

ISBBB 2018 Circular Economy for Bioproducts Innovation

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Radiation grafting on natural films

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RESALA

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CANADIAN IRRADIATION CENTRE

CIC

CENTRE D'IRRADIATION DU CANADA

Current global consumption of plastics

200 million tonnes/year

30% of these polymers
are related to food packaging

Development of biodegradable packagings

Currently, synthetic polymers are using as packaging materials which are a threat to the environment.

Efforts are in progress to develop alternative packaging materials witch are

environment-friendly,
cheap, light weight,
possess good thermo mechanical properties and
provides good moisture barrier.

Performance expected

Containing and protecting the food

Could be assured

by controlling and modifying their mechanical and barrier properties

The stability should be demonstrated

when in contact with the food

Materials under consideration

Proteins: Soya, Milk, corn zein, wheat gluten, collagen

Polyssacharides: Cellulose, Chitosan, Alginate

Lipids: fatty acids, vegetable oils

Waxes

Scientific Challenge

Improve the physico-chemical properties

The water resistance

Polymers should be biodegradable and produced at a competitive price

Irradiation should be applied in combination with other treatments like Chemical modification

or

development of new formulations should be done

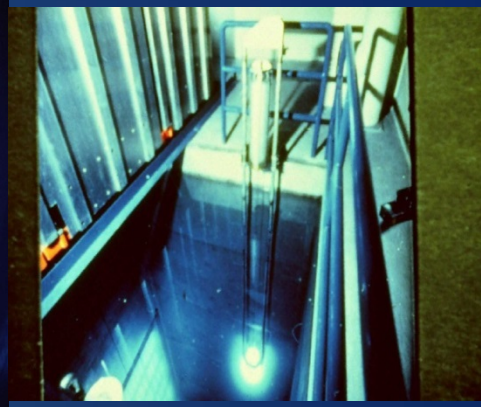
Gamma radiation

1. High frequency electromagnetic ionizing radiation
2. Advantages : easy process control, continuous operation, minimum time requirement, less atmospheric pollution
3. Production of radical reactions for polymer cross-linking in absence of solvents

⇒ Extended application in the preparation of polymer composites



A



B

Cobalt-60 source (A) and Underwater Calibrator (UC-15) (B) at Canadian Irradiation Center (CIC), Nordion Inc., Laval, QC.

Grafting and compatibilization: Advantages of irradiation

Formation of strong bridges between macromolecules

Compatibilization of polymer blend by high energy radiation

Addition of multifunction monomers and inomers to polymer blends in order to accelerate and increase the crosslinking degree in polymer blends

Biopolymer solution 1-5% (w/w)

1 - Stirring
2 - Degassing under vaccum

Monomer

3 - Flow of nitrogen

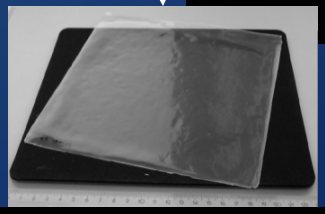
4 - Gamma irradiation



Plasticizer

^{60}Co source
Dose rate of 20 kGy/h

5 - Casting / Drying
(overnight at 60° C)

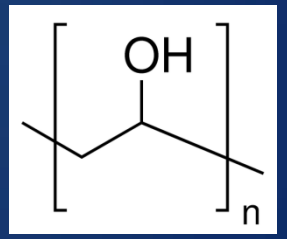


Grafted film
(thickness ~ 50-100 µm)

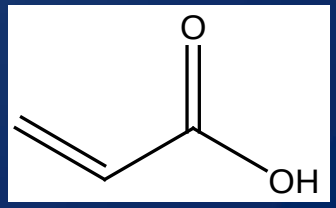
Zein/PVA-*g*-AAc films

- Zein : ⇒ 80% of total protein content in corn grains
  ⇒ Good thermoplasticity, hydrophobicity and impermeability to gases

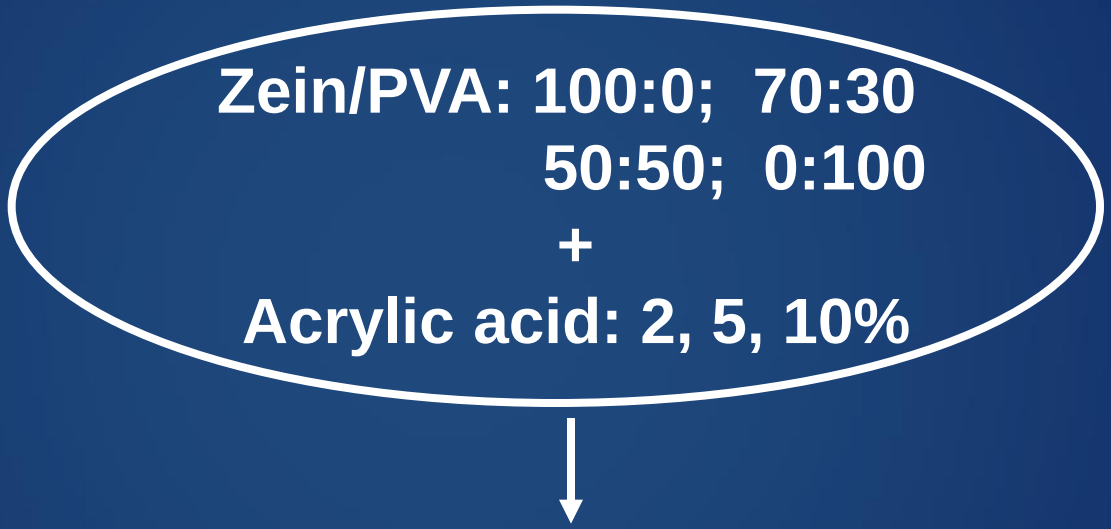
- PVA : ⇒ Versatile biodegradable polyol (many applications)
  ⇒ Used as a filler to enhance rheological performances of polymers
  (Mw 85,000-146,000, 87-89% hydrol.)

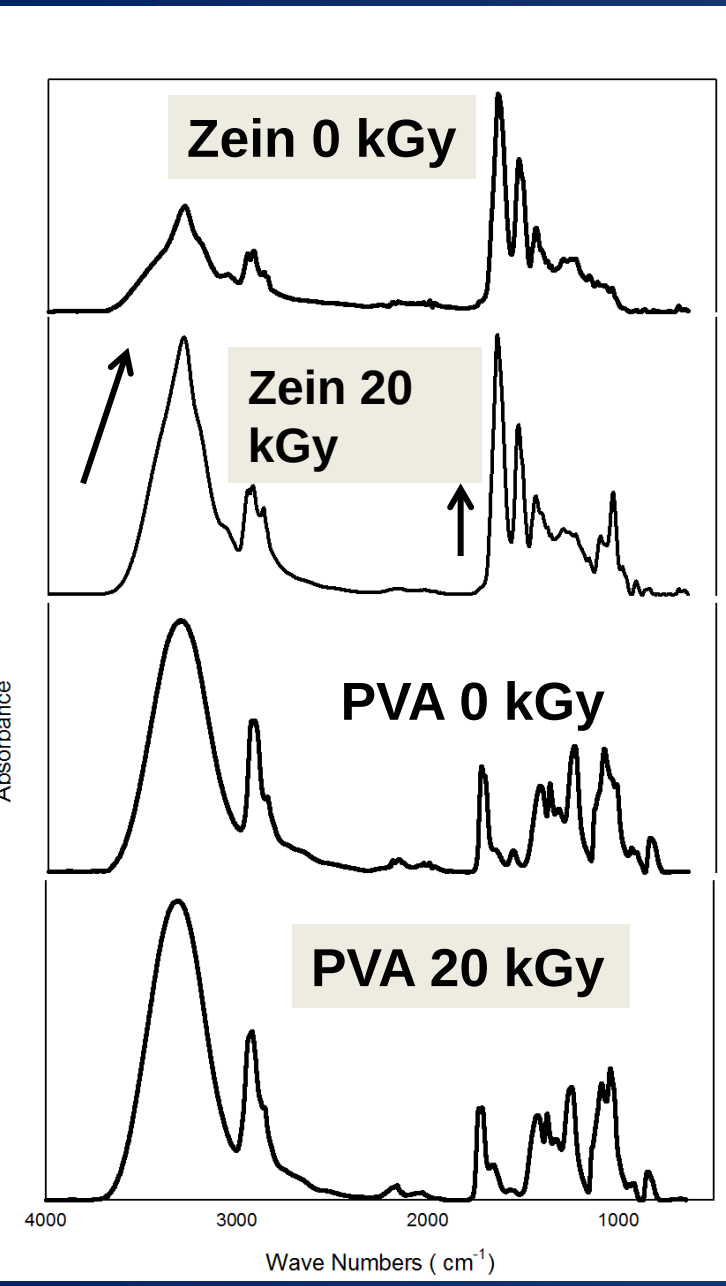


- AAc : ⇒ Highly reactive vinyl and carboxyl groups
  ⇒ Miscibility in water and solvents
  (applications in coatings,
  adhesives and elastomers).



Zein / PVA blending





FT-IR spectra of ZEIN/PVA

Zein: Amine I: 1640 cm^{-1}
Amine II: 1550 cm^{-1}
O-H stretching intraintermolecular Hydrogen bond: 3200-3570 cm^{-1}
alkyl stretching: 2850-3000 cm^{-1}
PVA: C-OH: 1100 cm^{-1}

slight increase in the amide I/II ratio : structure could be affected by irradiation

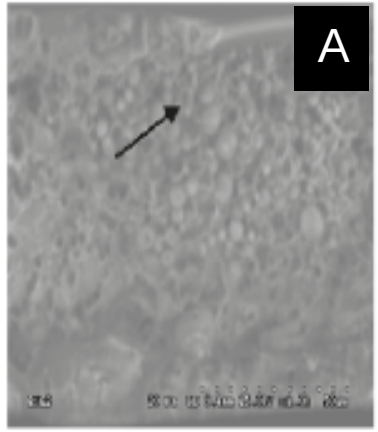
A shift of the C-OH stretching from 1100 cm^{-1} to 1000 cm^{-1} in irradiated PVA: may be a modification of degree of crystalinity

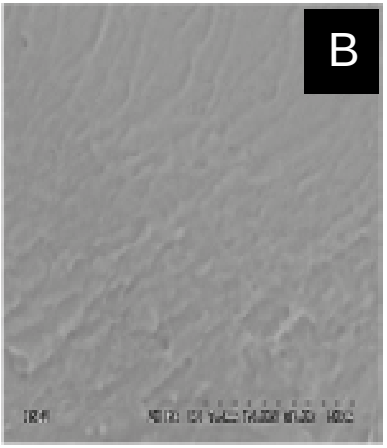
increase of the bands related to O-H stretching (3200-3500 cm-1) and C-H (2850-3000 cm-1) :
Possible fragmentation of the proteins

