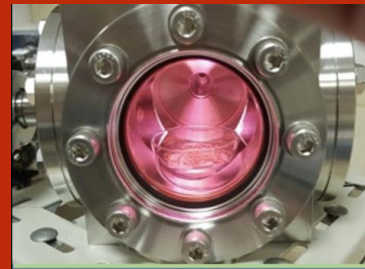


ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



3rd TRAINING SCHOOL PIAGRI COST ACTION CA19110

“Plasma applications for smart and sustainable agriculture”

PLASMAS FOR PLANT AND FOOD PROCESSING

11-14 June 2024, Faculty of Natural Sciences, Vytautas Magnus university, Kaunas, Lithuania



‘Cold plasma application for food stability and functionality’

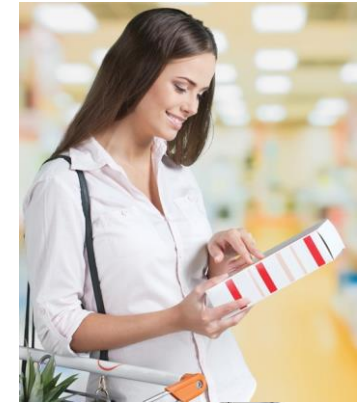
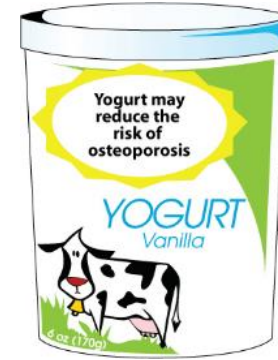
Prof. Silvia Tappi

DISTAL – Department of Agricultural and Food Sciences
– *Alma Mater Studiorum*, University of Bologna

silvia.tappi2@unibo.it

Motivation for emerging technologies in the food sector

- New products /consumer expectation
- Quality
 - Health benefits
 - Sensory attributes
- Food safety
- Economic advantage
 - cost (energy)
 - time
 - efficiency
- Waste reduction/reuse, sustainability



Application of cold plasma in the food sector

Presentation structure:

- Research on Cold Plasma in the Food Sector
- Main applications:
 - Food decontamination
 - microorganisms
 - viruses
 - mycotoxins
 - pesticides
 - allergens
 - Modification of enzymatic activity
 - Functionalization of food components (proteins, starches)
- Secondary effects: effects on quality and nutritional properties, lipid oxidation
- Legislative aspects



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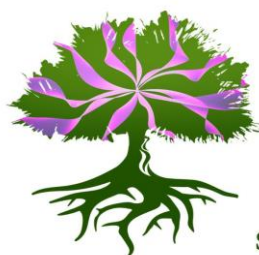
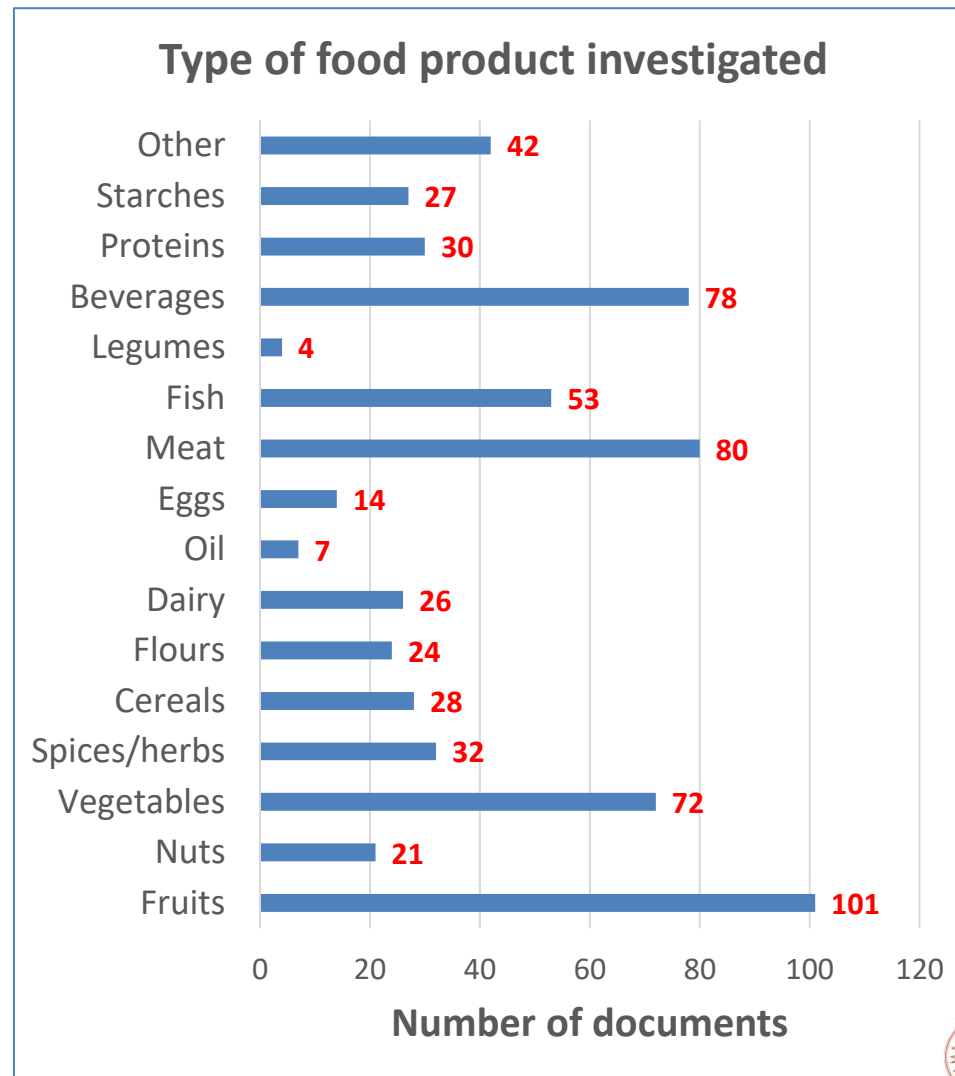
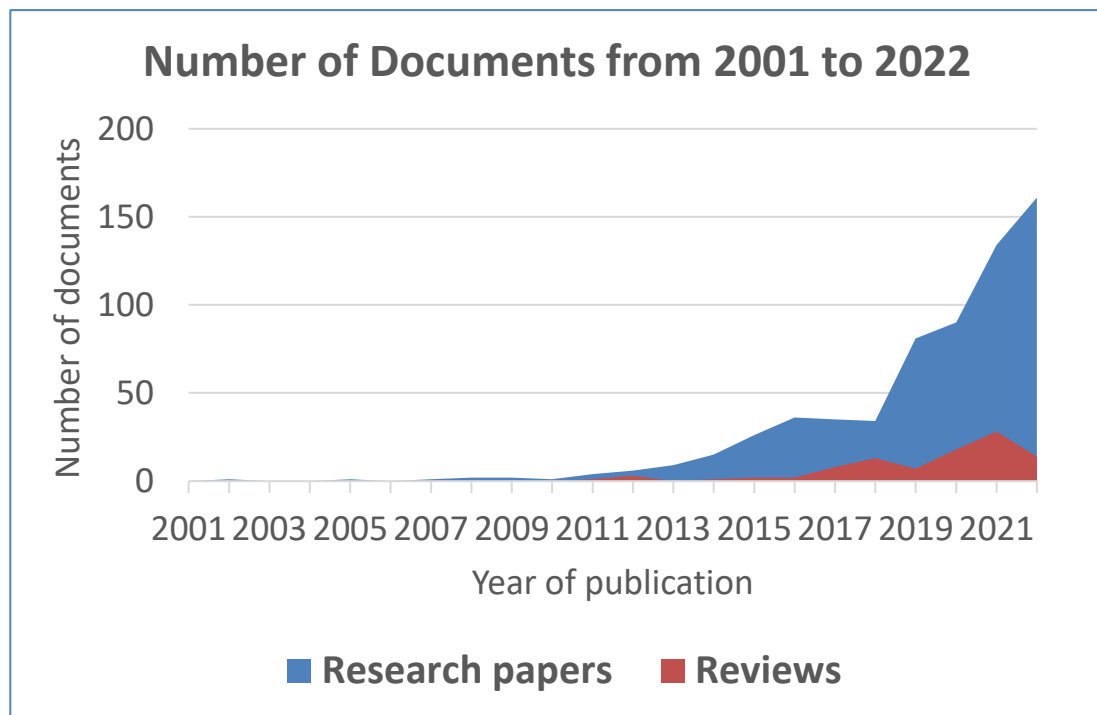
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Research on Cold Plasma in the Food Sector

Virtual Mobility Grant
Plasma effect on safety and quality of foods
 08/08/2023 to 20/10/2023

Source: Scopus
 738 bibliographic records (after pre-processing)

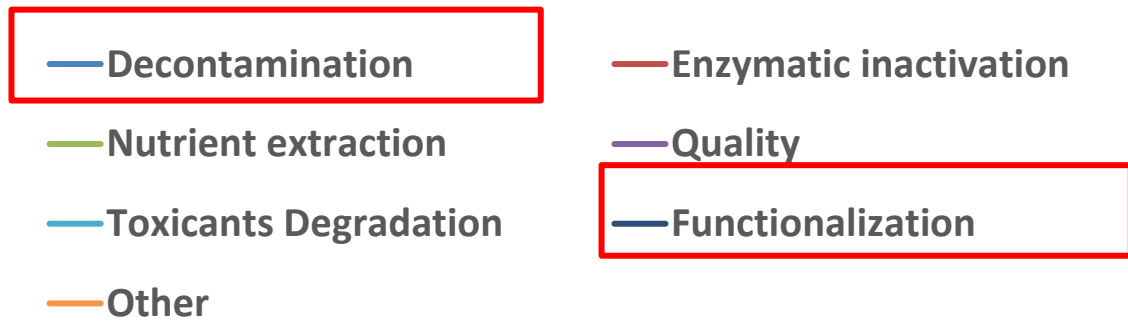
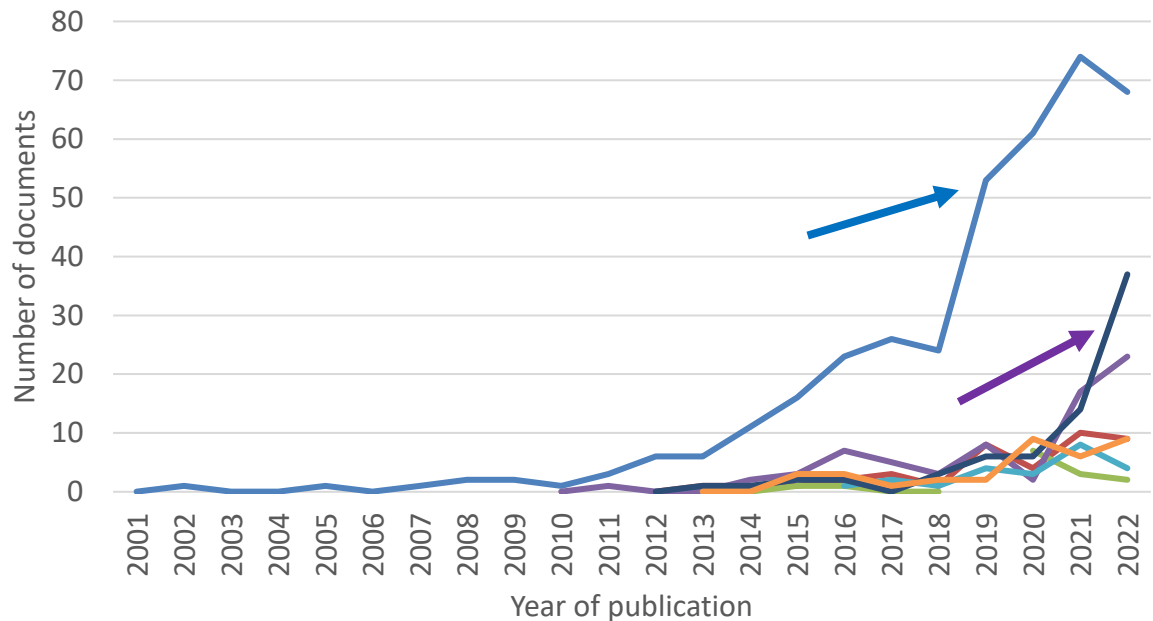


CA19110
 Plasma applications
 for smart and
 sustainable agriculture

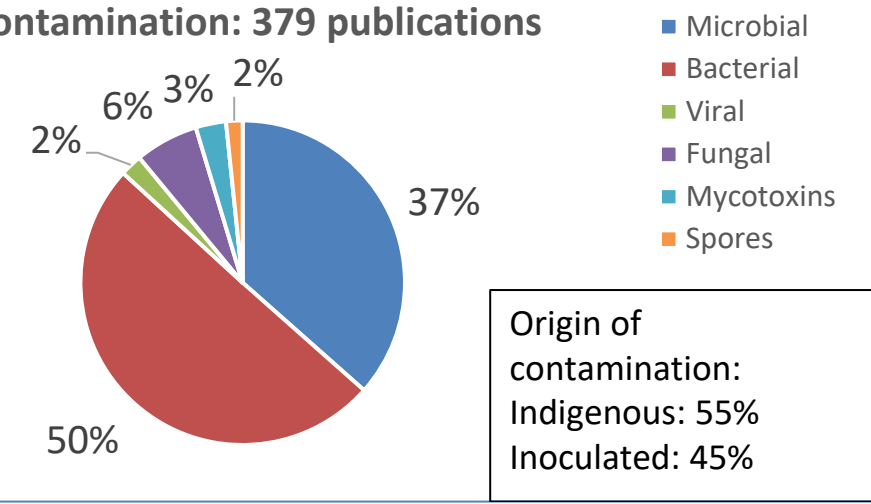


Research on Cold Plasma in the Food Sector

Applications of cold plasma

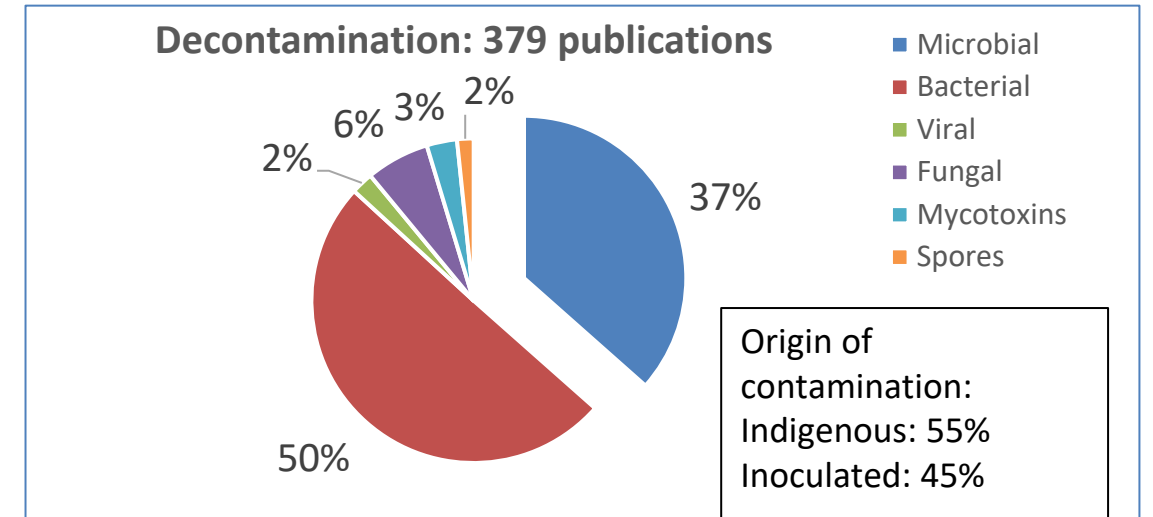
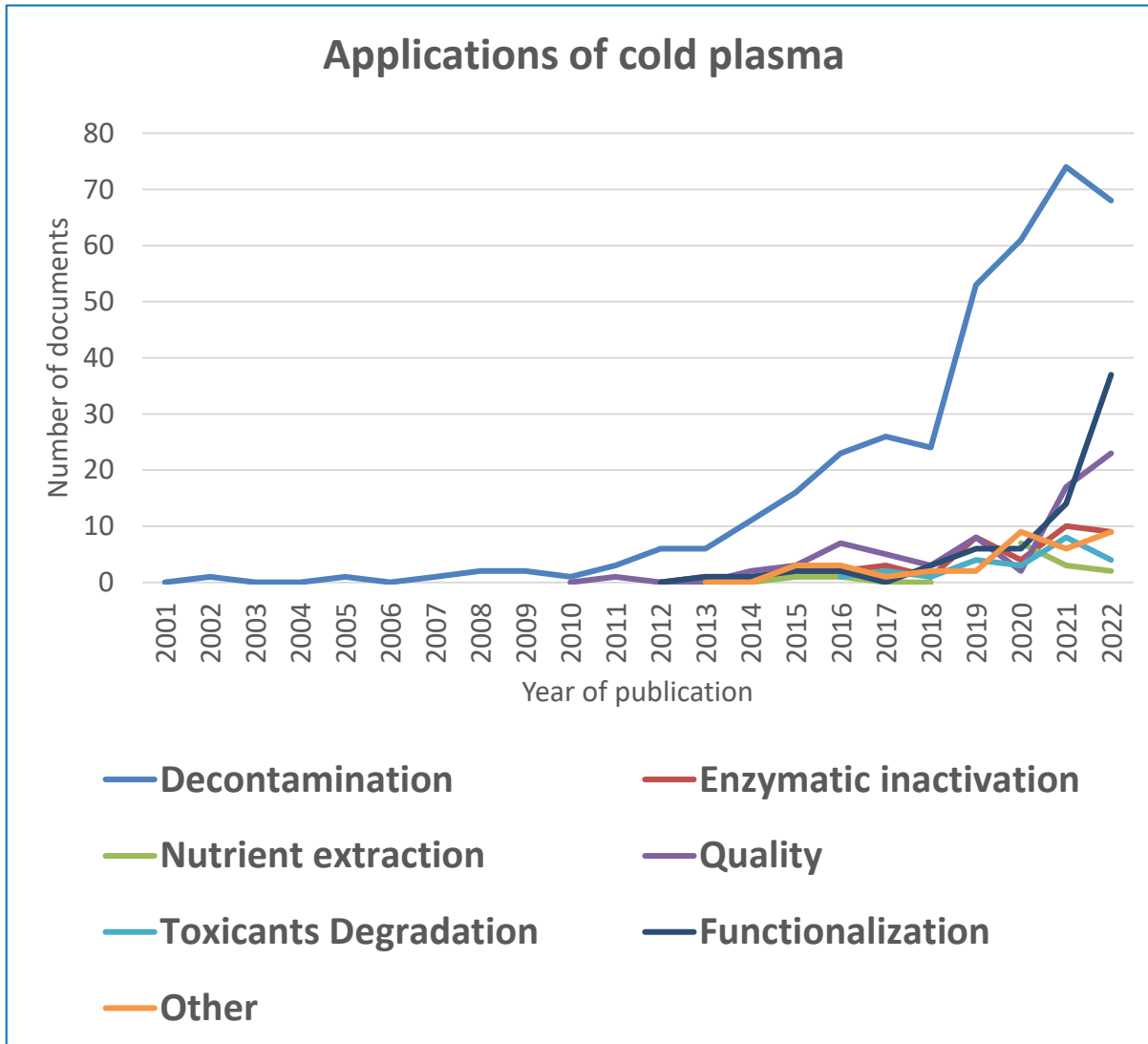


Decontamination: 379 publications



Total documents		379
Contamination agents	Number of documents	%
Total viable/mesophilic count	148	39.1
Yeast and moulds	59	15.6
Escherichia coli	95	25.1
Listeria	61	16.1
Salmonella spp	124	31
Saccharomyces cerevisiae	10	2.6
Aspergillus	31	9.2
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Norovirus	10	2.0
HAV	2	0.5
Parasites	1	0.2

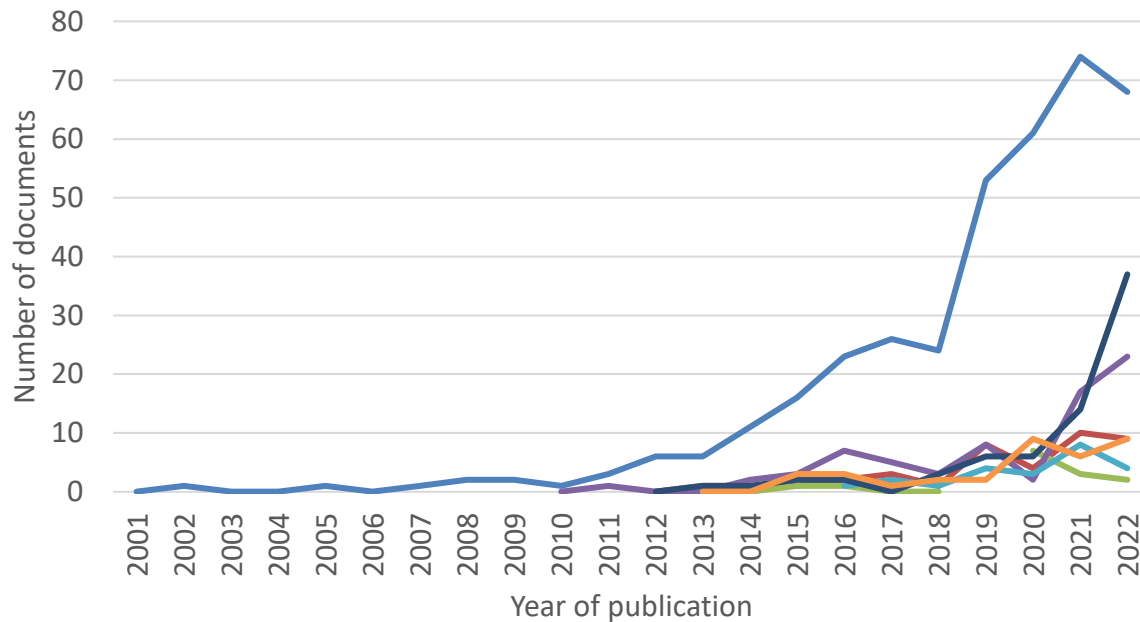
Research on Cold Plasma in the Food Sector



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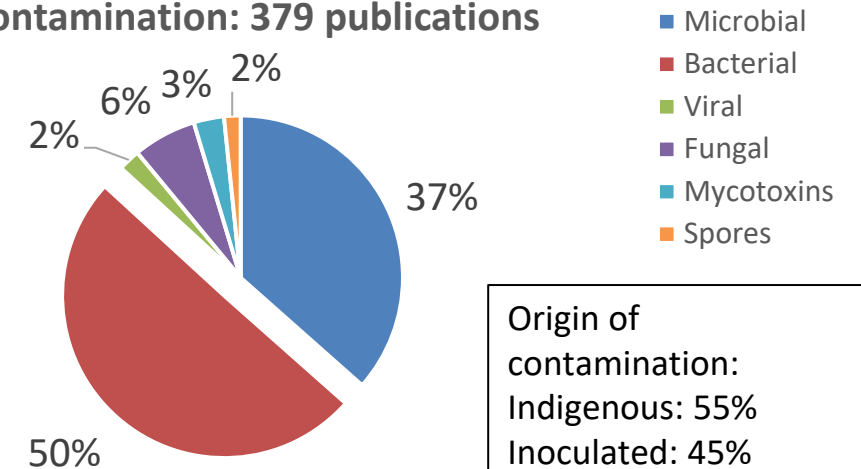
Research on Cold Plasma in the Food Sector

