

Overview of applications of radiation processing in combination with conventional treatments to assure food safety

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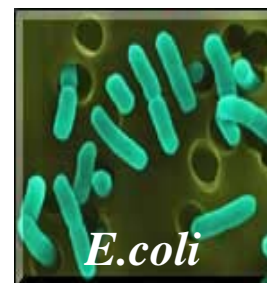
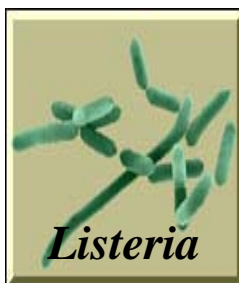
IAEA November 11, 2014

PROBLEMATIC ISSUES

The Center for Disease Control and Prevention (CDC) estimates that 48 million people get sick due to foodborne diseases in USA annually.

In Canada, the foodborne illness is estimated as more than 11 million episodes/year

→ Therefore, controlling of food pathogens in food products are very important.



Foodborne Illness in Canada



13 million cases/year

***Salmonella choleraesuis* : 630 000 cases/year,
\$ 98 Million**

***Staphylococcus aureus* : 100 000 cases/year**

***Campylobacter jejuni* : 16 000 cases/year**

***E. coli* O157:H7 : 13 000 cases/year**

***Shigella* : 19 000 cases/year**

***Listeria monocytogenes* : 2 800 cases/year**

Cost of Food Loss in Canada

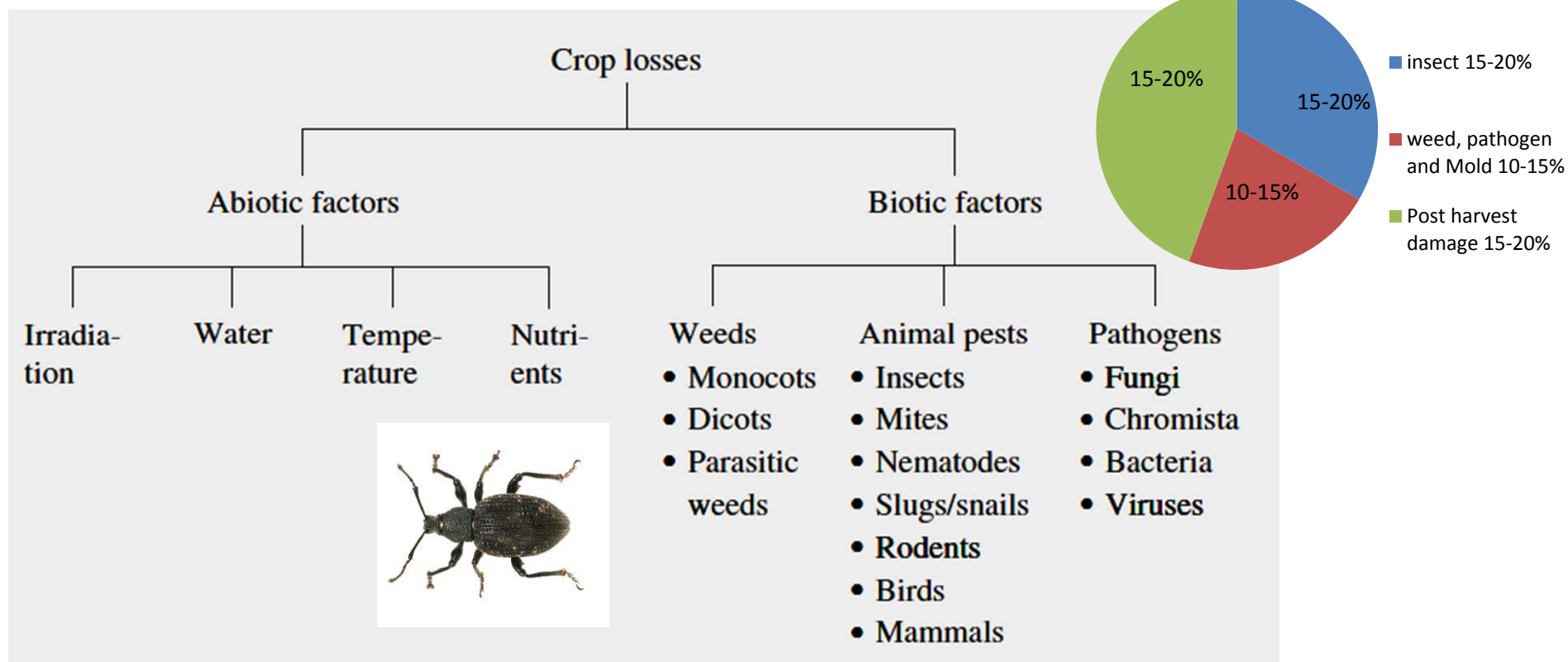
Meat, Poultry and Fishes: \$200 Million/year

In Canada, 50% of the mortality related to alimentary diseases is due to poultry consumption

Foodborne illness: \$ 1 Billion/year

Crop pests - a serious concern....

1. More than 40% crops are destroyed each year due to pests.
2. Preventing this loss could feed 3 billion malnourished people.
(Pimentel, D, 2009, Agricultural Sciences, Vol II, P-2)



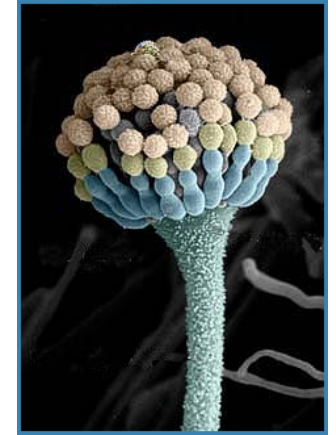
**More than 40% of food products could be rejected
due to molds contamination**

Aflatoxins produced by molds

-Toxic

- Mutagenic

- Carcinogenic



Occurrence of Aflatoxins in Food

Food	Country	Quantity	References
Rice	India	5-50 µg/kg	Rubsom 1997. <i>Food Chem.</i> 57-67.
	Chin	20 µg/kg	
Maize	USA	10-700 µg/kg	
	Denmark	5-174 µg/kg	
	France	> 20 µg/kg	
Oat/wheat/bardy	Sweden	50-400 µg/kg	Andrea <i>et al.</i> 2012. <i>Food Addit. Contam.</i> 276-280.
Meat	Egypt	6-40 µg/kg	
Dried figs	UK	10-40 µg/kg	
Cotton seed	Argentina	20-200 µg/kg	
Peanuts	Mexico	700 µg/kg	
	USA	24 µg/kg	Bhat <i>et al.</i> 2010. <i>Food Sci. Food Safety.</i> 57-81.
	Brazil	7.9-1496 µg/kg	
Nuts shelled	Brazil	16.5-1972 µg/kg	
Peanuts butter	USA	20-100 µg/kg	
	UK	38-535 µg/kg	
Raw drugs	China	1.29 µg/kg	Rodriguez Valasco <i>et al.</i> 2010. <i>Food Addit. Contam.</i> 276-280.
Medicine plant	China	10-160 µg/kg	
Eggs	China	3300 mg/Kg	
Milk	UE countries	28-1012 ng/kg	
	India	50 ng/L	
Red pepper	Ethiopia	250-525 µg/kg	Aydin <i>et al.</i> 2007. <i>Food Cont.</i> 1283- 1288.
	India	<10->100 µg/kg	
Red Paprika	Korea	5.40 µg/kg	
Wallnut	Malaysia	17.5 µg/kg	
Bakery product		24 µg/kg	
Coated nut products		113.1-514.7 µg/kg	Leong <i>et al.</i> 2010. <i>Food Cont.</i> 334-338.

Irradiation of any food must satisfy two objectives

1- Adequate kill of target micro-organisms and insects

2-Retention of high quality of product

3-Avoidance of radiation-induced sensory detriment

Challenge is to ensure the simultaneous attainment of these goals

Irradiation is a non-thermal technologies are emerging technologies that have the advantage to assure food safety without affecting the nutritional value and when done in combination with other technologies (ex: addition of antimicrobials and modified atmosphere packaging), can protect the physico-chemical and sensorial quality by increasing the bacterial sensitivity.

However, the efficiency of the process should be demonstrated *in situ*

Microorganisms Sensibility

Temperature

Bacteria level

Presence of oxygen

Water availability

Media composition

Other factor to consider

The increase of the consumer demand to eliminate the addition of additives in food system represent a challenge for the scientists

Natural antimicrobials characteristics like essential oils (EOs)

Low solubility in water

High volatility

they need to be encapsulated in appropriate delivery systems to promote their efficiency and assure a control release

The sensorial quality should be protected

Combination of Irradiation and EOs treatment

- **The ionizing radiation affects directly the microbial DNA molecules.**
- **The addition of EOs disintegrates the cell membrane, makes the cell impossible to repair the damage incurred by the action of both treatments (Oussalah et al., 2006 J. Food Prot. 69, 5, 1046-1055).**
- **Combined treatment lowers the doses required for individual treatment of EOs and Irradiation alone. (Takala et al., 2011, Rad. Phys. Chem., 80, 1414-18).**

- **Novel Functional Ingredients for Enhancing the Effectiveness of Irradiation in Food Processing**
- **Novel Encapsulation Technologies to Protect the Functional Ingredients and to Assure a control release of the functional ingredients**

Main results of our studies on bacterial and insect radiosensitization using combined treatments

Protection of the natural antimicrobial compounds used

Future opportunities and needs

Method used



**Fresh meat or ready-to-eat meat or vegetables
or rice**



with additive and test organism.