Zein/PVA-*g*-AAc films

Heterogenous aspect of ungrafted zein/PVA blend :A

Effect of grafting : B



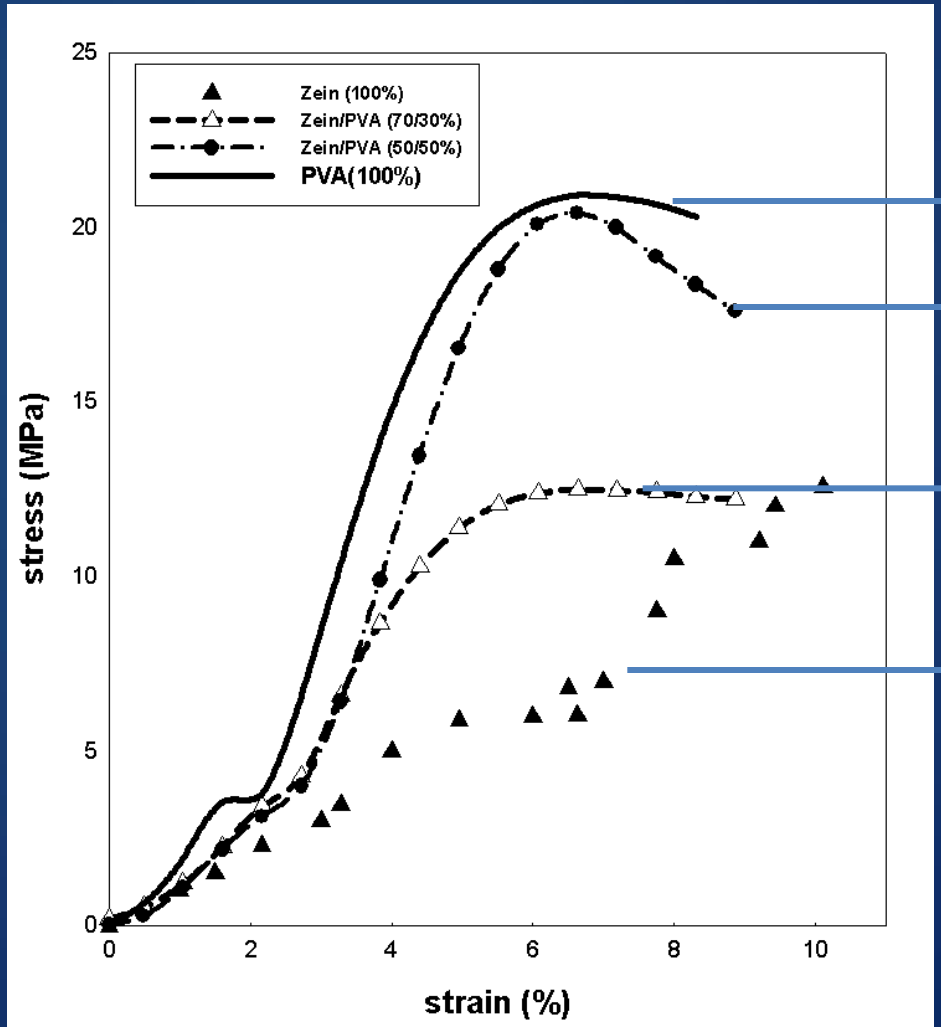


- **Reduction of the interfacial tension between the phases**
- **Higher distribution of PVA in zein matrix after AAc grafting**

⇒ **Compatibilization**

Stress-strain curves of unirradiated zein and PVA polymers and their blends of different ratio

The increase PVA ratio
Increase the toughness



Mechanical properties

Grafting of Aac ↗ PD and Compatibility

Inhibition of PVA crosslinking

Irradiation may produce crosslinking

Zein/PVA ratio (%)		PS ($N.nm^{-1}$)	PD (mm)
90/10 grafted with 5% AAc	20 kGy	56	3.0
70/30	0 kGy	29	2.6
70/30	20 kGy	68*	2.7
70/30 grafted with 5% AAc	20 kGy	40	6.5*
50/50	0 kGy	70	3.4
50/50	20 kGy	234*	5.5
50/50 grafted with 5% AAc	20 kGy	37	9.8*

Methylcellulose and 2-Hydroxyethyl-methacrylate (HEMA)

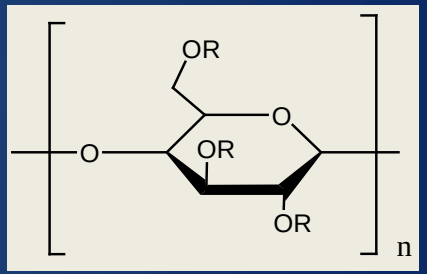
Methylcellulose (MC) is the derivative of cellulose which is biodegradable and biocompatible.

Hydroxyethyl methacrylate (HEMA) is a vinyl monomer converted to polymer by γ-irradiation.

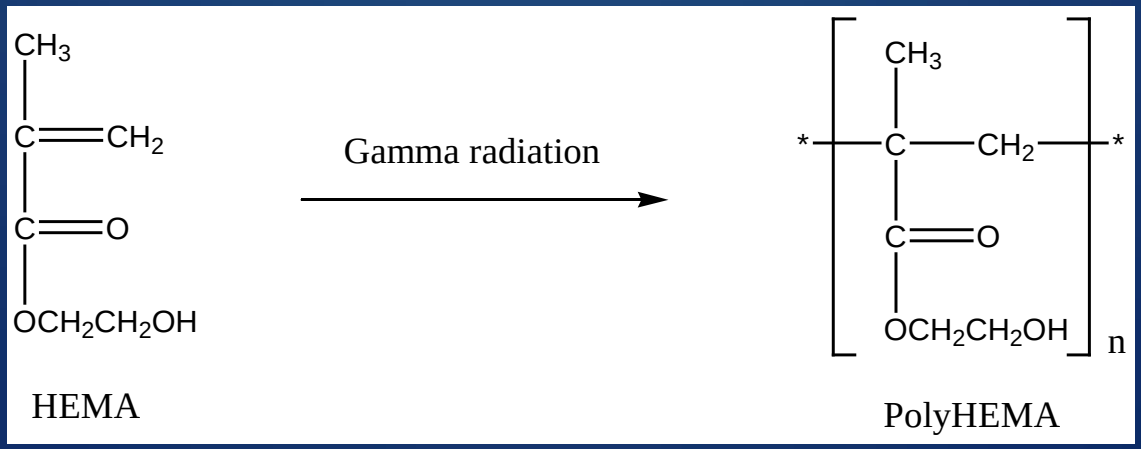
Poly(HEMA) has adequate biocompatibility and has many potential applications in medical sciences (drug delivery, biocompatib.lization).

MC-*g*-HEMA films

- MC : ⇒ Cellulose ether widely used to produce gels in pharmaceuticals, foods, paints and cosmetics
  ⇒ Good solubility in water at low temperatures

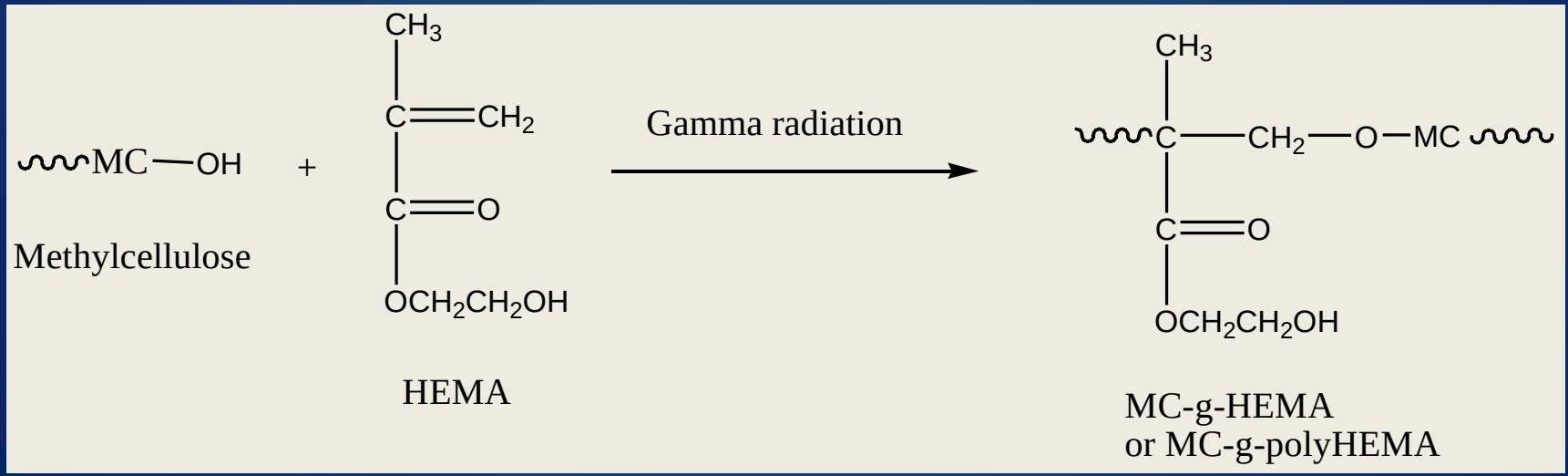


- HEMA : ⇒ Vinyl monomer soluble in water
  ⇒ Poly-HEMA hydrogels are biocompatible



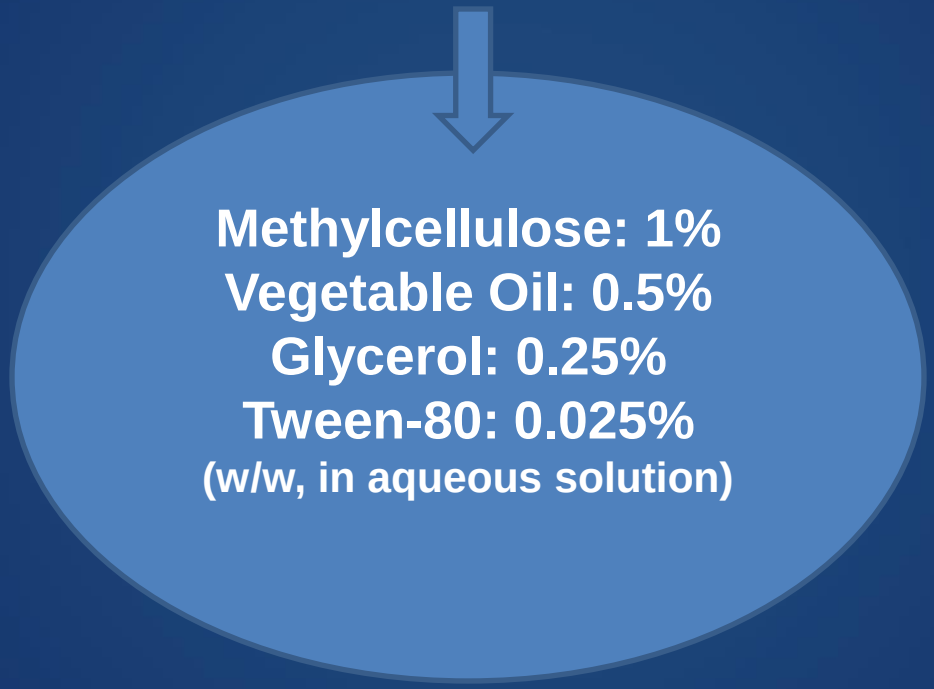
MC-*g*-HEMA films

Probable reaction mechanism of grafting reaction between MC and HEMA :



Effect of HEMA Monomer and Gamma Radiation on Mechanical Properties of MC-Based Films

HEMA (0.1-1%, w/w) was incorporated into the MC-based Formulation Followed by Gamma Radiation



Using the above formulation, HEMA containing MC-based films were prepared by casting.