Applications of cold plasma



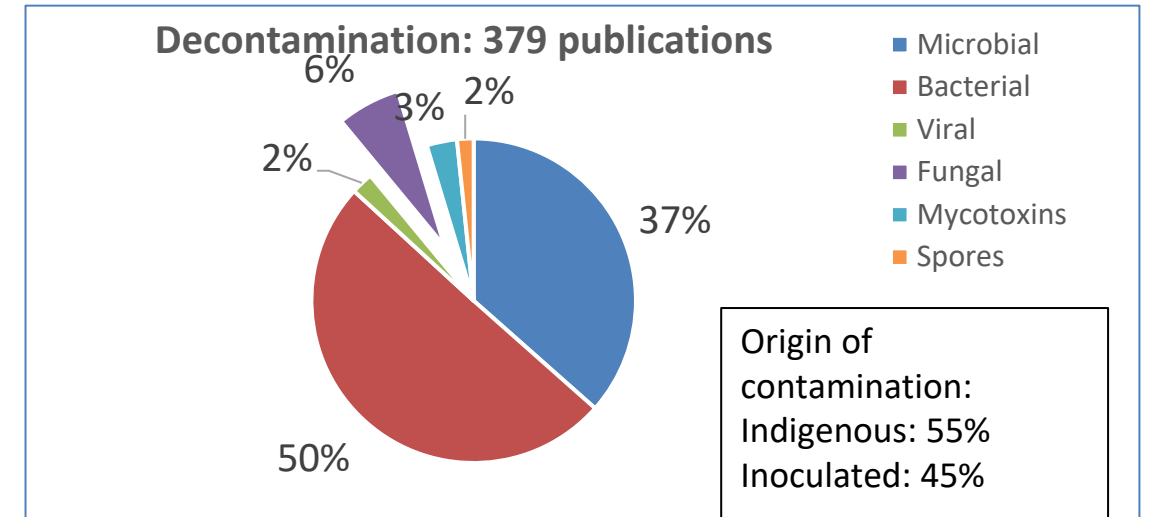
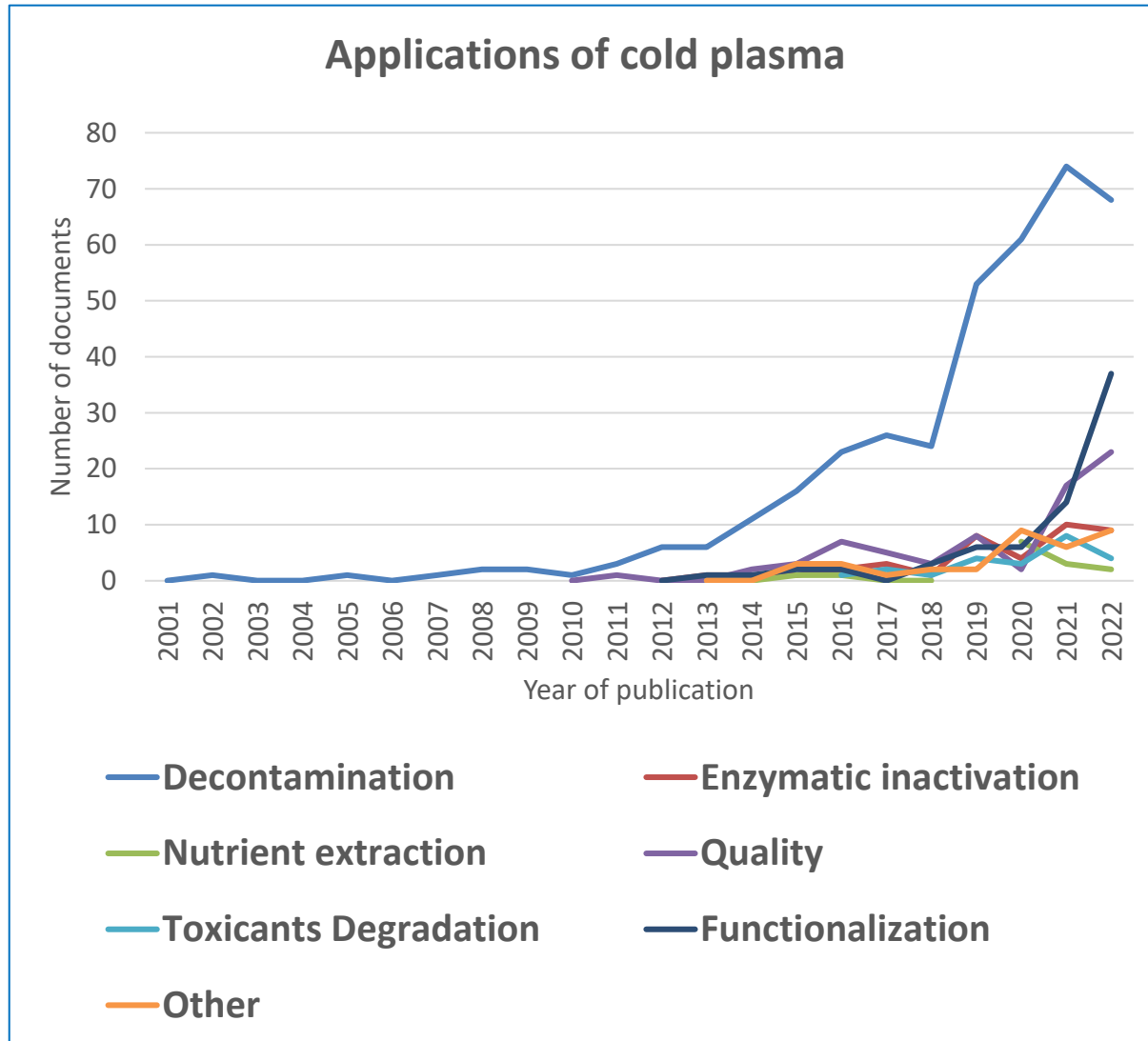
- Decontamination
- Enzymatic inactivation
- Nutrient extraction
- Quality
- Toxicants Degradation
- Functionalization
- Other

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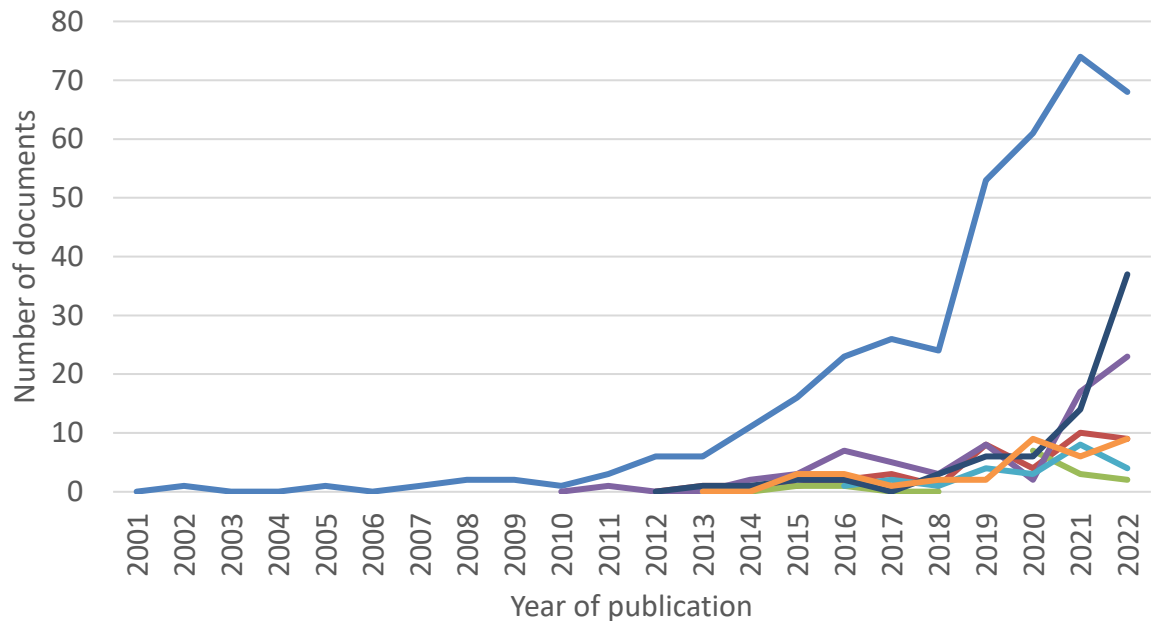
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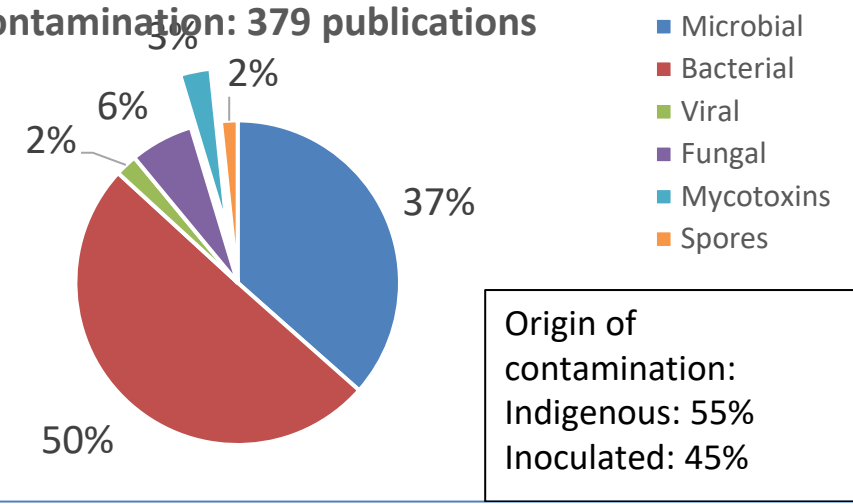
Research on Cold Plasma in the Food Sector

Applications of cold plasma



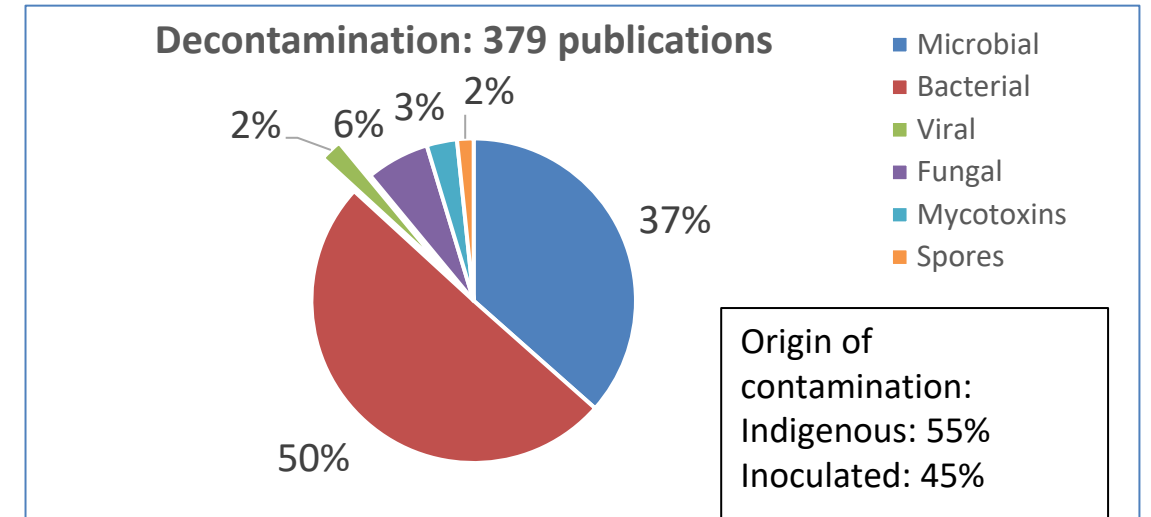
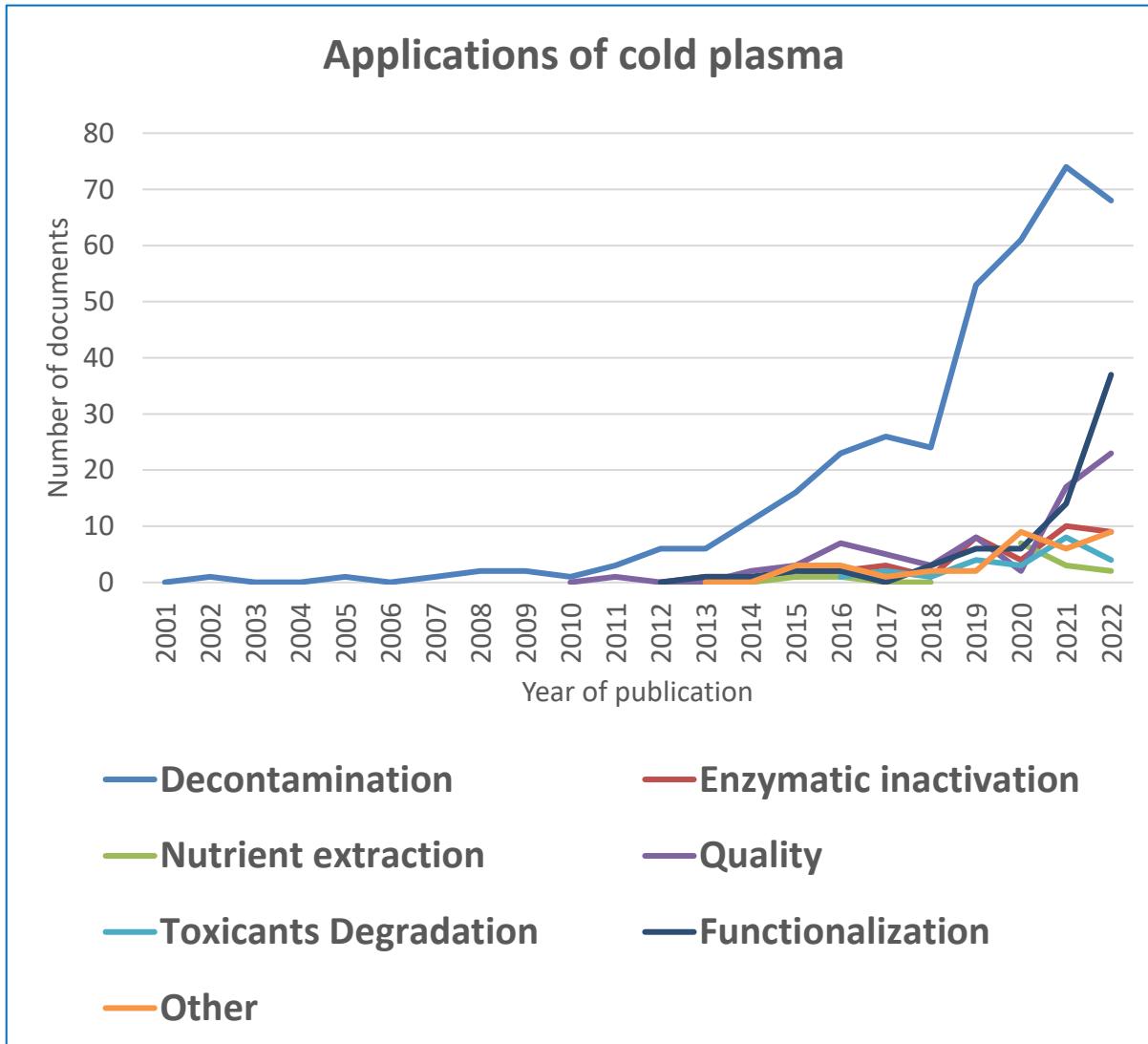
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Research on Cold Plasma in the Food Sector

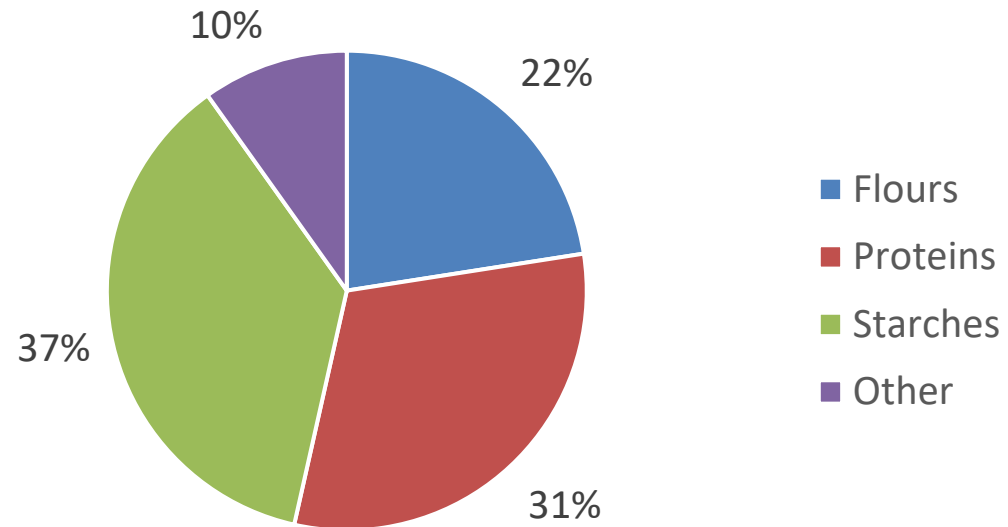


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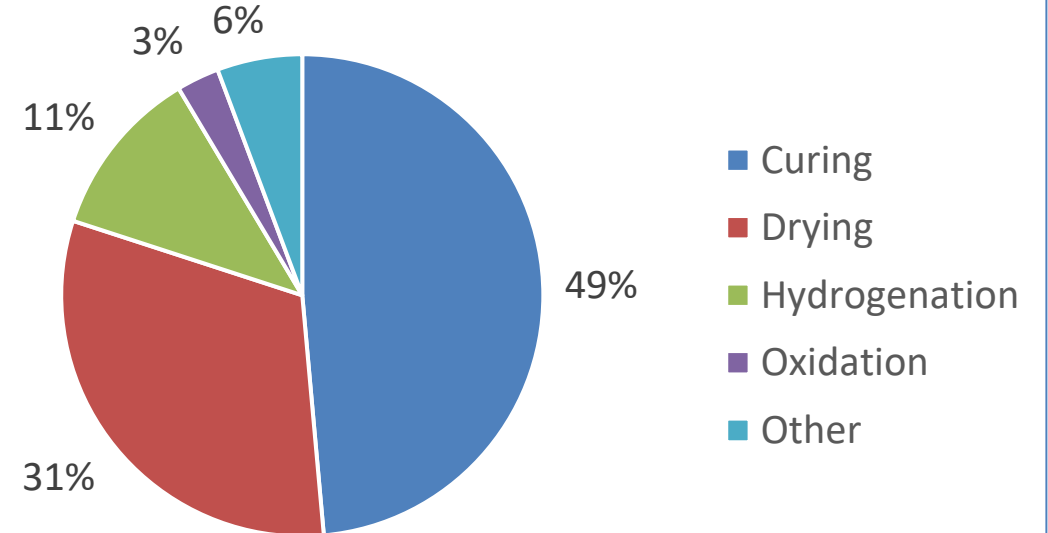
Research on Cold Plasma in the Food Sector

Emerging applications

Functionalization: 72 documents



Other applications: 36 publications



Application of cold plasma in the food sector

Presentation structure:

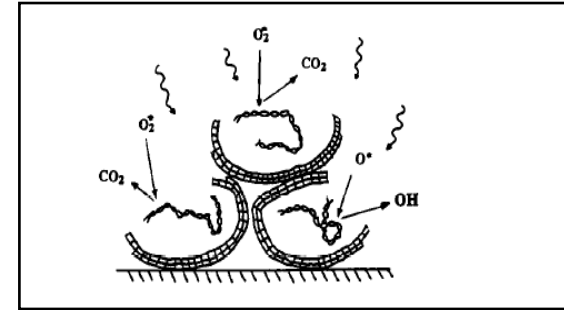
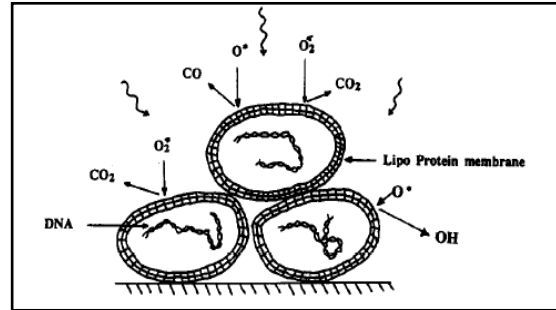
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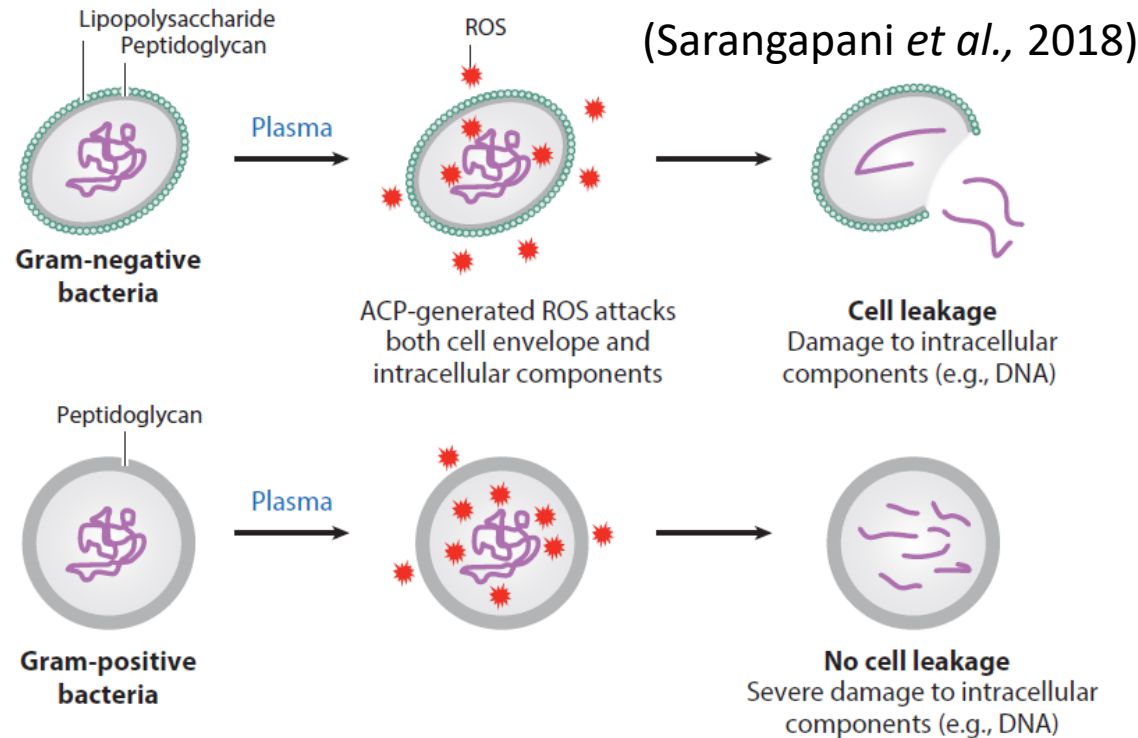
Hypothesis on the mechanism of microbial inactivation of plasma

