. Darling, A. J., Boose, J. A., & Spaltro, J. (1998). Virus assay methods: accuracy and validation. *Biologicals*, 26 : 105-110.
15. Elvis, A. M., and Ekta, J. S. (2011). Ozone therapy: A clinical review. *Journal of Natural Science, Biology and Medicine*, 2 :66.
16. Fan, X., and Sokorai, K. J. B. (2008). Retention of quality and nutritional value of 13 fresh-cut vegetables treated with low-dose radiation. *Journal of Food Science*, 73 : S367-S372.
17. Fan, X., Guan, W., and Sokorai, K. J. (2012A). Quality of fresh-cut Iceberg lettuce and spinach irradiated at doses up to 4 kGy. *Radiation Physics and Chemistry*, 81:1071-1075.
18. Fan, X., Sokorai, K. J., Niemira, B. A., Mills, R. S., and Zhen, M. Y. (2012B). Quality of gamma ray-irradiated iceberg lettuce and treatments to minimize irradiation-induced disorders. *HortScience*, 47:1108-1112.
19. Fan, X., Toivonen, P.M., Rajkowski, K. T., and Sokorai, K. J. (2003). Warm water treatment in combination with modified atmosphere packaging reduces undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. *Journal of Agricultural and Food Chemistry*, 51:1231-1236.
20. Feng, K., Divers, E., Ma, Y., and Li, J. (2011). Inactivation of human norovirus surrogate, human norovirus virus-like particle, and vesicular stomatitis virus by gamma irradiation: sensitivity and mechanism. *Applied and Environmental Microbiology,*77 :3507-3517.
21. Ferrante, A., and Maggiore, T. (2007). Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. *Postharvest Biology and Technology*, 45:73-80.
22. Franck, K. T., Lisby, M., Fonager, J., Schultz, A. C., Böttiger, B., Villif, A., ... and Ethelberg, S. (2014). Sources of calicivirus contamination in foodborne outbreaks in Denmark, 2005–2011—the role of the asymptomatic food handler. *The Journal of Infectious Diseases*, 211 :563-570.

23. Gibriel, A. Y., Al-Sayed, H. M., Rady, A. H., and Abdelaleem, M. A. (2013). Synergistic antibacterial activity of irradiated and nonirradiated cumin, thyme and rosemary essential oils. *Journal of Food Safety*, 33:222-228.
24. Giroux, M., Ouattara, B., Yefsah, R., Smoragiewicz, W., Saucier, L., and Lacroix, M. (2001). Combined effect of ascorbic acid and gamma irradiation on microbial and sensorial characteristics of beef patties during refrigerated storage. *Journal of Agricultural and Food Chemistry*, 49:919-925.
25. Heidelbaugh, N. D., and Giron, D. J. (1969). Effect of processing on recovery of polio virus from inoculated foods. *Journal of Food Science*, 34 : 239-239.
26. Hewitt, J., and Greening, G. E. (2004). Survival and persistence of norovirus, hepatitis A virus, and feline calicivirus in marinated mussels. *Journal of Food Protection*, 67 : 1743-1750.
27. Hirneisen, K. A., Markland, S. M., and Kniel, K. E. (2011). Ozone inactivation of norovirus surrogates on fresh produce. *Journal of Food Protection*, 74:836-839.
28. Jensen, H. D., Struve, C., Christensen, S. B., and Krogfelt, K. A. (2017). Cranberry juice and combinations of its organic acids are effective against experimental urinary tract infection. *Frontiers in Microbiology*, 8 :542.
29. Karaca, H., and Velioglu, Y. S. (2014). Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach and parsley. *Postharvest Biology and Technology*, 88:46-53.
30. Katzenelson, E., Kletter, B., and Shuval, H. I. (1974). Inactivation kinetics of viruses and bacteria in water by use of ozone. *Journal of the American Water Works Association*, 66:725-729.
31. Khadre, M. A., Yousef, A. E., and Kim, J. G. (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science*, 66 :1242-1252.
32. Kim BH, Jang A, Lee SO, Min JS, and Lee M. (2004). Combined effect of electron-beam (beta) irradiation and organic acids on shelf-life of pork loins during cold storage. *Journal of Food Protection,* 67:168–171
33. Kniel, K. E., Montville, T. J., and Matthews, K. R. (ed. 4). (2017). Food Microbiology: An Introduction. American Society Microbiology; 4th edition. Washington, DC.
34. Konowalchuk, J., and Speirs, J. I. (1975). Survival of enteric viruses on fresh vegetables. *Journal of Milk and Food Technology,* 38:469–472.
35. Koopmans, M., and Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90 : 23-41.
36. Koseki, S., Yoshida K, Isobe S, and Itoh. K. (2001). Decontamination of lettuce using electrolyzed water. *Journal of Food Protection,* 64:652-658.
37. Kurdziel, A. S., Wilkinson, N., Langton, S., and Cook, N. (2001). Survival of poliovirus on soft fruit and salad vegetables. *Journal of Food. Protection*, 64:706-709.

38. Lee, M. H., Yoo, S. H., Ha, S. D., and Choi, C. (2012). Inactivation of feline calicivirus and murine norovirus during Dongchimi fermentation. *Food Microbiology*, 31:210-214.
39. Lemoine, M. L., Civello, P. M., Chaves, A. R., and Martínez, G. A. (2008). Effect of combined treatment with hot air and UV-C on senescence and quality parameters of minimally processed broccoli (Brassica oleracea L. var. Italica). *Postharvest Biology and Technology,* 48:15-21.
40. Leuenberger, S., Widdowson, M. A., Feilchenfeldt, J., Egger, R., and Streuli, R. A. (2007). Norovirus outbreak in a district general hospital--new strain identified. *Swiss Medical Weekly*, 137 :57-61.
41. Lichtenthaler, H. K., and Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions,* 11:591 – 592.
42. MAPAQ. Bilan des toxi-infections alimentaires du 1er avril 2013 au 31 mars 2014 (2014). Available at : https://www.mapaq.gouv.qc.ca/fr/Publications/Bilan_Toxi-infection_2013-2014.pdf. Accessed 15 November 2019.
43. MAPAQ. Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire (2006). Available at: https://www.mapaq.gouv.qc.ca/fr/Publications/recueil.pdf. Accessed 12 August 2019.
44. Mescle, J-F. Zucca, J. and Bourgeois, C-M. (Ed.1). (1988). Microbiologie Alimentaire: Tome 1, Aspect microbiologique de la sécurité et de la qualité des aliments. *Tec & Doc*, Lavoisier, Apria. 1st edition. Paris, France.
45. Monk, J. D., Beuchat, L. R., and Doyle, M. P. (1995). Irradiation inactivation of food-borne microorganisms. *Journal of Food Protection*, 58 :197-208.
46. Nam, S., and Kwon, Y. (1997, October). Quality changes of hydroponically produced leaf lettuce (Lactuca sativa L., cv. Cheongchima) during postharvest storage. In *International Symposium on Vegetable Quality of Fresh and Fermented Vegetables,* 483: 173-184.
47. Park, S. Y., and Ha, S. D. (2017). Application of gamma radiation for the reduction of norovirus and the quality stability in optimally ripened cabbage kimchi. *Food Research International*, 100 :277-281.
48. Prakash, A., Guner, A. R., Caporaso, F., and Foley, D. M., (2000). Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. *Journal of Food Science*, 65:549-553.
49. Rabinowitch, E. I. (1965). The role of chlorophyll in photosynthesis. *Scientific American*, 213:74-83.
50. Sagong, H. G., Lee, S. Y., Chang, P. S., Heu, S., Ryu, S., Choi, Y. J., and Kang, D. H. (2011). Combined effect of ultrasound and organic acids to reduce Escherichia coli O157: H7, Salmonella

Typhimurium, and Listeria monocytogenes on organic fresh lettuce. *International Journal of Food Microbiology*, 145:287-292.

51. Shin, G. A., and Sobsey, M. D. (2003). Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. *Applied and Environmental Microbiology*, 69:3975-3978.
52. Su, X., and D'Souza, D. H. (2011). Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology,* 77: 3982-3987.
53. Su, X., Howell, A. B., and D'Souza, D. H. (2010). Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. *Food Microbiology*, 27 :985-991.
54. Su, X., and D'Souza, D. H. (2013). Grape seed extract for foodborne virus reduction on produce. *Food Microbiology*, 34 :1-6.
55. Theron, M. M., and Lues, J. R. (Ed. 1). (2010). *Organic acids and food preservation*. CRC Press. Florida, United States.
56. Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., and Gerba, C. P. (2005). Inactivation of enteric adenovirus and feline calicivirus by ozone. *Water Research*, 39: 3650-3656.
57. Vandekinderen, I., Van Camp, J., De Meulenaer, B., Veramme, K., Bernaert, N., Denon, Q., ... and Devlieghere, F. (2009). Moderate and high doses of sodium hypochlorite, neutral electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide did not affect the nutritional and sensory qualities of fresh-cut iceberg lettuce (Lactuca sativa Var. capitata L.) after washing. *Journal of Agricultural and Food Chemistry*, 57: 4195-4203.
58. Ward, B. K. & Irving, L. G. (1987). Virus survival on vegetables spray-irrigated with wastewater. *Water Research*, 21:57-63.
59. Widdowson, M.A., Cramer, E.H., Hadley, L., Bresee, J.S., Beard, R.S., Bulens, S.N., Charles, M., Chege, W., Isakbaeva, E., Wright, J.G., Mintz, E., Forney, D., Massey, J., Glass, R.I., and Monroe, S.S., (2004). Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of Norovirus -- United States, 2002. *Journal of Infectious Diseases,* 190: 27-36.
60. Wysok, B., Uradziński, J., and Gomólka-Pawlicka, M. (2006). Ozone as an alternative disinfectant-a review. *Polish Journal of Food and Nutrition Sciences*, 15:3.
61. Yuk, H. G., Yoo, M. Y., Yoon, J. W., Moon, K. D., Marshall, D. L., and Oh, D. H. (2006). Effect of combined ozone and organic acid treatment for control of Escherichia coli O157: H7 and Listeria monocytogenes on lettuce. *Journal of Food Science*, 71: M83-M87.
62. Zhang, L., Lu, Z., and Wang, H. (2006). Effect of gamma irradiation on microbial growth and sensory quality of fresh-cut lettuce. *International Journal of Food Microbiology*, 106:348-351.

Chapitre 5: Discussion générale

1.1. Discussion générale *in-vitro*

1.1.1. Traitements seuls

Au cours de cette étude, l'effet antiviral du JC et du BS, seul ou en combinaison avec l'irradiation-γ, contre le virus FCV-F9 à l'aide de cellules hôte CRFK a été étudié. L'activité antivirale du JC et du BS s'est avérée être dépendante de la concentration et puis une augmentation de la concentration semblait plus efficace contre le virus, ce qui est similaire aux résultats précédemment rapportés pour les acides organiques (Su *et al.,* 2010). Les mécanismes d'action des acides organiques et des polyphénols contre les analogues du NoV ne sont pas encore totalement compris, mais certaines hypothèses ont insinué que ce mécanisme pourrait être attribué à l'altération de la capside virale et de l'acide nucléique dû à l'acidité environnante et au bas pH (Cao, 2013).