Then films were irradiated (0.5-25 kGy dose).

Effect of ɣ-radiation on the PS and PD of MC-*g*-HEMA films.

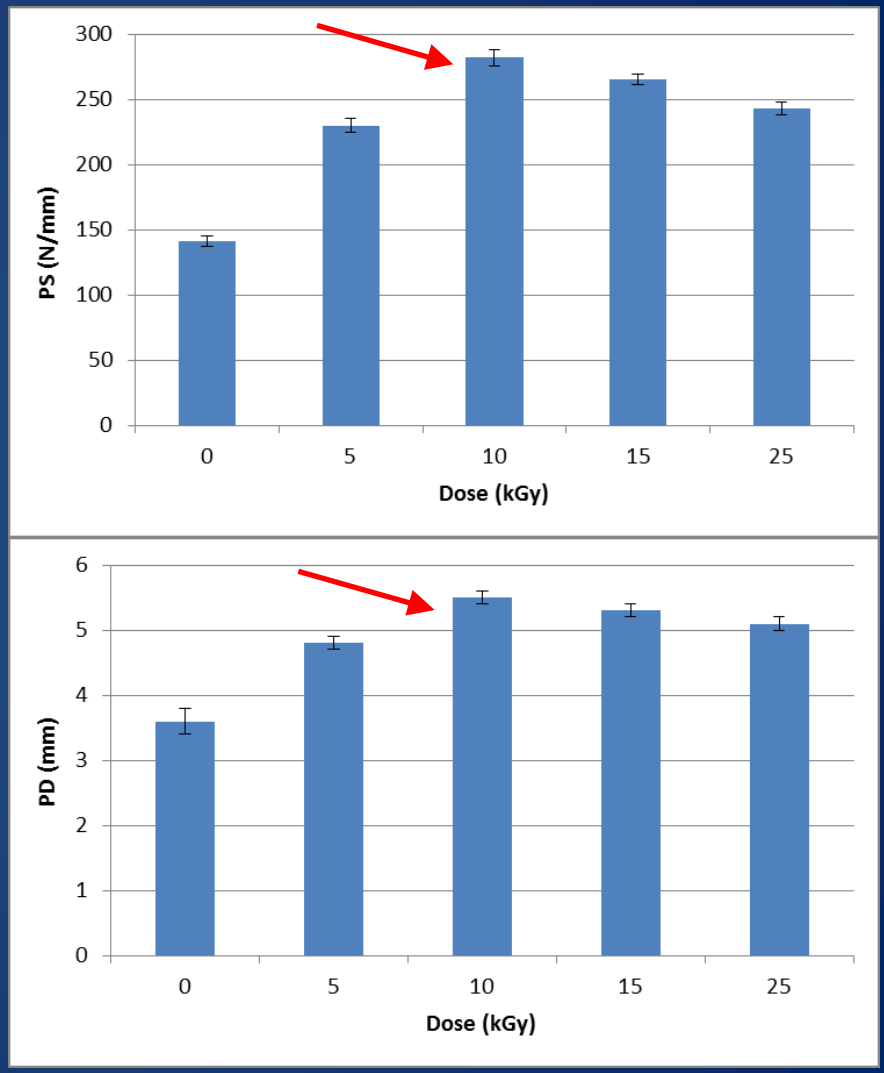
MC-*g*-HEMA films

<u>Mechanical properties</u>

- Highest PS value of 282 N/mm (92% improvement) at 10 kGy
- Highest PD value of 5.5 mm (59% improvement) at 10 kGy

⇒ Both PS and PD improved due to HEMA grafting on MC and polyHEMA formation after ɣ-radiation.

1% HEMA

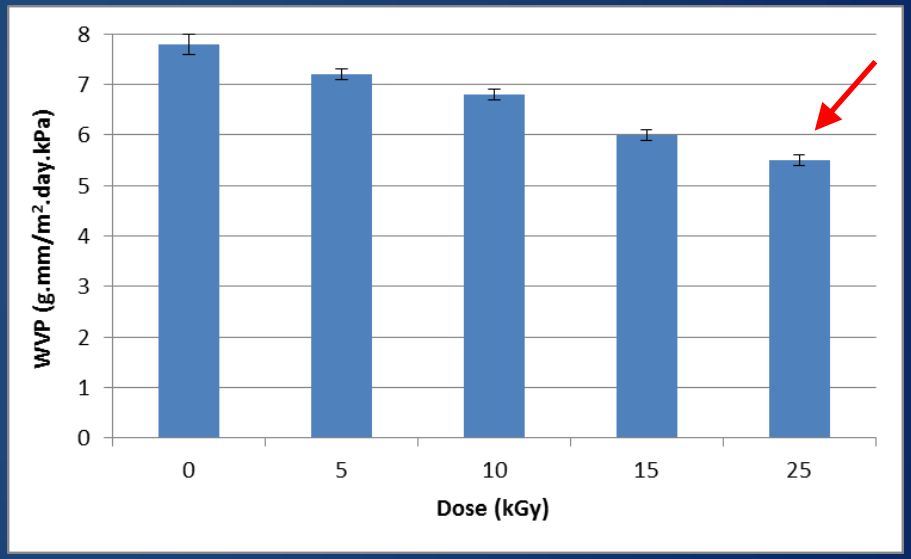


MC-*g*-HEMA films

Barrier properties

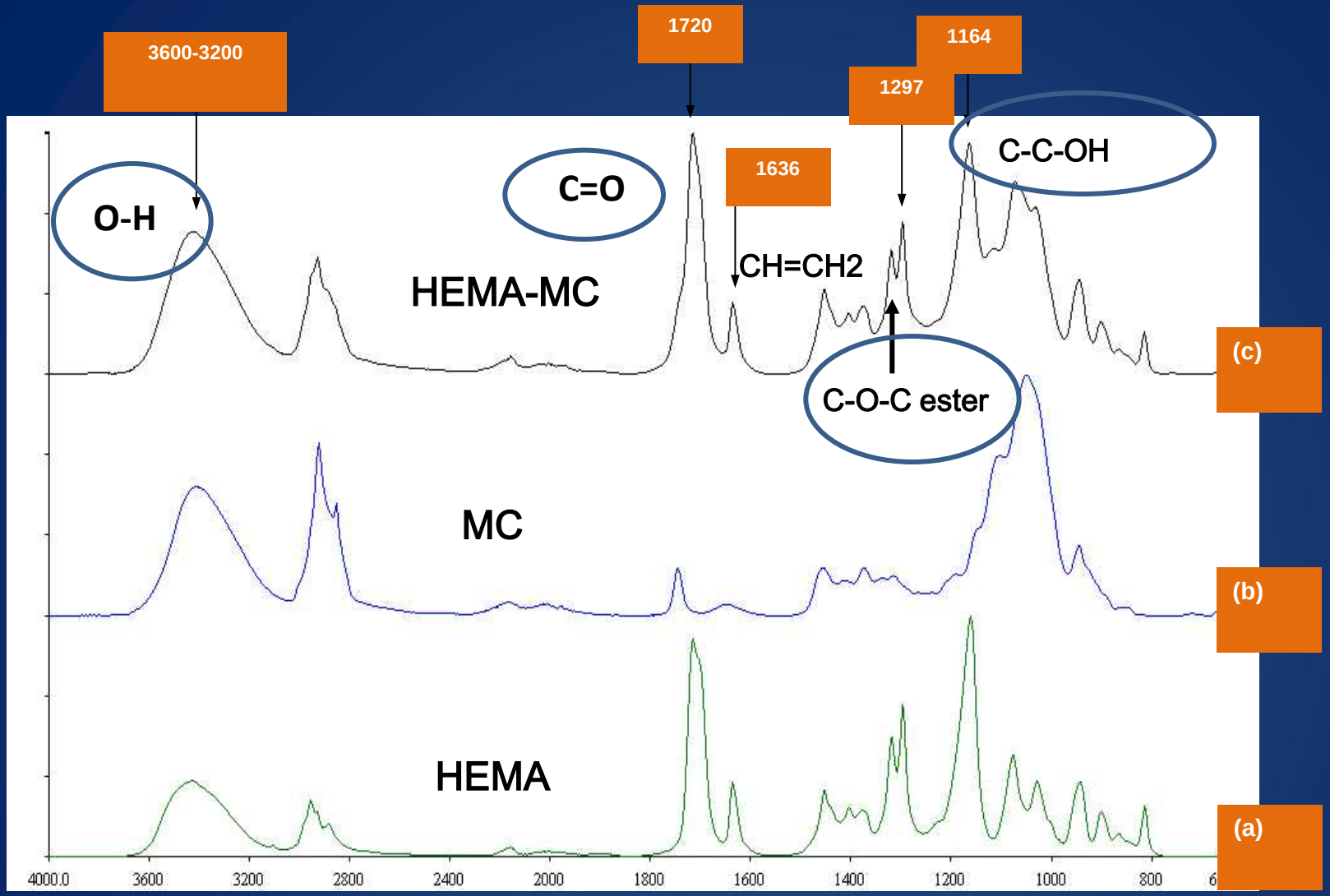
- ↘ of WVP with radiation dose
- Lowest WVP value of 5.5 g.mm/m^2.day.kPa (30% barrier improvement) at 25 kGy

⇒ Barrier properties to $H_2O_{(v)}$ improved due to grafted compled (MC-HEMA)



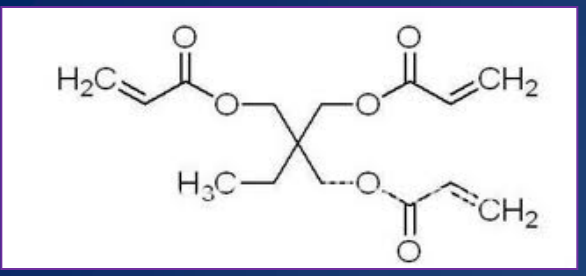
Effect of ɣ-radiation on the WVP of MC-*g*-HEMA films.