DIRECT DESTRUCTION OF DNA CAUSED BY UV RAYS

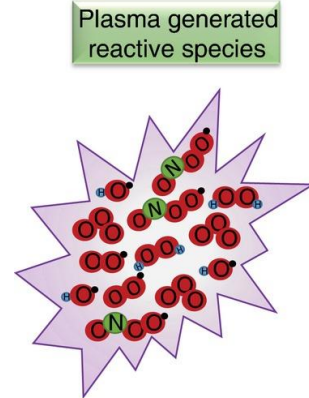
DEGRADATION OF CELL MEMBRANES



(Laroussi *et al.*, 2002)

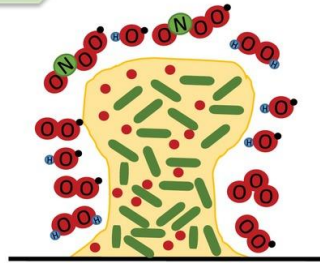


Mechanisms of cold plasma generated reactive species with respect to complexity of microbiological challenges

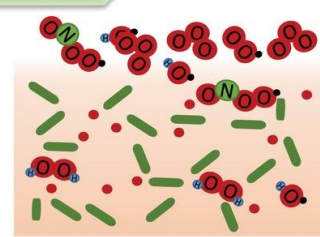


Extracellular challenges against reactive species

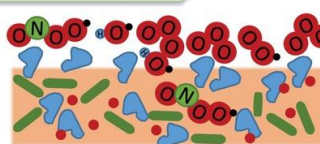
Biofilm



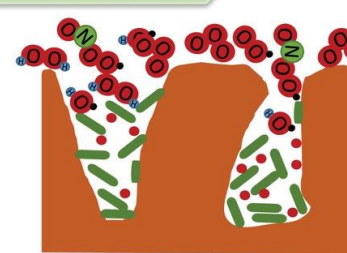
Liquid system



Nutrient rich system

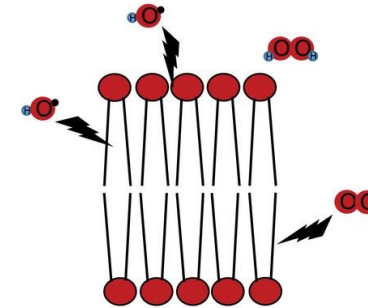


Structural protection



Intracellular challenges against reactive species

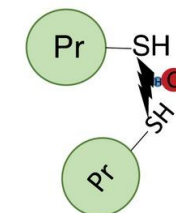
Lipid peroxidation



DNA damage

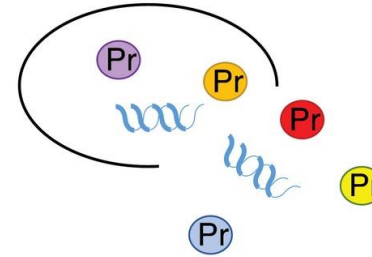


Protein disfunctioning

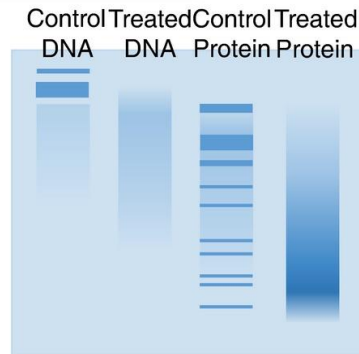


Inactivation effect caused by reactive species

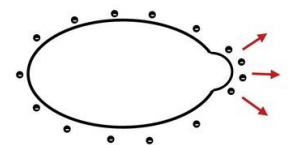
Cell leakage



Intracellular damage



Morphology changes

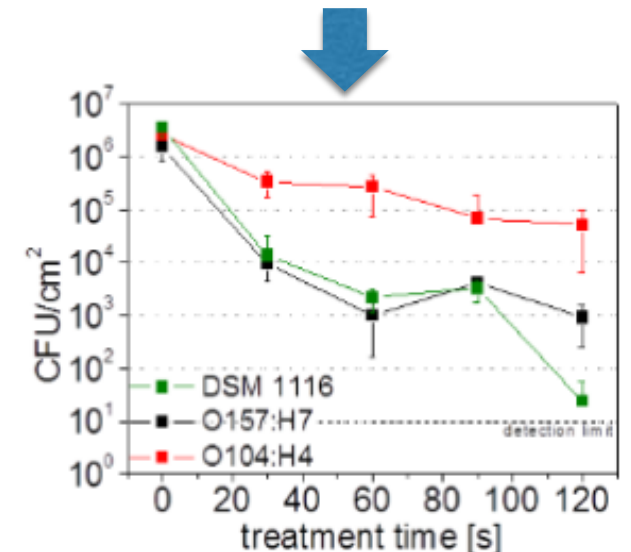
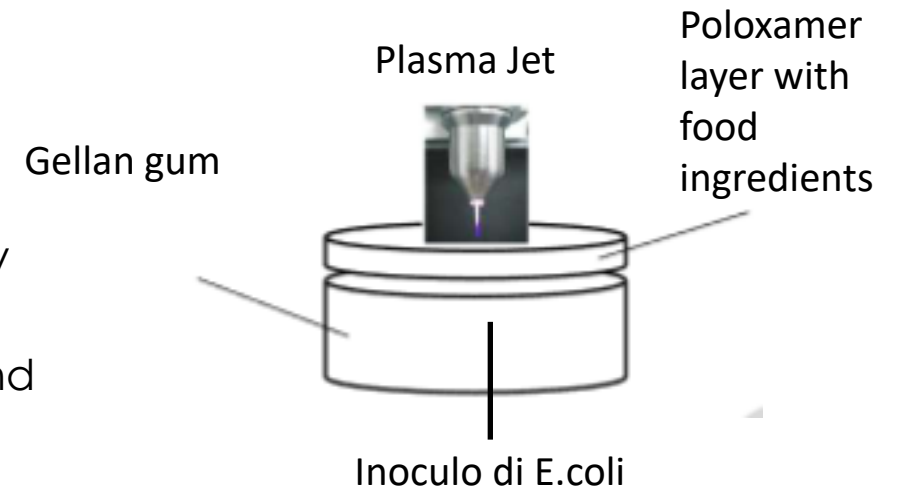


Bourke, P., Ziuzina, D., Han, L., Cullen, P. J., & Gilmore, B. F. (2017).

Studies on the mechanisms of microbial inactivation of plasma

Use of model systems

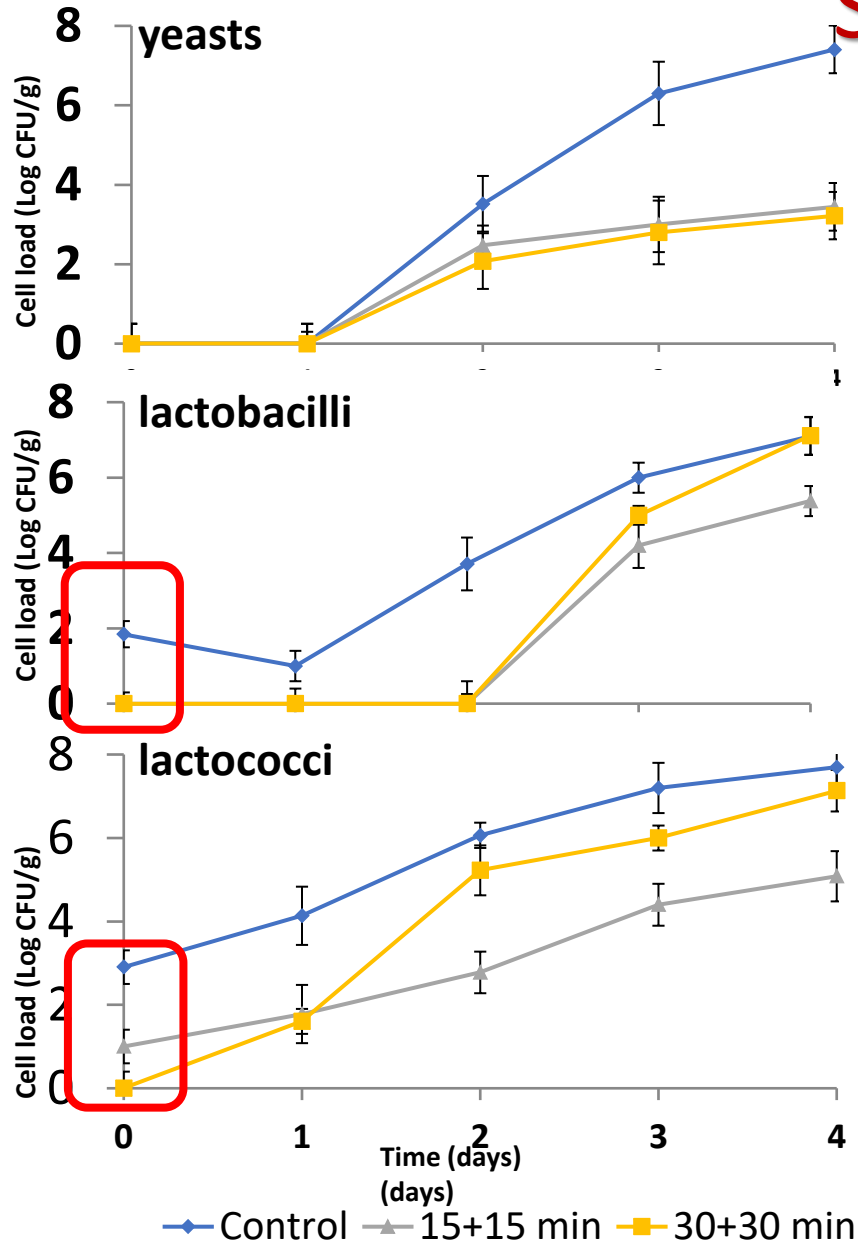
- useful aids for exploring efficient process parameters and efficacy limits for a single plasma device to be tested
- allow for homogeneous treatments and convenient extraction and provide reproducible results;
- As a simplified model system, large numbers of samples can be analyzed in the absence of drying stress, competing indigenous microflora or other inhibitory factors
- Generally consisting of a polysaccharide gel plate which contains some of the food ingredients, with a suitable pH at aw



ATTENTION: THE RESULTS ON REAL MATRICES COULD BE VERY DIFFERENT!!
MATRIX EFFECT

Plasma treatments for the decontamination of fresh-cut melon

Shelf-life study



Microbial group	Treatment time (min)	Shelf-life (giorni)**
Mesophiles	Control	2.3
	15+15 min	>4
	30+30 min	2.9
Psychrotrophes	Control	2.9
	15+15 min	>4
	30+30 min	2.6

**Time necessary to attain a cell count of 6 Log CFU g⁻¹, calculated using the predicted Gompertz parameters.

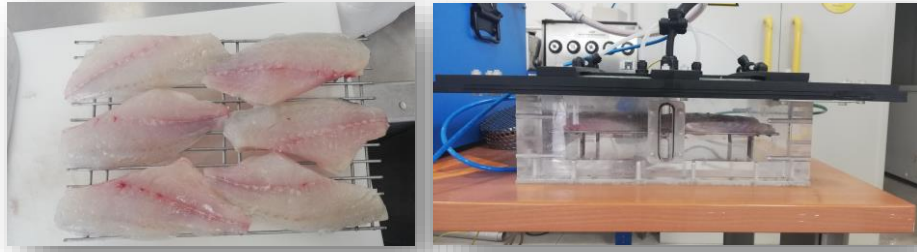
Microflora naturally present on the fruit pulp

Tappi et al., 2016

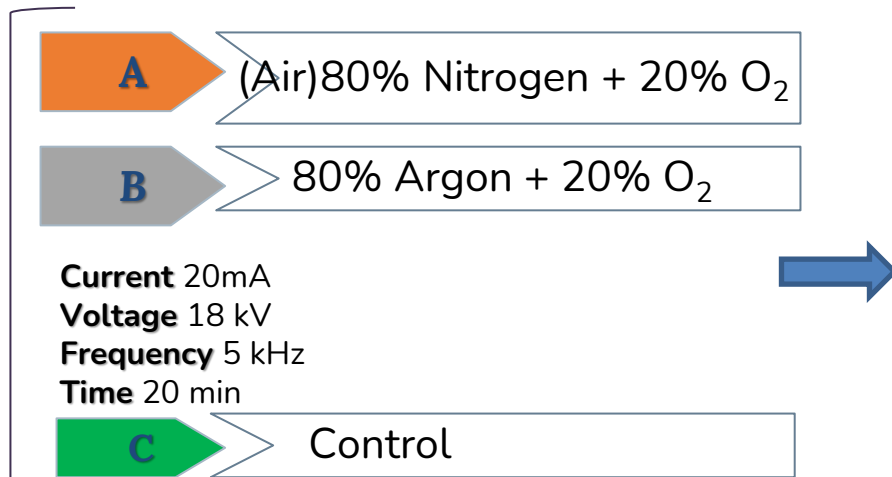


Cold plasma treatment to increase the shelf life of sea bream fillets

Seabreams from Kefalonia



PLASMA TREATMENT

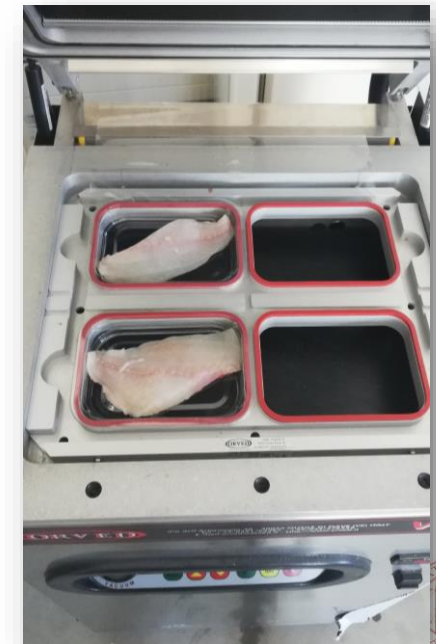


PACKAGING AND STORAGE



- Polypropilene (PP) trays and film
- Modified Atmosphere (MAP)
80% N₂ e 20% CO₂
- Refrigerated storage (4±1°) for 14 days

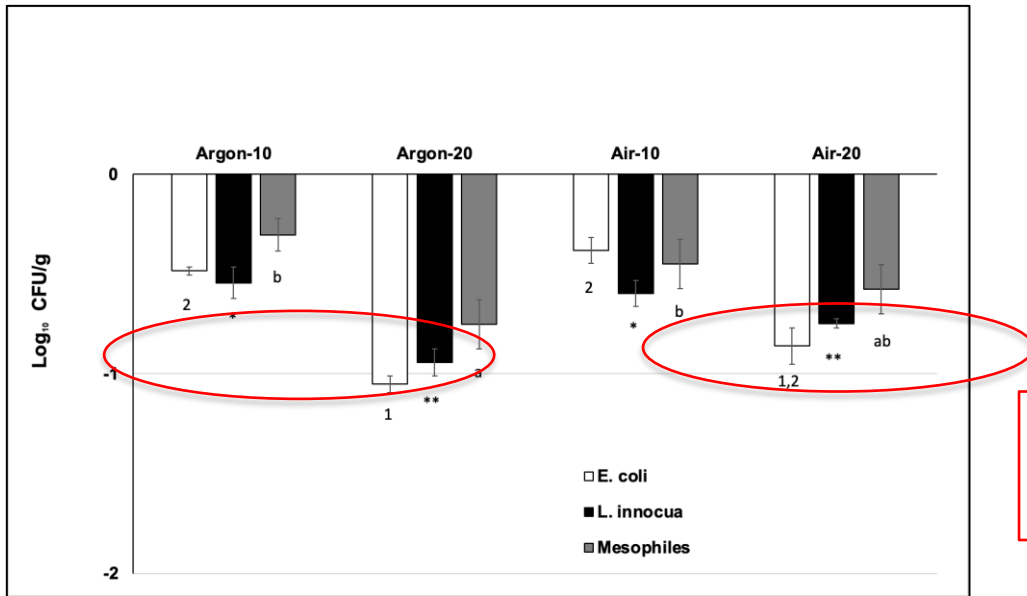
Sea bream



Cold plasma treatment to increase the shelf life of sea bream fillets

Inactivation of degradative and pathogenic bacteria

Sea bream



Latest acceptable data point →

Storage day	Control	Plasma Air
0	3.08	3.05
2	3.11	3.09
5	3.50	3.78
6	3.88	3.91
8	5.07	4.84
9	5.10	5.56
10	6.36	5.80
12	6.78	5.87
13	6.93	5.94
14	7.29	6.62

Increase of 4 days!



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



Tappi, S., Nissen, L., Casciano, F., Antonelli, G., Chiarello, E., Picone, G., ... & Rocculi, P. (2023).

Gaseous plasma treatment on shredded carrots and sliced apples inoculated with *Arcobacter* spp. or *E. coli*

Arcobacter spp.: Campylobacteriaceae family
emerging animal and human pathogen, underestimated
global prevalence
most likely source of *Arcobacter* spp. to fruits and
vegetables include irrigation water and contaminated soil



1-cm sliced apple



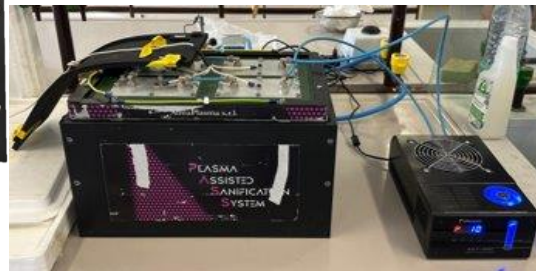
Shredded carrots



Prototype: PASS
Surface Dielectric Barrier
Discharge (SDBD)
Treatment times:
2, 5, 10, 20, 30 minutes
Gas regime: NO_x



SDBD



Teasted strains

E. coli 555

Arcobacter spp. (BZs 206) – strain 12

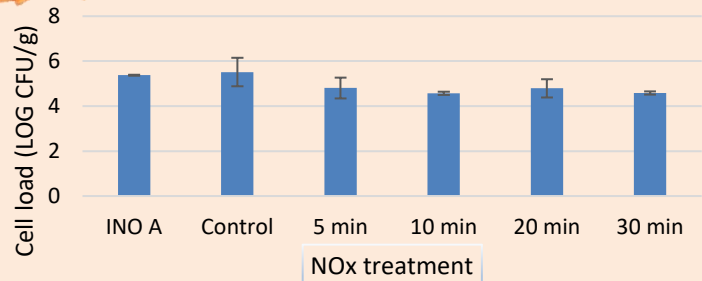
Arcobacter spp. (BZG 74) - strain 28

Gaseous plasma treatment on shredded carrots and sliced apples inoculated with *Arcobacter* spp. or *E. coli*

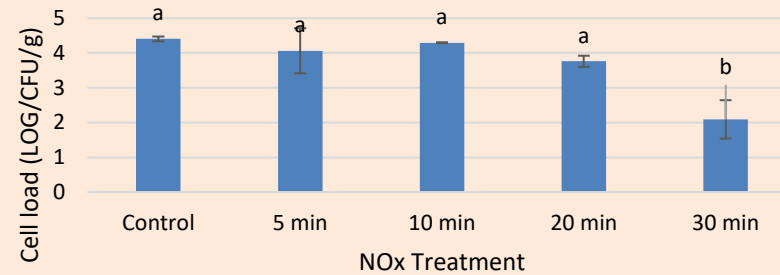
(Unpublished data)



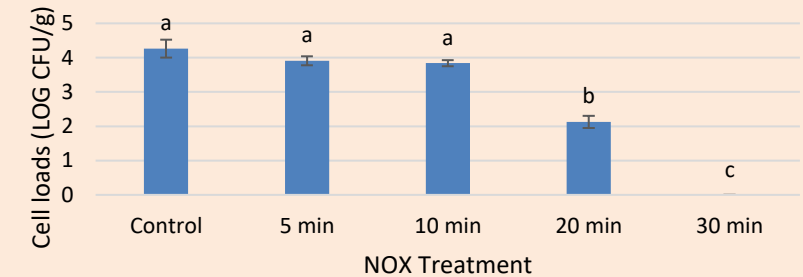
Shredded carrots inoculated with *E. coli* 555



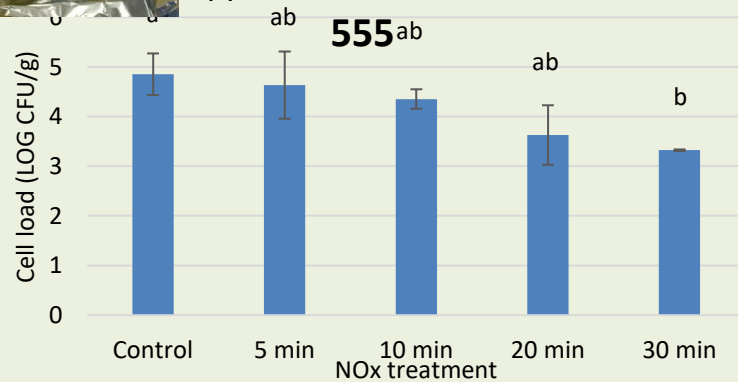
Shredded carrots inoculated with *Arcobacter* spp. BZS206 (strain12)



Shredded carrots inoculated with *Arcobacter* spp. BZS74 (strain 28)

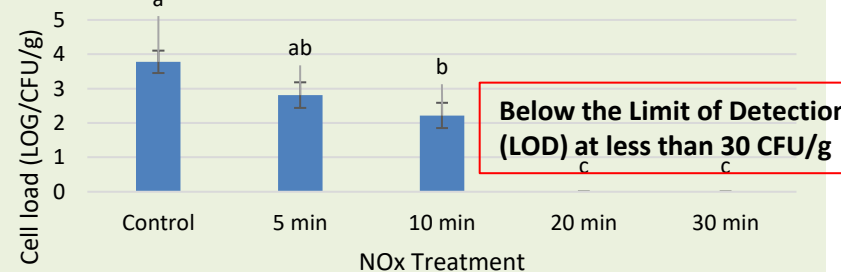


apples inoculated with *E. coli* 555



reduced by < 2.5 Log after 30 min

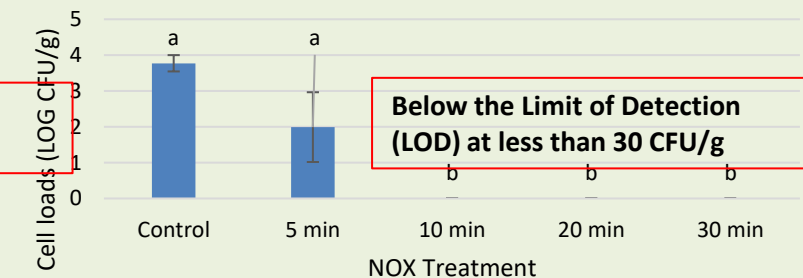
Sliced apples inoculated with *Arcobacter* BZS206 (strain 12)



Below the Limit of Detection (LOD) at less than 30 CFU/g

reduced by < 2 Log after 20 min

Sliced apples inoculated with *Arcobacter* spp. BZS74 (strain 28)