Lors de cette étude, une réduction de 2.20 log de $TCID_{50}$ / mL du titre viral initial de FCV-F9 a été obtenue à CJ-1% *in-vitro*. Su *et al.* (2010) ont testé les effets du JC sur le FCV-F9 à des temps d'exposition allant de 0 à 60 minutes à température ambiante. Une réduction du titre viral de 5 log_{10} UFP (Unité formatrice de plaque) / mL après 30 minutes de traitement par le JC (pH 2.7 et 7.0) a été observée. De plus, une réduction de 2.04 log $TCID_{50}$ / mL du titre viral FCV-F9 a été observée à une concentration de BS-0.1%. Le BS contient des acides organiques de type citrique, ascorbique et lactique. L'acide ascorbique (connu comme la vitamine C) peut inactiver un large spectre de virus *in-vitro*, tels que l'herpès simplex, la vaccine, la fièvre aphteuse, la rage, la mosaïque du tabac et les virus bactériens (Jungeblut, 1935). Macinga *et al.* (2008) ont aussi testé l'effet d'un désinfectant à base d'éthanol contenant un mélange synergique de polymère de polyquaternium (PQ-37) et d'acide organique (acide citrique). Ces résultats ont montré une réduction supérieure à 3 log_{10} UFP / mL après une exposition contre le MNV-1 et le FCV-F9 pendant 30 secondes. La PQ-37 et l'acide citrique maximisent ensemble l'activité contre les virus non-enveloppés des mammifères en raison de leur forte acidité. Les rayons-γ peuvent interagir avec les acides nucléiques via la production de radicaux libres, d'oxygène et de radicaux hydroxyles (Park and Ha, 2017). En conclusion, l'utilisation seule des trois traitements contre le FCV-F9 semblait efficace contre le virus et des concentrations optimales de JC et de BS ont été ensuite testées en combinaison avec l'irradiation-γ.

1.1.2. Traitements combinés

Les combinaisons de JC ou de BS avec l'irradiation-γ ont montré une synergie contre le FCV-F9 et plus la concentration de BS ou JC utilisée était faible et plus la valeur de la radiosensibilité était élevée. Aux concentrations les plus faibles d'acides organiques contre le virus, des radiorésistances étaient observés. La radiorésistance observée aux plus hautes concentrations d'acides organiques utilisées peut

être expliquée de plusieurs façons. Premièrement, les « *scavengers* » d'espèces réactives de l'oxygène (ROS) peuvent réagir avec les radicaux libres (R-OH) produits par irradiation-γ et ainsi protéger des dommages des rayons sur la cellule infectée par le virus normalement attaqué par les radicaux libres (Thornley, 1963). La radiorésistance peut également être causée par les composants chimiques présents dans le substrat. Par exemple, les alcools, les glucides et les protéines augmentent la radiorésistance des microorganismes (Lawrence and Stanton, 1968). Les levures sont également radiorésistantes en raison de leurs productions d'acides organiques tels que l'acide lactique, l'acide acétique et les alcools qui agissent comme des *scavengers* pour protéger les levures des radicaux libres produits par irradiation-γ (Aquino, 2011). Le JC utilisé seul est plus efficace que le BS utilisé seul contre le virus FCV-F9 *in-vitro*. De plus, la réduction du titre viral était maximale avec les concentrations plus élevées de JC (CJ-1%) ou de BS (BS-0.10%) utilisé seul.

1.2. Discussion générale *in-situ*

1.2.1. Traitements seuls antiviraux sans stockage à 4 °C

Les effets des traitements seuls avec l'irradiation-γ, le JC à plusieurs concentrations et l'O_3 à 5-ppm durant plusieurs temps d'exposition ont été évalués à la surface de la laitue sans entreposage à 4°C. Feng *et al.* (2011) ont testé l'effet d'une irradiation-γ à 5.6 kGy contre le virus de la stomatite vésiculaire (VSV) et le MNV-1 inoculé sur des aliments frais et ont constaté que le VSV était plus susceptible à l'irradiation que le MNV-1 en raison de la taille de son génome. Les tailles respectives du génome viral du VSV et du MNV-1 sont respectivement de 11 et 7 kpb. Les rayons-γ interagissent avec les acides nucléiques via les radicaux libres et via une interaction directe avec l'ADN. De plus, ce type de rayons peut briser des liaisons covalentes et non-covalentes comme les liaisons hydrogènes, les liaisons ioniques, les forces van der Waals et les liaisons hydrophobes, qui sont essentielles pour les structures protéiques (Park and Ha, 2017).

De plus, diverses concentrations de JC (de 0.1 % à 1.5 %) ont été utilisées contre le FCV-F9 sur une feuille de laitue iceberg. Le titre viral initial présent sur la laitue était de 5.70 log $TCID_{50}$ / mL. Après traitement, des réductions jusqu'à 5.12, 4.99, 4.99, 4.87, 4.85 à 4.80 log $TCID_{50}$ / mL avec des concentrations respectives de JC de CJ-0.1%, CJ-0.25%. CJ-0.50%, CJ-0.75%, CJ-1.0% et CJ-1.5% ont été observées. Le mécanisme d'action du JC contre les virus n'est pas encore totalement compris, mais pourrait être dû à l'altération des protéines de la capside à cause de l'acidité engendrée par le bas pH. Divers temps d'exposition variants de 0 à 15 minutes d'O_3 à une concentration de 5-ppm ont été utilisés pour réduire le titre viral du FCV sur la laitue iceberg. La charge virale initiale était de 5.54 log $TCID_{50}$ / mL à la surface de la laitue et, après traitement avec de l'O_3 aux temps de 2.5, 5.0, 7.5, 10 et 15 minutes, des réductions à 4.99, 4.74, 4.62, 4.57 et 4.41 log $TCID_{50}$ / mL ont été respectivement observées.

Hirneisen *et al.* (2011) ont montré qu'une exposition de 5 à 10 minutes avec 6.25 ppm d'O_3 (0.9 g d'O_3 / h à un débit de 2.4 L / min) était nécessaire pour réduire les titres viraux du FCV-F9 sur de la laitue et des oignons verts. Après un traitement avec de l'O_3 durant 10 minutes, le titre viral du FCV était réduit de 3.08 log $TCID_{50}$ / g sur la laitue et de 2.02 log $TCID_{50}$ / g sur les oignons verts. Les auteurs ont attribué ces résultats à la composition organique des oignons verts et de la laitue, car l'O_3 réagit avec les composés organiques complexes des aliments en raison de son potentiel d'oxydation élevé.

1.2.2. Traitements combinés antiviraux sans stockage à 4 °C

Par la suite, des combinaisons entre le JC et l'irradiation ont été testées sans entreposage contre le virus FCV-F9 à la surface de la laitue. Le CJ-0.25% a été utilisé en combinaison avec les doses d'irradiation de 0, 0.25, 0.5, 0.75, 1 et 1.5 kGy. La charge virale initiale sur la laitue était de 6.05 log $TCID_{50}$ / mL. Des réductions de 0.02 à 0.98 log $TCID_{50}$ / mL pour des doses allant de 0.25 à 1.5 kGy après traitement en combinaison avec CJ-0.25% ont été observées. Une réduction plus élevée a été observée à 1.5 kGy avec le CJ-0.1%. La valeur D_{10} du CJ-0.25% est 1.33 kGy et la valeur D_{10} du contrôle est 2.0 kGy, de sorte que la radiosensibilité relative est égale à 1.5. À ce jour, il n'existe pas beaucoup d'études associant des acides organiques et de l'irradiation γ contre le NoV à la surface d'aliments frais. Certaines études évaluent cette combinaison d'aliments contaminés par des bactéries. Une de ces études a montré que la combinaison de l'irradiation avec les acides organiques était préférable pour décontaminer la surface de la viande. En fait, les acides organiques et l'irradiation combinés étaient plus efficaces contre les numérations microbiennes lors du stockage de porc (Kim *et al.,* 2004).

1.2.3. Traitements seuls ou combinés antiviraux avec stockage à 4 °C

Après l'inoculation virale du virus FCV-F9 à ~ 7 log $TCID_{50}$ / mL sur la laitue iceberg, les traitements ont été effectués et testés durant le stockage durant 10 jours à 4 °C. En effet, au jour 0, la laitue sans traitement (contrôle) présentait des titres viraux significativement plus élevés ($p \leq 0.05$) que tous les autres traitements. Après le traitement avec du CJ-0.25% seul, le titre viral sur la laitue a été réduit de 1 log $TCID_{50}$ / mL. Lorsqu'il a été traité avec de l'O_3 à 5-ppm pendant 7.5 minutes, une réduction de 0.96 log $TCID_{50}$ / mL a été observée et quand le virus a été traité avec l'irradiation seule à 1.5 kGy, une réduction de 1.22 log $TCID_{50}$ / mL a été mesurée. Durant ce temps d'entreposage, la combinaison des trois traitements a montré la réduction la plus faible du titre viral, avec seulement 0.91 log $TCID_{50}$ / mL de réduction. Une étude sur de la laitue romaine a montré une réduction instantanée du titre viral du virus de l'hépatite A (HAV) inoculé initialement à 6.90 ± 0,04 log PFU / mL après irradiation à des doses allant de 1 à 10 kGy. Après irradiation à 1, 2, 3 kGy, des réductions de 6.90, 6.20 et 5.60 log PFU / mL de HAV ont été respectivement observées sur la laitue. La réduction la plus importante sur la laitue était à 10 kGy,

montrant un titre viral de 3.36 log PFU / mL, soit une réduction de 3.54 log PFU / mL (Bidawid *et al.,* 2000). Après 10 jours d'entreposage à 4 °C, les traitements les plus efficaces contre le FCV-F9 étaient la combinaison, l'irradiation seule, l'ozonation et finalement l'utilisation du JC. Des auteurs ont démontré que l'irradiation du kimchi avec des doses allant de 1 à 10 kGy induisait une réduction de 0.34 à 1.76 $\log_{10}$ UFP / mL du titre viral du MNV-1. L'irradiation γ est utilisée pour améliorer la qualité et la durée de conservation de produits frais comme le kimchi. Les auteurs ont attribué ces résultats à la génération de radicaux de type oxygène et hydroxyle par irradiation (Park and Ha, 2017).

1.2.4. Traitements seuls / combinés antiviraux testés contre la flore totale bactérienne durant l'entreposage

Dans la littérature, la combinaison de l'irradiation-γ à des doses de 1 ou 3 kGy avec du benzoate de sodium à 0.19 % (sel de l'acide benzoïque présent dans les canneberges) a prolongé la durée de conservation de la sole « Dover » conservée à une température de 5.6 à 6.7 °C. Des populations microbiennes aérobies de 10^6 / g ont été atteintes en 6 et 10 jours, alors qu'elles n'étaient irradiées que respectivement avec des doses de 1 et 3 kGy. Avec l'irradiation combinée à des doses de 1 ou 3 kGy, l'utilisation de benzoate de sodium a prolongé la durée de conservation à 9 et 19 jours, respectivement (Monk *et al.,* 1995). Le MAPAQ a émis un seuil de contamination par des bactéries aérobies mésophiles à 7 log CFU/mL. La durée de vie de la laitue iceberg sans aucun traitement était de 7 jours. La durée de conservation a été augmentée jusqu'à 10 jours après un traitement avec l'O_3 seul et le JC seul. L'irradiation seule à 1.5 kGy et l'utilisation des traitements combinés a maintenu la qualité de la laitue idéale même après 13 jours de stockage à 4 °C. Akbas et Olmez (2007) ont montré que les trempages dans de l'acide citrique et de l'acide lactique entraînaient des comptes mésophiles et psychrotrophes inférieurs à ceux des autres traitements de trempage pendant 12 jours de stockage de laitue iceberg fraîchement coupée. Les auteurs ont attribué ce mécanisme d'action antimicrobien à la réduction du pH, au rapport entre la fraction non dissociée de l'acide, à la longueur de la chaîne, à la physiologie cellulaire et au métabolisme. Il a aussi été démontré que l'O_3 inactive les bactéries grâce à des modifications oxydatives de nombreux composants cellulaires tels que les protéines, les lipides insaturés et les enzymes respiratoires dans les membranes cellulaires, les peptidoglycanes dans les enveloppes cellulaires et les enzymes (Yuk *et al.,* 2006).