FTIR spectra 10 kGy



Result of grafting: increase of the stretching band at 3600-3200 cm^{-1} : presence of hydroxyl groups of the grafted HEMA; increased vibrations of C=O, C–O–C and C–C–OH at 1720: ester linkage of HEMA; decrease of the vinyl vibration band from HEMA at 1636 cm^{-1} : polymerization of HEMA; aliphatic C–H stretching decreased: grafted HEMA layer covering MC surface: Presence of vinyl group (1636cm^{-1}) in grafted films : poly(HEMA) might be present as a co-product

CNC-reinforced MC-*g*-TMPTMA films



Grafting with TMPTMA monomer

MC + glycerol

↓

TMPTMA monomer (0.1-2% by wt)

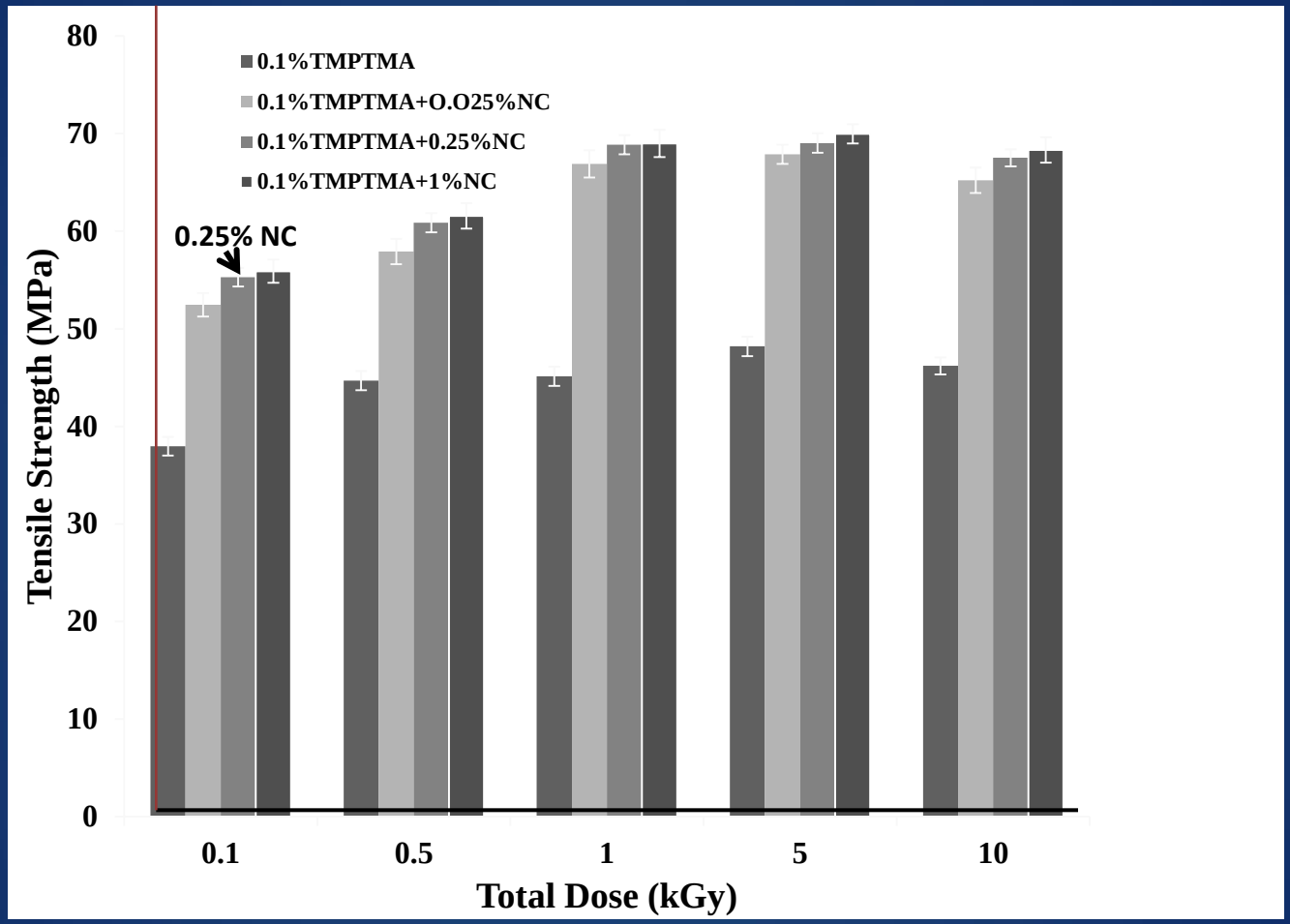
↓

NCC (0.1-1%)

↓

Casting and irradiation (0.1-10 kGy)

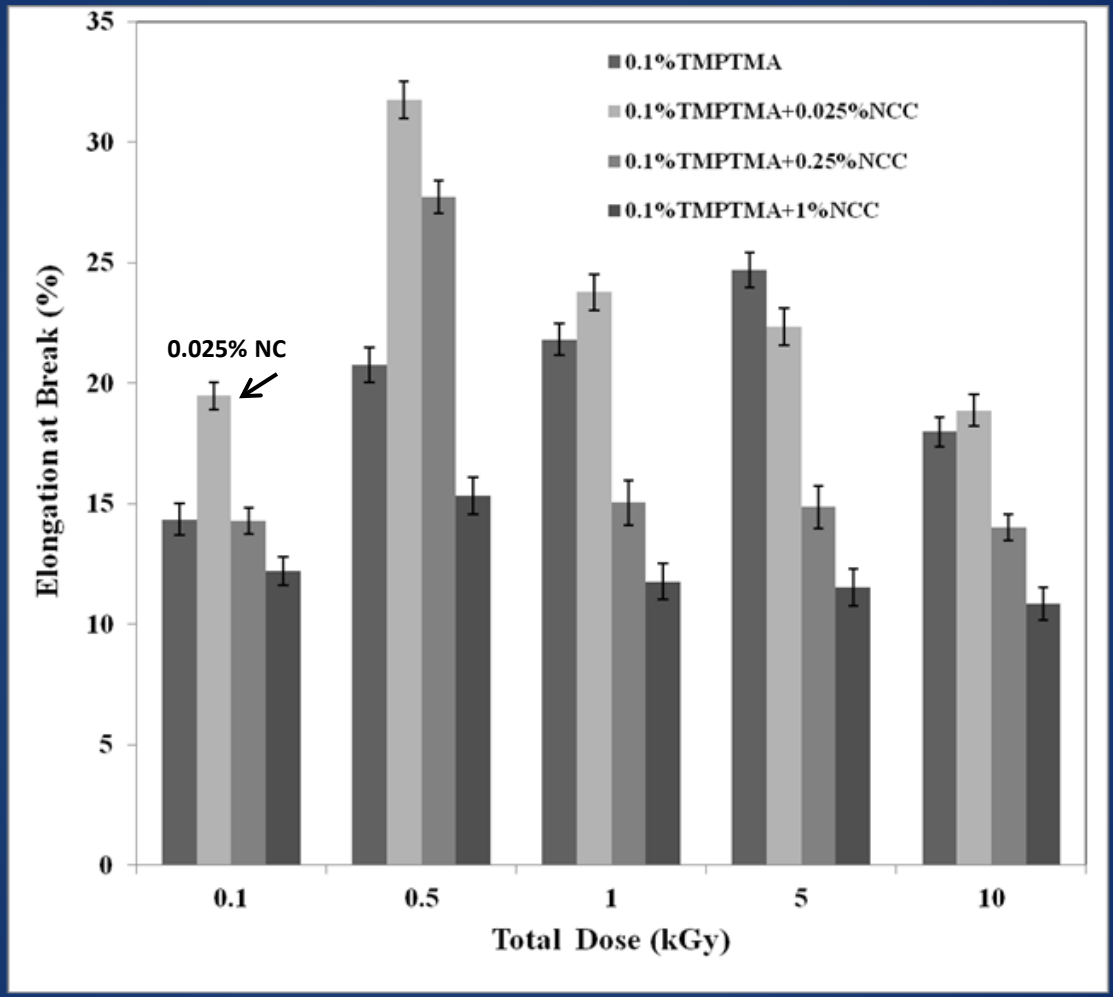
TS values of MC-NCC-TMPTMA films



Optimal concentration of TMPTMA: 0.1%
Optimal concentration of NC: 0.25%
Optimal dose of irradiation: 1 kGy

Effect of NC addition: ↗ PS by 3 times
Effect of irradiation: ↗ PS by 1.4 times

Elongation at break of MC-NCC-TMPTMA films



Optimal concentration of TMPTMA: 0.1%
Optimal concentration of NC: 0.025%
Optimal dose of irradiation: 0.5 kGy

Effect of TMPTMA: EB ↗ by 1.17 times
Effect of NC addition: EB ↗ by 1.4 times
Effect of irradiation: Eb ↗ by 1.4 times

Gallic Acid (AG)



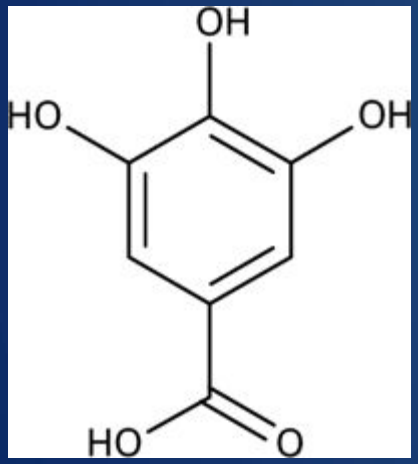


Fig.1. Molecule of gallic acid

Antioxidant

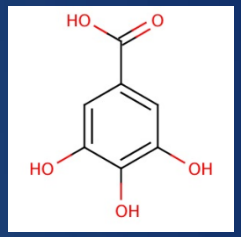
- **Antioxidant phenolic extracts from plants** (Madsen et Bertelsen, 1995)
- **Used in cosmetics and food industry** (Yen et al. 2002)
- **Prevents the discoloration of food** (Cholbi et al. 1991)

Antimicrobial

Decreased bacterial growth : *Salmonella typhi* , *Salmonella aureus*, *Escherichia coli*, *Listeria innocua* and *Bacillus subtilis* (Sun et al. 2014; Chanwitheesuk et al. 2007)

Sun et al. , 2014. Food Sci. Technol. 57, 83–89;
Chanwitheesuk, et al. 2007. Food Chem. 100, 1044–1048.

Modification of cellulose nanocrystals by grafting an antiradical molecule (gallic acid)



- Use of free radical grafting in presence of ascorbic acid and hydrogen peroxyde and by gamma-irradiation.