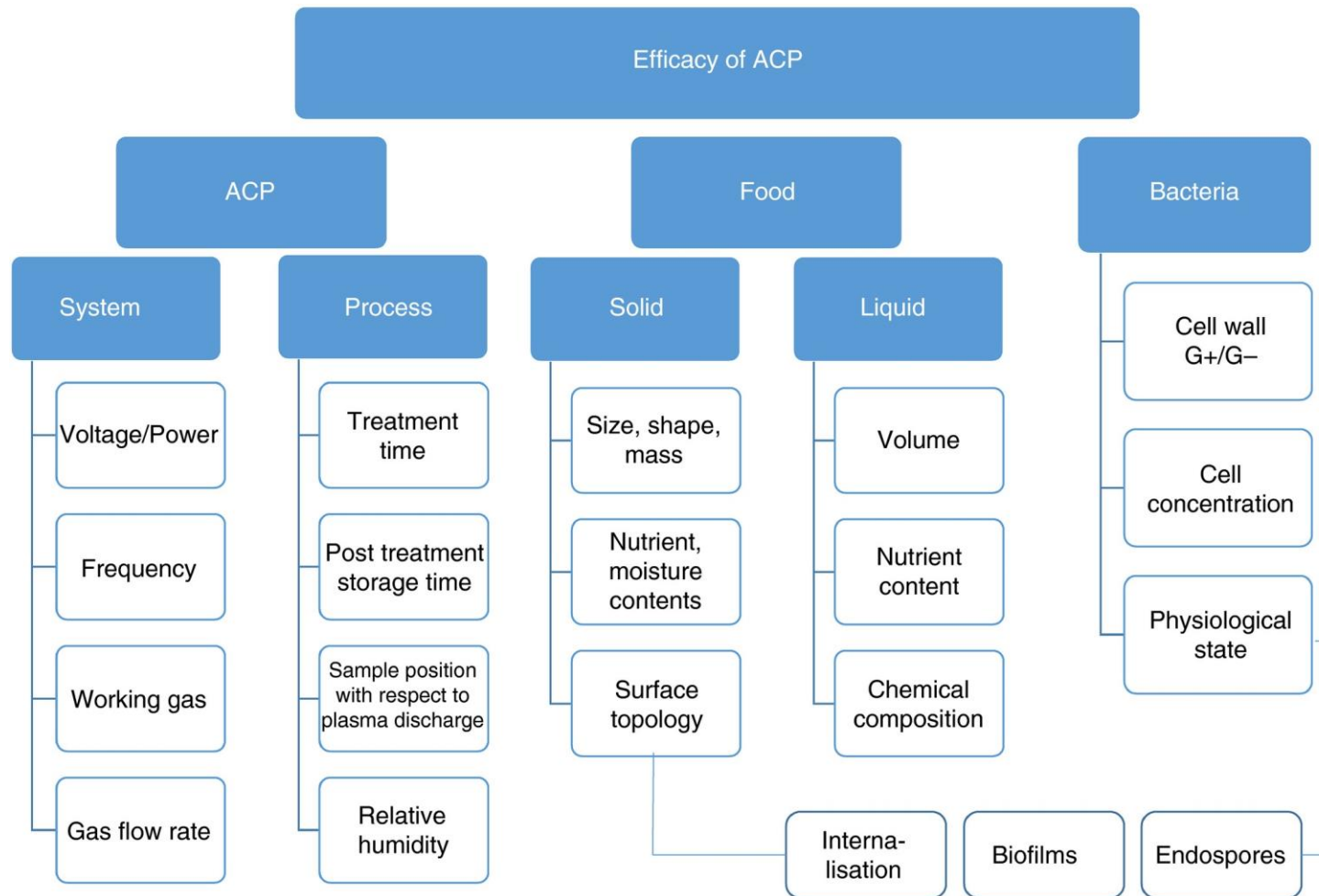
Below the Limit of Detection (LOD) at less than 30 CFU/g

reduction of approx. 1.5 Log after 30 min

reduced after 5 min of treatment (1 Log), max reduction after 30 min

reduced after 5 min, reduction under LOD after 10, 20 and 30 min

Parameters influencing plasma treatment decontamination efficacy



Necessary to 'tailor' the treatment to each specific situation, considering all variables!

Bourke, P., Ziuzina, D., Han, L., Cullen, P. J., & Gilmore, B. F. (2017). Microbiological interactions with cold plasma. *Journal of applied microbiology*, 123(2), 308-324.



Application of cold plasma in the food sector

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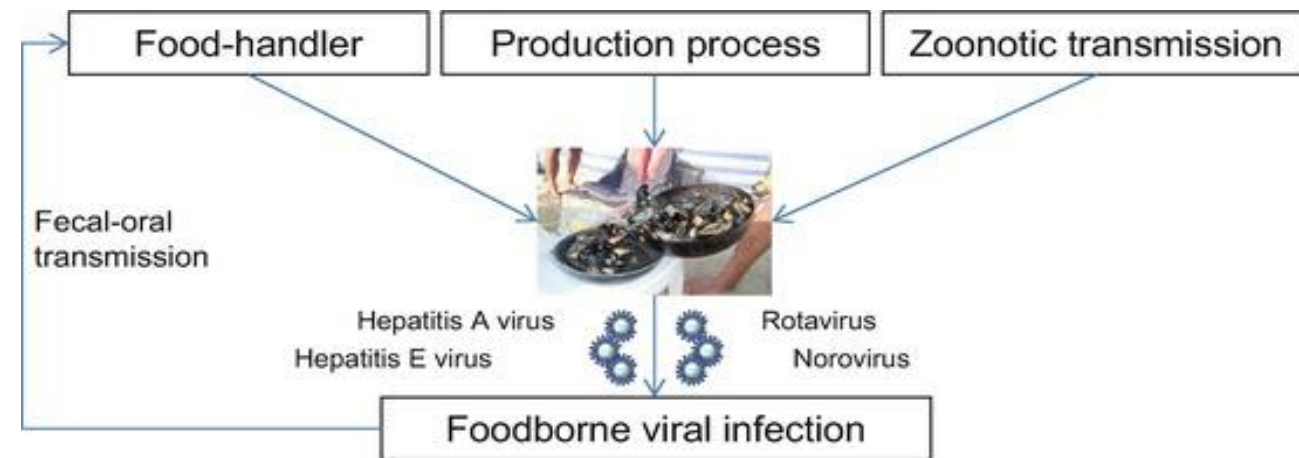
Foodborne viruses

Although viruses, unlike bacteria, cannot grow in or on foods, foodborne illnesses are associated with viruses due to contamination of fresh produce or processed foods by fecal matter containing viruses.



Commonly Reported Major Foodborne Illnesses
Due to:

- Norovirus,
- hepatitis A and E virus,
- rotavirus and
- astrovirus.



Characteristics: highly infectious nature and survival in drastic conditions such as high acid pH and low temperatures

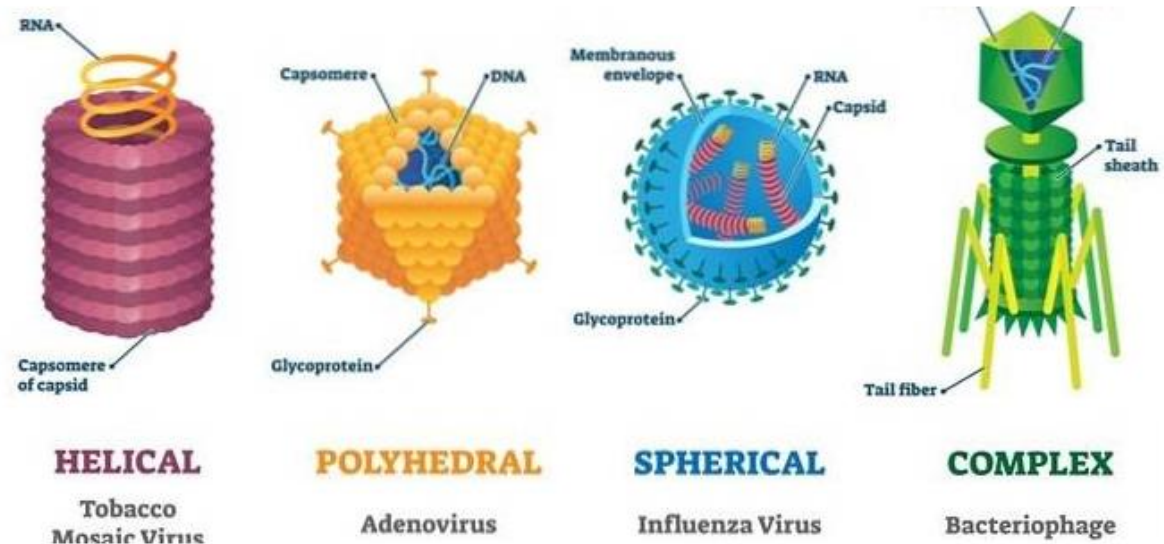


Structural elements of viruses

A complete virus particle, known as a **virion**, consists of **nucleic acid** surrounded by a protective coat of protein called a **capsid**. These are formed from protein subunits called capsomeres. Viruses can have a **lipid "envelope"** derived from the host cell membrane.

Some of the morphological types:

1. Helical
2. Icosahedral
3. Enveloped (SARS-CoV-2)
4. Complex



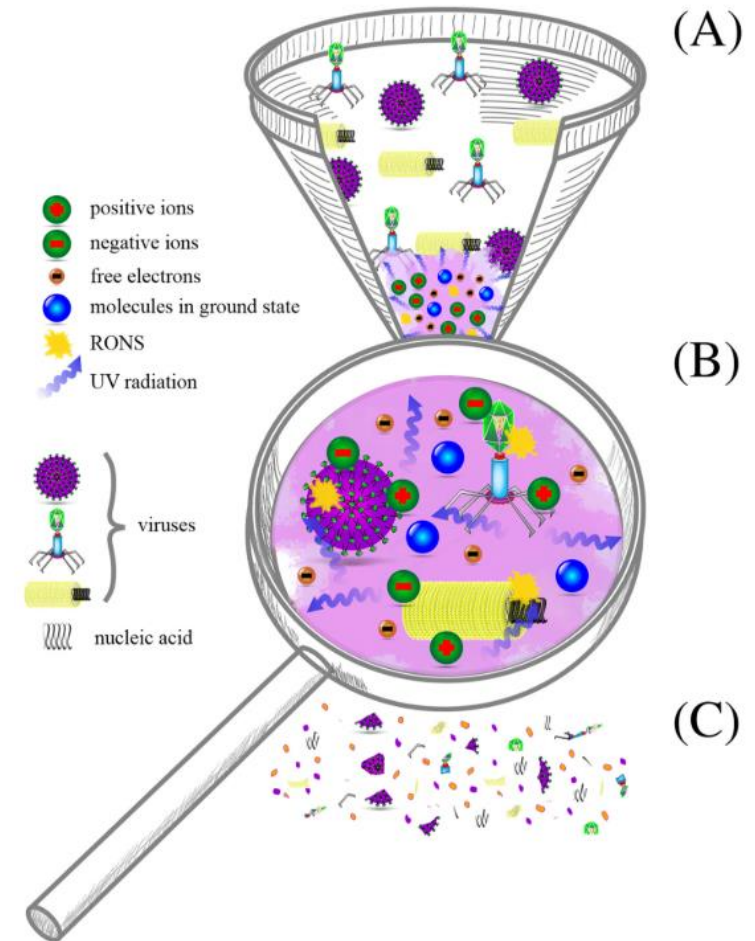
Plasma viral inactivation

Modes of virus inactivation

- Changes in lipid components from the envelope
- Degradation of the capsid proteins, which precedes the degradation of nucleic acids
- Disruption of virus integrity at both the structural and genomic levels by affecting both proteins and nucleic acids

- (A) Morphologically different viruses treated with CP
- (B) Close-up of CP properties responsible for virus inactivation
- (C) Residual virus particles and nucleic acids after CP treatment,

Filipić, A., Gutierrez-Aguirre, I., Primc, G., Mozetič, M., & Dobnik, D. (2020).

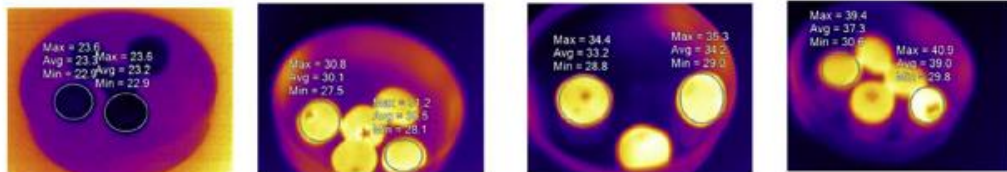


Plasma treatments for the decontamination of viruses

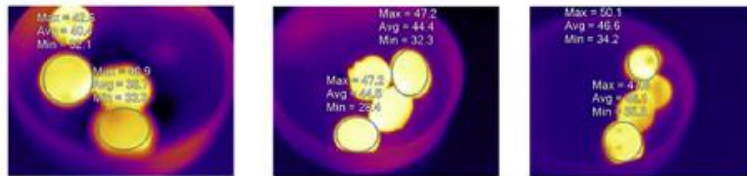
Lacombe et al. (2017)

Inoculus of Tulane virus and Norovirus on bluberries

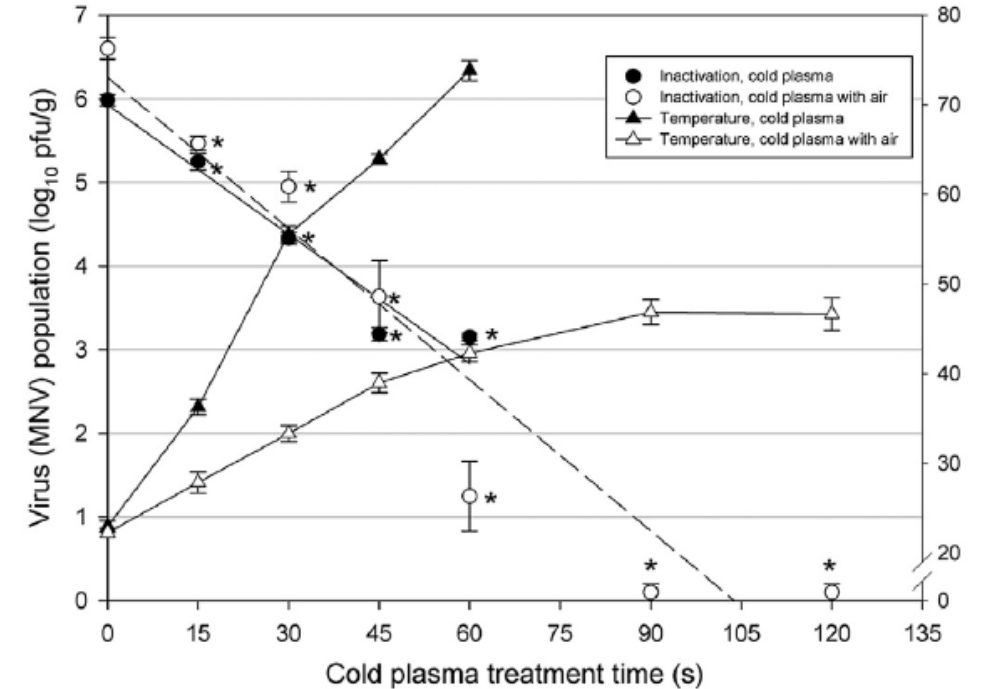
plasma jet
pulse frequency: 47 kHz
power consumption: 549W.



Control (23.2 °C) 15 sec (30.1 °C) 30 sec (34.2 °C) 45 sec (39.0 °C)



60 sec (40.4 °C) 90 sec (44.4 °C) 120 sec (46.6 °C)



Proposed mechanism:

ozone and ROS inactivate viruses
first damaging the polypeptide
chains causing a perforation in the
viral capsid

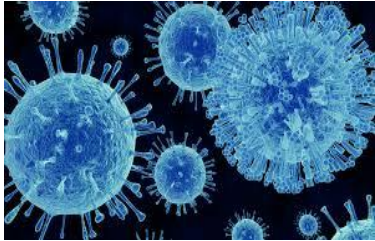


Thermal images of cold plasma treatment: 4 cfm
plasma jet plus 7 cfm air.

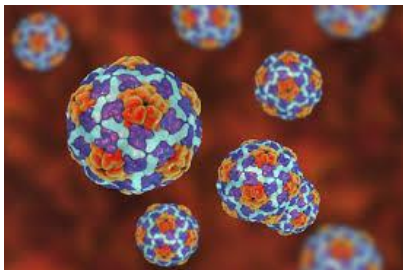
Plasma treatments for the decontamination of viruses



DIPARTIMENTO
SICUREZZA ALIMENTARE, NUTRIZIONE
E SANITÀ PUBBLICA VETERINARIA



NOROVIRUS
(MNV – strain MNV-1
3.5 log TCID₅₀/ml)



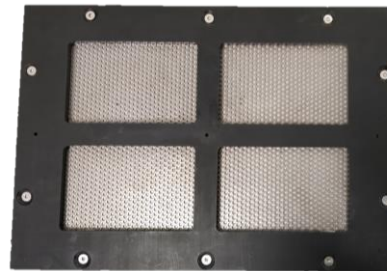
HEPATITIS A VIRUS
(HAV – strain HM 175
3.6 log TCID₅₀/ml)

Strawberries



Artificial
inoculum

SDBD



Surface Dielectric Barrier Discharge
(SDBD),

4 rectangular high-voltage electrodes
(115 cm² each)

Mica dielectric layer over 2 mm thick

Sinusoidal waveform, 6 kV, 23 kHz.

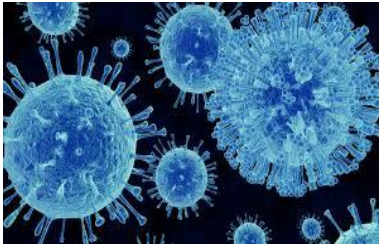
NOx-mode=332,9 W up to 30 min



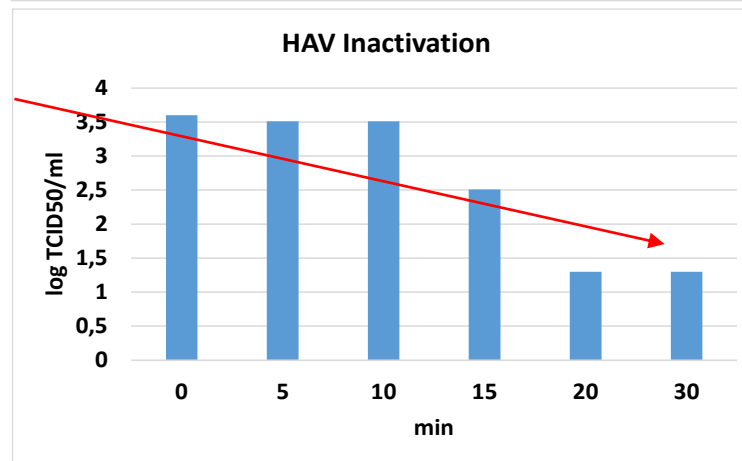
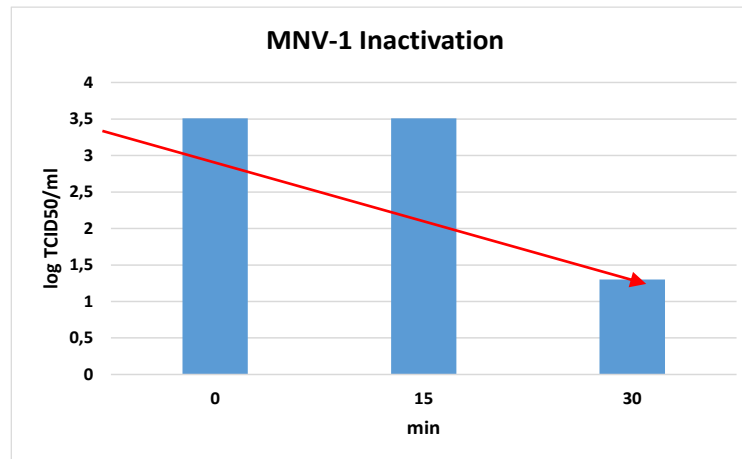
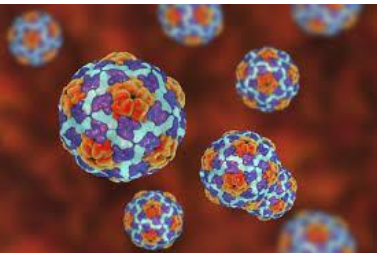
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Plasma treatments for the decontamination of viruses

NOROVIRUS



HEPATITIS A VIRUS



(Unpublished data)

Reduction below the detection level (20 TCID50/ml) was achieved corresponding to 1.2 log for MNV and to 2.3 log for HAV

Application of cold plasma in the food sector

Presentation structure:

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 - microorganisms
 - viruses
 - mycotoxins
 - pesticides
 - allergens
 - Modification of enzymatic activity
 - Functionalization of food components (proteins, starches)
- Secondary effects: effects on quality and nutritional properties, lipid oxidation
- Legislative aspects



Plasma treatments for the decontamination of mycotoxins

Many species of filamentous fungi have the ability to produce **toxic secondary metabolites** known as mycotoxins. The term mycotoxins is used only for toxic substances produced by fungi related to **food and feed products**; it does not include toxins produced by mushrooms

Today about **400 structurally different mycotoxins** have been discovered divided into the following main groups:

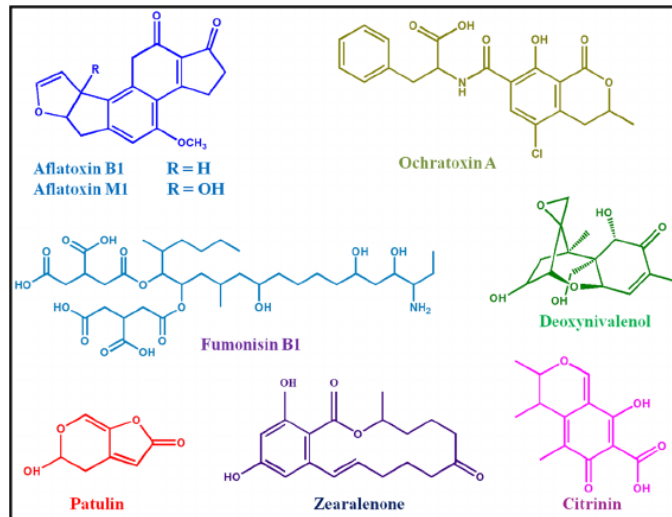
- (i) **aflatoxins** produced by *Aspergillus* species and **ochratoxins** produced by both *Aspergillus* and *Penicillium* species;
- (ii) **trichothecenes, zearalenone** and **fumonisin**s produced by *Fusarium* species;
- (iii) and ergot **alkaloids**, produced by *Claviceps* and other species