Irradiation to specified doses.



**Determine surviving microorganisms or insects as
function of radiation dose.**



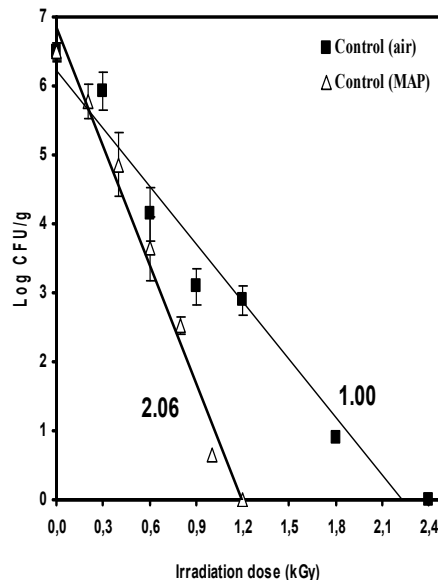
Plot radiation survival curves and calculate D_{10} .

Bacterial Radiosensitivity

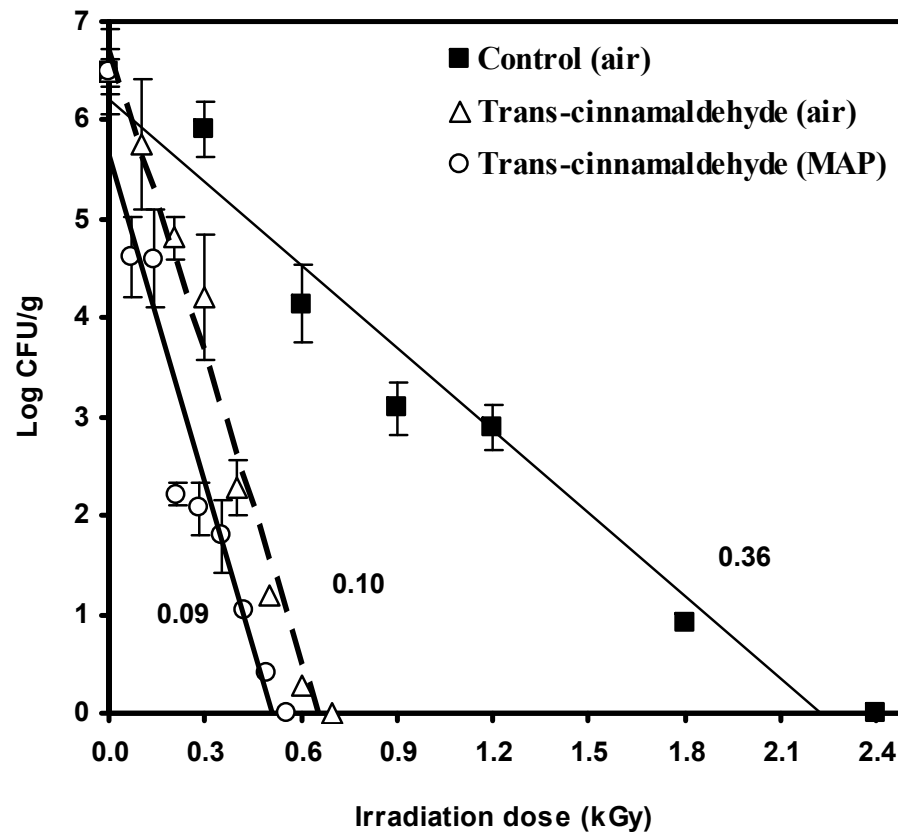
D_{10} (control) / D_{10} (treatment)

Modified atmosphere packaging (MAP) has been used to control bacterial populations in ready-to-used vegetables

Combination of MAP and irradiation without antimicrobial compounds can increase by 2 times the radiosensitization of *Listeria monocytogenes* in carrots



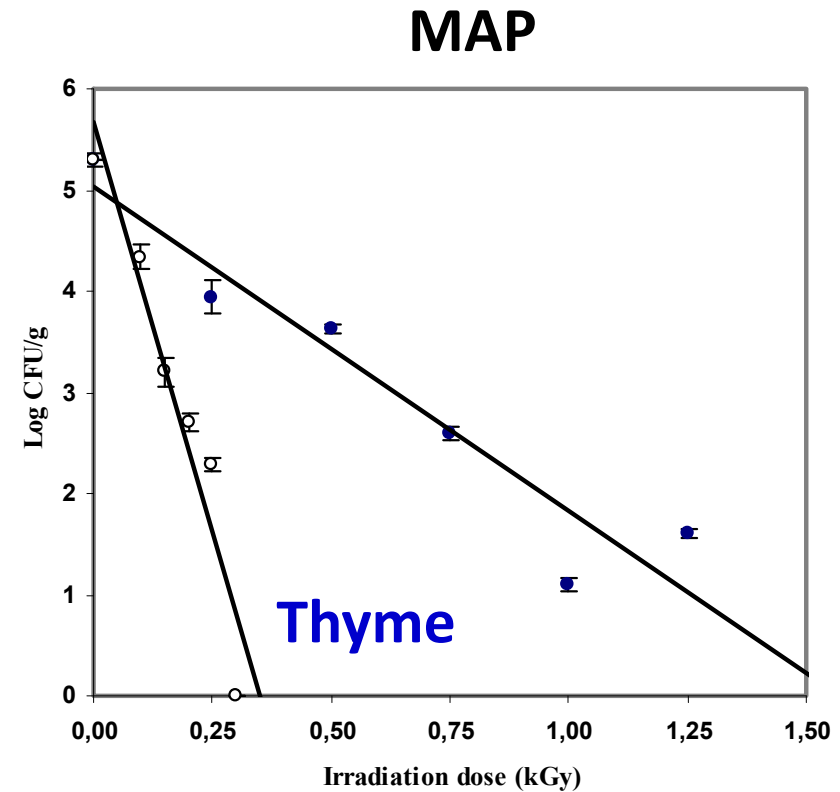
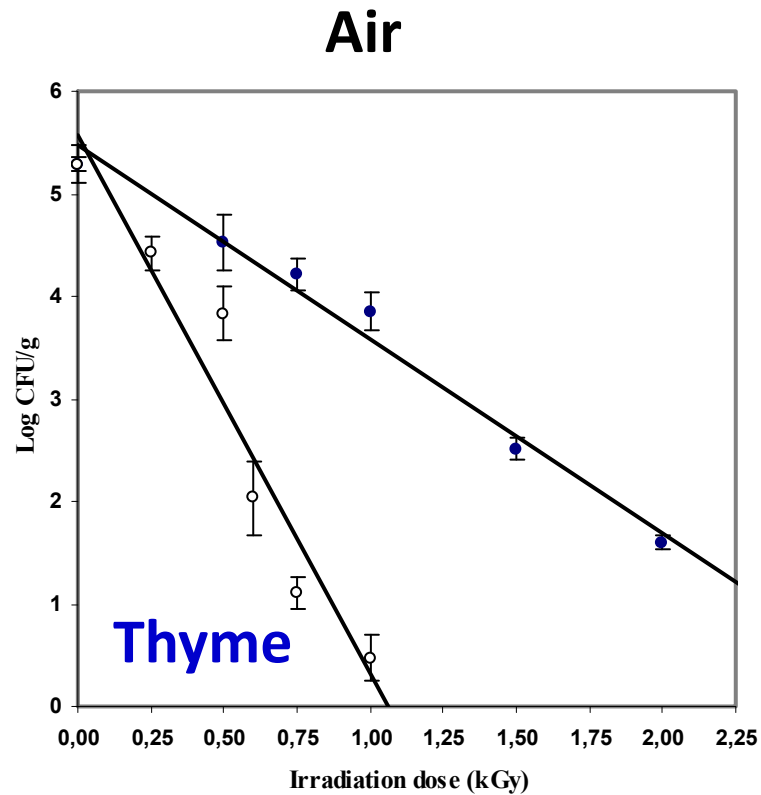
Listeria monocytogenes inhibition on ready to eat carrots in presence of natural antimicrobial compounds



HE + Ionisation
D₁₀ (air) = 0.1 kGy
D₁₀ (MAP) = 0.08 kGy
D₁₀ in presence of HE
Air = 3.7 et MAP = 4

***Salmonella* in Beef**

Bacterial Radiosensitization using spice extracts



Combinaison MAP and carvacrol on ground beef

E. coli

S.TYPHI

	D10 (kGy)	Relative <i>Sensitivity</i>	D ₁₀ (kGy)	sensitivity
<i>Contrôle</i>	0.126	1.00	0.526	1.00
<i>MAP</i>	0.086	1.9	0.221	4.15
<i>Ess. oils</i> + Air	0.055	2.29	0.254	2.2
<i>Ess. oils</i> + <i>MAP</i>	0.046	2.74	0.053	9.92