- Preparation of antiradical coatings and packaging in a polymeric matrix for mushroom application

- Determination of the antioxydant properties of grafted CNC

- Free radical grafting of gallic acid by a redox group on CNC surface according to (Spizzirri *et al*. 2009, Biomacromolecules 10, 1923-1930).
- Evaluation of different ratios of CNC / GA in antiradical properties
- Influence of grafting efficiency at different irradiation doses (5, 10, 20 kGy)
- Characterization of modified CNC and modified polymer matrices.
- Evaluation of different coating formulations in order to prevent mushroom browning.
- Evaluation of the mechanical properties of the films

CNC 2% (25 mL)

8 M H_2O_2 (0.567 mL)
+ 135 mg ascorbic acid

Free-radical reaction
(Step 1: initiation by ·OH radicals)

CNC radicals
Ascorbate radicals

GAMMA IRRADIATION: 5, 10, 20 kGy

Gallic Acid
$R_{CNC/GA}$=0.66 and 8

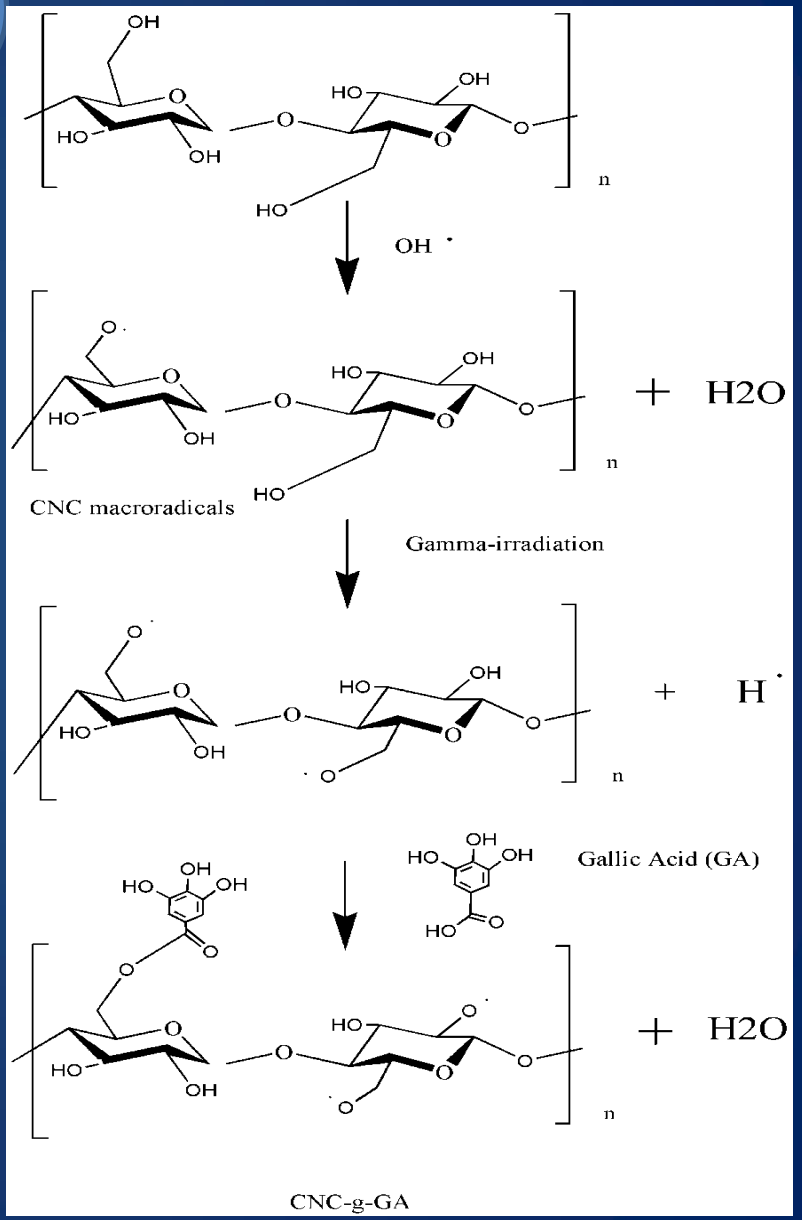
Dialysis

By-products : ascorbic acid, dehydroascorbic acid, gallic acid

CNC-g-GA conjugate

Covalent insertion of GA onto CNC backbone
(Step 2: termination via ether and alkyl linkage)

Film preparation



Antioxidant film preparation

Polymer 2% (w/v) gellan gum
Glycerol 1% (w/v)

→

modified and non-modified CNC (5, 10 and 20% (w/w polymer, dry basis) was added.

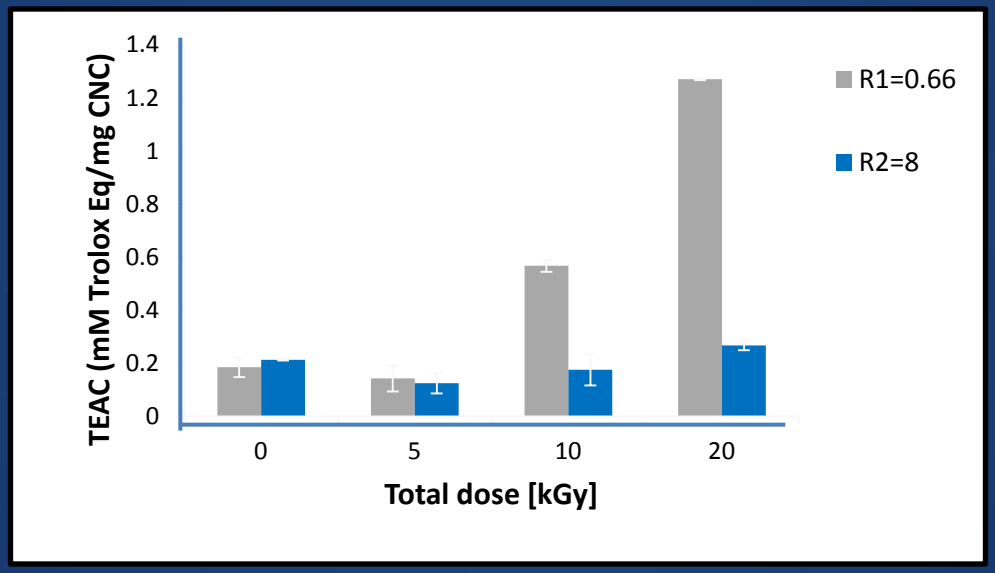
↓

20 ml of film solution was spread on petri dishes

after 3 day drying at 25°C

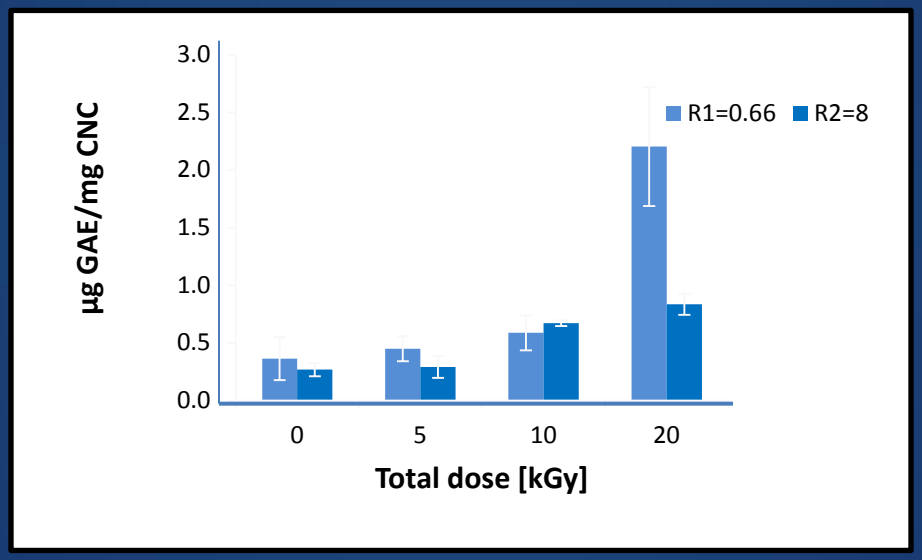
2 ml of calcium chloride solution was added on the pre-dried film.

Influence of gamma irradiation at different CNC/gallic acid ratios on radical DPPD inhibition (mM Trolox equivalent)



- Grafting process of gallic acid on CNC: is favored when using higher doses of gamma-irradiation.
- New antiradical properties are observed in CNC at Rcnc/GA=0.66

Influence of gamma irradiation at different polymer/gallic acid ratios on mM Gallic acid equivalent



- Increase of phenolic compound attachments correlates the increase of antiradical properties of modified CNC.

	mM Trolox Eq/mg CNC	standard deviation	-COOH Mm/kg CNC
CNC	0,024	0,001	49
CNC-γ	1,92	0,065	134
CNC-H_20_2-AA	0,317	0,050	128
CNC-H_20_2-AA-γ	0,934	0,058	121
CNC-g-GA H_20_2-AA	1,642	0,050	
CNC-g-GA H_20_2-AA-γ	8.20	0,130	132
GA	2,90	0,004	

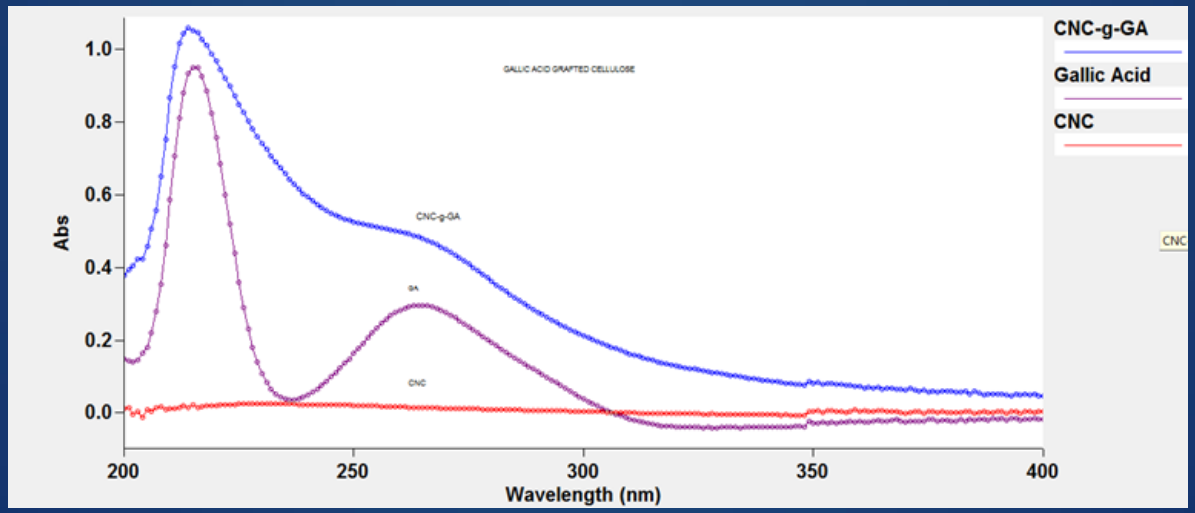
Degradation of CNC by irradiation producing a rise of aldehyde groups following decreasing of molecular weight of CNC

Aldehyde has antiradical properties

Treatment with redox group , ɣ-irradiation or ɣ-irradiation followed with Gallic acid exposition showed an increase of carboxylic group -COOH.