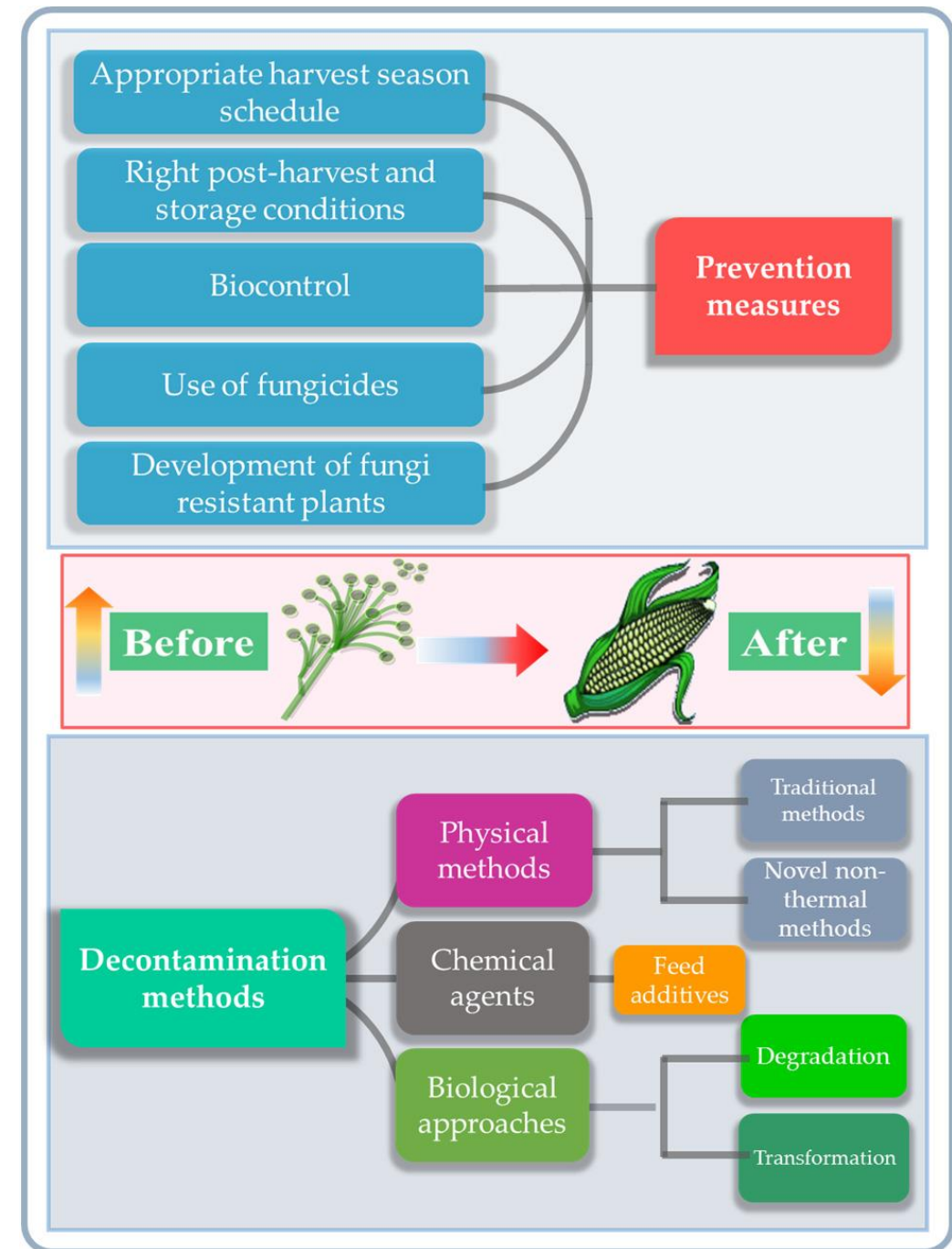
In favorable environmental conditions the *Aspergillus* spores germinate and subsequently colonize various types of foods, such as **grains, corn, peanuts and other oil seeds.**



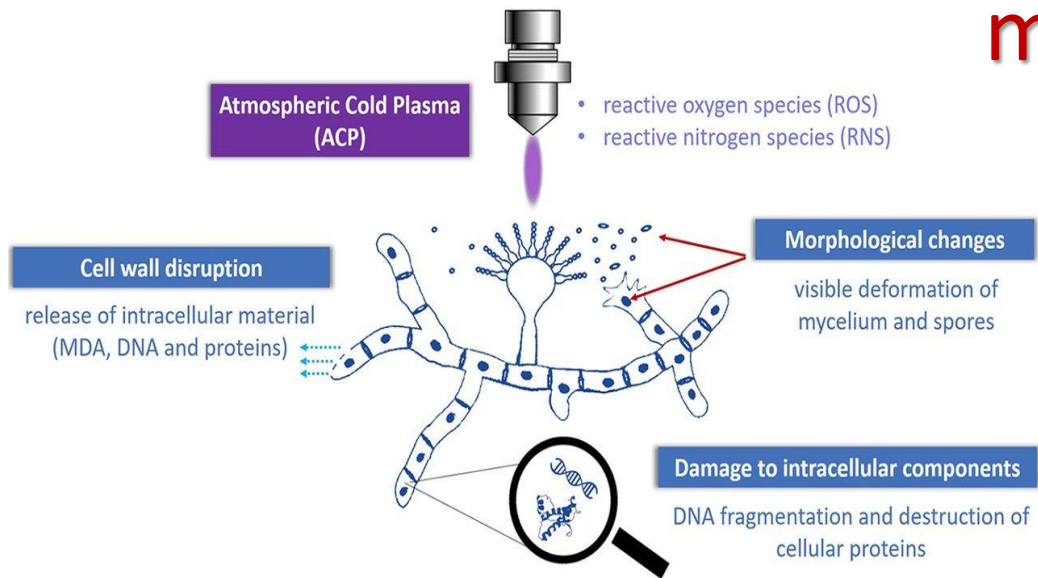
High levels of aflatoxins induce acute hepatic necrosis, liver carcinoma, and are classified as mutagenic and carcinogenic, being able to intercalate with DNA and being able to alkylate its bases through its more oxidized metabolites.



Overview of currently available mycotoxin prevention and decontamination measures taken before and after contamination of food by fungi and mycotoxins.



Plasma treatments for the decontamination of mycotoxins



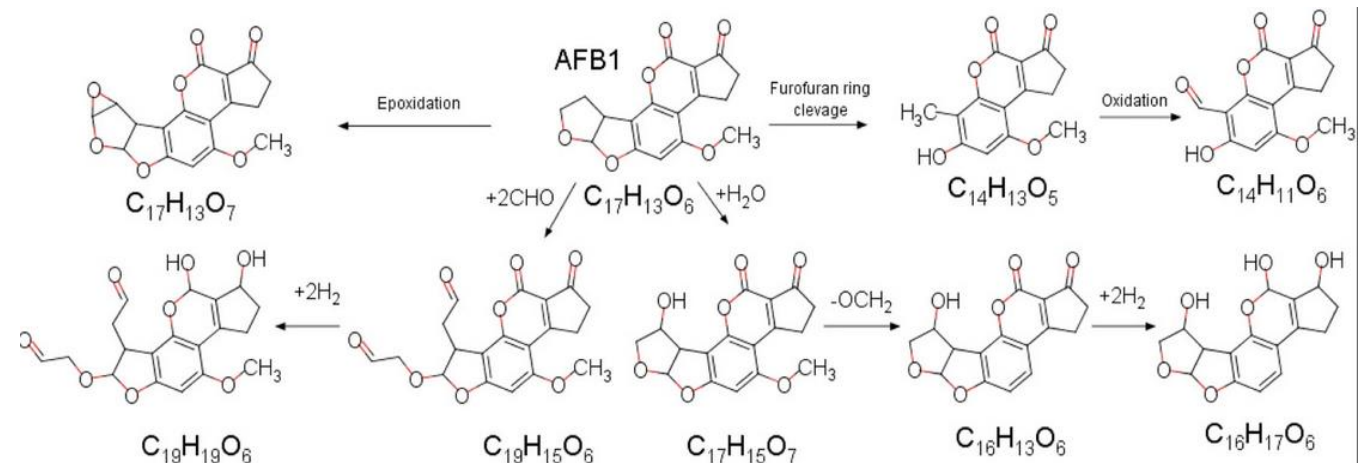
Approach 1: inactivation of fungal species

Various mechanisms related to structural and metabolic changes

Approach 2: chemical degradation of mycotoxins

Structure modification during plasma treatment: related to the presence of UV photons, ozone or reactive ions and electrons.

Possible significant contributions of different reactive species or to the synergistic action of these species → the effectiveness of mycotoxin degradation of cold plasma technology is greater than that of ozone or UV treatments only



Degradation pathways of aflatoxin (AFB1) on exposure to high voltage atmospheric cold plasma. Adapted from Shi et al. (2017).

Plasma treatments for the decontamination of mycotoxins

Reduction of 97 and 99% in the growth of *Aspergillus parasiticus* and *Aspergillus flavus*.

40 W 15 min and 60 W 12 min plasma treated samples: more than 70% and 90% reduction in aflatoxin B1



Devi et al. 2017

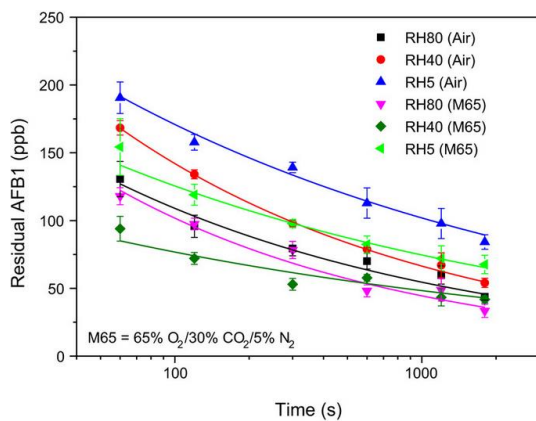
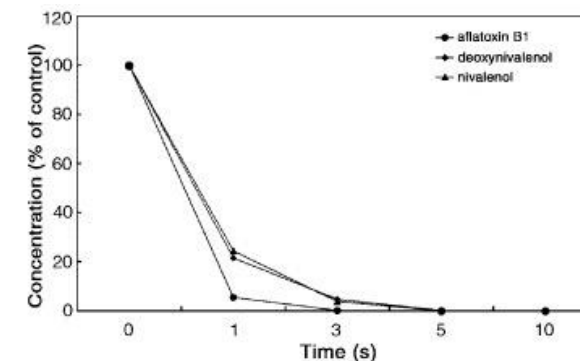


Reduction of 3-log for both *Aspergillus* spp. and *Penicillium* spp. 15 min of SF6 plasma on dry legumes

Selcuk et al. 2015

Complete inactivation of aflatoxin B1 (AFB1), deoxynivalenol (DON) and nivalenol (NIV) with a microwave plasma using Argon at atmospheric pressure

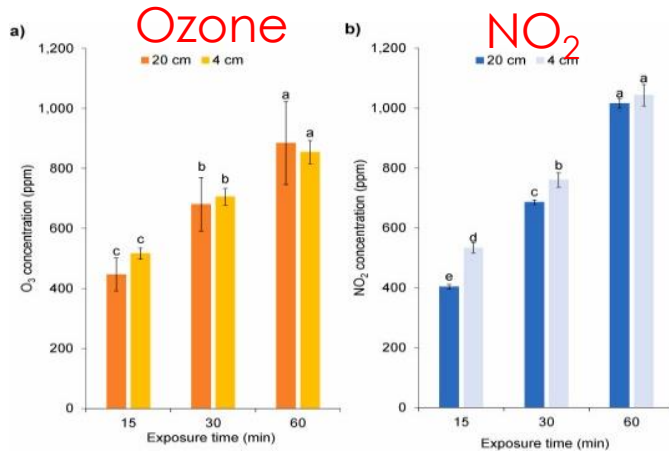
Park et al. 2007



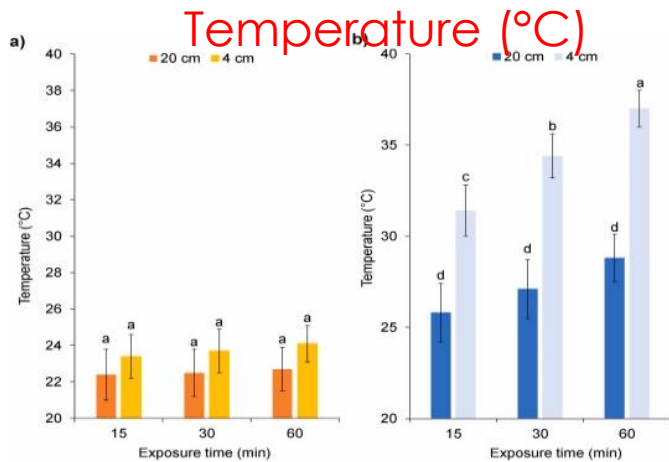
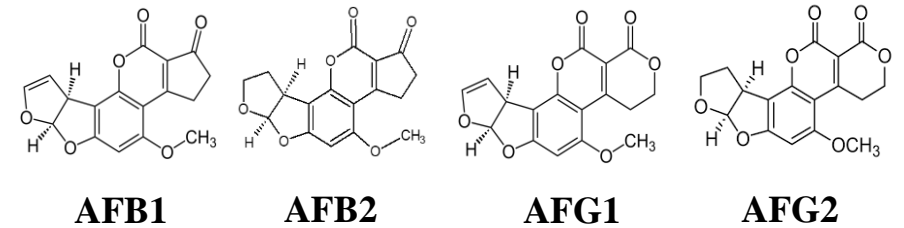
Degradation of aflatoxin in contaminated corn kernels subjected to atmospheric pressure cold plasma DBD working at 90 kV, discharge gap 44 mm
Shi et al (2017)



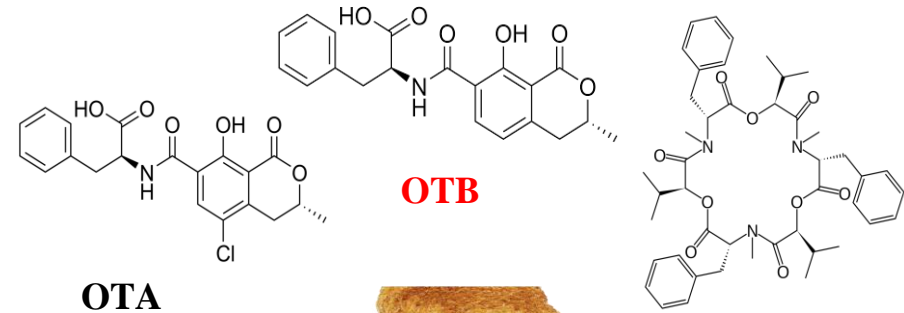
Degradation of mycotoxins in pistachio kernels



- Surface Dielectric Barrier Discharge (SDBD)
 - sinusoidal waveform,
 - peak voltage: 6 kV
 - fixed frequency: 23 kHz
- NO_x regime:** duty cycle equal to 100% (425.35 ± 25.79 W), **O₃ regime:** duty cycle equal to 10% (42.54 ± 2.58 W)



Exposure time: 15, 30, 60 min
Distance from source: 4 cm, 20 cm

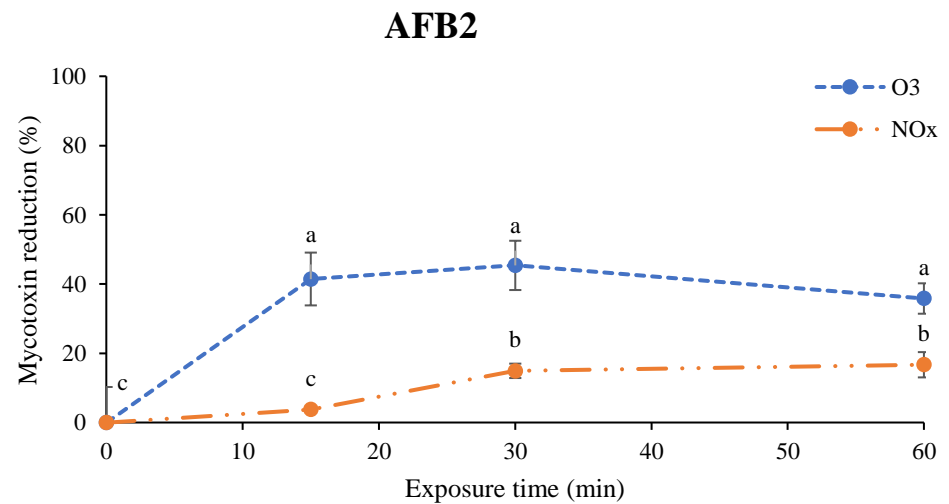
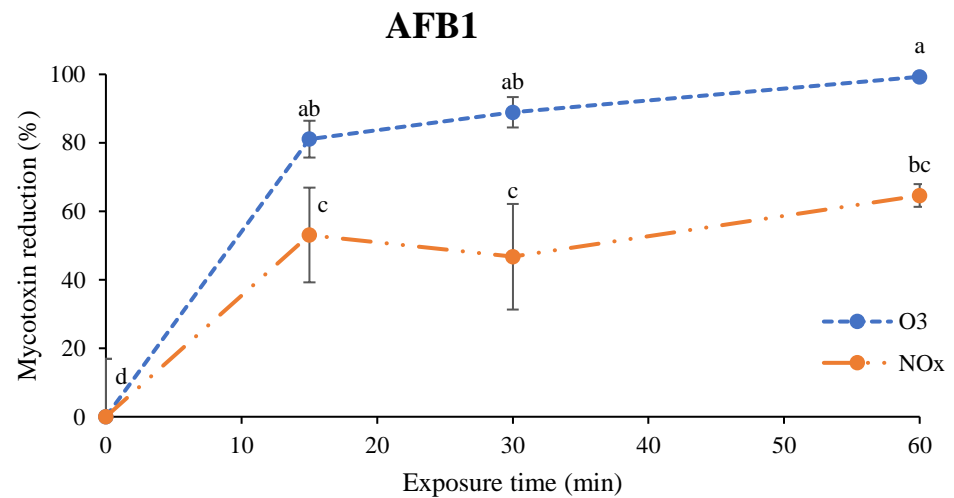
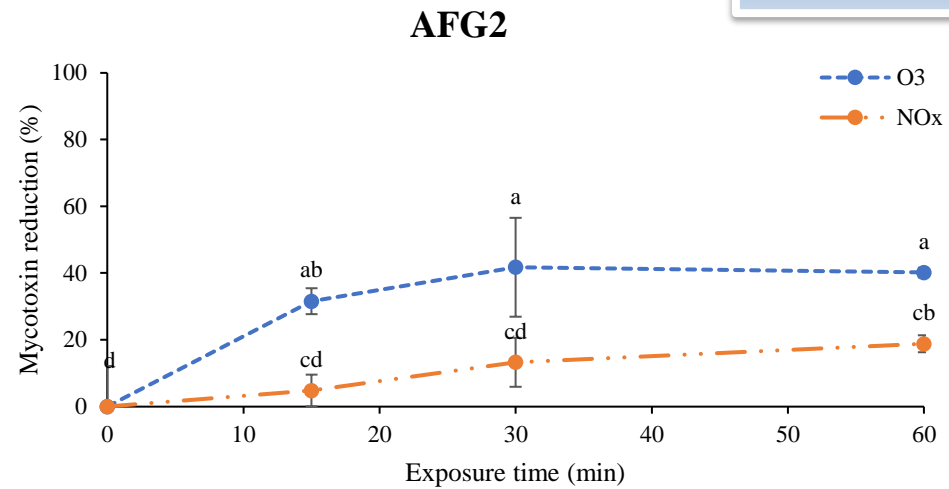
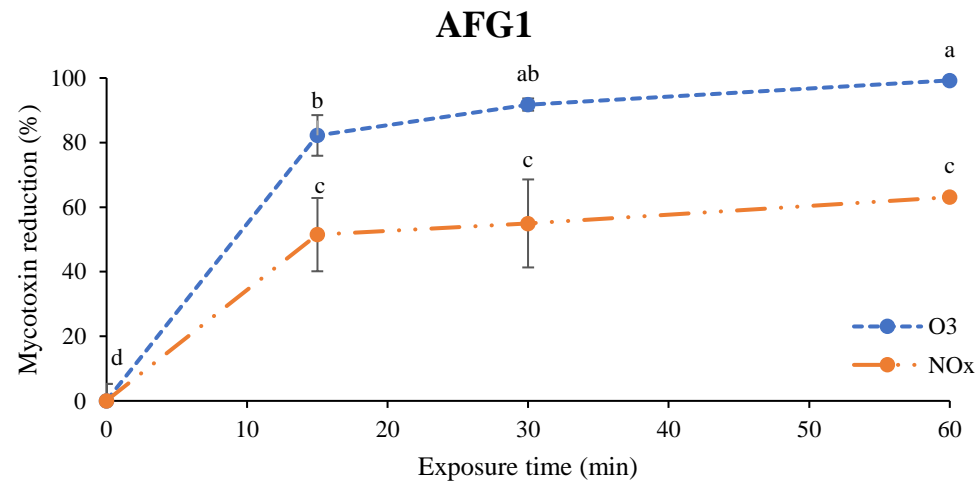


Laika, J., Viteritti, E., Molina-Hernandez, J. B., Sergi, M., Neri, L., Laurita, R., Tappi, S., Ricci, A. & Chaves-López, C. (2024).