1.2.5. Effets sur la colorimétrie, la chlorophylle, la texture et l'analyse sensorielle durant l'entreposage

Les traitements effectués ont apporté de faibles changements sur la colorimétrie des feuilles après 10 jours d'entreposage à 4 °C. Malgré l'absence de différences visibles à l'œil nu, l'appareil a dénoté quelques changements significatifs. La feuille s'est éclaircie 10 jours après le traitement avec le JC-0.25% seul comparativement aux autres. Le niveau de vert a diminué significativement dans le cas des feuilles

traitées avec l'irradiation seule à 1.5 kGy et avec l'O_3 à 5-ppm. Finalement, un jaunissement significatif a été observé après le traitement avec le CJ-0.25% et la combinaison des trois traitements. Bolin *et al.* (1977) ont montré que la température d'entreposage influençait les paramètres de colorimétrie de la laitue. Ils ont montré que le stockage à haute température (10 °C) assombrissait l'échantillon de laitue par rapport à la laitue stockée à une température plus froide (2 °C). En plus de l'obscurcissement, une perte de pigment vert (augmentation a*) a été observée. Au cours de cette étude, nous avons stocké la laitue à la température du réfrigérateur (4 °C), ce qui explique pourquoi le paramètre a* a augmenté de manière significative et pourquoi la chlorophylle a chuté. La chlorophylle représente le pigment vert qui capte l'énergie de la lumière et la transforme en énergie chimique par la photosynthèse. La chlorophylle a été évalué aux jours 0, 6 et 10 d'entreposage à 4 °C après les traitements effectués sur les laitues iceberg. Une baisse dans le temps a été observée pour le contrôle, l'O_3 et l'irradiation seule. Une faible remontée du pigment a été observée au jour 6 et au jour 10 après le traitement avec le JC seul. En spéculant avec les résultats montrés dans l'article de Agüero *et al.* (2008), il serait possible que les mouvements et pertes d'eaux aient pu concentrer la chlorophylle dans le haut de la feuille après le traitement avec le JC agissant comme un facteur environnemental stressant et ainsi démontré une hausse du pigment durant l'entreposage à 4 °C. Les combinaisons de traitements ont maintenu le niveau de chlorophylle stable tout au long de l'entreposage. Certaines études ont démontré que la température de stockage (4 °C ou 10 °C) de la salade *Valeriana* entraînait une réduction significative de la chlorophylle. Après 8 jours de stockage, une diminution de 22 % de la chlorophylle initiale a été observée sur des laitues conservées à 4 °C, tandis qu'une réduction de 35 % était observée pendant un stockage à 10 °C. Dans notre étude, après 6 jours de stockage à 4 °C, la chlorophylle totale a été réduite de plus de 12 %, il est donc en corrélation avec cette recherche d'observer une diminution naturelle lors du stockage à 4 °C (Ferrante and Maggiore, 2007). La production d'éthylène pendant le stockage de la laitue peut augmenter l'activité de la chlorophyllase, ce qui peut entraîner une diminution naturelle de la chlorophylle pendant le stockage à 4 °C (Agüero *et al.,* 2008). La texture n'a pas été affecté par aucun traitement tout au long du stockage. Une faible différence statistique a cependant été observée entre le contrôle et l'O_3 au jour 6. Baur *et al. (*2004) ont montré qu'après 9 jours de stockage de laitue iceberg, aucun changement significatif de texture n'avait été observé après le lavage des aliments avec de l'eau, du chlore et de l'eau et de l'eau ozonée. De plus, Sagong *et al.* (2011) ont montré que la combinaison d'acides organiques et d'ultrasons ne modifiait pas de manière significative la texture de la laitue pendant plus de 7 jours de stockage à 4 °C ($p > 0.05$) dans tous les échantillons, seuls ou combinés. Les acides organiques utilisés étaient l'acide malique, l'acide lactique et l'acide citrique et des ultrasons de 5 L à 40 kHz (Sagong *et al.,* 2011). Finalement, les analyses sensorielles sur un vingtaine de panelistes a démontré qu'après 5 jours d'entreposage des laitues traités à : 1) contrôle, 2) CJ-0.25% seul et 3) la combinaison des traitements, le traitement avec le JC seul était le

préféré en termes de goût, de texture, d'odeur et de couleur. Une différence statistique a été noté entre le jour 0 et le jour 5 pour le contrôle pour les paramètres de couleur et de goût. Les panelistes lui ont attribué des notes inférieures. Zhang *et al.* (2006) ont montré que l'irradiation-γ à 1.5 kGy affectait un peu la couleur de la laitue après 8 jours de stockage à 4 °C. La laitue préférée au jour 8 a été observée après le traitement à 1 kGy. Les doses d'irradiation de 0 et 0.5 kGy sur la laitue ont été les moins appréciées. Les auteurs ont montré que 1.0 kGy avait les effets optimaux sur la qualité sensorielle de la laitue.

Bibliographie

1. Agüero, M. V., Barg, M. V., Yommi, A., Camelo, A., and Roura, S. I. (2008). Postharvest changes in water status and chlorophyll content of lettuce (Lactuca sativa L.) and their relationship with overall visual quality. *Journal of Food Science*, 73 : 47-55.
2. Akbas, M. Y., and Ölmez, H. (2007). Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. *Journal of the Science of Food and Agriculture*, 87 : 2609-2616.
3. Aquino, S. (2011). Gamma radiation against toxigenic fungi in food, medicinal and aromatic herbs. *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, Mendez-Vilas, A. (Ed.)*, 272-281.
4. Baur, S., Klaiber, R., Hammes, W. P., and Carle, R. (2004). Sensory and microbiological quality of shredded, packaged iceberg lettuce as affected by pre-washing procedures with chlorinated and ozonated water. *Innovative Food Science & Emerging Technologies*, 5 : 45-55.
5. Bidawid, S., Farber, J. M., and Sattar, S. A. (2000). Rapid concentration and detection of hepatitis A virus from lettuce and strawberries. *Journal of Virological Methods*, 88 : 175-185.
6. Bolin, H. R., Stafford, A. E., King Jr, A. D., and Huxsoll, C. C. (1977). Factors affecting the storage stability of shredded lettuce. *Journal of Food Science*, 42: 1319-1321.
7. Cao, C. (2013). Chemical Disinfectants for Inactivation of Human Norovirus Surrogates.
8. Feng, K., Divers, E., Ma, Y., and Li, J. (2011). Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*, 77 : 3507-3517.
9. Ferrante, A., and Maggiore, T. (2007). Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. *Postharvest Biology and Technology*, 45: 73-80.
10. Hirneisen, K. A., Markland, S. M., and Kniel, K. E. (2011). Ozone inactivation of norovirus surrogates on fresh produce. *Journal of Food Protection*, 74: 836-839.

11. Jungeblut, C. W. (1935). Inactivation of poliomyelitis virus in vitro by crystalline vitamin C (ascorbic acid). *Journal of Experimental Medicine*, 62 : 517-521.
12. Kim, B. H., Jang, A., Lee, S. O., Min, J.S., and Lee, M. (2004). Combined effect of electron-beam (beta) irradiation and organic acids on shelf-life of pork loins during cold storage. *Journal of Food Protection,* 67 :168–171.
13. Lawrence, C. A. (Carl Adam)., Block, S. Stanton. (1968). *Disinfection, sterilization, and preservation: [edited by] Carl A. Lawrence [and] Seymour S. Block.* Philadelphia: Lea & Febiger.
14. Macinga, D. R., Sattar, S. A., Jaykus, L. A., and Arbogast, J. W. (2008). Improved inactivation of nonenveloped enteric viruses and their surrogates by a novel alcohol-based hand sanitizer. *Applied and Environmental Microbiology*, 74 : 5047-5052.
15. Monk, J. D., Beuchat, L. R., and Doyle, M. P. (1995). Irradiation inactivation of food-borne microorganisms. *Journal of Food Protection*, 58 : 197-208.
16. Park, S. Y., and Ha, S. D. (2017). Application of gamma radiation for the reduction of norovirus and the quality stability in optimally ripened cabbage kimchi. *Food Research International*, 100 : 277-281.
17. Sagong, H. G., Lee, S. Y., Chang, P. S., Heu, S., Ryu, S., Choi, Y. J., and Kang, D. H. (2011). Combined effect of ultrasound and organic acids to reduce Escherichia coli O157: H7, Salmonella Typhimurium, and Listeria monocytogenes on organic fresh lettuce. *International Journal of Food Microbiology*, 145 : 287-292.
18. Su, X., Howell, A. B., and D'Souza, D. H. (2010). Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. *Food Microbiology*, 27 : 985-991.
19. Thornley, M. J. (1963). Radiation resistance among bacteria. *Journal of Applied Bacteriology (England)*, 26.
20. Yuk, H. G., Yoo, M. Y., Yoon, J. W., Moon, K. D., Marshall, D. L., and Oh, D. H. (2006). Effect of combined ozone and organic acid treatment for control of Escherichia coli O157: H7 and Listeria monocytogenes on lettuce. *Journal of Food Science*, 71 : M83-M87.
21. Zhang, L., Lu, Z., and Wang, H. (2006). Effect of gamma irradiation on microbial growth and sensory quality of fresh-cut lettuce. *International Journal of Food Microbiology*, 106 : 348-351.

Chapitre 6: Conclusion générale

Dans ce mémoire, nous nous sommes intéressés au développement de traitement en combinaison avec l'irradiation, l'ozonation et l'utilisation de jus de canneberge à la surface de la laitue iceberg contre le virus alimentaire FCV, responsable de la gastroentérite chez les humains. Le processus du développement était d'évaluer les effets seuls des traitements *in-vitro* et *in-situ* et aussi leurs effets sans entreposage à 4 °C. L'hypothèse était que la combinaison de traitements allait réduire la dose d'irradiation requise pour éliminer le virus tout en gardant les propriétés physico-chimiques et sensorielles de la laitue à travers l'entreposage à 4 °C.

Après l'entreposage de la laitue iceberg à 4 °C traitée avec l'ozone 5-ppm durant 7.5 minutes, l'irradiation-γ à 1.5 kGy et le CJ-0.25% utilisés seuls ou en combinaison, les résultats ont démontré que les virus étaient plus facilement éliminés avec la combinaison des trois traitements. La flore totale bactérienne est significativement diminuée après un traitement avec l'irradiation seule et complètement éliminée après la combinaison des trois traitements après 13 jours d'entreposage à 4 °C. Peu d'effets visuels sont observés sur les feuilles de laitues malgré un éclaircissement des feuilles après l'utilisation du JC. Aucun effet sur la texture n'a été observé après 10 jours d'entreposage des laitues avec tous les traitements. La chlorophylle est restée stable dans les feuilles traitées avec la combinaison de traitement après 10 jours de stockage. Le traitement favori en termes de goût, de texture, d'odeur et de couleur était le JC après 5 jours d'entreposage à 4 °C.