1. MAP : 60% O₂ - 30% CO₂ - 10% N₂ ;

Ess. oils mix: Carvacrol (1 %) + TSPP (0.1%)}

3. Relative Sensitivity = ratio du D₁₀(contrôle) : D₁₀(treatment)

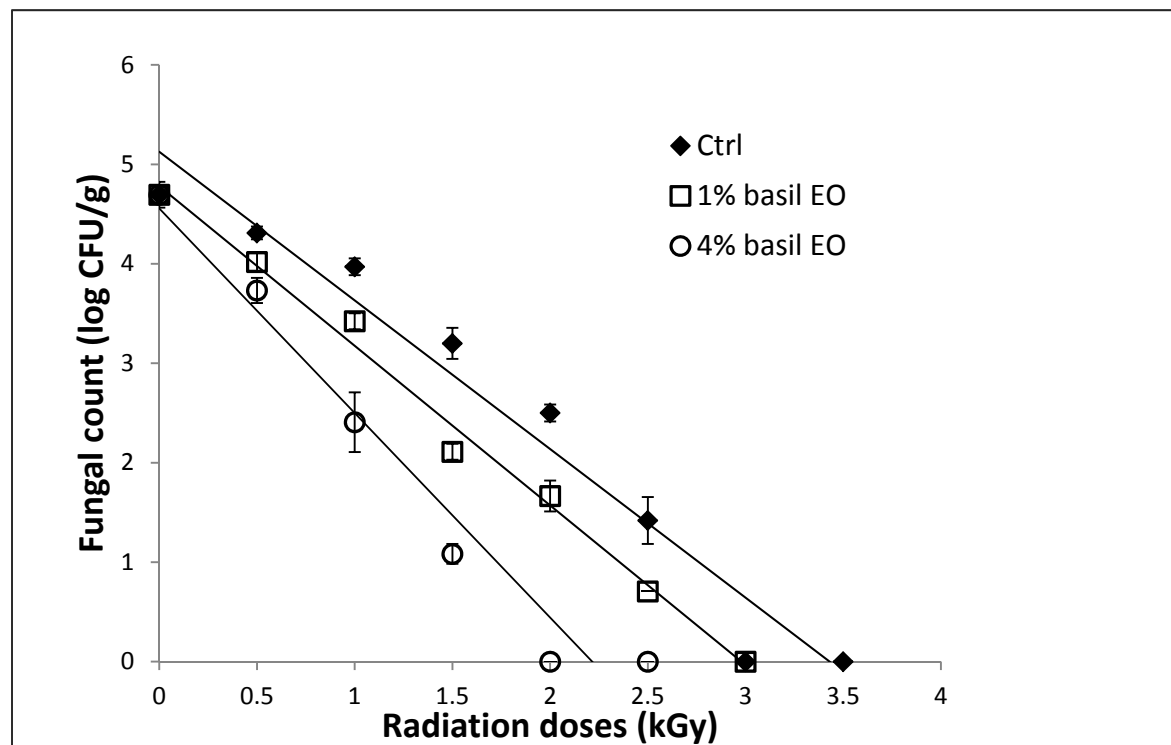
Shelf life extension of ground beef mesophilic aerobic count (\log_{10})

Treatment/Day	1	3	7	14
AIR				
0 kGy	7.3	>7	>7	>7
1.5 kGy	2.6 (4.7 log reduction)	3.5	4.3	>7
0 kGy Mustard 0.075%	7.4	>7	>7	>7
1.5 kGy Mustard 0.075%	2.6	2.4	3.8	>7
MAP				
1.5 kGy	<10	<10	<10	<10 even after 28 days of storage
0 kGy Mustard 0.075%	5.6 (1.7 log reduction)	7.1	>7	>7
1.5 kGy Mustard 0.075%	<1	<1	<1	<10 even after 28 days of storage

***Listeria* content in ready to eat carrots**

Treatment	Day 1 (Log CFU/gr)	Day 7 (Log CFU/gr)
Control	3	3
0.25 kGy Air	2.6	2.4
0.50 kGy Air	1.9	1.15
0.25 kGy MAP	1.75	1.4
0.50 kGy MAP	<1	<1
Cinnamon 0.25 kGy Air	2.59	1.72
Cinnamon 0.5 kGy Air	2.04	<1
Cinnamon 0.25 kGy MAP	2.1	<1
Cinnamon 0.5 kGy MAP	<1	<1

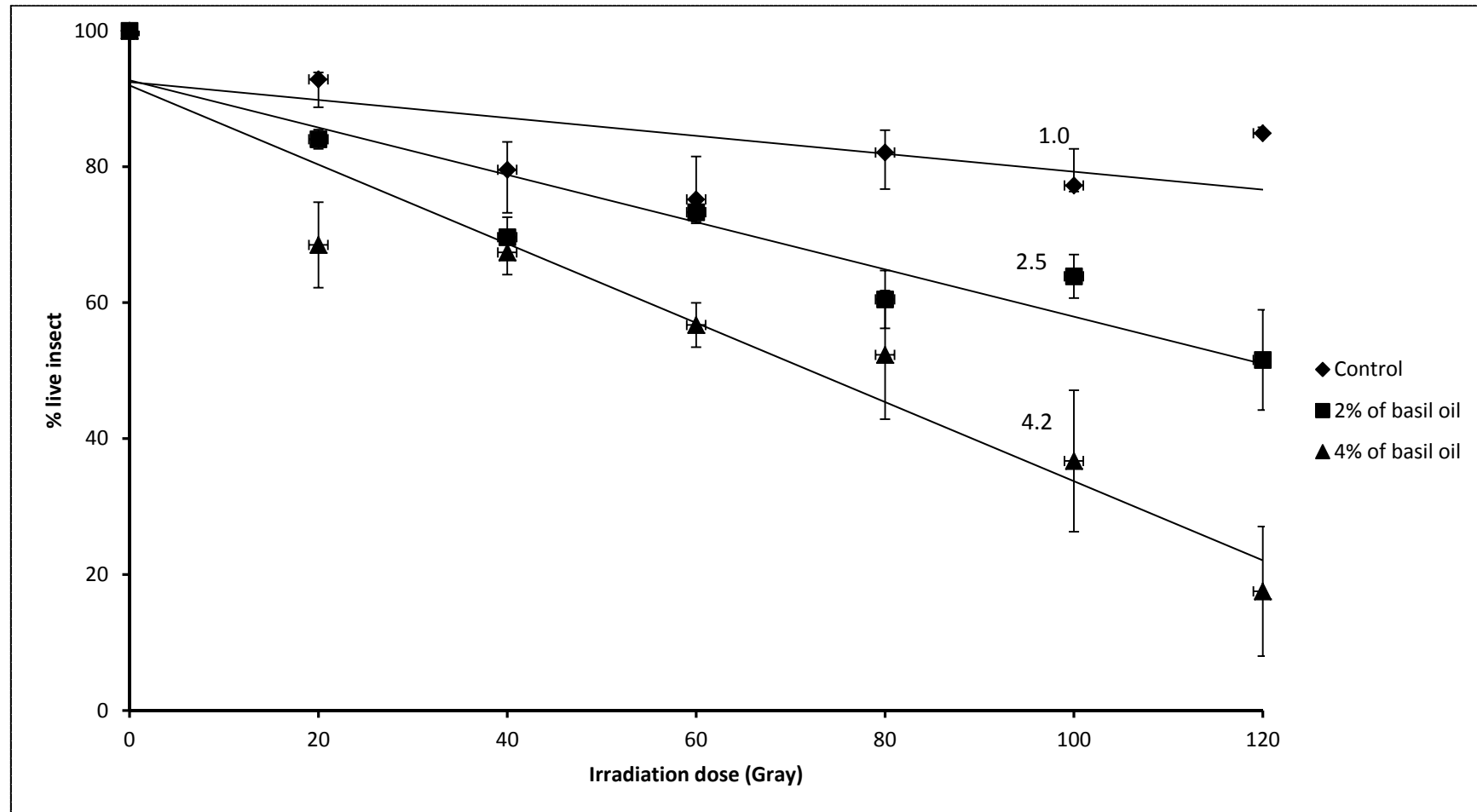
***In situ* Irradiation Experiment to Eliminate *A. niger* in rice**



Relative sensitivity (RS) of *A. niger*

The D_{10} value decreased to 0.49 kGy and RS increased 1.38 times by combining 4% basil EO and irradiation treatment ($p \leq 0.05$) as compared to control.