Degradation of mycotoxins in pistachio kernels

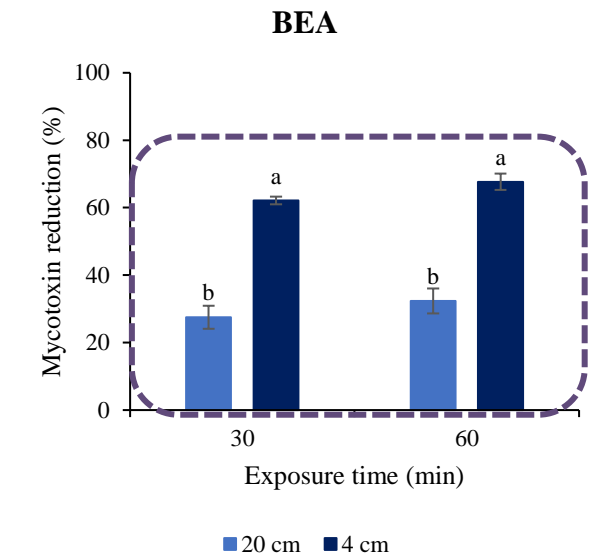
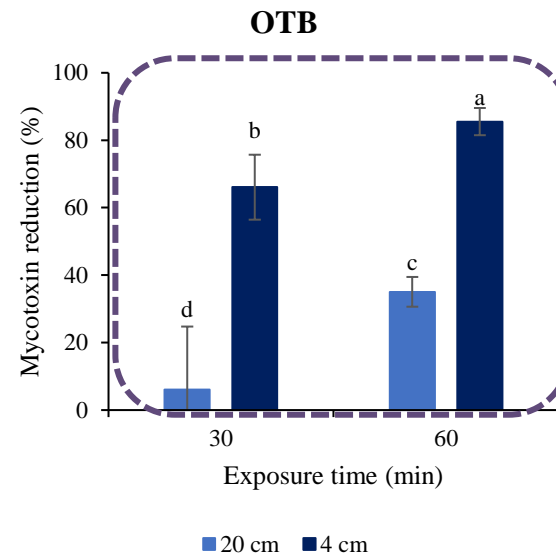
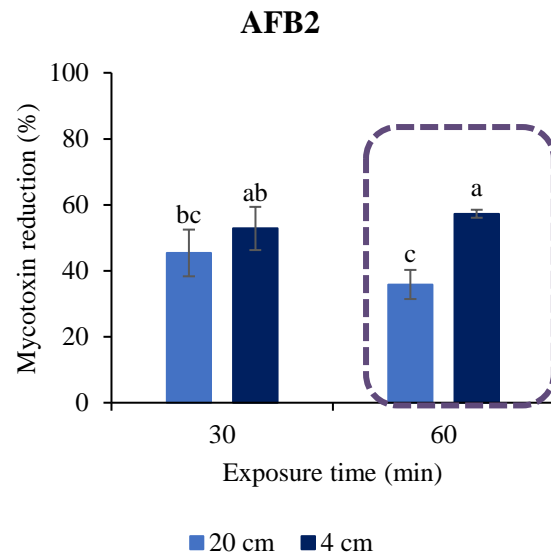
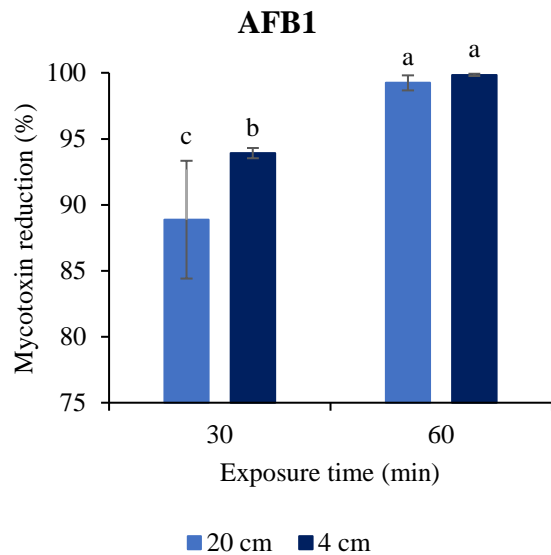
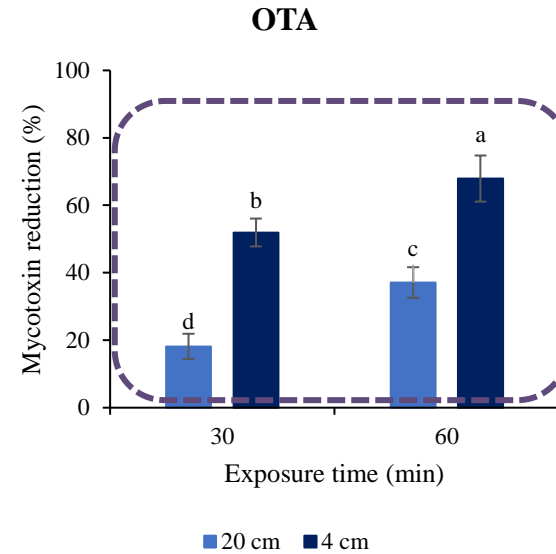
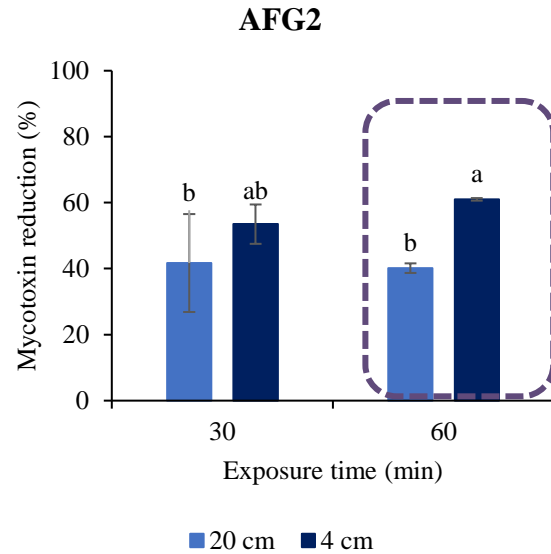
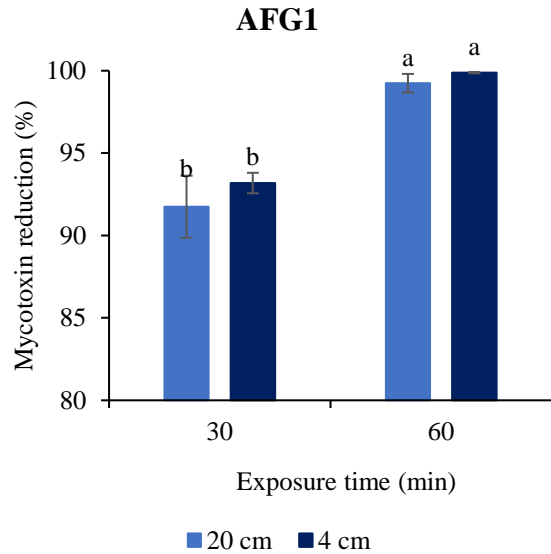
Effect of the plasma regime

O₃ – REGIME
MORE
EFFICIENT

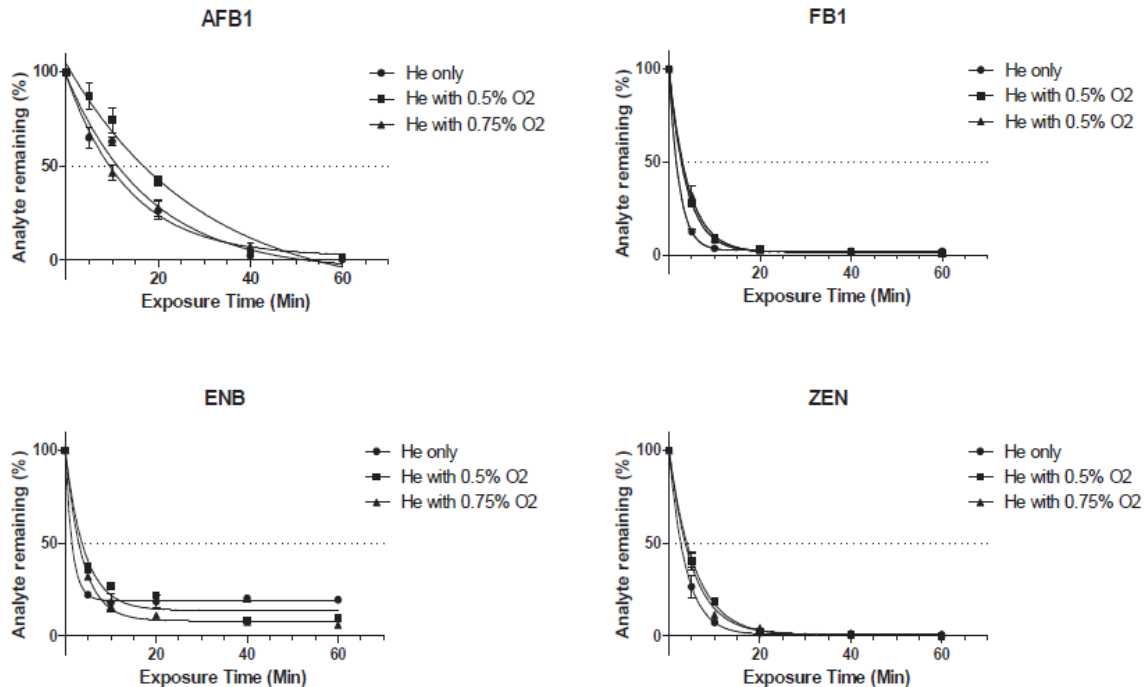


Degradation of mycotoxins in pistachio kernels

Effect of distance from source



Plasma treatments for the decontamination of mycotoxins



Degradation products
untargeted UPLC-HRMS analysis



Cytotoxicity
human hepatocarcinoma (HepG2)

Wielogorska et al. (2019).

Results

no increase in cellular toxicity in HepG2 cell line after CAPP treatment, possible increased toxicity should still be investigated in other cell assays or in vivo to fully confirm CAPP safety, especially in produce destined for human consumption.



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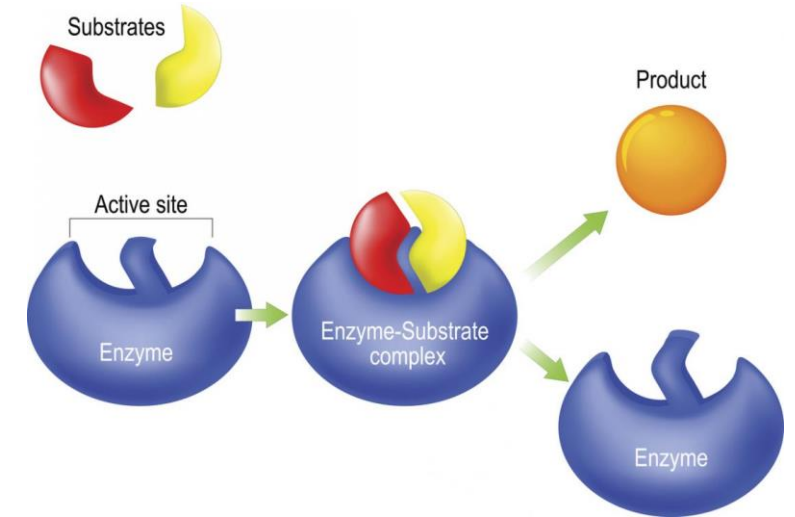


Enzymes

Enzymes are **protein** molecules that are present in all living things.

They speed up and target **chemical reactions**, in many cases increasing the rate of reaction millions of times.

For example, they aid digestion, metabolise and eliminate waste in humans and animals, and play a crucial role in muscle contraction.

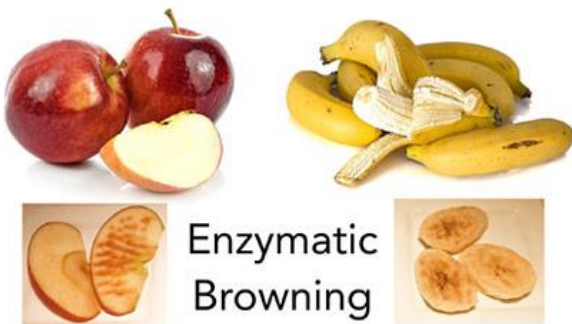


Enzymes in food

Enzymes can be **favourably exploited** by the food industry.

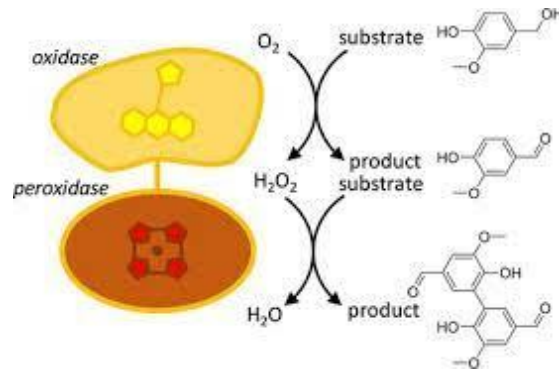
However, the **residual activity** of many enzymes is **detrimental to the quality** of foods, resulting in effects such as **browning**, **off-flavour** and **loss of vitamins**.

In certain cases, validation of processing conditions can only be achieved when the critical parameters of plasma processes assure inactivation of not only micro-organisms but also enzymes

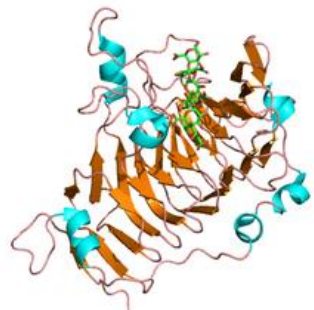


Examples of degradative enzymes

Polyphenol oxidase (PPO; also polyphenol oxidase i, chloroplastic), an enzyme involved in fruit browning, PPO catalyses the rapid polymerization of o-quinones to produce black, brown or red pigments (polyphenols).



Peroxidase is a common, heat stable enzyme that is found in plants and plant products, including fruits, vegetables and grains. There is a direct relationship between the peroxidase activity and the development of off-flavours, off-odours and off-colours in food

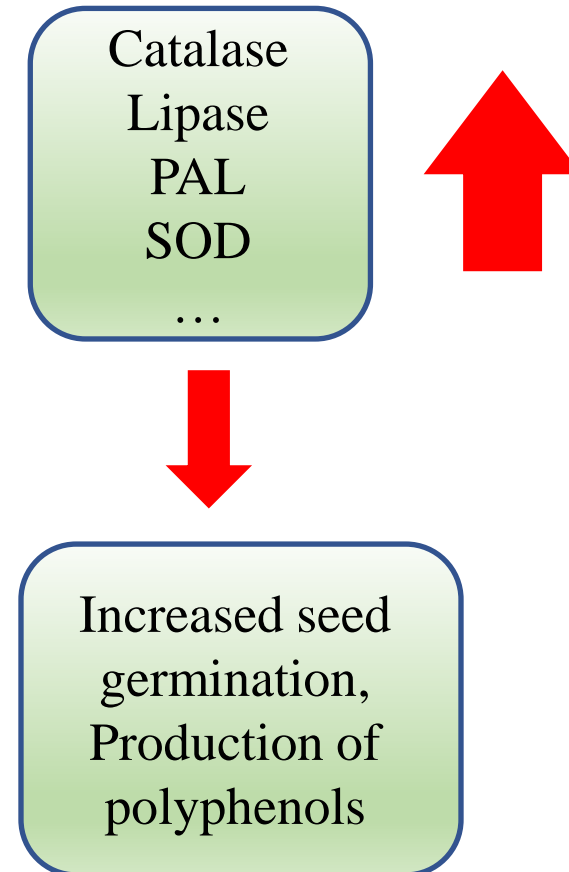
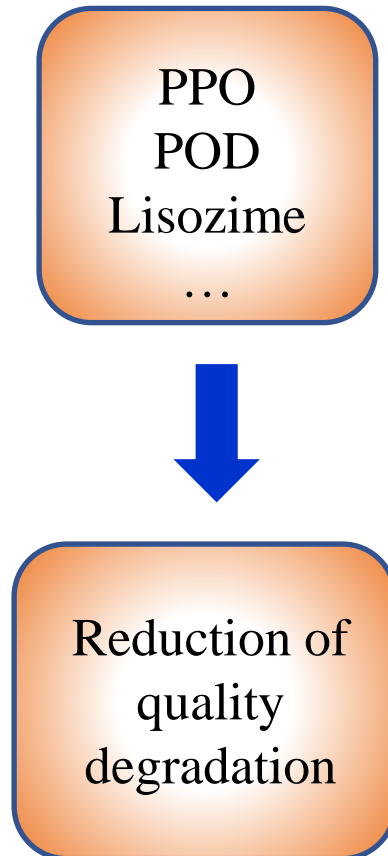
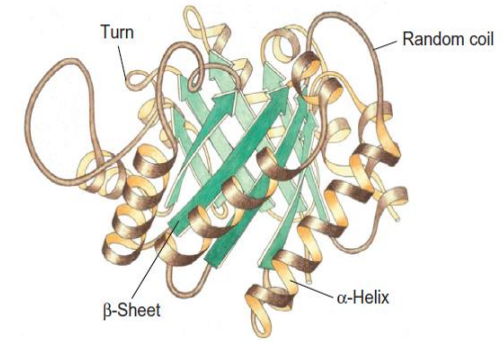


Pectinesterase is a ubiquitous cell-wall-associated enzyme that presents several isoforms that facilitate plant cell wall modification and subsequent breakdown.



Effect on enzymatic activity

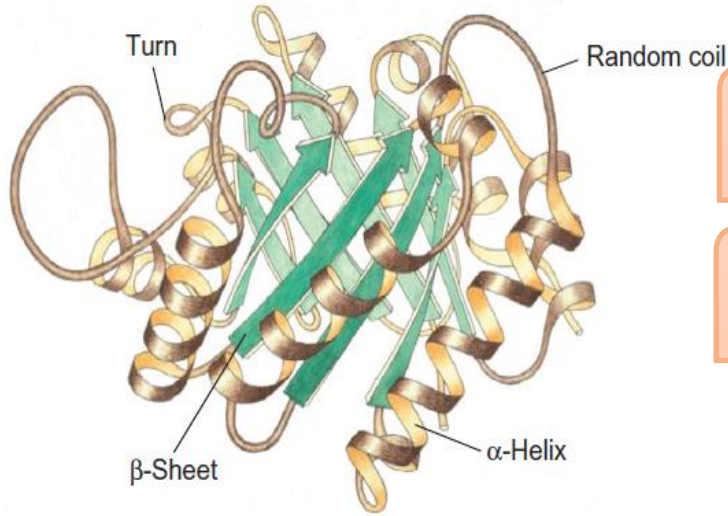
**PLASMA
TREATMENT
ROS, RNS**



This contrary effect can thus be utilized to achieve different purposes during food processing.



ROS, RNS



OXIDATION OF LATERAL AMINO ACIDS

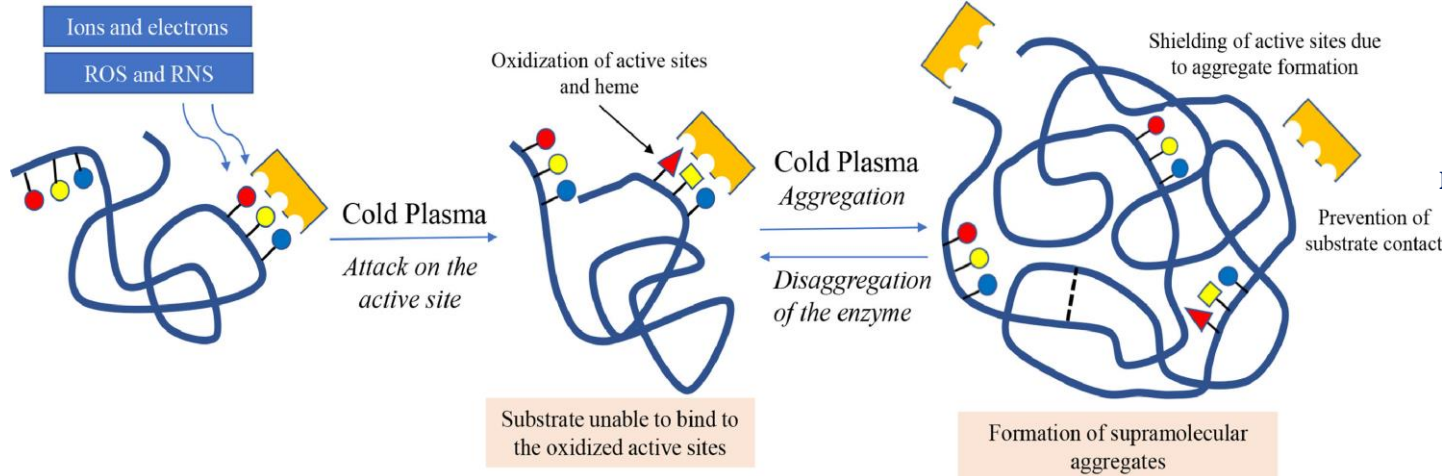
CHANGES OR LOSS OF THE TERTIARY STRUCTURE

Hypothesis on the enzymatic inactivation mechanism

(Misra et al., 2016)

hydroxyl radicals (OH), superoxide anionic radicals (O₂⁻), hydroperoxy radicals (HOO) and nitric oxide (NO) → chemical modifications of the most reactive amino acids in the side chains of the protein (cysteine, aromatic rings of phenylalanine, tyrosine and tryptophan) → alteration of the structure → loss of enzymatic activity

(Takai et al., 2012)



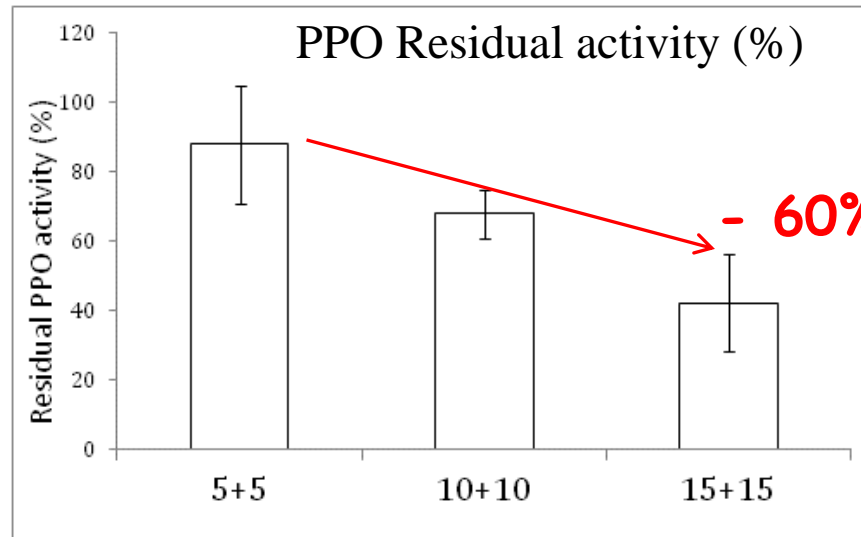
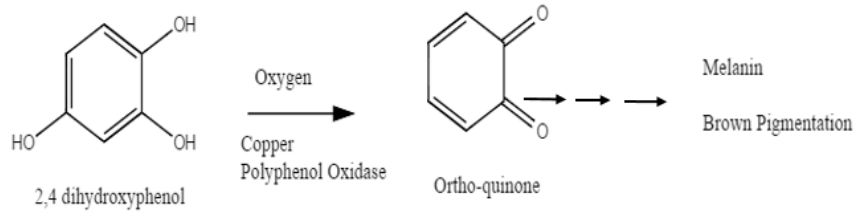
decomposition of C-H, C-N and N-H bonds in proteins resulting from oxidation by reactive oxygen-containing species

→ loss of structure β

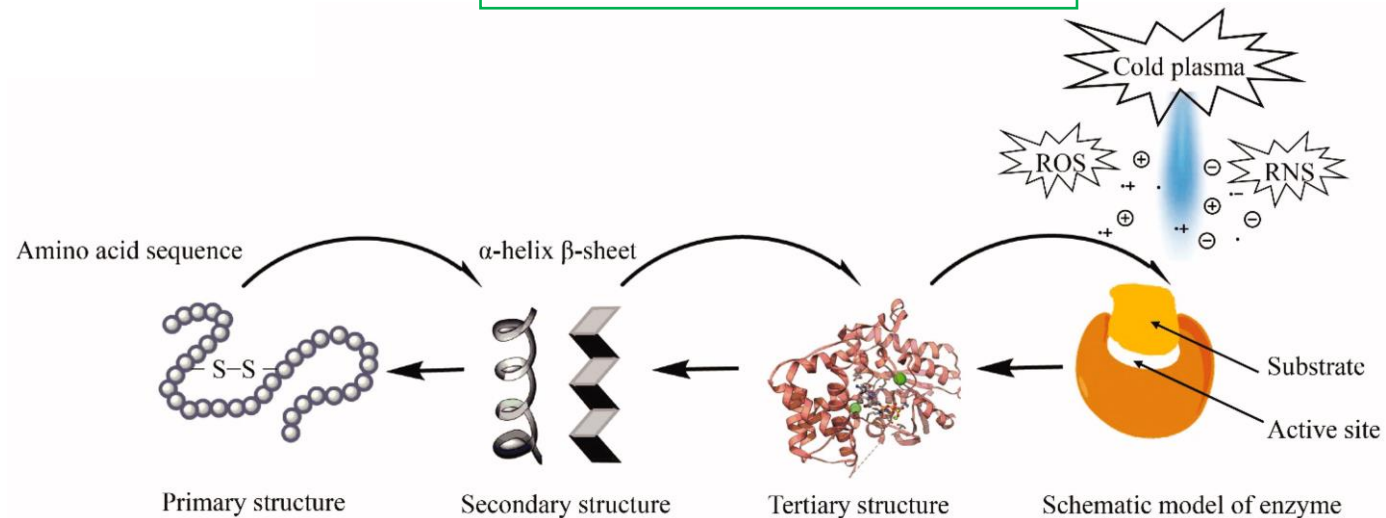
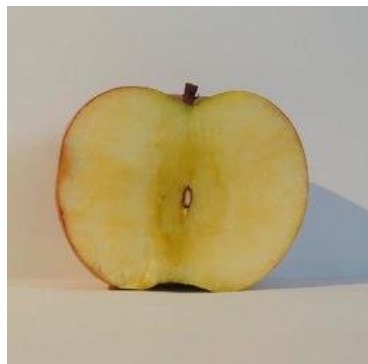
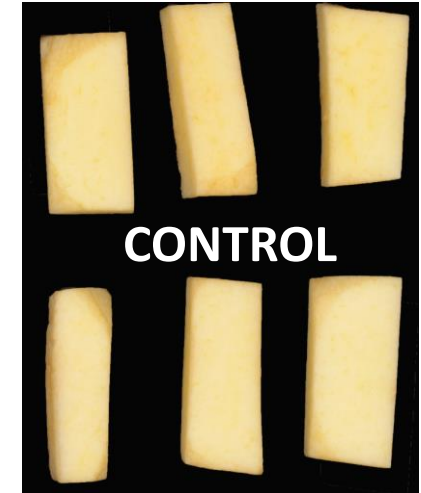
(Misra et al. 2016)