Finalement, des combinaisons de traitements avec l'irradiation-γ ont permis d'augmenter la radiosensibilisation virale sans toutefois affecter les propriétés sensorielles et nutritionnelles de l'aliment. À ce jour, dans le secteur de l'agroalimentaire, très peu d'études concernent l'utilisation combinée d'acides organiques, d'ozonation et d'irradiation-γ contre les virus alimentaires justifiant ainsi ce projet de recherche. Par ces travaux, nous avons donc pu déterminer s'il existait une synergie entre ces traitements, permettant d'abaisser la dose d'irradiation employée sur l'aliment frais tout en maintenant les propriétés physico-chimiques et nutritives.

Bibliographie complète du mémoire

1. Aboubakr, H. A., El-Banna, A. A., Youssef, M. M., Al-Sohaimy, S. A., and Goyal, S. M. (2014). Antiviral effects of Lactococcus lactis on feline calicivirus, a human norovirus surrogate. *Food and Environmental Virology*, 6 : 282-289.
2. Aboubakr, H. A., Nauertz, A., Luong, N. T., Agrawal, S., El-Sohaimy, S. A., Youssef, M. M., and Goyal, S.M. (2016). In vitro antiviral activity of clove and ginger aqueous extracts against feline calicivirus, a surrogate for human norovirus. *Journal of Food Protection*, 79 :1001-1012.
3. Adams, M. R., Moss, M. O., and McClure, P. (2016). Food Microbiology. Royal Society of Chemistry, 4th edition. Cambridge, UK.
4. Adams, M. R., and Nicolaides, L. (1997). Review of the sensitivity of different foodborne pathogens to fermentation. *Food Control*, 8: 227-239.
5. Agriculture - Province of Manitoba. (2019). Ozonation in Food Applications. Available at : https://www.gov.mb.ca/agriculture/food-safety/at-the-food-processor/ozonation.html. Accessed 17 March 2019.
6. Agüero, M. V., Barg, M. V., Yommi, A., Camelo, A., and Roura, S. I. (2008). Postharvest changes in water status and chlorophyll content of lettuce (Lactuca sativa L.) and their relationship with overall visual quality. *Journal of Food Science*, 73: S47-S55.
7. Ahmed, S. M., Hall, A. J., Robinson, A. E., Verhoef, L., Premkumar, P., Parashar, U. D., ... and Lopman, B. A. (2014). Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, 14: 725-730.
8. Akbas, M. Y., and Ölmez, H. (2007). Effectiveness of organic acid, ozonated water and chlorine dippings on microbial reduction and storage quality of fresh-cut iceberg lettuce. *Journal of the Science of Food and Agriculture*, 87:2609-2616.
9. Alinkina, E. S., Misharina, T. A., and Fatkullina, L. D. (2013). Antiradical properties of oregano, thyme, and savory essential oils. *Applied Biochemistry and Microbiology*, 49 : 73-78.
10. Alvaro, J. E., Moreno, S., Dianez, F., Santos, M., Carrasco, G., and Urrestarazu, M. (2009). Effects of peracetic acid disinfectant on the postharvest of some fresh vegetables. *Journal of Food Engineering*, 95 : 11-15.
11. Aquino, S. (2011). Gamma radiation against toxigenic fungi in food, medicinal and aromatic herbs. *Science Against Microbial Pathogens: Communicating Current Research and Technological Advances, Mendez-Vilas, A. (Ed.)*, 272-281.
12. Arthur, S. E., and Gibson, K. E. (2015). Physicochemical stability profile of Tulane virus: a human norovirus surrogate. *Journal of Applied Microbiology*, 119 : 868-875.

13. Assemand, E., Lacroix, M., and Mateescu, M. A. (2003). Caeruloplasmin sterilized by γ-irradiation in the presence of l-tyrosine maintains structural and catalytic characteristics. *Biotechnology and Applied Biochemistry*, 38: 143-149.
14. ATCC, (2014). *Q&A, ATCC- Excellence in Research Webinar "ATCC-Influenza Research Materials"*. [online] Available at: https://www.atcc.org/~/media/PDFs/webinars/QA/2014/QA_Webinar_Influenza.ashx [Accessed 16 Nov. 2019]. Virginia, USA.
15. Aviram, M., and Rosenblat, M. (2012). Pomegranate protection against cardiovascular diseases. *Evidence-Based Complementary and Alternative Medicine*, Volume 2012 : 20 p.
16. Aw, T. G., Wengert, S., and Rose, J. B. (2016). Metagenomic analysis of viruses associated with field-grown and retail lettuce identifies human and animal viruses. *International Journal of Food Microbiology*, 223:50-56.
17. Azizkhani, M., and Tooryan, F. (2016). Effect of Cinnamon, Rosemary and Zataria Essential Oils on Food-borne Gastroenteritis Viruses. *Journal of Mazandaran University of Medical Sciences*, 26: 8-15.
18. Bahadoran, Z., Mirmiran, P., and Azizi, F. (2013). Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *Journal of Diabetes and Metabolic Disorders,* 12: 43.
19. Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and Chemical Toxicology*, 46 : 446-475.
20. Barker, J., Stevens, D., and Bloomfield, S. F. (2001). Spread and prevention of some common viral infections in community facilities and domestic homes. *Journal of Applied Microbiology*, 91: 7-21.
21. Barker, J., Vipond, I. B., and Bloomfield, S. F. (2004). Effects of cleaning and disinfection in reducing the spread of Norovirus contamination via environmental surfaces. *Journal of Hospital Infection*, 58 : 42-49.
22. Baur, S., Klaiber, R., Hammes, W. P., and Carle, R. (2004). Sensory and microbiological quality of shredded, packaged iceberg lettuce as affected by pre-washing procedures with chlorinated and ozonated water. *Innovative Food Science & Emerging Technologies*, 5:45-55.
23. Belliot, G., Lavaux, A., Souihel, D., Agnello, D., and Pothier, P. (2008). Use of murine norovirus as a surrogate to evaluate resistance of human norovirus to disinfectants. *Applied and Environmental Microbiology*, 74 : 3315-3318.

24. Berger, C. N., Sodha, S. V., Shaw, R. K., Griffin, P. M., Pink, D., Hand, P., and Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology,* 12: 2385–2397.
25. Beuchat, L. R. (1992). Surface disinfection of raw produce. *Dairy, Food and Environmental Sanitation*, 12: 6-9.
26. Beuchat, L. R. (1998). Efficacy of spray application of chlorinated water in killing pathogenic bacteria on raw apples, tomatoes and lettuce. *Journal of Food Protection*, 61: 1305–1311.
27. Bidawid, S., Farber, J. M., and Sattar, S. A. (2000). Inactivation of hepatitis A virus (HAV) in fruits and vegetables by gamma irradiation. *International Journal of Food Microbiology*, 57 : 91-97.
28. Bidawid, S., Farber, J. M., and Sattar, S. A. (2000). Rapid concentration and detection of hepatitis A virus from lettuce and strawberries. *Journal of Virological Methods*, 88*:*175-185.
29. Biswas, N., Balac, P., Narlakanti, S. K., Haque, M. D. E., and Hassan, M. D. M. (2013). Identification of phenolic compounds in processed cranberries by HPLC method. *Journal of Nutrition & Food Sciences*, 3.
30. Block, S. S. (Ed.). (2001). *Disinfection, Sterilization, and Preservation*. Lippincott Williams & Wilkins.
31. Block, G., Patterson, B., and Subar, A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer*, 18 : 1-29.
32. Bolin, H. R., and Huxsoll, C. C. (1991). Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *Journal of Food Science*, 56:60-62.
33. Bolin, H. R., Stafford, A. E., King Jr, A. D., and Huxsoll, C. C. (1977). Factors affecting the storage stability of shredded lettuce. *Journal of Food Science*, 42:1319-1321.
34. Boumail, A., Salmieri, S., and Lacroix, M. (2016). Combined effect of antimicrobial coatings, gamma radiation and negative air ionization with ozone on Listeria innocua, Escherichia coli and mesophilic bacteria on ready-to-eat cauliflower florets. *Postharvest Biology and Technology*, 118 : 134-140.
35. Boutekedjiret, C., Bentahar, F., Belabbes, R., and Bessiere, J. M. (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. *Flavour and Fragrance Journal*, 18 : 481-484.
36. Brié, A., Razafimahefa, R., Loutreul, J., Robert, A., Gantzer, C., Boudaud, N., and Bertrand, I. (2017). The effect of heat and free chlorine treatments on the surface properties of murine norovirus. *Food and Environmental Virology*, 9 :149-158.

37. Buckow, R., Isbarn, S., Knorr, D., Heinz, V., and Lehmacher, A. (2008). Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Applied and Environmental Microbiology*, 74 :1030-1038.
38. Burt, S. A., and Reinders, R. D. (2003). Antibacterial activity of selected plant essential oils against Escherichia coli O157: H7. *Letters in Applied Microbiology*, 36: 162-167.
39. Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94: 223-253.
40. Butot, S., T. Putallaz, R. Amoroso, and G. Sanchez. (2009). Inactivation of enteric viruses in minimally processed berries and herbs. *Applied and Environmental Microbiology,* 75: 4155–4161.
41. Cadet, J., and Douki, T. (2018). Formation of UV-induced DNA damage contributing to skin cancer development. *Photochemical & Photobiological Sciences*, 17 :1816-1841.
42. Caillet, S., Côté, J., Doyon, G., Sylvain, J. F., and Lacroix, M. (2011). Antioxidant and antiradical properties of cranberry juice and extracts. *Food Research International*, 44: 1408-1413.
43. Canadian Food Inspection Agency. (2019). *Irradiated foods*. Available at: http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/labelling/for-industry/irradiated-foods/eng/1334594151161/1334596074872#tc_fi. Accessed 17 Mar. 2019.
44. Cann, A. J. (2005). Principles of molecular virology. Elsevier Academic Press, 4th Edition. University of Leicester, UK.
45. Cannon, J. L., Papafragkou, E., Park, G. W., Osborne, J., Jaykus, L. A., and Vinjé, J. (2006). Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *Journal of Food Protection*, 69 : 2761-2765.
46. Cao, C. (2013). Chemical Disinfectants for Inactivation of Human Norovirus Surrogates.
47. Cayot, N. (2007). Sensory quality of traditional foods. *Food Chemistry*, 102: 445-453.
48. Centers for Disease Control and Prevention (CDC). (2019). Available at: https://www.cdc.gov/norovirus/preventing-infection.html. Accessed 9 May 2019.
49. Cheesbrough, J. S., Green, J., Gallimore, C. I., Wright, P. A., and Brown, D. W. G. (2000). Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiology & Infection*, 125 : 93-98.
50. Chen, H., Hoover, D. G., and Kingsley, D. H. (2005). Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection*, 68: 2389-2394.
51. Cheong, S., Lee, C., Song, S. W., Choi, W. C., Lee, C. H. and Kim, S. J. (2009). Enteric viruses in raw vegetables and groundwater used for irrigation in South Korea. *Applied and Environmental Microbiology*, 75: 7745-7751.