Radiosensitization of *Sitophilus oryzae* in rice



Encapsulation

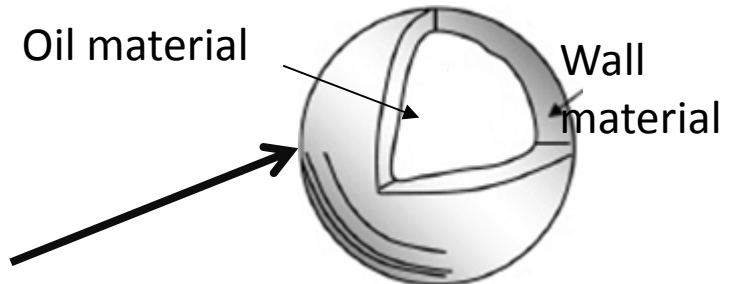
Core materials
(bioactive agents
Flavor, antioxidant,
living cell)

Wall materials

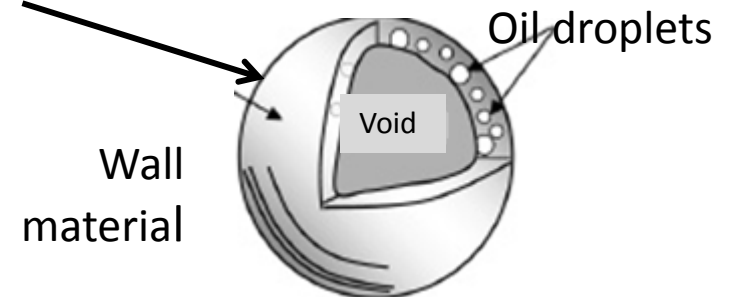
- Carbohydrate (starch, sucrose)
- Protein(Gelatin)
- Cellulose (MC, CMC)

ENCAPSULATION

SINGLE CORE



MULTIPLE CORE



Encapsulated product

Benefits:

- Prevents degradative reactions
- Allows mixing of incompatible components
- Ensures control release to the target site.

Natural polymer in agriculture.

- Natural polymers such as starches, chitosan, cellulose derivatives can be convenient for agricultural sector.

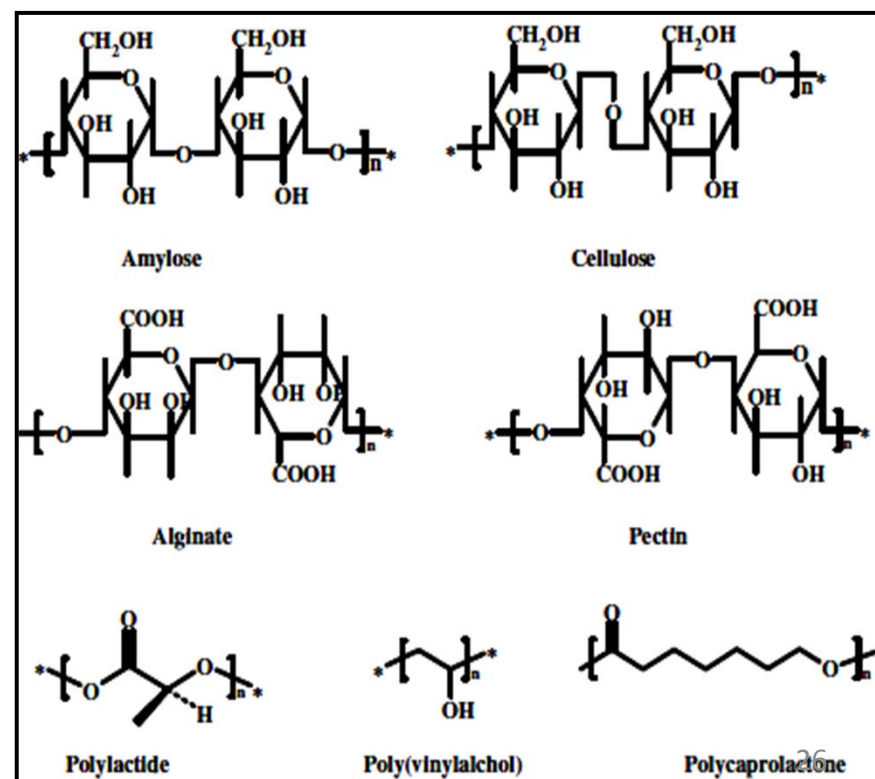
- Challenge

The properties of these polymers do not fit specific applications.

- Alternative

Blending, mixing and modifying with other polymers can regulate their morphologies and properties easily and efficiently.

(Puoci, et al., 2008, Am. J. Agric and Biol. Sci. V.3. 299-314)



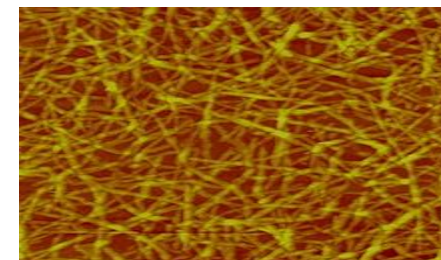
**Active coating and Nanoscale encapsulation have
been proposed as**

Innovative approaches

**Nanoscale encapsulation of natural antimicrobial compounds
inside the active coating formulation can increase the
concentration of bioactive compounds in food areas where
microorganisms are preferably located**

**The active coating can be applied on vegetables to minimize or
prevent the growth of pathogenic microorganisms**

Crystalline nanocellulose (CNC)

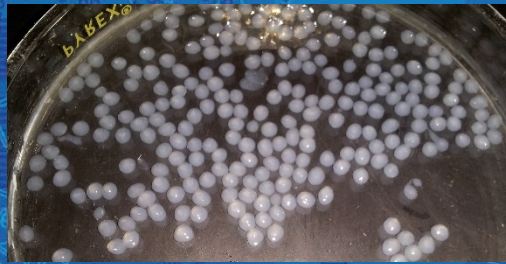


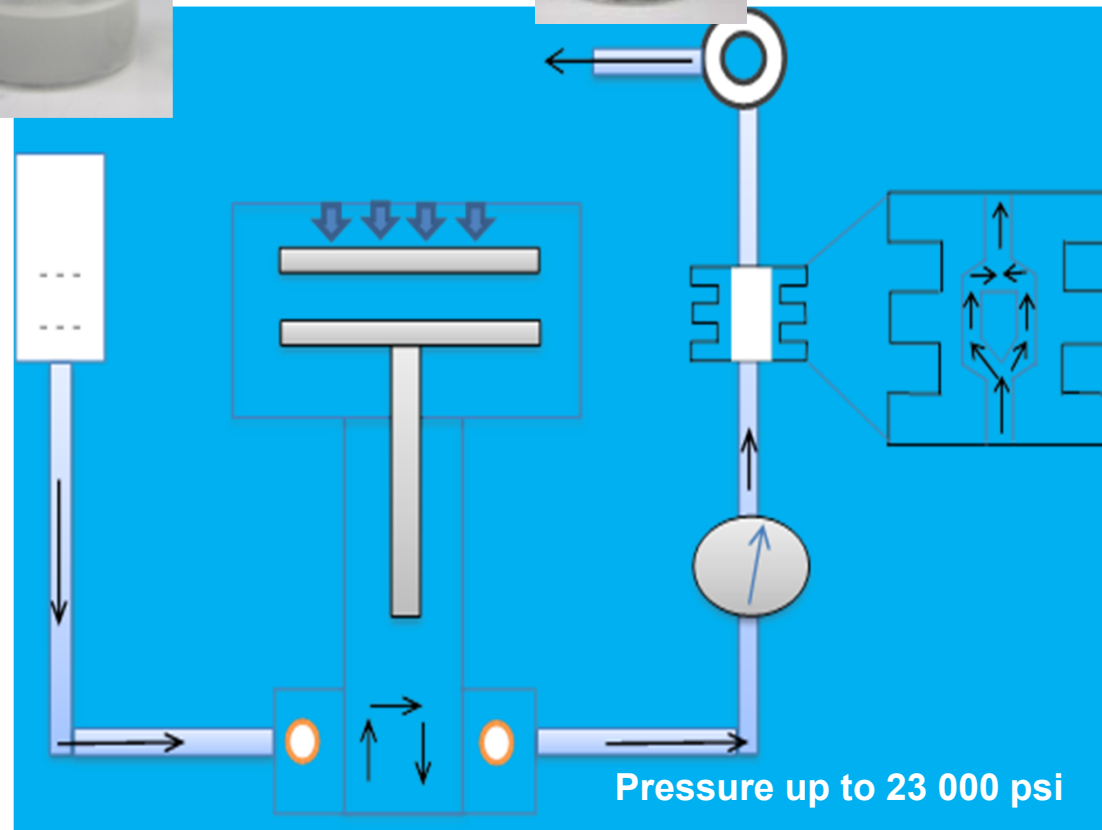
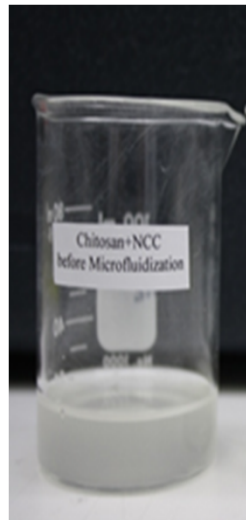
CNC

- **Fillers for thermoplastic polymers due to their outstanding mechanical and barrier properties.**
- **Can increase the stability of encapsulated bioactive agents into polymer matrices and ensure controlled release.**

(Khan et al., 2010. J. Agric. Food Chem., 58, 7878-85),
(Boumail et al., 2013. J. Agric. Food Chem. 61, 811-21),
Jamshidian et al., 2010, Comprehensive Reviews in Food Science and Food Safety, 9, 552-571)

Development of Cellulose Nanocrystal (CNC) Reinforced Bio polymeric Matrix for Encapsulation of Bioactive Compounds