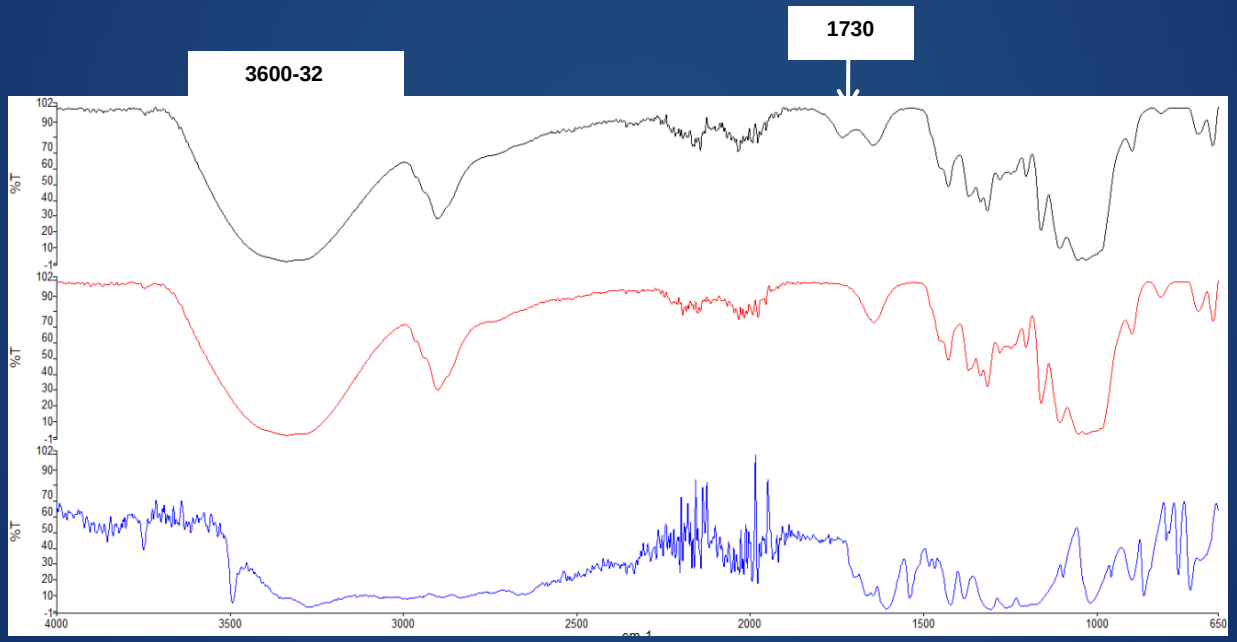
CNC did not show any antiradical properties (0,024 Mm Trolox Eq/mg CNC)
results OF 0,317 Mm Trolox Eq/mg CNC were obtained after the addition of H_20_2 and ascorbic acid.
After γ-irradiation at 20 kGy without gallic acid, CNC had a little antiradical effect of 0.934 mM Trolox Eq/mg CNC
After γ-irradiation at 20 kGy with gallic acid, CNC showed an antiradical effect of 1.642 mM Trolox Eq/mg CNC
After γ-irradiation at 20 kGy with gallic acid, H_20_2-AA, showed an antiradical effect of 8.20 mM Trolox Eq/mg CNC

UV-vis spectra of CNC; Gallic acid and CNC-g-GA H_20_2-AA-γ



GA at wavelength of 214 and 260 nm

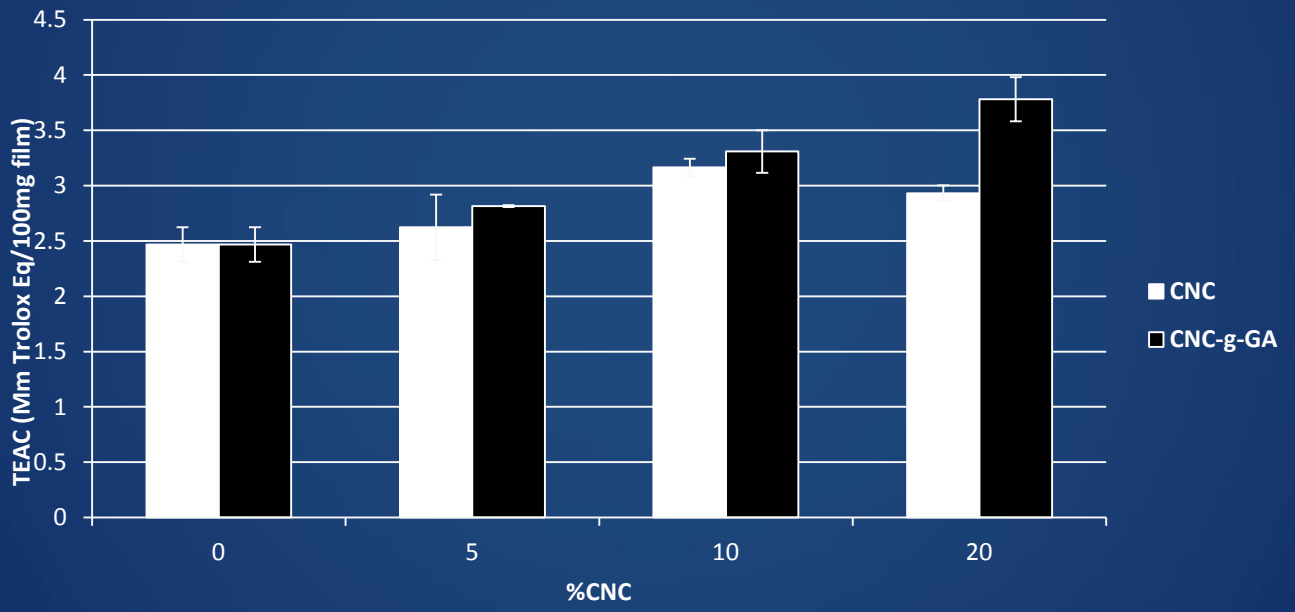
FT-IR



➢After gallic acid grafting: -New C=O peak at 1730 $nm^{-1}$of ester carbonyl stretching (linkage (C=O) from aromatic compounds) may be related to GA covalent attachment. -Displacement of ester linkage compared to 1603 nm^{-1} found in gallic acid.

➢3496 $nm^{-1}$of O-H band in Gallic acid; 1603 $nm^{-1}$of –COOH band in Gallic acid; 3600-3200 $nm^{-1}$of native CNC.

Films preparations in gellan gum at different concentrations of CNC and CNC-g-GA



- Increase of TEAC values up to 20% CNC-g-GA (w/w polymer, dry basis) ($p \leq 0.05$)
- Small increase of TEAC values were found when native CNC is added ($p \leq 0.05$)

Mushroom coating formulation

1% (w/v) gellan gum solution , 1% (w/v) of glycerol and modified and non-modified CNC (5-20% (w/w polymer, dry basis)



Dipping of pre-coated mushroom

1.5 % (w/v) $CaCl_2$ t = 2 min



drying at 25°C

Mushrooms were stored at 4°C and 90% RH in a plastic bag for next analysis

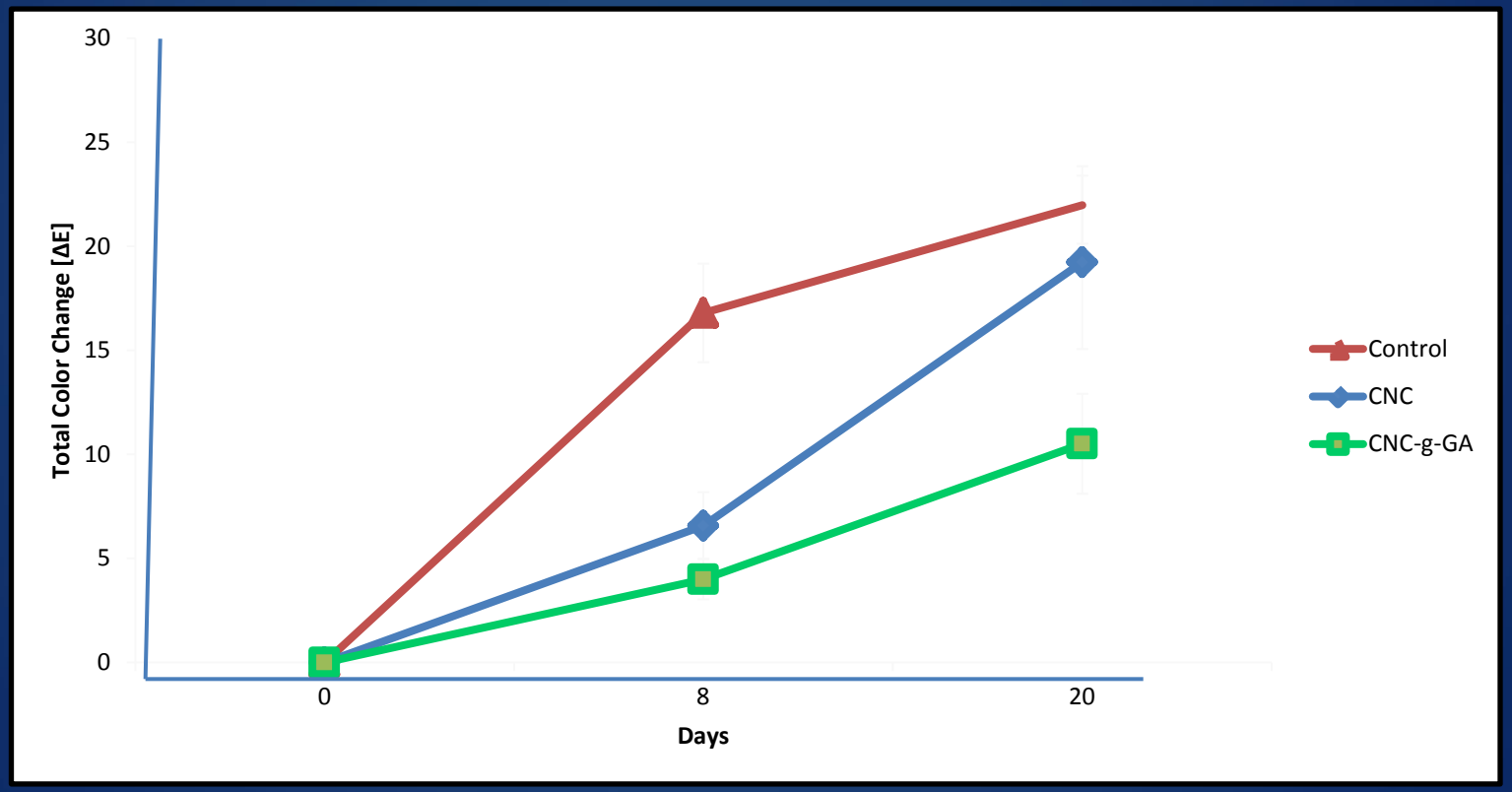
Water loss for mushrooms at different concentrations of CNC-g-GA in gellan films