52. Ciesek, S., von Hahn, T., Colpitts, C. C., Schang, L. M., Friesland, M., Steinmann, J., ... and Pietschmann, T. (2011). The green tea polyphenol, epigallocatechin-3-gallate, inhibits hepatitis C virus entry. *Hepatology*, 54: 1947-1955.
53. Cleland, M. R., and Stichelbaut, F. (2013). Radiation processing with high-energy X-rays. *Radiation Physics and Chemistry*, 84: 91-99.
54. Cliver, D. O. (1997). Virus transmission via food. *World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales*, 50 :90-101.
55. Coppola, E. D., Conrad, E. C., and Cotter, R. (1978). High pressure liquid chromatographic determination of major organic acids in cranberry juice. *Journal Association of Official Analatylical Chemists*, 61 : 1490-1492.
56. Côté, J., Caillet, S., Doyon, G., Sylvain, J. F., and Lacroix, M. (2010). Bioactive compounds in cranberries and their biological properties. *Critical Reviews in Food Science and Nutrition*, 50: 666-679.
57. Côté, J., Caillet, S., Doyon, G., Dussault, D., Salmieri, S., Lorenzo, G., ... and Lacroix, M. (2011A). Effects of juice processing on cranberry antioxidant properties. *Food Research International*, 44: 2907-2914.
58. Côté, J., Caillet, S., Doyon, G., Dussault, D., Sylvain, J. F., and Lacroix, M. (2011B). Antimicrobial effect of cranberry juice and extracts. *Food Control*, 22: 1413-1418.
59. Côté, J., Caillet, S., Dussault, D., Sylvain, J. F., and Lacroix, M. (2011C). Effect of juice processing on cranberry antibacterial properties. *Food Research International*, 44: 2922-2929.
60. Cromeans, T., Park, G. W., Costantini, V., Lee, D., Wang, Q., Farkas, T., ... and Vinjé, J. (2014). Comprehensive comparison of cultivable norovirus surrogates in response to different inactivation and disinfection treatments. *Applied and Environmental Microbiology*, 80 : 5743-5751.
61. Dai, T., Vrahas, M. S., Murray, C. K., and Hamblin, M. R. (2012). Ultraviolet C irradiation: an alternative antimicrobial approach to localized infections? *Expert Review of Anti-Infective Therapy*, 10 : 185-195.
62. Darling, A. J., Boose, J. A., and Spaltro, J. (1998). Virus assay methods: accuracy and validation. *Biologicals*, 26 : 105-110.
63. De Roda Husman, A. M., Bijkerk, P., Lodder, W., Van Den Berg, H., Pribil, W., Cabaj, A., ... and Duizer, E. (2004). Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (gamma) radiation. *Applied and Environmental Microbiology*, 70 : 5089-5093.
64. Diehl, J. F. (1992). Food irradiation: is it an alternative to chemical preservatives? *Food Additives & Contaminants*, 9 : 409-416.

65. Diels, A. M., Callewaert, L., Wuytack, E. Y., Masschalck, B., and Michiels, C. W. (2004). Moderate Temperatures Affect Escherichia coli Inactivation by High-Pressure Homogenization Only through Fluid Viscosity. *Biotechnology Progress*, 20 : 1512-1517.
66. Dixon, R. A., Xie, D. Y., and Sharma, S. B. (2005). Proanthocyanidins–a final frontier in flavonoid research? *New Phytologist*, 165: 9-28.
67. Donaldson, E. F., Lindesmith, L. C., Lobue, A. D., and Baric, R. S. (2008). Norovirus pathogenesis: mechanisms of persistence and immune evasion in human populations. *Immunological Reviews*, 225 : 190-211.
68. Dortu, C., and Thonart, P. (2009). Les bactériocines des bactéries lactiques: caractéristiques et intérêts pour la bioconservation des produits alimentaires/Bacteriocins from lactic acid bacteria: interest for food products biopreservation. *Biotechnologie, Agronomie, Société et Environnement*, 13: 143.
69. Doyle, M. P., and M. C. Erickson. (2008). Summer meeting 2007—the problems with fresh produce: an overview. *Journal of Applied Microbiology*, 105: 317–330.
70. D'Souza, D.H. (2014). Phytocompounds for the control of human enteric viruses. *Current Opinions in Virology*, 4: 44–49.
71. D'Souza, D. H., Su, X., Roach, A., and Harte, F. (2009). High-pressure homogenization for the inactivation of human enteric virus surrogates. *Journal of Food Protection*, 72: 2418-2422.
72. Du, J., Han, Y., and Linton, R. H. (2002). Inactivation by chlorine dioxide gas (ClO2) of Listeria monocytogenes spotted onto different apple surfaces. *Food Microbiology*, 19: 481-490.
73. Duizer, E., Bijkerk, P., Rockx, B., De Groot, A., Twisk, F., and Koopmans, M. (2004). Inactivation of caliciviruses. *Applied and Environmental Microbiology*, 70: 4538-4543.
74. Dybowska, B. E. (2005). Emulsion reproducibility in two-stage homogenizer Niro Soavi type Panda. *Milchwissenschaft*, 60 : 367-370.
75. Ehling, S., and Cole, S. (2011). Analysis of organic acids in fruit juices by liquid chromatography− mass spectrometry: an enhanced tool for authenticity testing. *Journal of Agricultural and Food Chemistry*, 59 : 2229-2234.
76. Elizaquível, P., Azizkhani, M., Aznar, R., and Sánchez, G. (2013). The effect of essential oils on norovirus surrogates. *Food Control*, 32: 275-278.
77. Elvis, A. M., and Ekta, J. S. (2011). Ozone therapy: A clinical review. *Journal of Natural Science, Biology, and Medicine*, 2 : 66.
78. Esseili, M. A., Saif, L. J., Farkas, T., and Wang, Q. (2015). Feline calicivirus, murine norovirus, porcine sapovirus, and Tulane virus survival on postharvest lettuce. *Applied and Environmental Microbiology*, 81: 5085-5092.

79. Fan, X., and Sokorai, K. J. B. (2008). Retention of quality and nutritional value of 13 fresh-cut vegetables treated with low-dose radiation. *Journal of Food Science*, 73 : S367-S372.
80. Fan, X., Guan, W., and Sokorai, K. J. (2012A). Quality of fresh-cut Iceberg lettuce and spinach irradiated at doses up to 4 kGy. *Radiation Physics and Chemistry*, 81:1071-1075.
81. Fan, X., Sokorai, K. J., Niemira, B. A., Mills, R. S., and Zhen, M. Y. (2012B). Quality of gamma ray-irradiated iceberg lettuce and treatments to minimize irradiation-induced disorders. *HortScience*, 47:1108-1112.
82. Fan, X., Toivonen, P.M., Rajkowski, K. T., and Sokorai, K. J. (2003). Warm water treatment in combination with modified atmosphere packaging reduces undesirable effects of irradiation on the quality of fresh-cut iceberg lettuce. *Journal of Agricultural and Food Chemistry*, 51:1231-1236.
83. Fankhauser, R. L., Monroe, S. S., Noel, J. S., Humphrey, C. D., Bresee, J. S., Parashar, U. D., ... and Glass, R. I. (2002). Epidemiologic and molecular trends of "Norwalk-like viruses" associated with outbreaks of gastroenteritis in the United States. *Journal of Infectious Diseases*, 186 : 1-7.
84. Farkas, J. (1989). Microbiological safety of irradiated foods. *International Journal of Food Microbiology*, 9: 1-15.
85. Farkas, J., and Mohácsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science & Technology*, 22 : 121-126.
86. Feng, K., Divers, E., Ma, Y., and Li, J. (2011). Inactivation of a human norovirus surrogate, human norovirus virus-like particles, and vesicular stomatitis virus by gamma irradiation. *Applied and Environmental Microbiology*, 77: 3507-3517.
87. Ferrante, A., and Maggiore, T. (2007). Chlorophyll a fluorescence measurements to evaluate storage time and temperature of Valeriana leafy vegetables. *Postharvest Biology and Technology*, 45:73-80.
88. Fields, B. N., Knipe, D. M., Howley, P. M., Everiss, K. D., and Kung, H. J. (1996). *Fundamental Virology* Philadelphia ePA PA: Lippincott-Raven, pp. 401-402.
89. Fraisse, A., Temmam, S., Deboosere, N., Guillier, L., Delobel, A., Maris, P., and Perelle, S. (2011). Comparison of chlorine and peroxyacetic-based disinfectant to inactivate Feline calicivirus, Murine norovirus and Hepatitis A virus on lettuce. *International Journal of Food Microbiology*, 151: 98-104.
90. Franck, K. T., Lisby, M., Fonager, J., Schultz, A. C., Böttiger, B., Villif, A., ... and Ethelberg, S. (2014). Sources of calicivirus contamination in foodborne outbreaks in Denmark, 2005–2011—the role of the asymptomatic food handler. *The Journal of Infectious Diseases*, 211 :563-570.
91. Fretz-Männel, R., Baumgartner, A., Lüthi, Schmid, H., Schorr, D., Svoboda, P., Witschi, A. (2005). Norovirus- Caractéristiques biologiques, épidémiologie, tableau clinique, prévention

Recommandations pour la gestion des épidémies. Édition Office fédéral de la santé publique. Berne, Suisse.

92. Ghabraie, M., Vu, K. D., Tata, L., Salmieri, S., and Lacroix, M. (2016). Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. *LWT-Food Science and Technology*, 66 : 332-339.
93. Gibson, K. E., and Schwab, K. J. (2011). Thermal inactivation of human norovirus surrogates. *Food and Environmental Virology*, 3 : 74.
94. Gibriel, A. Y., Al-Sayed, H. M., Rady, A. H., and Abdelaleem, M. A. (2013). Synergistic antibacterial activity of irradiated and nonirradiated cumin, thyme and rosemary essential oils. *Journal of Food Safety*, 33:222-228.
95. Gilling, D. H., Kitajima, M., Torrey, J. R., and Bright, K. R. (2014A). Mechanisms of antiviral action of plant antimicrobials against murine norovirus. *Applied and Environmental Microbiology*, 80 : 4898-4910.
96. Gilling, D. H., Kitajima, M., Torrey, J. R., and Bright, K. R. (2014B). Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *Journal of Applied Microbiology*, 116: 1149-1163.
97. Giroux, M., Ouattara, B., Yefsah, R., Smoragiewicz, W., Saucier, L., and Lacroix, M. (2001). Combined effect of ascorbic acid and gamma irradiation on microbial and sensorial characteristics of beef patties during refrigerated storage. *Journal of Agricultural and Food Chemistry*, 49:919-925.
98. Glass, R. I., Parashar, U. D., and Estes, M. K. (2009). Norovirus gastroenteritis. *New England Journal of Medicine*, 361: 1776-1785.
99. Götz, M. E., Künig, G., Riederer, P., and Youdim, M. B. (1994). Oxidative stress: free radical production in neural degeneration. *Pharmacology & Therapeutics*, 63 : 37-122.
100. Guzel-Seydim, Z. B., Greene, A. K., and Seydim, A. C. (2004). Use of ozone in the food industry. *LWT-Food Science and Technology*, 37: 453-460.
101. Hall, A. J., Wikswo, M. E., Pringle, K., Gould, L. H., and Parashar, U. D. (2014). Vital signs: foodborne norovirus outbreaks—United States, 2009–2012. *MMWR. Morbidity and Mortality Weekly Report*, 63: 491.
102. Heaton, J. C., and K. Jones. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: a review. *Journal of Applied Microbiology* 104:613–626.
103. Heidelbaugh, N. D., and Giron, D. J. (1969). Effect of processing on recovery of polio virus from inoculated foods. *Journal of Food Science*, 34 : 239-239.