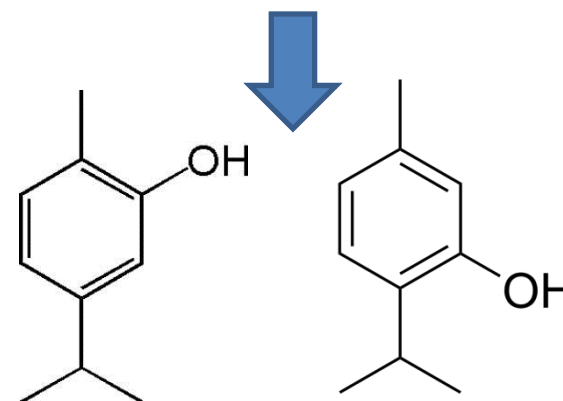




Essential Oils as antimicrobial compounds

- Aromatic oily liquids
- Extracted from plant
- Complex mixtures of comparatively volatile substances
- Widely used as an antimicrobial compounds against pathogenic bacteria

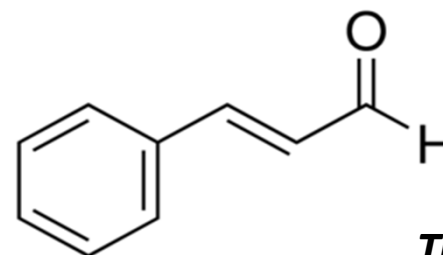
Origanum Compactum



Carvacrol

Thymol

Cinnamomum Cassia

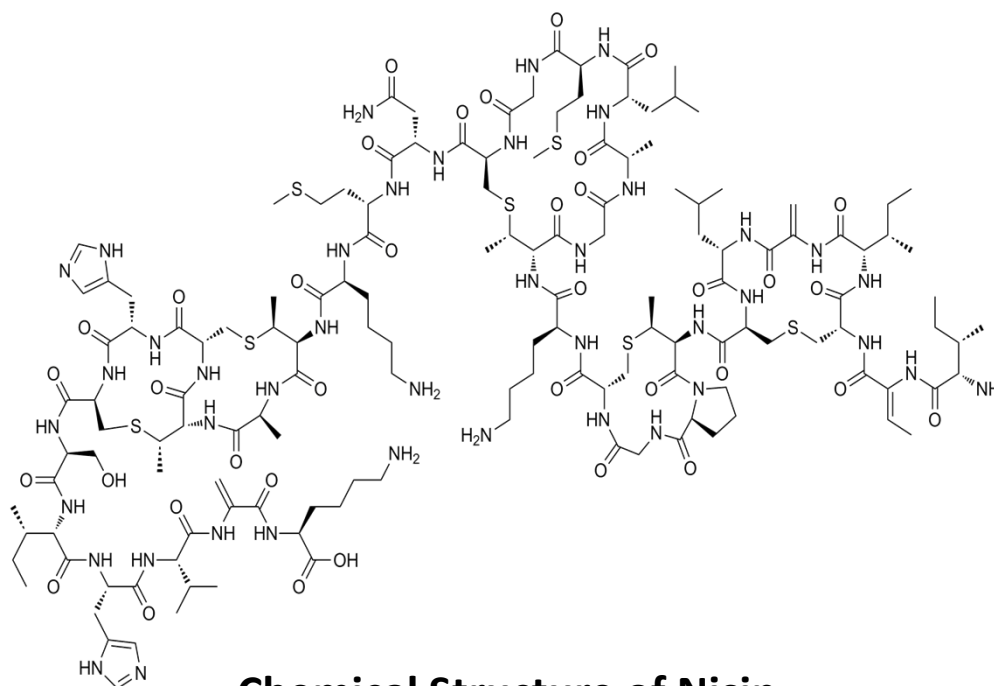


Trans-cinnamaldehyde

Nisin as an antimicrobial compound

- An antimicrobial peptide and produced from *Lactococcus lactis*
- Recognized as GRAS by the United States Food and Drug Administration (FDA)
- Showed antimicrobial activity against pathogenic bacteria (*Listeria monocytogenes*)

activity
bacteria



Chemical Structure of Nisin

Cutter and Siragusa, 1997. *Food Microbiology* 14: 425–430.

***In situ* Analysis (Ready-to-eat, RTE Meat)**



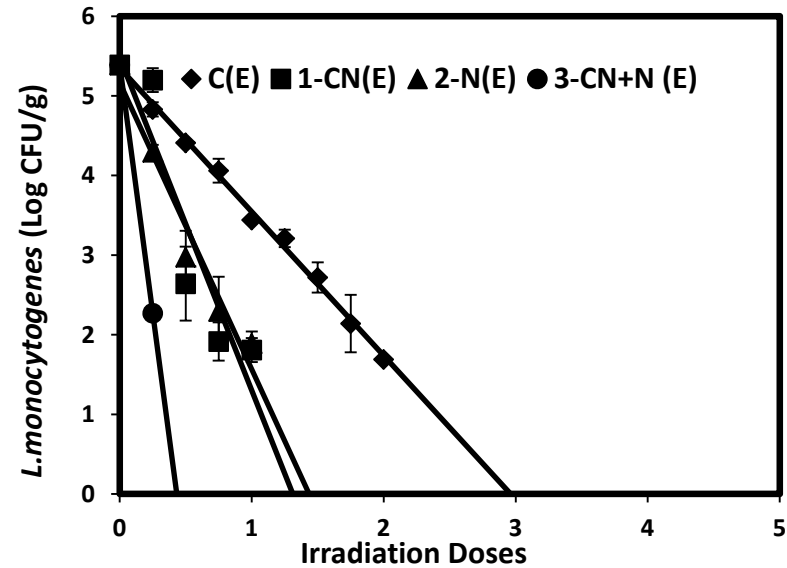
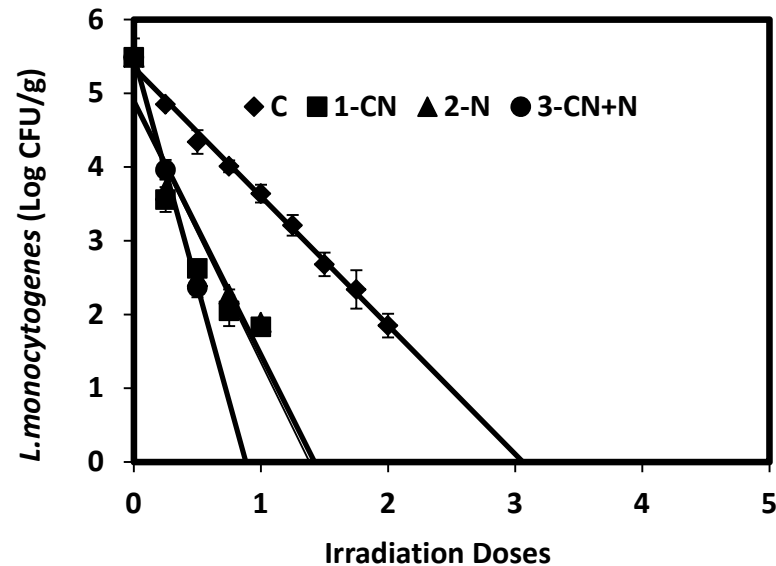
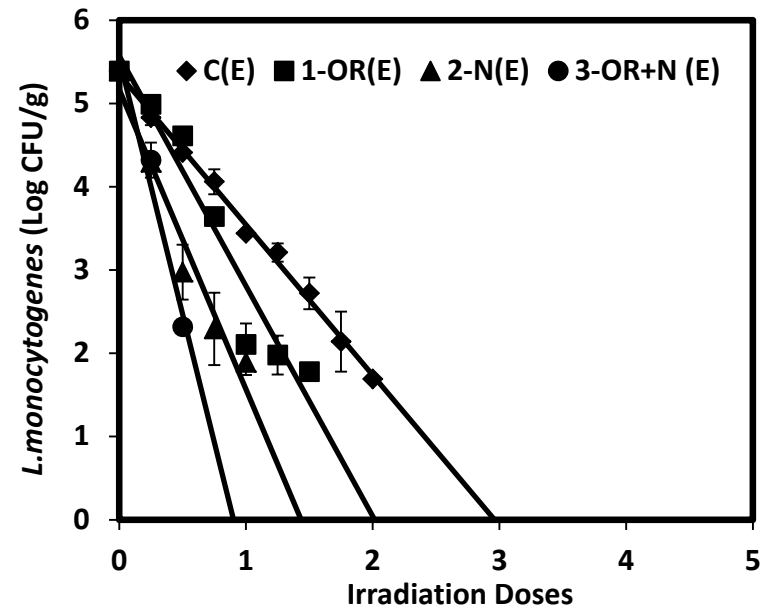
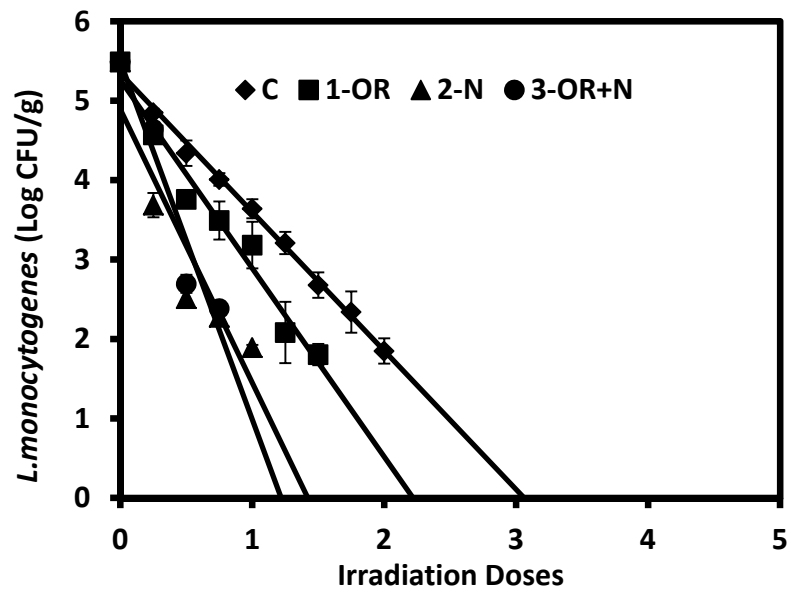
- Ground lean pork
- Sodium chloride (13.95g), triphosphate (4.59g), erythorbate (0.80g) and nitrite salt (0.83g)
- Cooked at about 1hr at 162.7° C