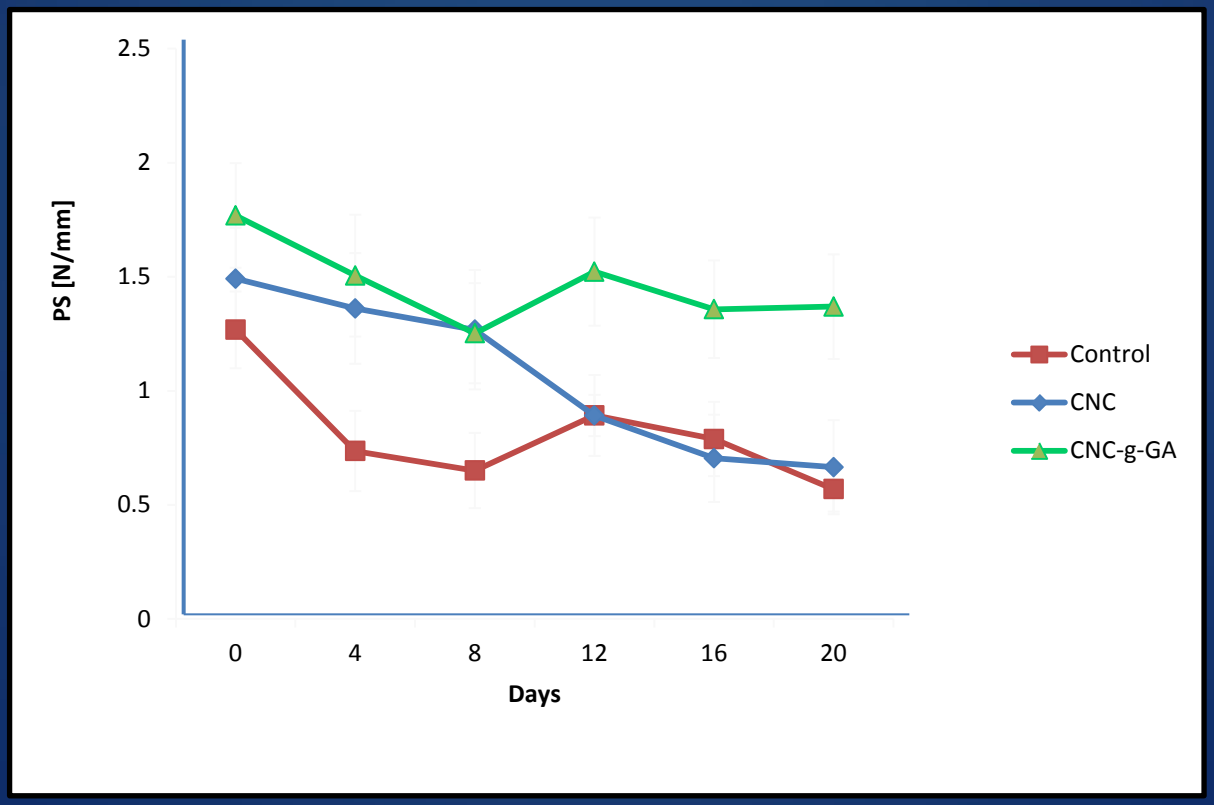
- More hydrated mushrooms can be kept when CNC is modified with gallic acid compared to coating without any addition of antiradical molecule.

- Difference on water loss of 3.7% compared to 5.3% of non-modified CNC at day 20 ($p \leq 0.01$) can attribute to gallic acid grafted to CNC

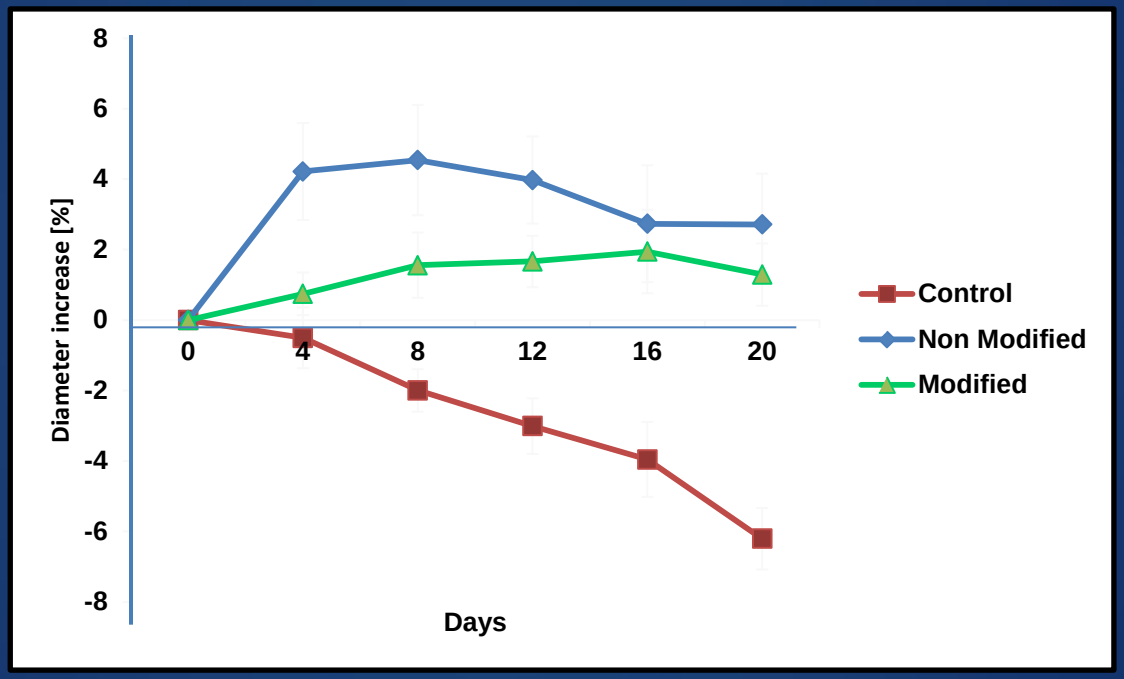
Total Color Change (ΔE) OF MUSHROOMS



TEXTURE OF COATED MUSHROOMS

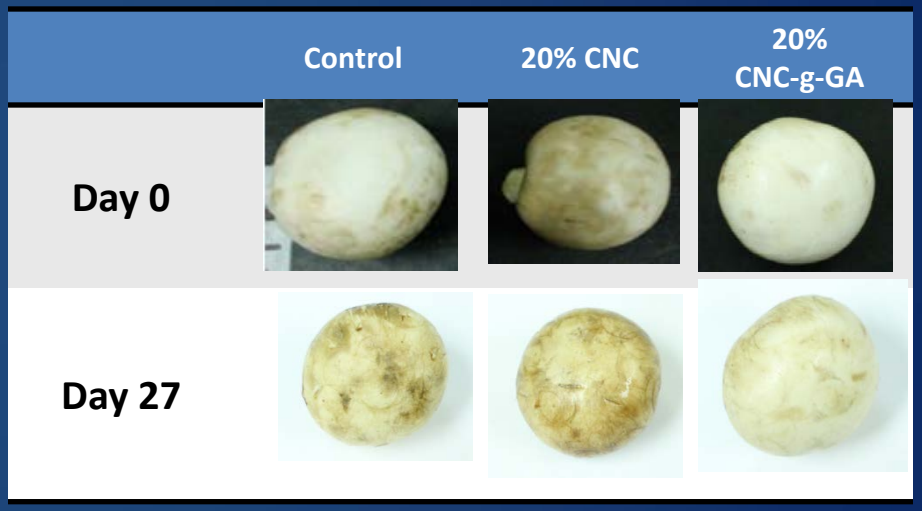


CAP DIAMETER OF COATED MUSHROOMS



Browning of mushrooms after CNC based gellan coating

- Coated mushrooms appearance was kept hydrated compared to the control mushrooms which tended to peel.
- Browning appeared on mushrooms with non-modified CNC.
- A delay of browning was observed on coated mushrooms with CNC after 20 days.



Summary and Challenges

Nanotechnology has potential for the development of active coating and packaging

New Advances are Focused on Intelligent and Active Packaging (control of microbial growth, oxydation, moisture, flavor and volatiles migration)

However, films should be stable in contact of the food

Ref: Sanchez-Garcia et al., 2010, Trends in Food Science & Technology 21, 528-536

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Effectiveness of Silane Monomer on Chitosan based-Films

Chitosan (1-30%)

↓

Silane monomer (0.25%)

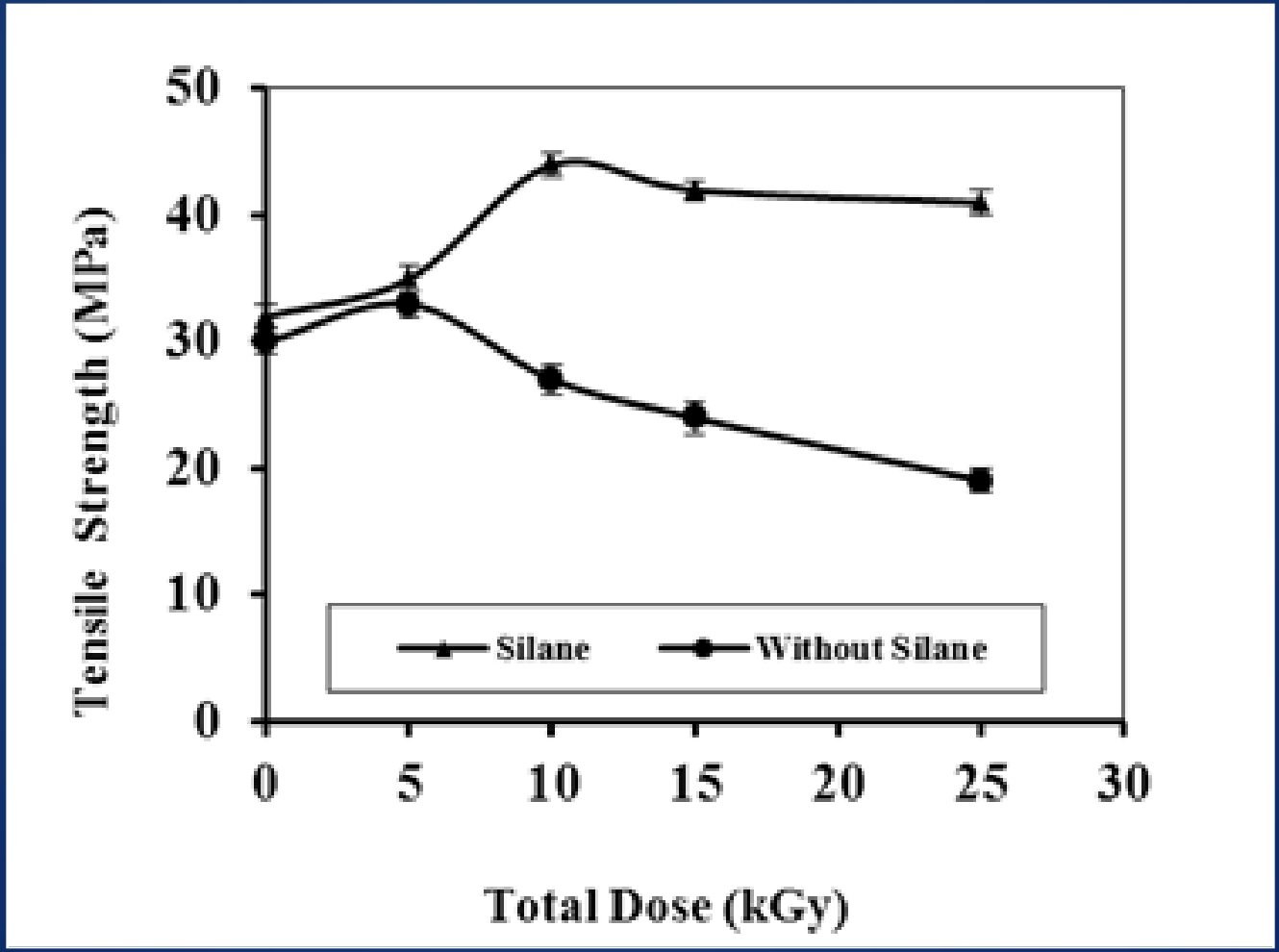
↓

Mechanical properties:

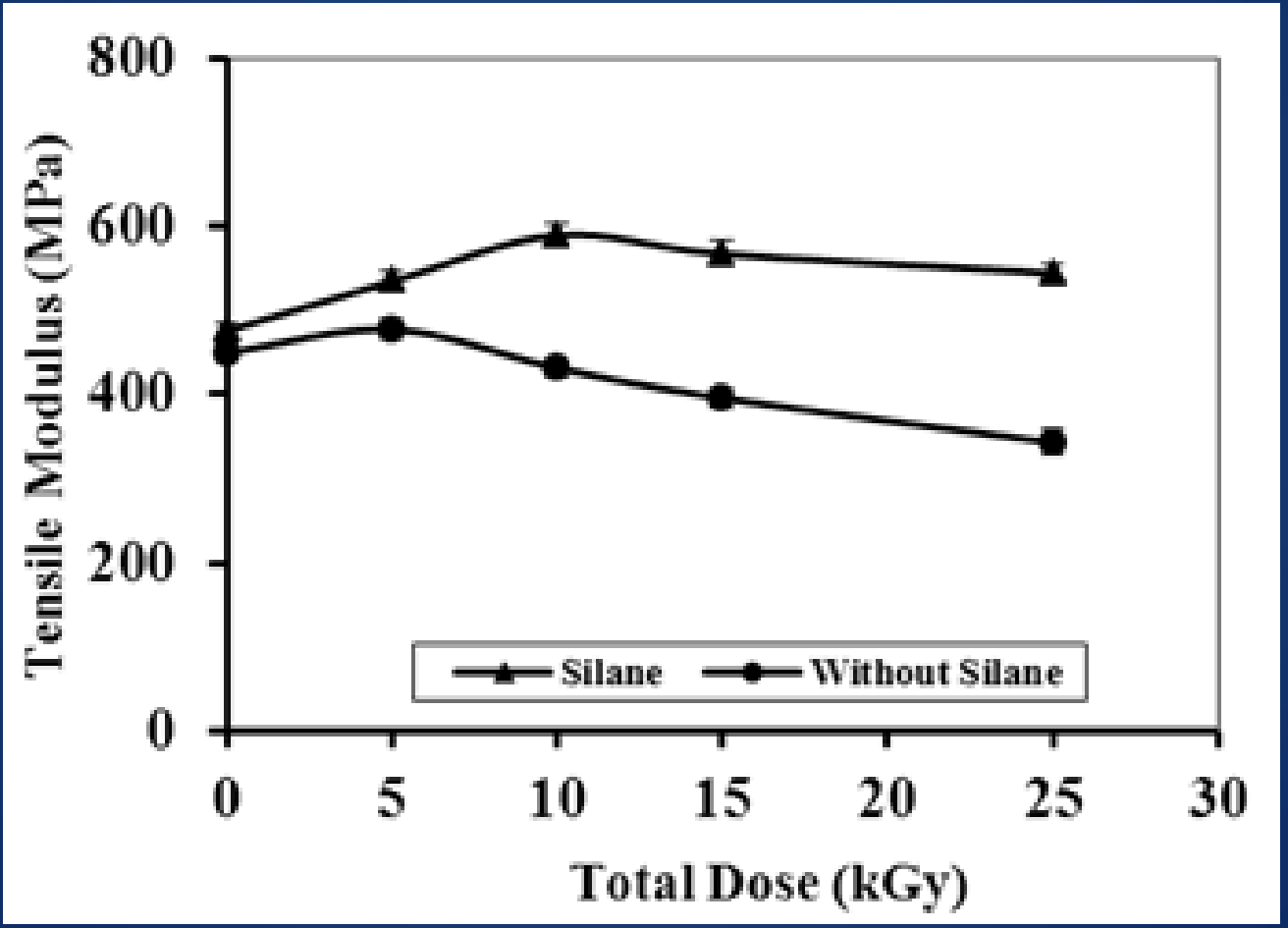
Effect of gamma irradiation

SEM

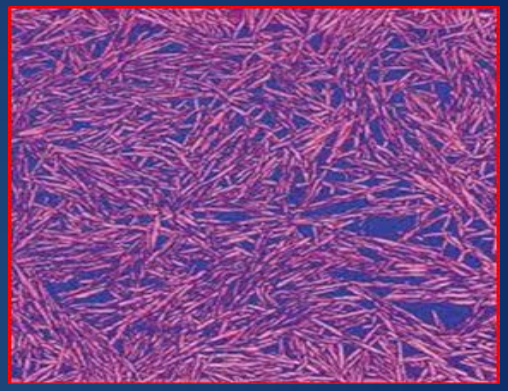
Effect of gamma radiation on TS values of chitosan films and silane grafted chitosan films.



Tensile modulus of chitosan and silane grafted chitosan films against gamma radiation.



CNC-reinforced MC-*g*-TMPTMA films



- Properties of CNC

- 2-20 nm ∅ ; 100-500 nm length
- Biodegradable, non-toxic
- Tensile strength 7.5 GPa ; Young's modulus 137 GPa
- Excellent fillers for thermoplastic polymers due to their outstanding mechanical and barrier properties (Khan *et al.*, 2010. *J. Agric. Food Chem.*, 58, 7878-85)
- Control gas exchange through « environment-food product » packaging interface (Khan *et al.*, 2012, *J. Appl. Polym. Sci.*, 123, 1690-7)
- ↗ the stability of encapsulated bioactive agents into polymer matrices (Boumail *et al.*, 2013. *J. Agric. Food Chem.*, 61, 811-21)

Active packaging to prevent the contamination of food with pathogens and extend their shelf life



Fresh Meat



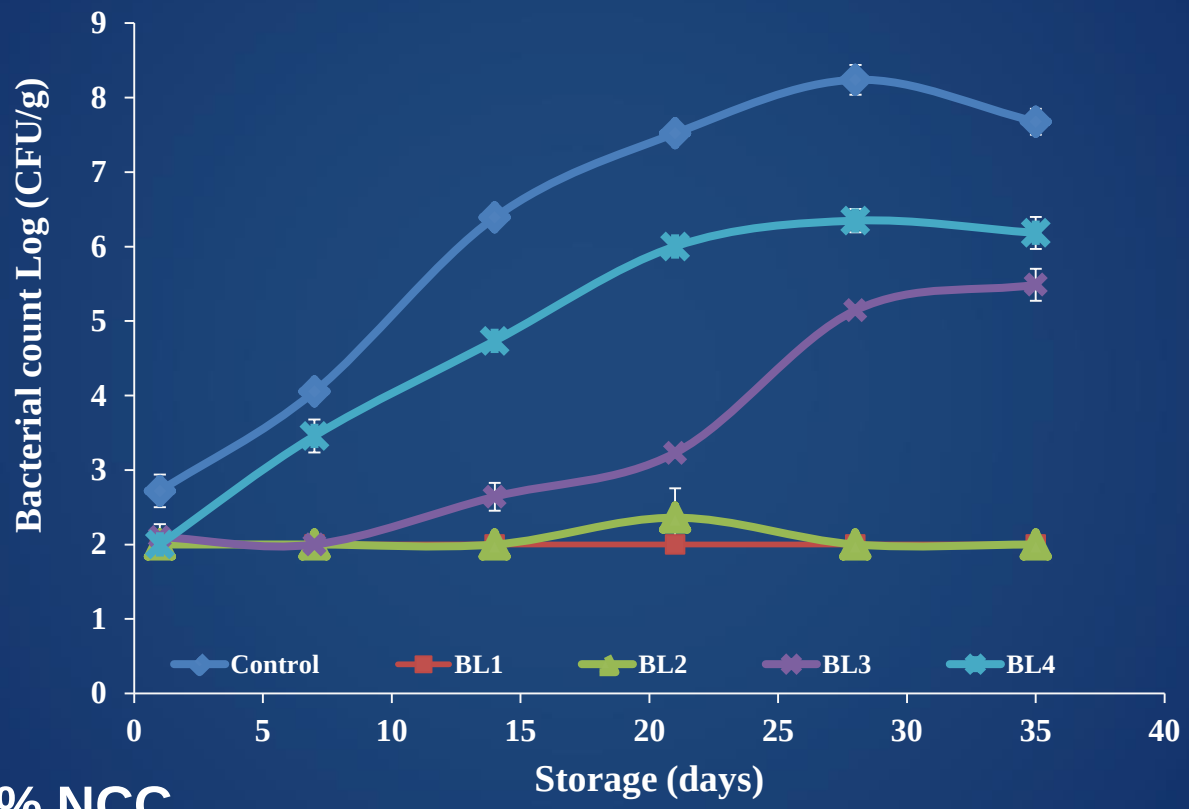
Fresh ready to eat vegetables



Active packaging allows to interact with food product and the environment and play a dynamic role in food protection

Bilayer films based on chitosan, NC and MC – bacteriocin

Efficiency of *Listeria monocytogenes* inhibition on ham



Control: 0.025% NCC

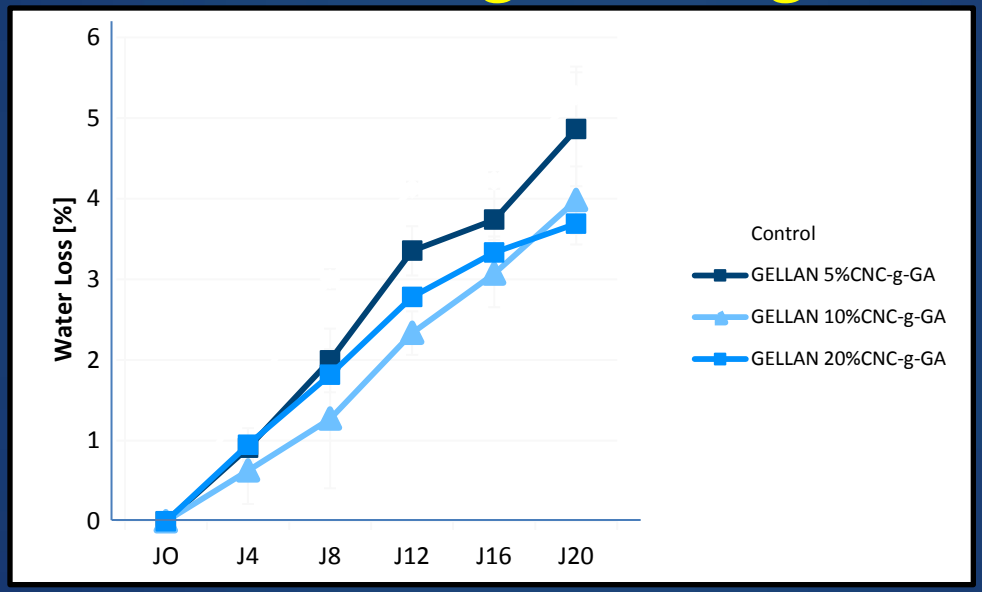
BL1: 0.025%NCC+ 18.65 µg/cm² of nisin

BL2:0.025%NCC+ 9.33 µg/cm² of nisin

BL3: 0.025%NCC+ 4.66 µg/cm² of nisin

BL4:0.025%NCC+ 2.33 µg/cm² of nisin

Water loss for mushrooms at different concentrations of CNC-g-GA in gellan films



- Water loss of mushrooms decreases during storage conditions (4°C) when concentrations of CNC-g-GA is added to gellan films.
- A smaller water loss behaviour for 20% CNC-g-GA coated mushrooms at J8 and J12.
- A smaller water loss behaviour for 5% CNC-g-GA was observed as compared to the control during all the experience ($p \leq 0.01$)