Inhibition of enzymatic browning



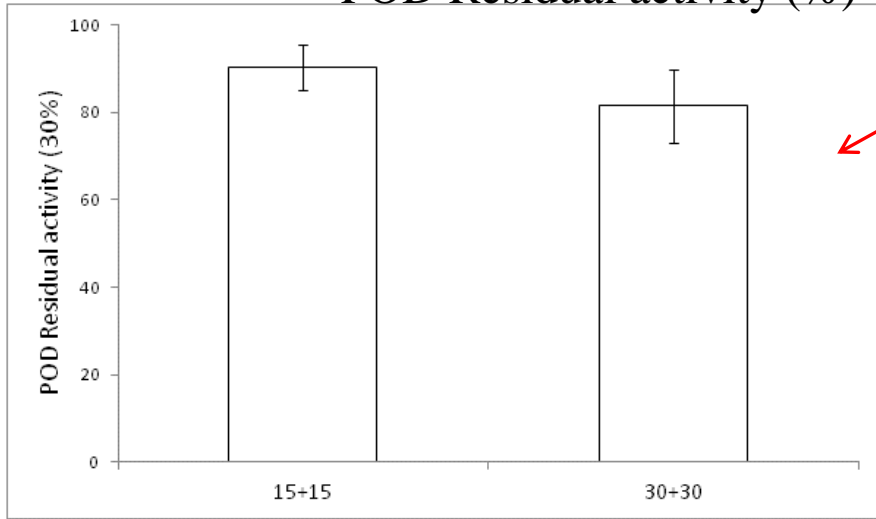
Tappi et al., 2014



From Han, Y., Cheng, J. H., & Sun, D. W. (2019)

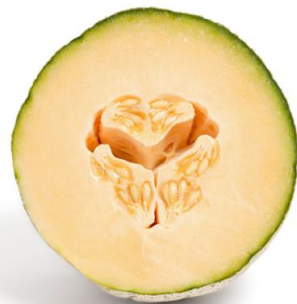
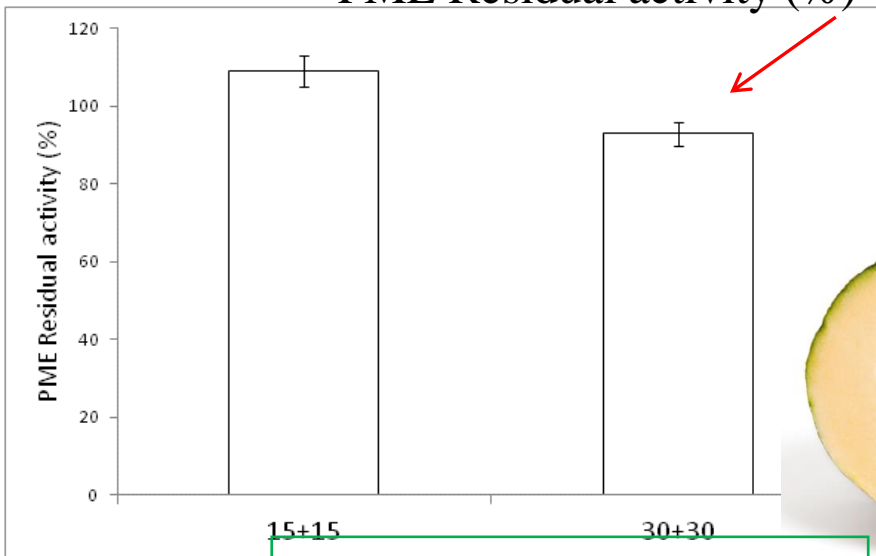
Inhibition of enzymatic activity in fruit

POD Residual activity (%)



- 17,4%

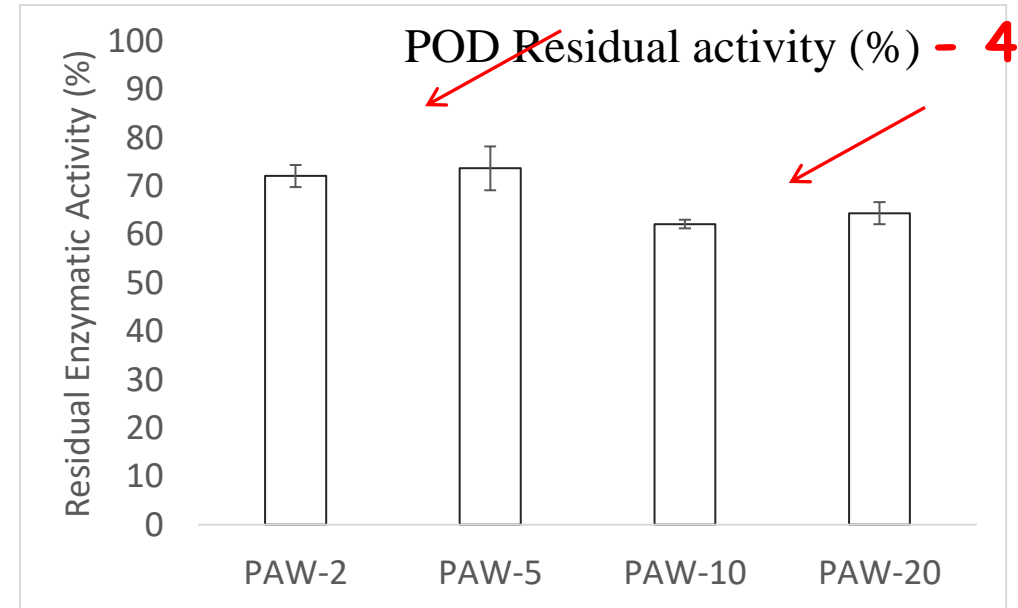
PME Residual activity (%) - 6%



Tappi et al., 2016

- 30%

POD Residual activity (%) - 40%



High variability depending on type of enzyme and on the matrix!!



Role of the matrix on enzymatic inactivation

Laika, J., Sacchetti, G., Sabatucci, A.,
Molina-Hernandez, J. B., Ricci, A., Laurita,
R., ... & Neri, L. (2023).

effect of different CAP exposures on the activity of **horseradish peroxidase (HRP)**

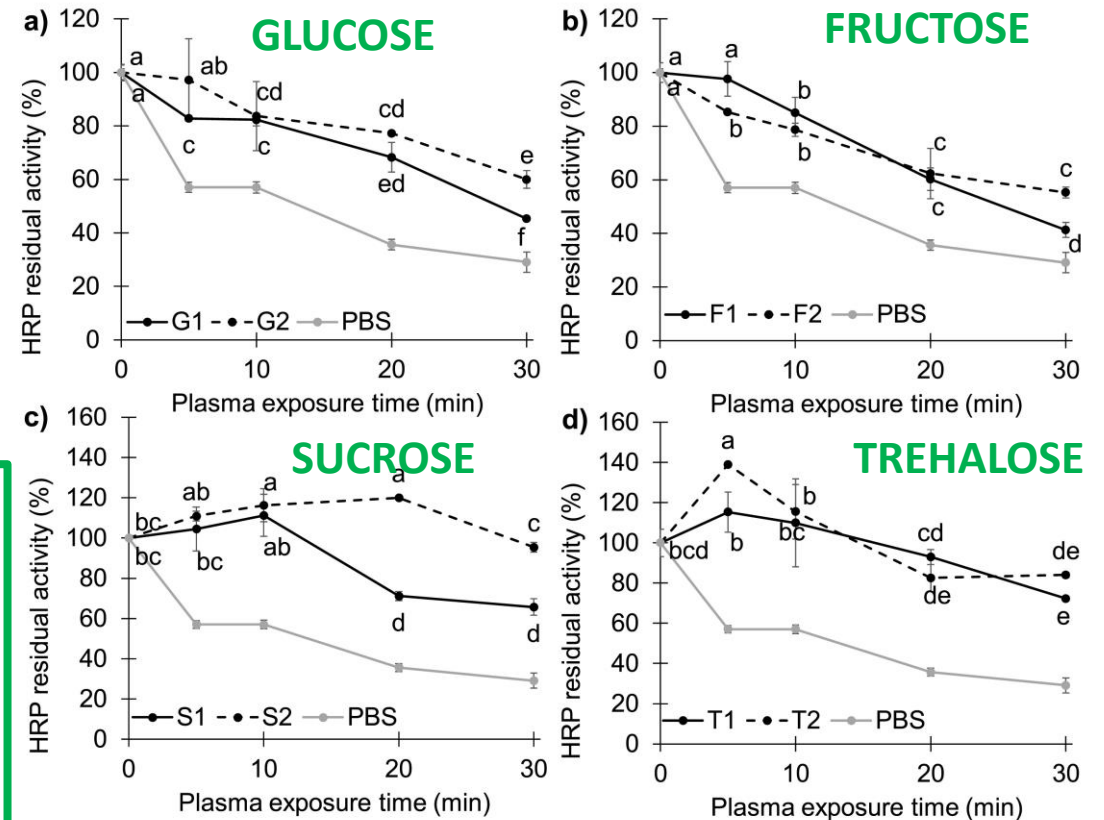
- phosphate buffer and in model systems
- with different concentrations of monosaccharides and disaccharides

mono and disaccharides: naturally found in fruits and vegetables and widely employed in food and beverage industries

diverse protective effects of sugars on HRP

- ability of sugars, and especially of disaccharides, to scavenge free radicals with a concentration-dependent effect
- prevent denaturation phenomena through the suppression of perturbation of the hydration structure of the protein

HRP inactivation kinetic in PBS system and sugar model systems.



Enhancement of enzymatic activity

Reactive species (ROS and/or RNS) can be generated and interact with the surfaces of foods. In this condition, a **physiological response** occurs.

A **complex antioxidant enzymatic system** converts these reactive species into less harmful compounds for vegetable cells.

significant increase in the activity and genes expression of

- ascorbate peroxidase,
- Catalase (CAT) , and
- superoxide dismutase (SOD)
- phenylalanine ammoniumlyase (PAL)



**activation of the physiological
antioxidant system**

during storage of different fruits treated with plasma



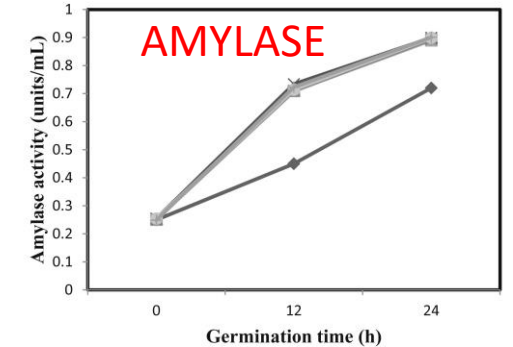
Increased seed germination

Cold atmospheric plasma treatment for 1 min showed a strongly improved chickpea seed germination (89.2%) and speed of germination (Mitra et al., 2014)

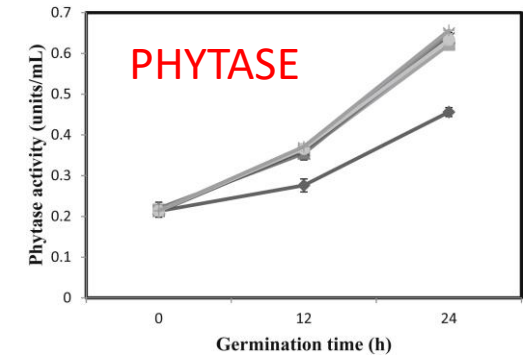
Cold plasma enhance the seed germination and seedling growth (Bormashenko et al., 2012, Sera et al., 2008, Zhou et al., 2011)

Plasma enhanced the seed germination and seedling growth rates which might be associated with the increased water uptake of seeds (Ling et al., 2014)

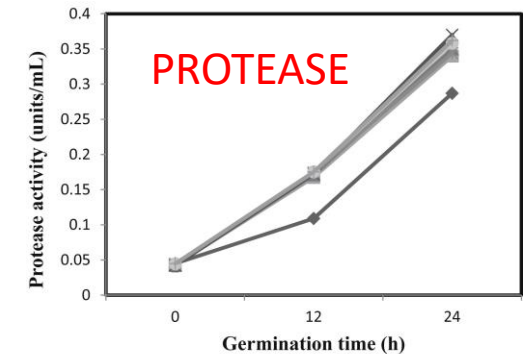
The plasma treatment can increase the roughness of the seed surface, and finally lead to increase in the hydrophilicity of the seed by changing the chemical structure, which may account for the increase in water uptake of seed (Bormashenko et al., 2012)



(A)



(B)



(C)

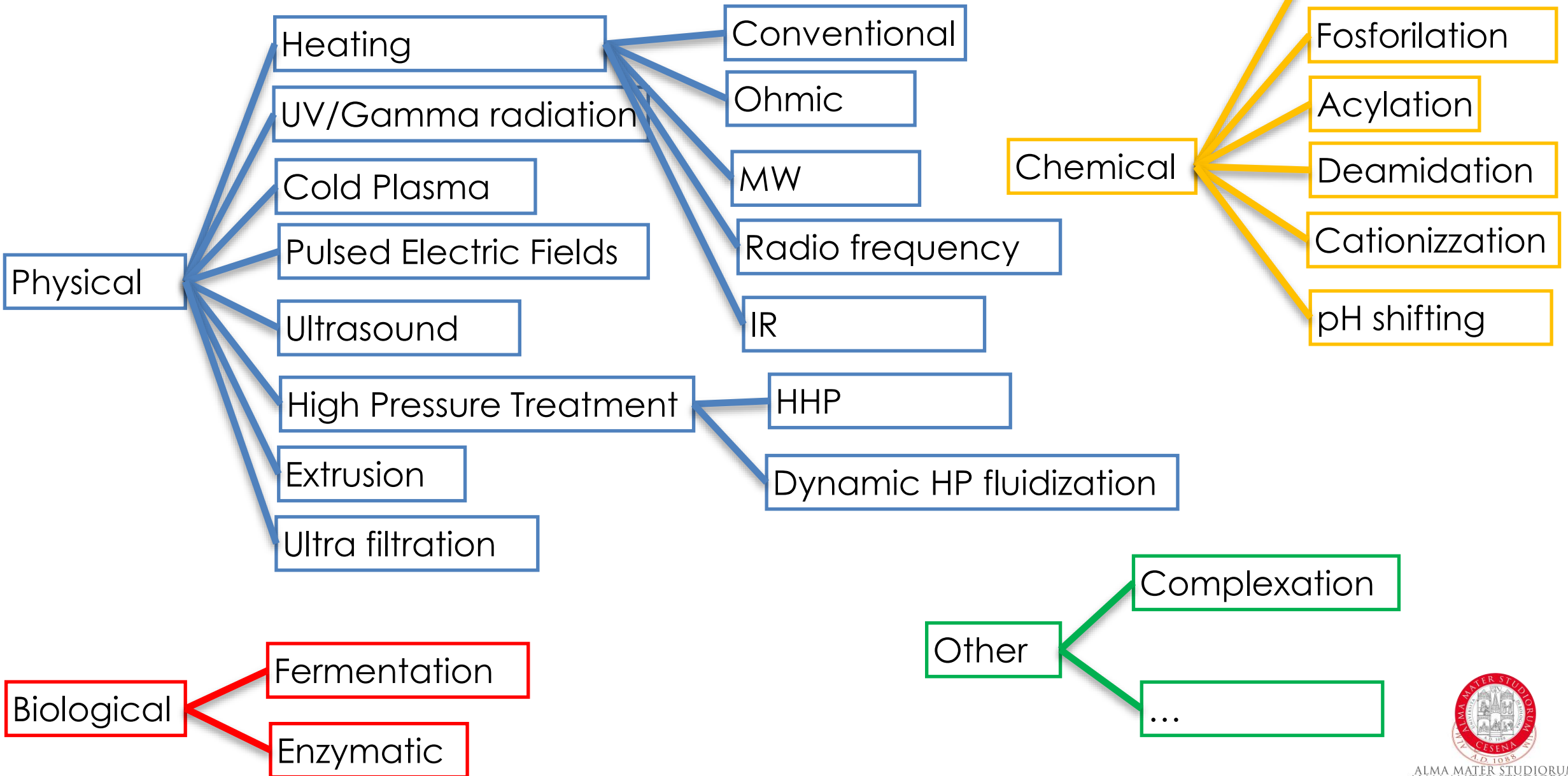
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Approaches for ingredients modification



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Effect of cold plasma on food quality

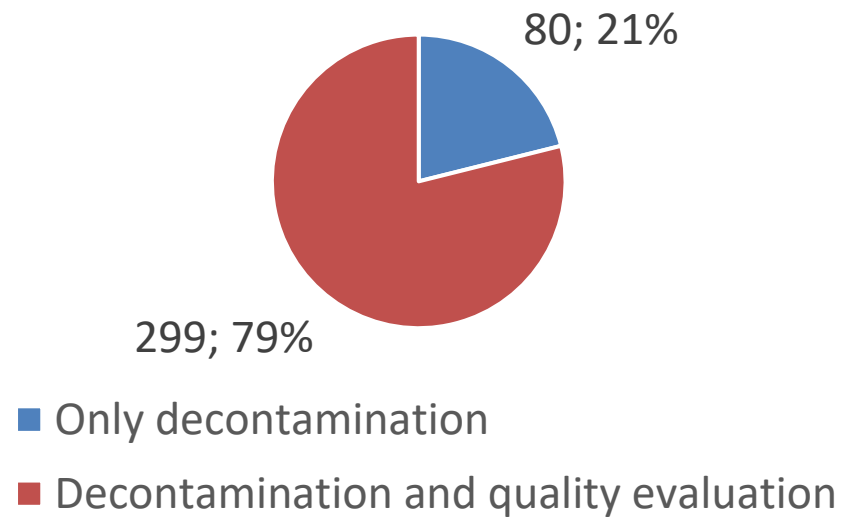
Food Safety
vs
Food Quality



Quality of foods:

- Organoleptic properties
- Nutritional value
- Techno-functional properties
- Physico-chemical properties

Presence of quality analysis of plasma treated foods



CA19110
Plasma applications
for smart and
sustainable agriculture



Virtual Mobility Grant
**Plasma effect on safety and
quality of foods**
08/08/2023 to 20/10/2023

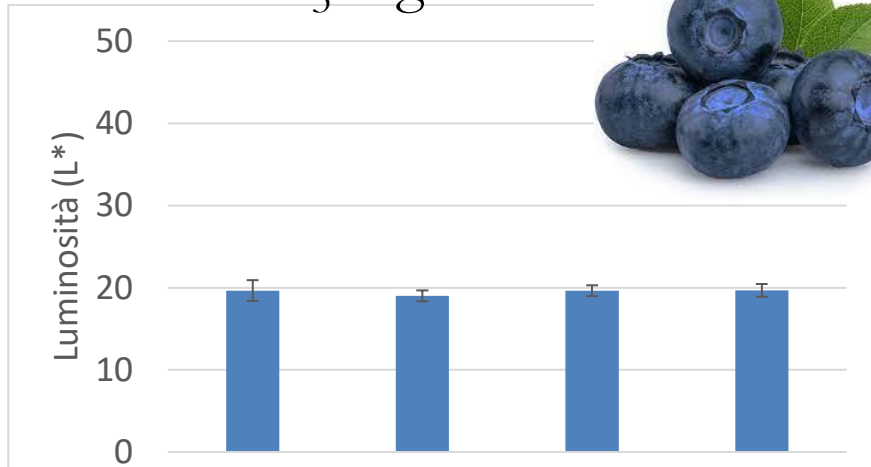


Effect of plasma on colour of food products

PAW immersion

PASS prototype

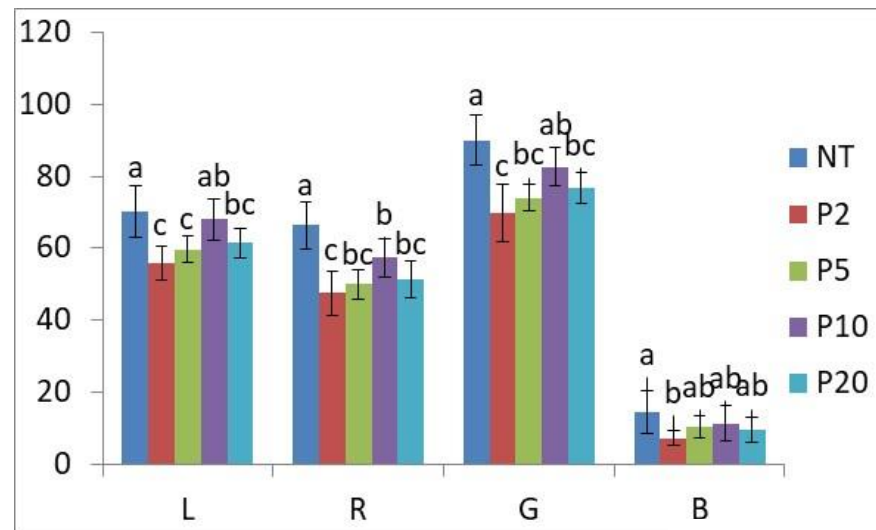
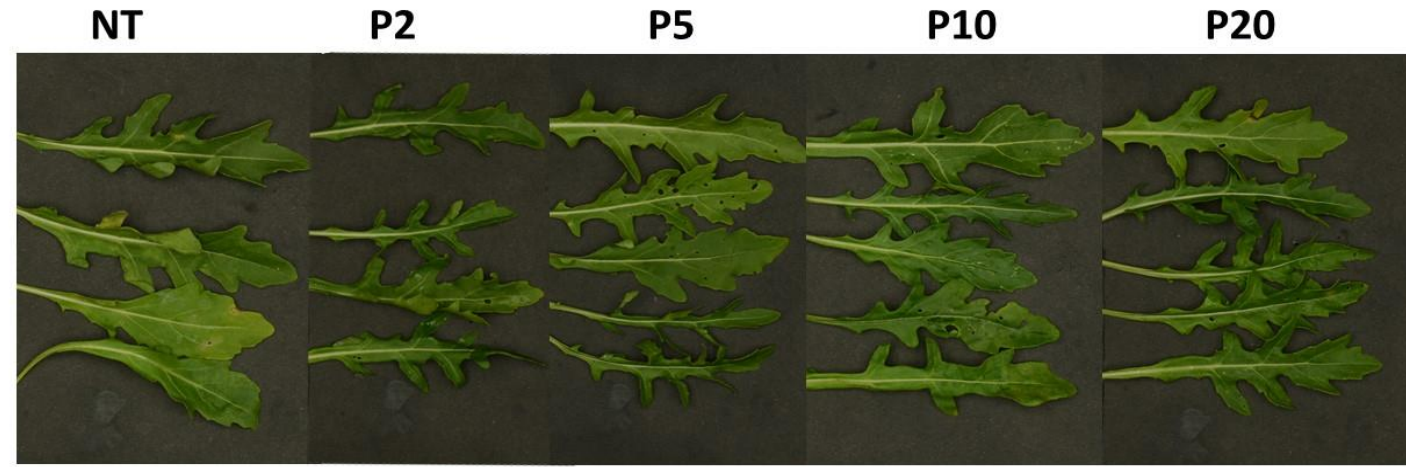
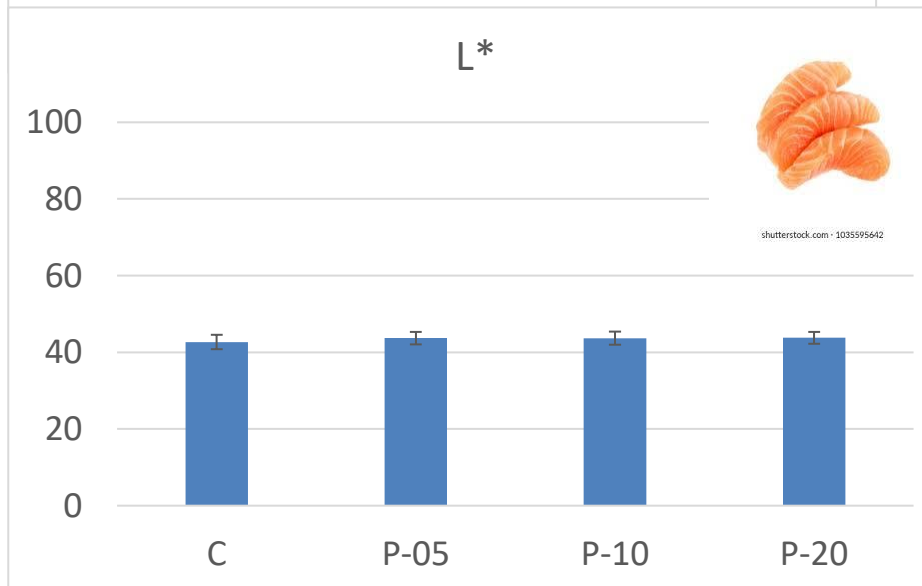
O₃ regime