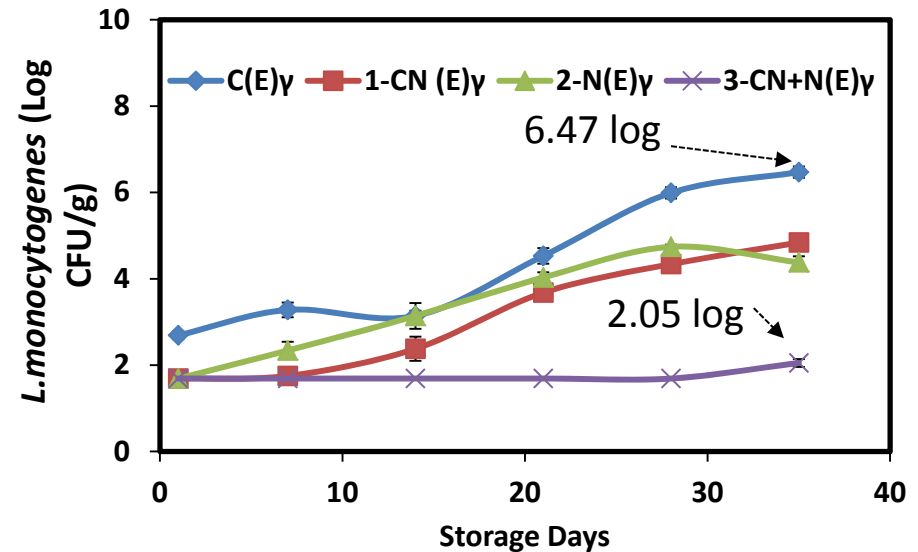
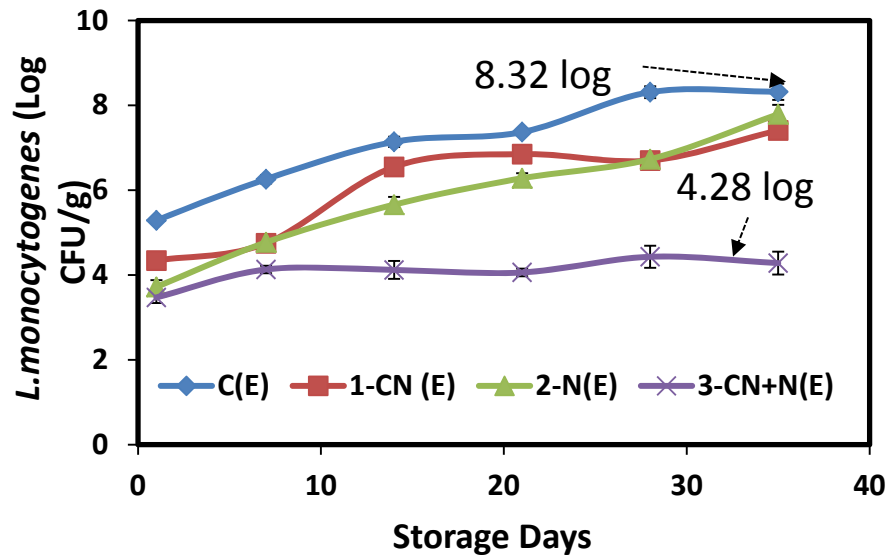
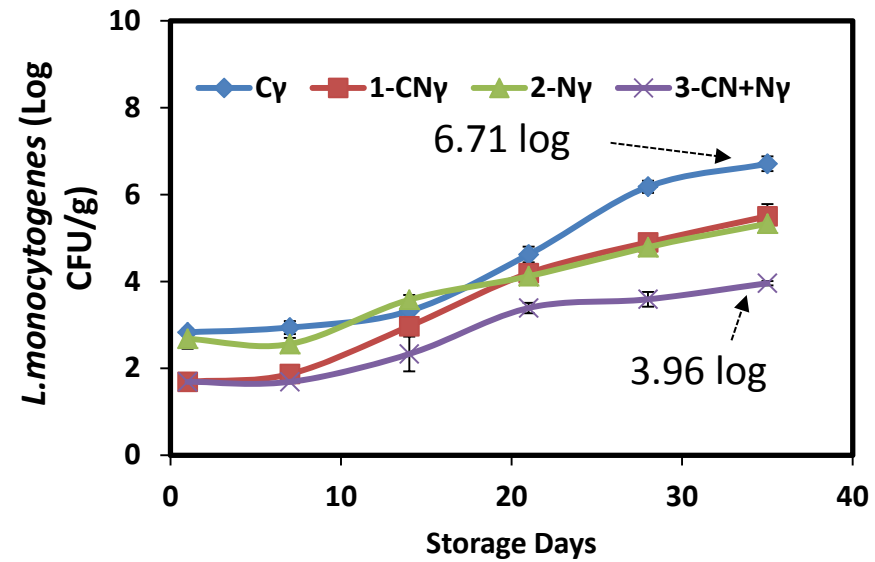
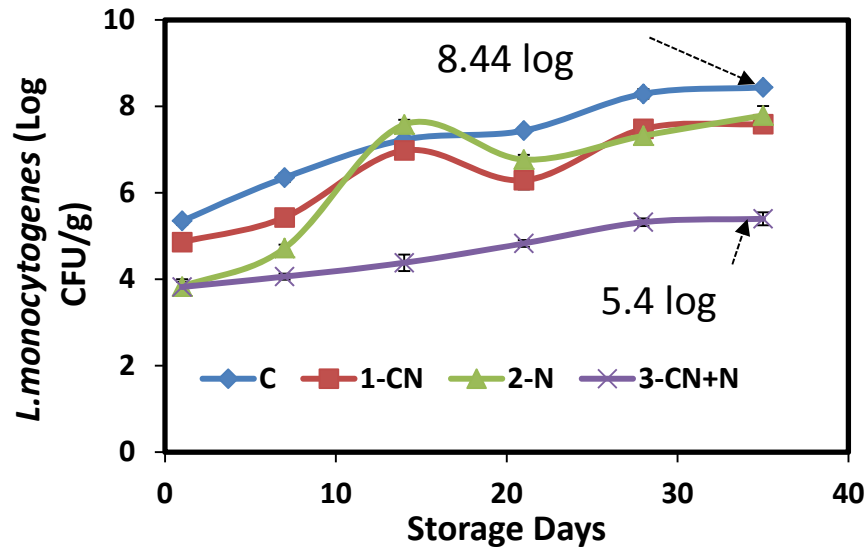
**Without microencapsulated
ham**



**Microencapsulated nisin In
alginate/CNC based polymer**



Radiosensitization of *L.monocytogenes* on RTE meat containing non and microencapsulated *Origanum Compactum* (0.25% w/v), *Cinnamomum Cassia* (0.25% w/v) and Nisin (0.125% w/w)