104. Heinonen, M. (2007). Antioxidant activity and antimicrobial effect of berry phenolics–a Finnish perspective. *Molecular Nutrition & Food Research*, 51: 684-691.
105. Herwaldt, B. L., Lew, J. F., Moe, C. L., Lewis, D. C., Humphrey, C. D., Monroe, S. S., ... and Glass, R. I. (1994). Characterization of a variant strain of Norwalk virus from a food-borne outbreak of gastroenteritis on a cruise ship in Hawaii. *Journal of Clinical Microbiology*, 32 : 861-866.
106. Hewitt, J., and Greening, G. E. (2004). Survival and persistence of norovirus, hepatitis A virus, and feline calicivirus in marinated mussels. *Journal of Food Protection*, 67 : 1743-1750.
107. Hirneisen, K. A., Markland, S. M., and Kniel, K. E. (2011). Ozone inactivation of norovirus surrogates on fresh produce. *Journal of Food Protection*, 74: 836-839.
108. Hirneisen, K. A., and Kniel, K. E. (2013). Comparing human norovirus surrogates: murine norovirus and Tulane virus. *Journal of Food Protection*, 76: 139-143.
109. Holley, R. A. (2014) Food irradiation adds cost but makes sense. Canadian Food Insights 2:28-30.
110. Husman, A.M.D., Bijkerk, P., Lodder, W., van den Berg, H., Pribil, W., Cabaj, A., Gehringer, P., Sommer, R., and Duizer, E. (2004). Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (Gamma) radiation. *Applied Environmental Microbiology,* 70: 5089-5093.
111. Jariwalla, R. J., and Harakeh, S. (1996). Antiviral and immunomodulatory activities of ascorbic acid. *Subcellular Biochemistry*, 215-231.
112. Jensen, H. D., Struve, C., Christensen, S. B., and Krogfelt, K. A. (2017). Cranberry juice and combinations of its organic acids are effective against experimental urinary tract infection. *Frontiers in Microbiology*, 8 :542.
113. Joshi, S. S., Howell, A. B., and D'Souza, D. H. (2016). Reduction of enteric viruses by blueberry juice and blueberry proanthocyanidins. *Food and Environmental Virology*, 8 : 235-243.
114. Joshi, S., Howell, A. B., and D'Souza, D. H. (2017). Blueberry proanthocyanidins against human norovirus surrogates in model foods and under simulated gastric conditions. *Food Microbiology*, 63: 263-267.
115. Joshi, S.S., Howell, A.B., and D'Souza, D.H. (2019). Antiviral effects of blueberry proanthocyanidins against Aichi virus. *Food Microbiol*ogy, 82: 202-208.
116. Jung, P. M., Park, J. S., Park, J. G., Park, J. N., Han, I. J., Song, B. S., ... and Chung, Y. J. (2009). Radiation sensitivity of poliovirus, a model for norovirus, inoculated in oyster (Crassostrea gigas) and culture broth under different conditions. *Radiation Physics and Chemistry*, 78 : 597-599.
117. Jungeblut, C. W. (1935). Inactivation of poliomyelitis virus in vitro by crystalline vitamin C (ascorbic acid). *The Journal of Experimental Medicine*, 62 : 517-521.

118. Kang, S., Park, S. Y., and Ha, S. D. (2016). Application of gamma irradiation for the reduction of norovirus in traditional Korean half-dried seafood products during storage. *LWT Food Science and Technology*, 65 : 739-745.
119. Karaca, H., and Velioglu, Y. S. (2014). Effects of ozone treatments on microbial quality and some chemical properties of lettuce, spinach and parsley. *Postharvest Biology and Technology*, 88:46-53.
120. Katzenelson, E., Kletter, B., and Shuval, H. I. (1974). Inactivation kinetics of viruses and bacteria in water by use of ozone. *Journal of the American Water Works Association*, 66:725-729.
121. Khadre, M. A., Yousef, A. E., and Kim, J. G. (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science*, 66 : 1242-1252.
122. Khadre, M. A., and Yousef, A. E. (2002). Susceptibility of human rotavirus to ozone, high pressure, and pulsed electric field. *Journal of Food Protection*, 65 : 1441-1446.
123. Khurana, S., Venkataraman, K., Hollingsworth, A., Piche, M., and Tai, T. C. (2013). Polyphenols: benefits to the cardiovascular system in health and in aging. *Nutrients*, 5: 3779–3827.
124. Kim, B. H., Jang, A., Lee, S.O., Min, J.S., and Lee, M. (2004). Combined effect of electron-beam (beta) irradiation and organic acids on shelf-life of pork loins during cold storage. *Journal of Food Protection,* 67:168–171
125. Kim, H.W., Chung, D.H., Kim, S.A., and Rhee, M.S. (2019). Synergistic cranberry juice combinations with natural-borne antimicrobials for the eradication of uropathogenic Escherichia coli biofilm within a short time. *Letters in Applied Microbiology,* 68: 321-328.
126. Kim, J. G., Yousef, A. E., and Khadre, M. A. (2003). Ozone and its current and future application in the food industry. *Advances in Food and Nutrition Research*, 45: 167-218.
127. Kim, S. H., Shahbaz, H. M., Park, D., Chun, S., Lee, W., Oh, J. W., … and Park, J. (2017). A combined treatment of UV-assisted TiO2 photocatalysis and high hydrostatic pressure to inactivate internalized murine norovirus. *Innovative Food Science & Emerging Technologies*, 39 : 188-196.
128. Kim, Y. S., Zheng, Z. B., and Shin, D. H. (2008). Growth inhibitory effects of kimchi (Korean traditional fermented vegetable product) against Bacillus cereus, Listeria monocytogenes, and Staphylococcus aureus. *Journal of Food Protection*, 71 : 325-332.
129. Kirk, J. R., and Mitchell, S. K. (1980). Risks and benefits associated with chlorine in the food industry. *Water Chlorination. Environ-Chlorination of Organic Compounds,* 273 : 218-225.
130. Kniel, K. E., Montville, T. J., and Matthews, K. R. (ed. 4). (2017). Food Microbiology: An Introduction. American Society Microbiology; 4th edition. Washington, DC.

131. Komanapalli, I. R., and Lau, B. H. S. (1998). Inactivation of bacteriophage k, Escherichia coli, and Candida albicans by ozone. *Applied Microbiology and Biotechnology*, 49 : 766-769.
132. Komolprasert, V., Bailey, A., and Machuga, E. (2008). Irradiation of Food Packaging Materials: Regulatory Report. FDA. *Center for Food Safety and Applied Nutrition.*
133. Konowalchuk, J., and Speirs, J. I. (1975). Survival of enteric viruses on fresh vegetables. *Journal of Milk and Food Technology,* 38:469–472.
134. Konowalchuk, J., and Speirs, J. I. (1976). Antiviral activity of fruit extracts. *Journal of Food Science*, 41 : 1013-1017.
135. Koopmans, M., and Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90 : 23-41.
136. Koseki, S., Yoshida K, Isobe S, and Itoh. K. (2001). Decontamination of lettuce using electrolyzed water. *Journal of Food Protection,* 64:652-658.
137. Kurdziel, A. S., Wilkinson, N., Langton, S., and Cook, N. (2001). Survival of poliovirus on soft fruit and salad vegetables. *Journal of Food. Protection*, 64:706-709.
138. Kuusi, M., Nuorti, J. P., Maunula, L., Minh, N. T., Ratia, M., Karlsson, J., and Von Bonsdorff, C. H. (2002). A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. *Epidemiology and. Infection*, 129 : 133-138.
139. Lacombe, A., Niemira, B. A., Gurtler, J. B., Sites, J., Boyd, G., Kingsley, D. H., and Chen, H. (2017). Nonthermal inactivation of norovirus surrogates on blueberries using atmospheric cold plasma. *Food Microbiology*, 63 : 1-5.
140. Lacroix, M., and Ouattara, B. (2000). Combined industrial processes with irradiation to assure innocuity and preservation of food products—a review. *Food Research International*, 33 : 719-724.
141. Lacroix, M., and Vigneault, C. (2007). Irradiation treatment for increasing fruit and vegetable quality. *Stewart Postharvest Review*, 3: 1-8.
142. Lankinen, M. H., Vilpo, L. M., and Vilpo, J. A. (1996). UV-and γ-irradiation-induced DNA single-strand breaks and their repair in human blood granulocytes and lymphocytes. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 352: 31-38.
143. Lawrence, C. A., Block, S. S. (1968). *Disinfection, sterilization, and preservation: [edited by] Carl A. Lawrence [and] Seymour S. Block.* Philadelphia: Lea and Febiger.
144. Lee, C. H. (1997). Lactic acid fermented foods and their benefits in Asia. *Food Control*, 8 : 259-269.

145. Lee, M. H., Yoo, S. H., Ha, S. D., and Choi, C. (2012). Inactivation of feline calicivirus and murine norovirus during Dongchimi fermentation. *Food Microbiology*, 31: 210-214.
146. Lee, J. H., min Lee, H., Kim, S. H., Eun, J. B., and Ha, J. H. (2017). Recovery of intact human norovirus from cabbage Kimchi stored at 4° C and 10° C during fermentation. *LWT-Food Science and Technology*, 78: 258-264.
147. Lemay, G. (2017). *MCB 2994, virologie et pathogenèse virale*. Montréal: Librairie de l'Université de Montréal. Montréal, Canada.
148. Lemoine, M. L., Civello, P. M., Chaves, A. R., and Martínez, G. A. (2008). Effect of combined treatment with hot air and UV-C on senescence and quality parameters of minimally processed broccoli (Brassica oleracea L. var. Italica). *Postharvest Biology and Technology,* 48:15-21.
149. Leuenberger, S., Widdowson, M. A., Feilchenfeldt, J., Egger, R., and Streuli, R. A. (2007). Norovirus outbreak in a district general hospital--new strain identified. *Swiss Medical Weekly*, 137 :57-61.
150. Li, D., Baert, L., and Uyttendaele, M. (2013). Inactivation of food-borne viruses using natural biochemical substances. *Food Microbiology,* 35*:* 1–9.
151. Li, J. W., Xin, Z. T., Wang, X. W., Zheng, J. L., and Chao, F. H. (2004). Mechanisms of inactivation of hepatitis A virus in water by chlorine dioxide. *Water Research*, 38 : 1514-1519.
152. Li, X., and Chen, H. (2015). Evaluation of the porcine gastric mucin binding assay for high-pressure-inactivation studies using murine norovirus and tulane virus. *Applied and Environmental Microbiology*, 81: 515-521.
153. Lichtenthaler, H. K., and Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions,* 11:591 – 592.
154. Lin, C., Yu, Y., Zhao, H. G., Yang, A., Yan, H., and Cui, Y. (2012). Combination of quercetin with radiotherapy enhances tumor radiosensitivity in vitro and in vivo. *Radiotherapy and Oncology*, 104 : 395-400.
155. Lipson, S. M., Gordon, R. E., Karthikeyan, L., Singh, M., Burdowski, A., Roy, M., and Stotzky, G. (2010). Cranberry and Grape Juice Drinks Affect Infectivity, Integrity, and Pathology of Enteric Viruses in an Animal Model. In *ACS symposium series* (Vol. 1035, pp. 177-195). Oxford University Press.
156. Liu, Y., Gallardo-Moreno, A. M., Pinzon-Arango, P. A., Reynolds, Y., Rodriguez, G., and Camesano, T. A. (2008). Cranberry changes the physicochemical surface properties of E. coli and adhesion with uroepithelial cells. *Colloids and Surfaces B : Biointerfaces*, 65 : 35-42.