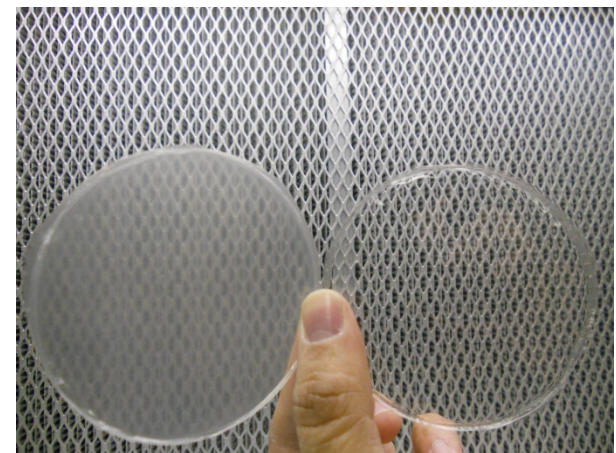
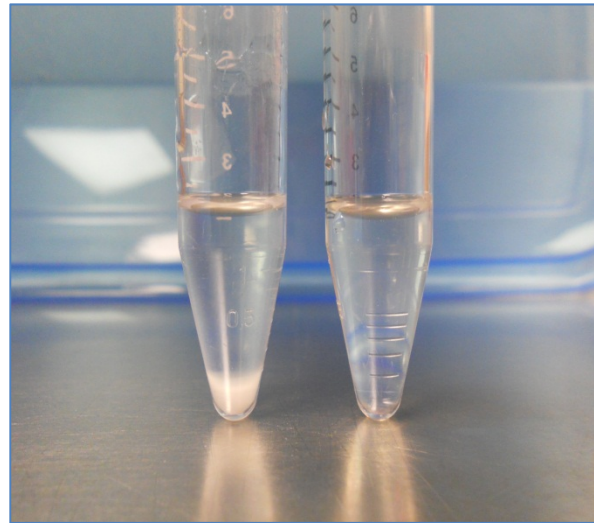
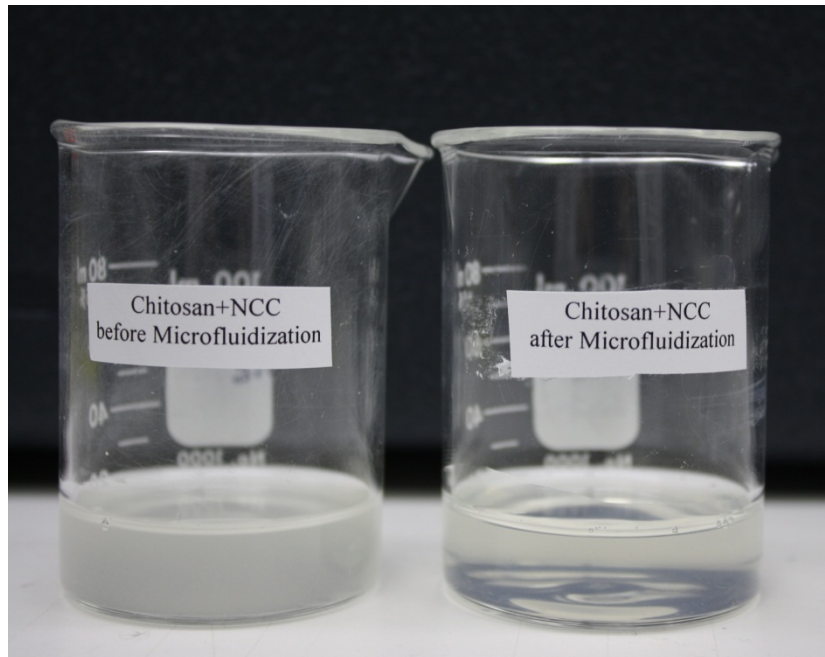
***L. monocytogenes* with and without microencapsulated essential oils and nisin with gamma irradiation (at 1.5 kGy) on RTE meat during storage against *L. monocytogenes*.**

D₁₀ and Radiosensitivity (RS) for non and microencapsulated antimicrobial microbeads against *L.monocytogenes**

	D ₁₀ (kGy)	RS
C	0.57±0.031 ^a	1±0.00 ^a
OR	0.49±0.044 ^b	1.16±0.09 ^{ab}
CN	0.26±0.035 ^d	2.18±0.28 ^{cd}
N	0.29±0.018 ^{cd}	1.94±0.06 ^c
OR+N	0.20±0.036^e	2.91±0.74^{ef}
CN+N	0.16±0.001^e	3.57±0.15^g
C(E)	0.55±0.015 ^a	1±0.00 ^a
OR(E)	0.34±0.048 ^c	1.65±0.30 ^{bc}
CN(E)	0.21±0.028 ^e	2.62±0.34 ^{de}
N (E)	0.29±0.034 ^d	1.95±0.26 ^c
OR+N(E)	0.16±0.003^e	3.40±0.17^{fg}
CN+N (E)	0.08±0.002^f	6.89±0.30^h

*Values are means ± standard deviations. Means with the same letter are not significantly different (P > 0.05).

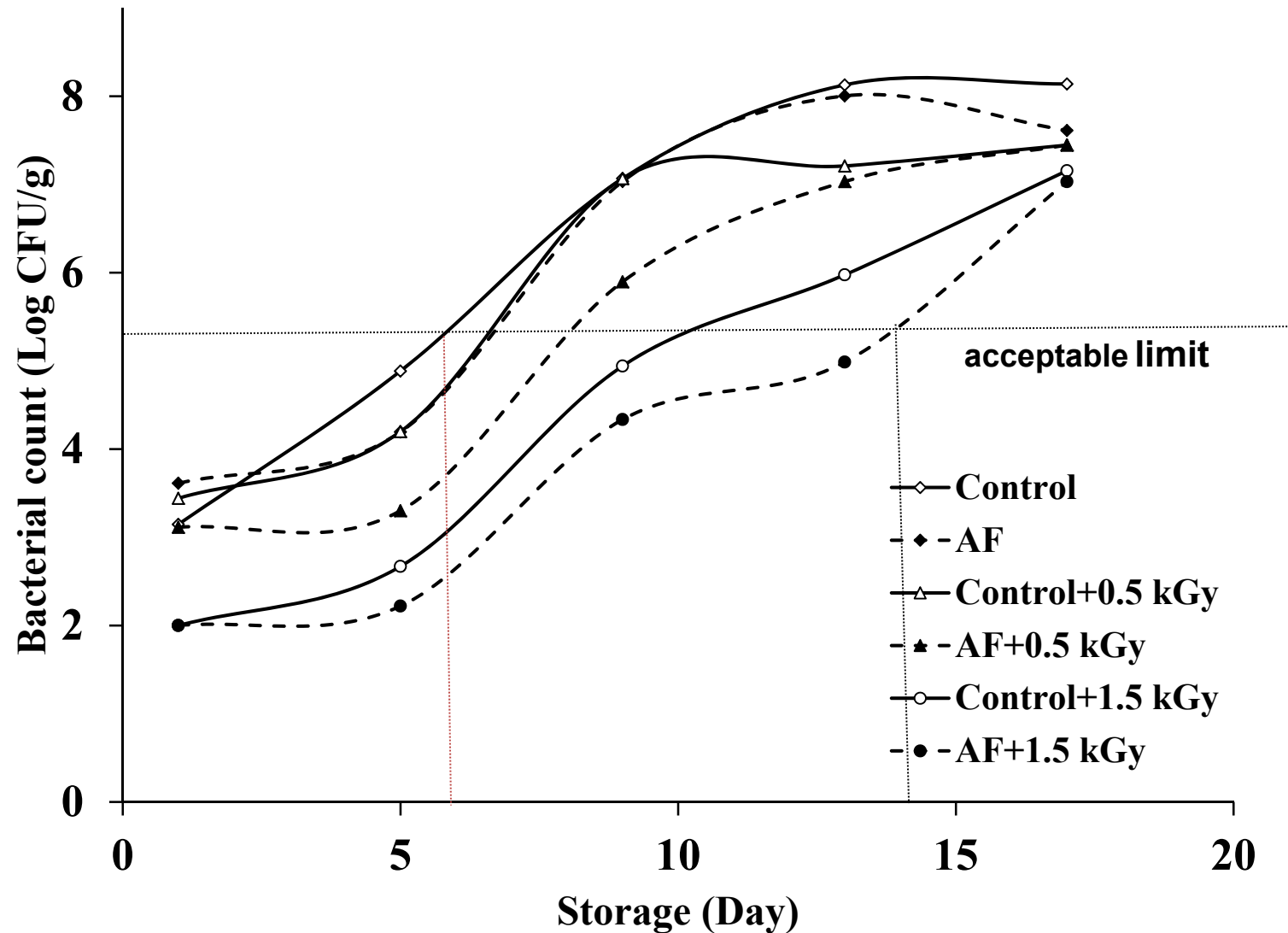
Microfluidisation of chitosan-NCC based solution and film formation



Active chitosan-NCC based film



Effect of active films based on chitosan-NCC and irradiation on mesophilic bacteria in RTE meat



Coating application on ready to eat vegetables and irradiation treatment



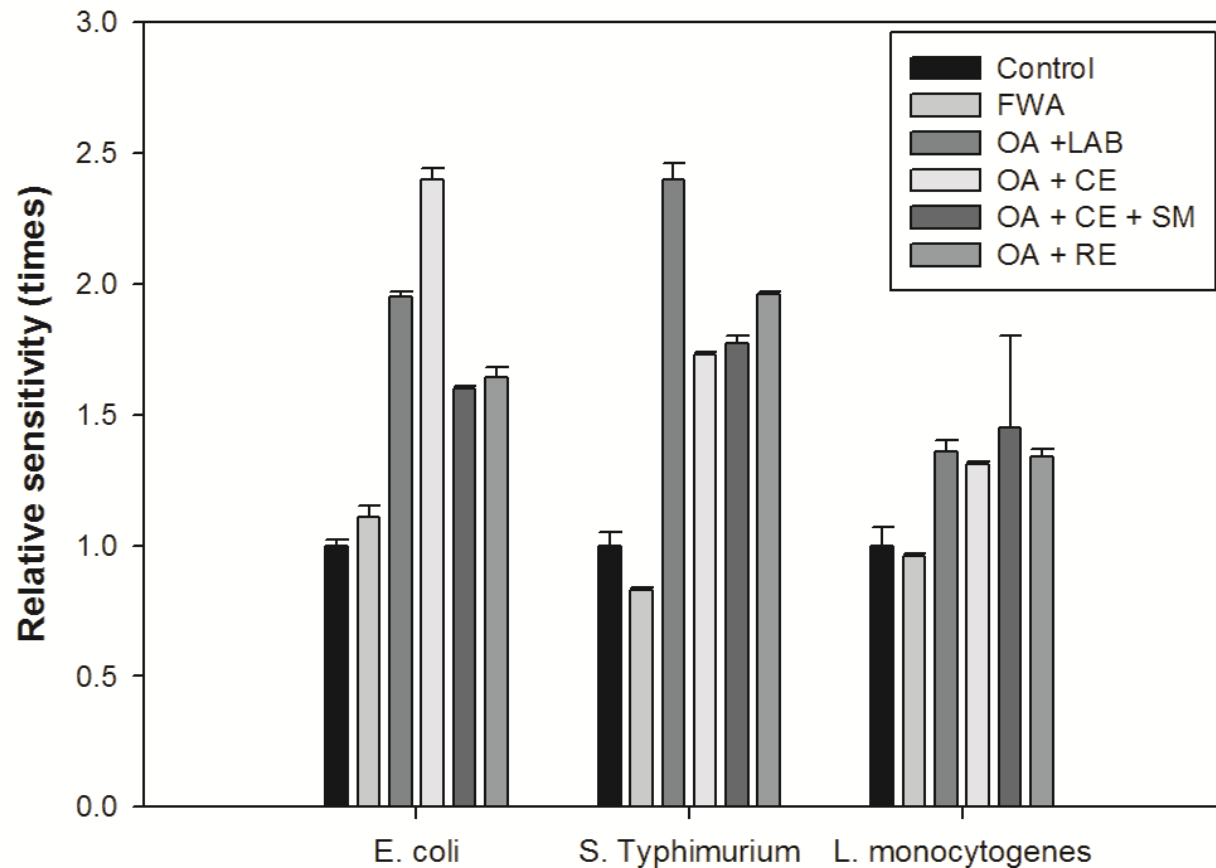
D₁₀ values of selected pathogens and total microflora in broccoli florets coated with active coating

Bacteria	Control	OA/LAB metabolites	OA/FE	OA/FE/SM	OA/SE
<i>L. monocytogenes</i>	0.4	0.29	0.3	0.27	0.3
<i>E. coli</i>	0.38	0.2*	0.16*	0.24	0.23
<i>S. Typhimurium</i>	0.50	0.2*	0.29*	0.28*	0.25*
Aerobic flora	0.57	0.36*	0.32*	0.38	0.33

OA: organic acid mixture; LAB: mixture of LAB ferment; FE: fruit extracts;
SM: spice mixture; SE: spice extract

Irradiation treatment from 0 to 3.3 kGy

Bacterial radiosensitization irradiation and active edible coatings



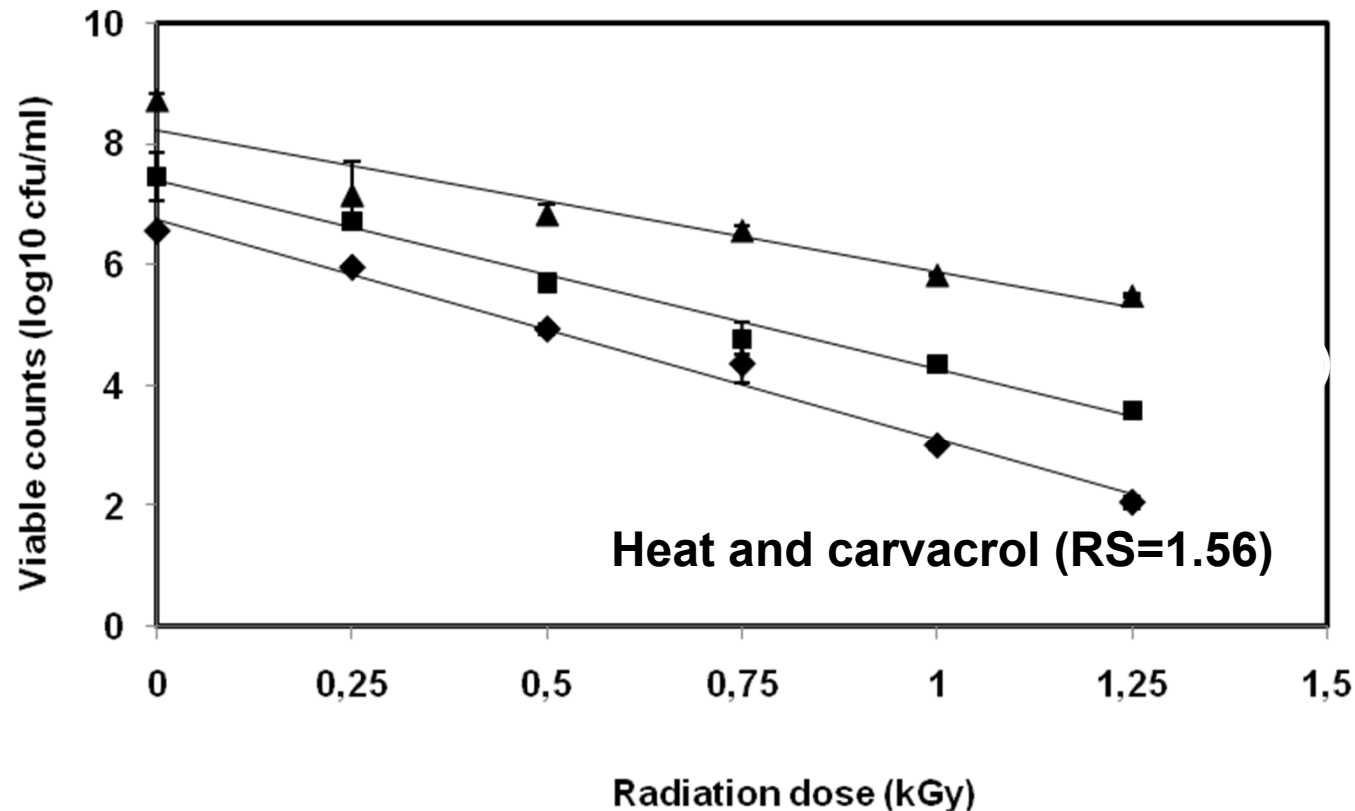
Takala et al., 2011 J. Food Prot. 74, 7, 1065-1069.

**Effect of bioactive coating in combination with modified atmosphere packaging and gamma irradiation (0.25 kGy)
on population of *E. coli* on green beans samples during storage at 4 °C**

	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Control	2.98 ^A _a	3.03 ^A _a	3.10 ^{AB} _a	3.14 ^{AB} _a	3.18 ^B _a	3.41 ^C _a	3.95 ^D _a
MAP	3.02 ^A _a	3.19 ^A _a	3.05 ^{AB} _a	3.01 ^{ABa}	2.80 ^B _b	2.98 ^{AB} _b	3.01 ^{AB} _b
Coating (air)	2.45 ^{AB} _b	2.15 ^A _b	2.57 ^B _b	1.40 ^C _b	1.25 ^C _c	ND	ND
Coating+MAP	2.64 ^A _b	2.59 ^{AB} _c	2.30 ^B _b	1.66 ^C _b	1.19 ^D _c	ND	ND
γ (air)	1.71 ^A _c	1.26 ^B _d	1.18 ^B _c	ND	ND	ND	ND
γ +MAP	1.62 ^A _{cd}	1.45 ^B _e	1.19 ^C _c	ND	ND	ND	ND
γ+coating (air)	1.30 ^A _d	1.35 ^A _{de}	1.25 ^A _c	ND	ND	ND	ND
γ+coating+MAP	ND	ND	ND	ND	ND	ND	ND

Values are means ± standard deviations. Means with different lowercase letters within the same column are significantly different ($P \leq 0.05$), while means with different uppercase letters within each treatment lot are significantly different ($P \leq 0.05$)

Inactivation curve of *B. cereus* in culture media by gamma irradiation in combined treatments with mild treatment and natural antimicrobials



Control untreated cells (▲), cells treated with mild heat for 10 min at 45°C (■), cells treated with 312 ppm of carvacrol for 10 min at 30°C (◆).

CONCLUSIONS

Microorganisms and pests have the capability to develop significant increase of the relative radiation sensitivity (D_{10}) value in presence of natural antimicrobials and/or in combination with heat treatment.

Combination of irradiation and natural antimicrobials can be used to control pathogens, fungi and insect pest, in order to increase the shelf life and protect the safety and the quality of food.

Active edible coating or packaging can also act in synergy with irradiation treatment to protect food quality and safety but assure a better control of the safety during the whole storage.

Thank you

**Monique Lacroix, Ph.D.
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