157. Lopman, B., Gastanaduy, P., Park, G. W., Hall, A. J., Parashar, U. D. and Vinjé, J. (2012). Environmental transmission of norovirus gastroenteritis. *Current Opinion in Virology*, 2 : 96-102.
158. Lu, Y., and Wu, C. (2010). Reduction of Salmonella enterica contamination on grape tomatoes by washing with thyme oil, thymol, and carvacrol as compared with chlorine treatment. *Journal of Food Protection*, 73: 2270-2275.
159. Macinga, D. R., Sattar, S. A., Jaykus, L. A., and Arbogast, J. W. (2008). Improved inactivation of nonenveloped enteric viruses and their surrogates by a novel alcohol-based hand sanitizer. *Applied and Environmental Microbiology*, 74 : 5047-5052.
160. Maki, K.C., Kaspar, K.L., Khoo, C., Derrig, L.H., Schild, A.L., and Gupta, K. (2016) Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. The *American Journal of Clinical Nutrition*, 103: 1434-1442.
161. Maki, K.C., Nieman, K.M., Schild, A.L., Kaspar, K.L., and Khoo, C. (2018). The effect of cranberry juice consumption on the recurrence of urinary tract infection: relationship to baseline risk factors. The *Journal of American College of Nutrition*, 37: 121-126.
162. Manach, C., Scalbert, A., Morand, C., Rémésy, C., and Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition*, 79 : 727-747.
163. MAPAQ. Bilan des toxi-infections alimentaires du 1er avril 2013 au 31 mars 2014 (2014). Available at : https://www.mapaq.gouv.qc.ca/fr/Publications/Bilan_Toxi-infection_2013-2014.pdf. Accessed 15 November 2019.
164. MAPAQ. Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire (2006). Available at: https://www.mapaq.gouv.qc.ca/fr/Publications/recueil.pdf. Accessed 12 August 2019.
165. Mara, D., and Sleigh, A. (2010). Estimation of norovirus infection risks to consumers of wastewater-irrigated food crops eaten raw. *Journal of Water and Health*, 8: 39-43.
166. Mattison, K., Karthikeyan, K., Abebe, M., Malik, N., Sattar, S. A., Farber, J. M., and Bidawid, S. (2007). Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *Journal of Food Protection*, 70 : 500-503.
167. McDonnell, G., and Russell, A. D. (1999). Antiseptics and disinfectants: activity, action, and resistance. *Clinical Microbiology Reviews*, 12: 147-179.
168. McLinden, T., Sargeant, J. M., Thomas, M. K., Papadopoulos, A., and Fazil, A. (2014). Component costs of foodborne illness: a scoping review. *BMC Public Health*, 14: 509.

169. Mescle, J-F. Zucca, J. and Bourgeois, C-M. (Ed.1). (1988). Microbiologie Alimentaire: Tome 1, Aspect microbiologique de la sécurité et de la qualité des aliments. *Tec & Doc*, Lavoisier, Apria. 1st edition. Paris, France.

170. Methot, Y., Essler-Gravesen, D., and Talbot, J. (2017). Citrus-based antimicrobial composition. US Patent 2017/0079281 A1.

171. Monk, J. D., Beuchat, L. R., and Doyle, M. P. (1995). Irradiation inactivation of food-borne microorganisms. *Journal of Food Protection*, 58 : 197-208.

172. Montazeri, N., Manuel, C., Moorman, E., Khatiwada, J. R., Williams, L. L., and Jaykus, L. A. (2017). Virucidal Activity of Fogged Chlorine Dioxide-and Hydrogen Peroxide-Based Disinfectants against Human Norovirus and Its Surrogate, Feline Calicivirus, on Hard-to-Reach Surfaces. *Frontiers in Microbiology*, 8 : 1031.

173. Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., and Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, 25 : 361-366.

174. Nam, S., and Kwon, Y. (1997, October). Quality changes of hydroponically produced leaf lettuce (Lactuca sativa L., cv. Cheongchima) during postharvest storage. In *International Symposium on Vegetable Quality of Fresh and Fermented Vegetables,* 483: 173-184.

175. Ndoti-Nembe, A., Vu, K. D., Han, J., Doucet, N., and Lacroix, M. (2015). Antimicrobial Effects of Nisin, Essential Oil, and γ-Irradiation Treatments against High Load of Salmonella typhimurium on Mini-carrots. *Journal of Food Science*, 80 : 1544-1548.

176. Nostro, A., and Papalia, T. (2012). Antimicrobial activity of carvacrol: current progress and future prospectives. *Recent patents on anti-infective drug discovery*, 7 : 28-35.

177. Nout, M. R., Rombouts, F. M., and Havelaar, A. (1989). Effect of accelerated natural lactic fermentation of infant good ingredients on some pathogenic microorganisms. *International Journal of Food Microbiology*, 8: 351-361.

178. Nuanualsuwan, S., and Cliver, D. O. (2003). Capsid functions of inactivated human picornaviruses and feline calicivirus. *Applied and Environmental Microbiology*, 69 : 350-357.

179. Oh, M., Bae, S. Y., Lee, J. H., Cho, K. J., Kim, K. H., and Chung, M. S. (2012). Antiviral effects of black raspberry (*Rubus coreanus*) juice on foodborne viral surrogates. *Foodborne Pathogens and Disease,* 9: 915–921.

180. Oh, M., and Chung, M. S. (2014). Effects of oils and essential oils from seeds of Zanthoxylum schinifolium against foodborne viral surrogates. *Evidence-Based Complementary and Alternative Medicine*, Volume 2014.

181. Oldstone, M. B. (2009). *Viruses, plagues, and history: past, present and future*. Oxford University Press.
182. Oussalah, M., Caillet, S., and Lacroix, M. (2006). Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of Escherichia coli O157: H7 and Listeria monocytogenes. *Journal of Food Protection*, 69 : 1046-1055.
183. Park, S. Y., and Ha, S. D. (2017). Application of gamma radiation for the reduction of norovirus and the quality stability in optimally ripened cabbage kimchi. *Food Research International*, 100 : 277-281.
184. Pate, K. M., Rogers, M., Reed, J. W., van der Munnik, N., Vance, S. Z., and Moss, M. A. (2017). Anthoxanthin Polyphenols Attenuate Aβ Oligomer-induced Neuronal Responses Associated with Alzheimer's Disease. *CNS Neuroscience & Therapeutics*, 23 : 135-144.
185. Patel, M. M., Widdowson, M. A., Glass, R. I., Akazawa, K., Vinjé, J., and Parashar, U. D. (2008). Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerging Infectious Diseases*, 14: 1224.
186. Perumalla, A. V. S., and Hettiarachchy, N. S. (2011). Green tea and grape seed extracts—Potential applications in food safety and quality. *Food Research International*, 44 : 827-839.
187. Picart, L., Thiebaud, M., René, M., Guiraud, J. P., Cheftel, J. C., and Dumay, E. (2006). Effects of high pressure homogenisation of raw bovine milk on alkaline phosphatase and microbial inactivation. A comparison with continuous short-time thermal treatments. *Journal of Dairy Research*, 73: 454-463.
188. Pinzón-Arango, P. A., Liu, Y., and Camesano, T. A. (2009). Role of cranberry on bacterial adhesion forces and implications for Escherichia coli–uroepithelial cell attachment. *Journal of Medicinal Food*, 12 : 259-270.
189. Prakash, A., Guner, A. R., Caporaso, F., and Foley, D. M., (2000). Effects of low-dose gamma irradiation on the shelf life and quality characteristics of cut romaine lettuce packaged under modified atmosphere. *Journal of Food Science*, 65:549-553.
190. Predmore, A., and Li, J. (2011). Enhanced removal of a human norovirus surrogate from fresh vegetables and fruits by a combination of surfactants and sanitizers. *Applied and Environmental Microbiology*, 77: 4829-4838.
191. Predmore, A., Sanglay, G., Li, J., and Lee, K. (2015). Control of human norovirus surrogates in fresh foods by gaseous ozone and a proposed mechanism of inactivation. *Food Microbiology*, 50 : 118-125.
192. Rabinowitch, E. I. (1965). The role of chlorophyll in photosynthesis. *Scientific American*, 213:74-83.

193. Rahimi, E., Faghihi, R., Baradaran-Ghahfarokhi, M.,Alavaian-Ghavinini, A., Baradaran-Ghahfarokhi, H. R., Siavashpour, S., … and Rafie, F. (2013). Effects of gamma irradiation on microbial load and quality characteristics of veal. *Advanced Biomedical Research*, Vol 2.
194. Reichling, J., Schnitzler, P., Suschke, U., and Saller, R. (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties–an overview. *Complementary Medicine Research*, 16 : 79-90.
195. Rice, R. G., Graham, D. M., and Lowe, M. T. (2002). Recent ozone applications in food processing and sanitation. *Food Safety Magazine*, 8:10-17.
196. Ross, R. P., Morgan, S., and Hill, C. (2002). Preservation and fermentation: past, present and future. *International Journal of Food Microbiology*, 79: 3-16.
197. Ryu, S., You, H. J., Kim, Y. W., Lee, A., Ko, G. P., Lee, S. J. and Song, M. J. (2015). Inactivation of norovirus and surrogates by natural phytochemicals and bioactive substances. *Molecular Nutrition & Food Research*, 59: 65-74.
198. Rzeżutka, A., and Cook, N. (2004). Survival of human enteric viruses in the environment and food. *FEMS Microbiology Reviews*, 28 : 441-453.
199. Sagong, H. G., Lee, S. Y., Chang, P. S., Heu, S., Ryu, S., Choi, Y. J., and Kang, D. H. (2011). Combined effect of ultrasound and organic acids to reduce Escherichia coli O157: H7, Salmonella Typhimurium, and Listeria monocytogenes on organic fresh lettuce. *International Journal of Food Microbiology*, 145:287-292.
200. Sair, A. I., D'Souza, D. H., Moe, C. L., and Jaykus, L. A. (2002). Improved detection of human enteric viruses in foods by RT-PCR. *Journal of Virological Methods*, 100: 57-69.
201. Sánchez, C., Aznar, R., and Sánchez, G. (2015). The effect of carvacrol on enteric viruses. *International Journal of Food Microbiology*, 192: 72-76.
202. Scalbert, A. (1991). Antimicrobial properties of tannins. *Phytochemistry*, 30 : 3875-3883.
203. Scharff, R. L. (2010). Health-related costs from foodborne illness in the United States. Produce Safety Project, Georgetown University, Washington, DC.
204. Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection*, 75: 123-131.
205. Seo, D. J. and C. Choi (2017). Inhibition of Murine Norovirus and Feline Calicivirus by Edible Herbal Extracts. *Food Environmental Virology,* 9: 35-44.
206. Seo, D. J., and Choi, C. (2017). Inhibitory mechanism of five natural flavonoids against murine norovirus. *Phytomedicine*, 30 : 59-66.

207. Seo, D. J., Jeon, S. B., Oh, H., Lee, B. H., Lee, S. Y., Oh, S. H., ... and Choi, C. (2016). Comparison of the antiviral activity of flavonoids against murine norovirus and feline calicivirus. *Food Control*, 60 : 25-30.

208. Seo, D. J., Lee, M. H., Seo, J., Ha, S. D., and Choi, C. (2014). Inactivation of murine norovirus and feline calicivirus during oyster fermentation. *Food Microbiology*, 44 : 81-86.

209. Seo, D. J., Lee, M., Jeon, S. B., Park, H., Jeong, S., Lee, B. H., and Choi, C. (2017). Antiviral activity of herbal extracts against the hepatitis A virus. *Food Control*, 72 : 9-13.

210. Severino, R., Ferrari, G., Vu, K. D., Donsì, F., Salmieri, S., and Lacroix, M. (2015). Antimicrobial effects of modified chitosan-based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against Escherichia coli O157: H7 and Salmonella Typhimurium on green beans. *Food Control*, 50 : 215-222.

211. Seymour, I. J., and Appleton, H. (2001). Foodborne viruses and fresh produce. *Journal of Applied Microbiology*, 91: 759-773.

212. Shahidi, F., and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of Functional Foods*, 18 : 820-897.

213. Shang, C., Cheung, L. M., and Liu, W. (2007). MS2 coliphage inactivation with UV irradiation and free chlorine/monochloramine. *Environmental Engineering Science*, 24 : 1321-1332.

214. Sharma, M., Shearer, A.E.H., Hoover, D.G., Liu, M.N., Solomon, M.B., Kniel, K.E. (2008). Comparison of hydrostatic and hydrodynamic pressure to inactivate foodborne viruses. *Innovative Food Science and Emerging Technologies*, 9: 418-422.

215. Shin, G. A., and Sobsey, M. D. (2003). Reduction of Norwalk virus, poliovirus 1, and bacteriophage MS2 by ozone disinfection of water. *Applied and Environmental Microbiology*, 69:3975-3978.

216. Silindir, M. and Özer, A. Y. (2009). Sterilization methods and the comparison of E-beam sterilization with gamma radiation sterilization. *Fabad Journal of Pharmacology* Sciences, 34: 43.

217. Simek, M., and Clupek, M. (2002). Efficiency of ozone production by pulsed positive corona discharge in synthetic air. *Journal of Physics D: Applied Physics*, 35 : 1171.

218. Smith, W. L. (1962). Chemical treatments to reduce postharvest spoilage of fruits and vegetables. *The Botanical Review*, 28 : 411-445.

219. Sommers, C. H., and Fan, X. (2002). Antioxidant power, lipid oxidation, color, and viability of Listeria monocytogenes in beef bologna treated with gamma radiation and containing various levels of glucose. *Journal of Food Protection*, 65 : 1750-1755.

220. Su, X., Howell, A. B., and D'Souza, D. H. (2010). Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates–a time dependence study in vitro. *Food Microbiology,* 27: 985-991.

221. Su, X., Sangster, M. Y., and D'Souza, D. H. (2011A). Time-dependent effects of pomegranate juice and pomegranate polyphenols on foodborne viral reduction. *Foodborne Pathogens and Disease*, 8: 1177-1183.

222. Su, X., and D'Souza, D. H. (2011B). Grape seed extract for control of human enteric viruses. *Applied and Environmental Microbiology*, 77: 3982-3987.

223. Su, X., and D'Souza, D. H. (2013). Grape seed extract for foodborne virus reduction on produce. *Food Microbiology*, 34 :1-6.

224. Su, X., and D'Souza, D. H. (2013). Naturally occurring flavonoids against human norovirus surrogates. *Food and environmental virology*, 5: 97-102.

225. Suzuki, M., Sasaki, K., Yoshizaki, F., Fujisawa, M., Oguchi, K., and Cyong, J. C. (2005). Anti-hepatitis C virus effect of citrus unshiu peel and its active ingredient nobiletin. *The American Journal of Chinese Medicine,* 33: 87-94.

226. Terio, V., Bottaro, M., Pavoni, E., Losio, M. N., Serraino, A., Giacometti, F., ... and Tantillo, G. (2017). Occurrence of hepatitis A and E and norovirus GI and GII in ready-to-eat vegetables in Italy. *International Journal of Food Microbiology*, 249: 61-65.

227. Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B., and Erim, F. B. (2009). Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry*, 115 : 873-877.

228. Theron, M. M., and Lues, J. R. (Ed. 1). (2010). *Organic acids and food preservation*. CRC Press. Florida, United States.

229. Thornley, M. J. (1963). Radiation resistance among bacteria. *Journal of Applied Bacteriology (England)*, *26*.

230. Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., and Gerba, C. P. (2003). Chlorine inactivation of adenovirus type 40 and feline calicivirus. *Applied and Environmental Microbiology*, 69 : 3979-3985.

231. Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., and Gerba, C. P.(2005). Inactivation of enteric adenovirus and feline calicivirus by ozone. *Water Research*, 39 : 3650-3656.

232. Trindade, M. A., Kushida, M. M., Montes Villanueva, N. D., Pereira, D. U. D. S., and de Oliveira, C. E. L. (2012). Comparison of ozone and chlorine in low concentrations as sanitizing agents of chicken carcasses in the water immersion chiller. *Journal of Food Protection*, 75 : 1139-1143.

233. Tsen, S. W. D., Chapa, T., Beatty, W., Tsen, K. T., Yu, D., and Achilefu, S. (2012). Inactivation of enveloped virus by laser-driven protein aggregation. *Journal of Biomedical Optics*, 17: 128002.

234. Ultee, A., Slump, R. A.., Steging, G., and Smid, E. J. (2000). Antimicrobial activity of carvacrol toward Bacillus cereus on rice. *Journal of Food Protection*, 63: 620–4.

235. Urakami, H., Ikarashi, K., Okamoto, K., Abe, Y., Ikarashi, T., Kono, T., and Tanaka, N. (2007). Chlorine sensitivity of feline calicivirus, a norovirus surrogate. *Applied and Environmental Microbiology*, 73: 5679-5682.

236. Vandekinderen, I., Van Camp, J., De Meulenaer, B., Veramme, K., Bernaert, N., Denon, Q., ... and Devlieghere, F. (2009). Moderate and high doses of sodium hypochlorite, neutral electrolyzed oxidizing water, peroxyacetic acid, and gaseous chlorine dioxide did not affect the nutritional and sensory qualities of fresh-cut iceberg lettuce (Lactuca sativa Var. capitata L.) after washing. *Journal of Agricultural and Food Chemistry*, 57: 4195-4203.

237. Vercauteren, J., and Chèze, C. (1998). *Polyphenols 96: 18th International Conference on Polyphenols, Bordeaux (France), July 15-18, 1996* (No. 87). Editions Quae.

238. Vorsa, N., Vvedenskaya, I. O., Huang, M. T., and Rosen, R. T. (2007). *U.S. Patent No. 7,270,837*. Washington, DC: U.S. Patent and Trademark Office.

239. Vreeswijk, M. P., Van Hoffen, A., Westland, B. E., Vrieling, H., Van Zeeland, A. A., and Mullenders, L. H. (1994). Analysis of repair of cyclobutane pyrimidine dimers and pyrimidine 6-4 pyrimidone photoproducts in transcriptionally active and inactive genes in Chinese hamster cells. *Journal of Biological Chemistry*, 269 : 31858-31863.

240. Ward, B. K. & Irving, L. G. (1987). Virus survival on vegetables spray-irrigated with wastewater. *Water Research*, 21:57-63.

241. Watanabe, Y., Miyata, H., and Sato, H. (1989). Inactivation of laboratory animal RNA-viruses by physicochemical treatment. *Experimental Animals*, 38: 305-311.

242. Wei, J., and Kniel, K. E. (2010). Pre-harvest viral contamination of crops originating from fecal matter. *Food and Environmental Virology*, 2: 195-206.

243. Whitehead, K., and McCue, K. A. (2010). Virucidal efficacy of disinfectant actives against feline calicivirus, a surrogate for norovirus, in a short contact time. *The American Journal of Infection Control,* 38: 26-30.

244. Widdowson, M. A., Cramer, E. H., Hadley, L., Bresee, J. S., Beard, R. S., Bulens, S. N., ... and Mintz, E. (2004). Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United States, 2002. *Journal of Infectious Diseases*, 190 : 27-36.

245. Wobus, C. E., Thackray, L. B., and Virgin, H. W. (2006). Murine norovirus: a model system to study norovirus biology and pathogenesis. *Journal of Virology,* 80: 5104-5112.
246. Wolff, S. P., Garner, A., and Dean, R. T. (1986). Free radicals, lipids and protein degradation. *Trends in Biochemical Sciences*, 11 : 27-31.
247. Wondrak GT, Jacobson MK, and Jacobson EL. (2006). Endogenous UVA-photosensitizers: mediators of skin photodamage and novel targets for skin photoprotection. *Photochemical and Photobiological Sciences,* 5:215-237.
248. Woo, L., and Sandford, C. L. (2002). Comparison of electron beam irradiation with gamma processing for medical packaging materials. *Radiation Physics and Chemistry*, 63 : 845-850.
249. Wu, V. C., Qiu, X., Benildo, G., Lin, C. S., and Pan, Y. (2009). Application of cranberry concentrate (Vaccinium macrocarpon) to control Escherichia coli O157: H7 in ground beef and its antimicrobial mechanism related to the downregulated slp, hdeA and cfa. *Food Microbiology*, 26: 32-38.
250. Wysok, B., Uradziñski, J., and Gomólka-Pawlicka, M. (2006). Ozone as an alternative disinfectant-a review. *Polish Journal of Food and Nutrition Sciences*, 15:3.
251. Xie, Y., Hajdok, C., Mittal, G. S., and Warriner, K. (2008). Inactivation of MS2 F (+) coliphage on lettuce by a combination of UV light and hydrogen peroxide. *Journal of Food Protection*, 71 : 903-907.
252. Yuk, H. G., Yoo, M. Y., Yoon, J. W., Moon, K. D., Marshall, D. L., and Oh, D. H. (2006). Effect of combined ozone and organic acid treatment for control of Escherichia coli O157: H7 and Listeria monocytogenes on lettuce. *Journal of Food Science*, 71: M83-M87.
253. Zakaryan, H., Arabyan, E., Oo, A., and Zandi, K. (2017). Flavonoids: promising natural compounds against viral infections. *Archives of Virology*, 162 : 2539-2551.
254. Zhang, L., Lu, Z., and Wang, H. (2006). Effect of gamma irradiation on microbial growth and sensory quality of fresh-cut lettuce. *International Journal of Food Microbiology*, 106:348-351.
255. Zhang, X. F., Dai, Y. C., Zhong, W., Tan, M., Lv, Z. P., Zhou, Y. C., and Jiang, X. (2012). Tannic acid inhibited norovirus binding to HBGA receptors, a study of 50 Chinese medicinal herbs. *Bioorganic & Medicinal Chemistry*, 20 :1616-1623.
256. Zhao, S., Liu, H., and Gu, L. (2018). American cranberries and health benefits–an evolving story of 25 years. *Journal of the Science of Food Agriculture,* 2018.