L*



shutterstock.com • 1035595642



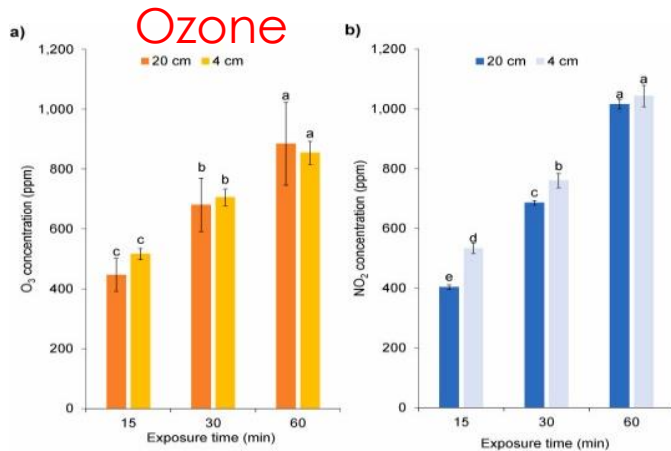
Significant changes
in colour
coordinates

Perceptible by
human senses?

Laurita et al., 2021



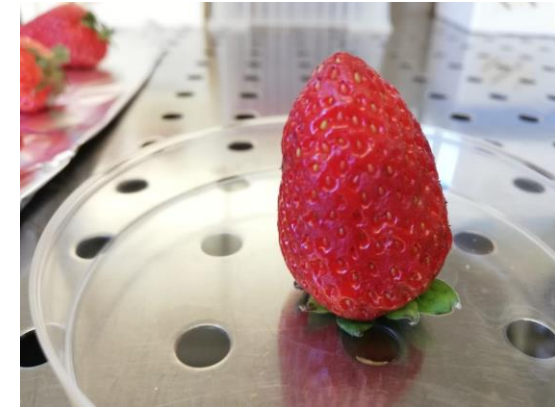
Effect of plasma on visual quality of food products



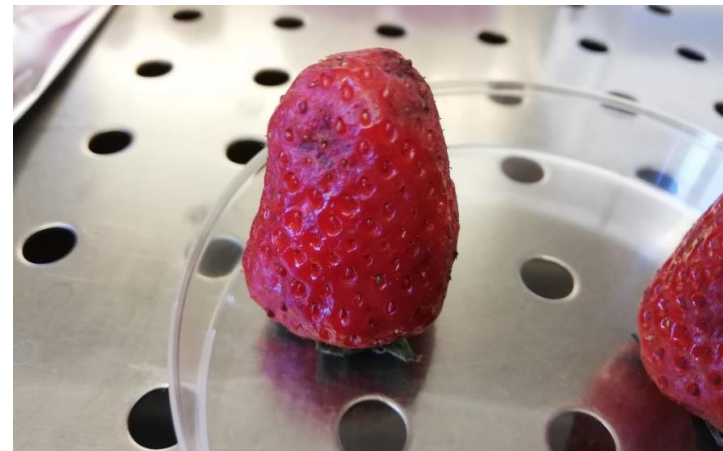
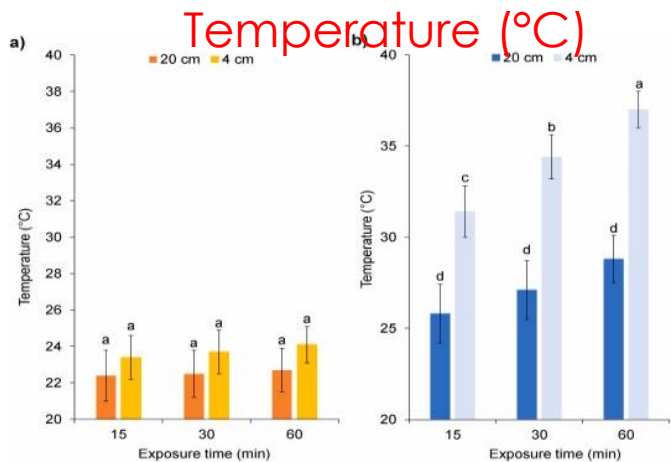
- Surface Dielectric Barrier Discharge (SDBD)
- sinusoidal waveform,
- peak voltage: 6 kV
- fixed frequency: 23 kHz

NO_x regime: duty cycle equal to 100% (425.35 ± 25.79 W), **O₃ regime:** duty cycle equal to 10% (42.54 ± 2.58 W)

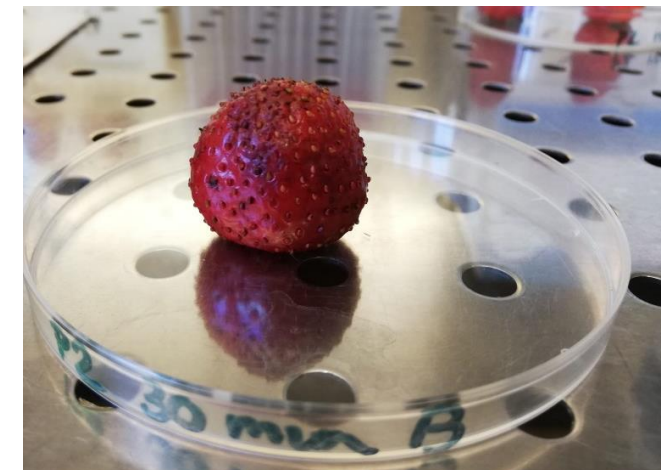
Distance from source: **20 cm**



Untreated sample



60 min of O₃ treatment



60 min of Nox treatment



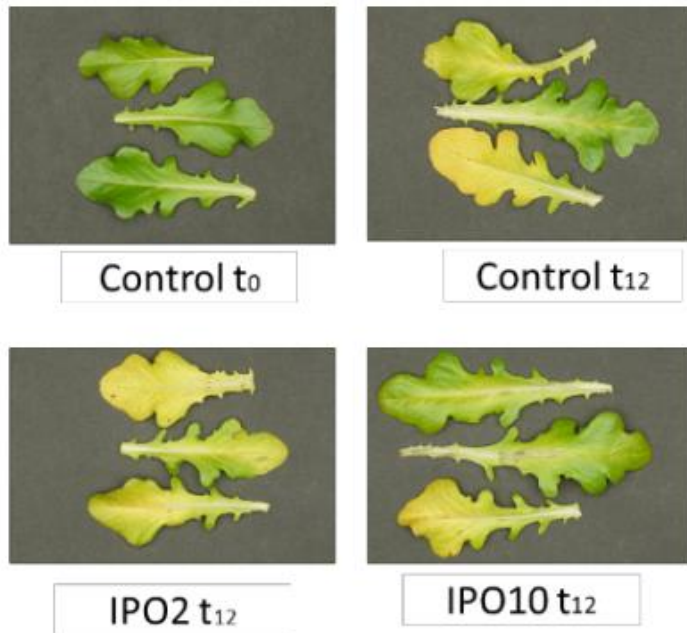
Effect on visual quality of baby leaves treated with PAW during storage

Appearances of salads treated and untreated during storage period
(4 °C until day 4, and 15 °C until day 12)

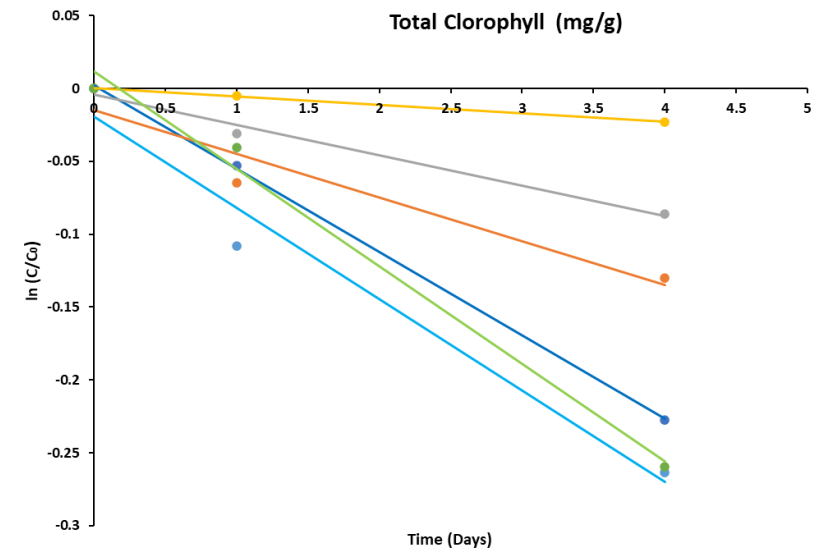


This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101000852

(Unpublished data)



P2, and P10 had less effect in visual quality compared to NaOCL after 12 days



Total Chlorophyll degradation

Treatment	k/day ⁻¹	R ²
Control	0.051	0.9997
IPO2	0.9234	0.9234
IPO10	0.0208	0.9861
P2	0.0057	0.9999
P10	0.0626	0.9904
P20	0.0668	0.9691

Green color degradation

Treatment	k/day ⁻¹	R ²
Control	0.0026	0.994
IPO2	0.0046	0.9715
IPO10	0.0053	0.999
P2	0.0019	0.9994
P10	0.0046	0.9715
P20	0.0033	0.9906

Bioactive compounds in foods



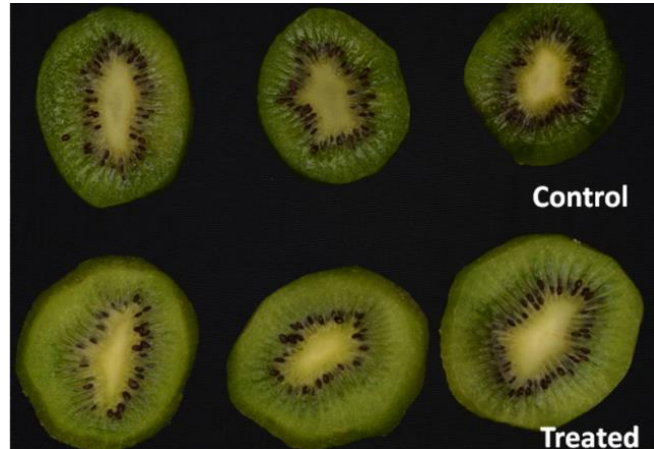
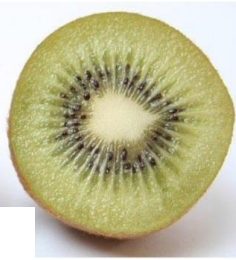
Challenge: improve products shelf life
preserving of bioactive compounds

Many compounds are sensitive to oxidation

TREATMENT OPTIMIZATION

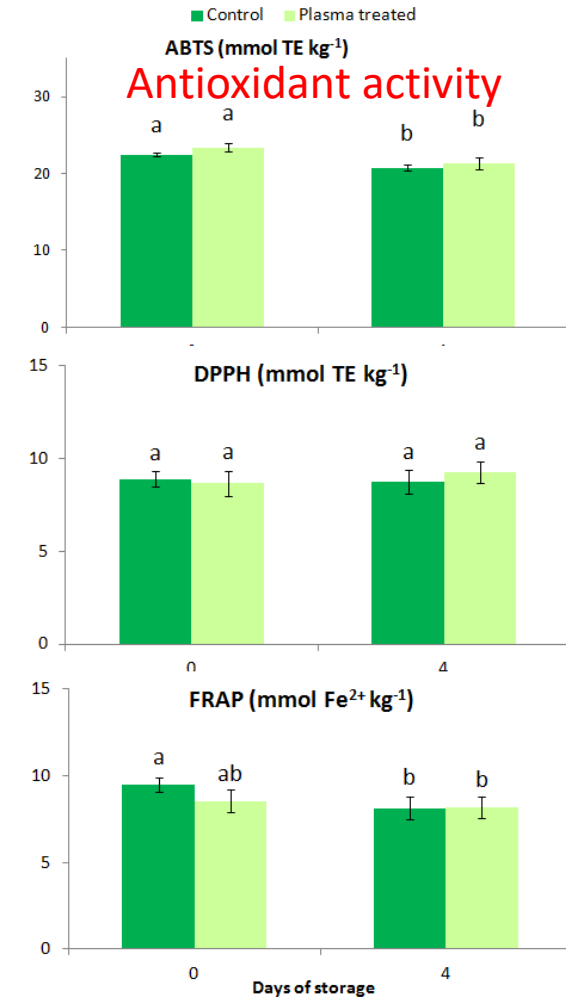
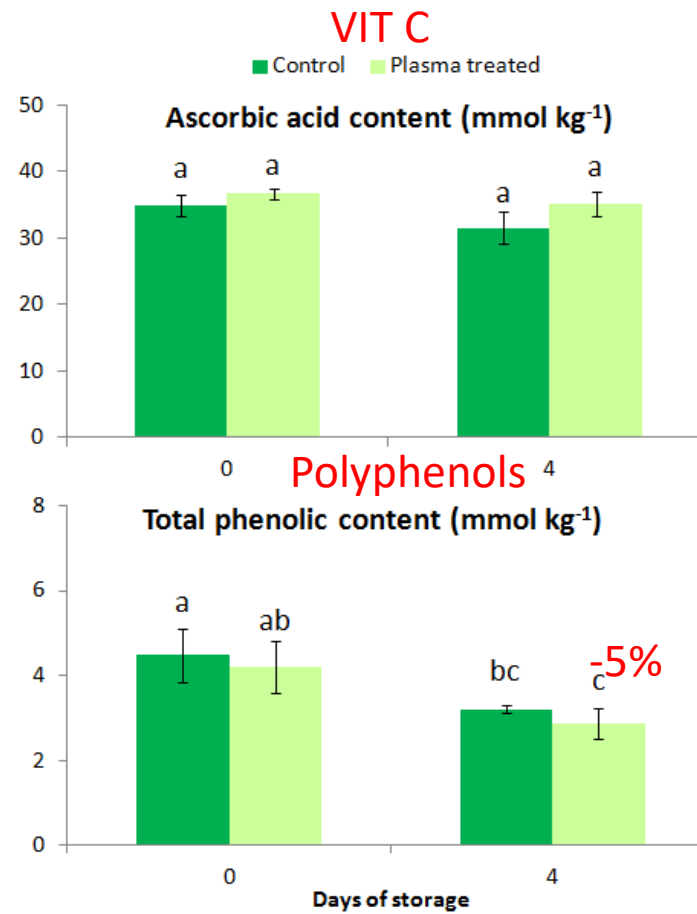


Bioactive compounds in kiwifruit treated with DBD plasma

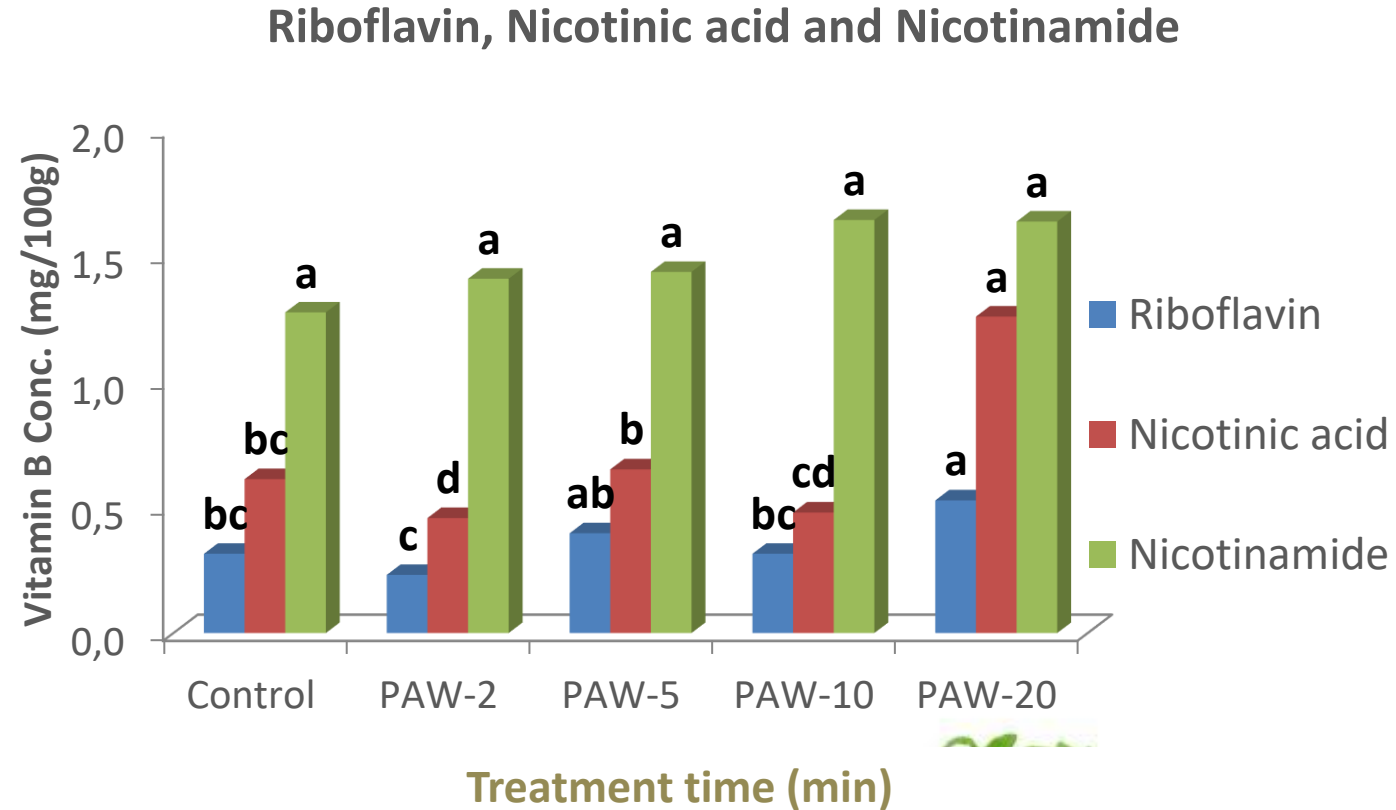
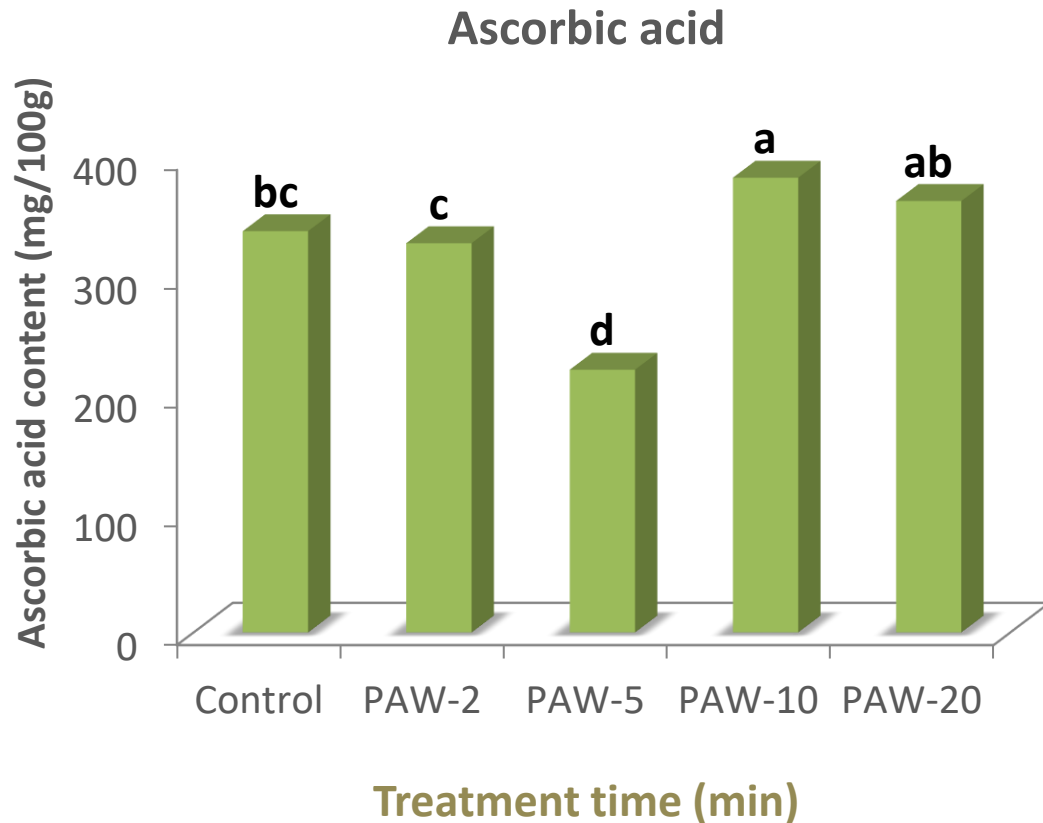


Kiwifruit slices subjected to 20 + 20 min DBD gas plasma treatment and control ones after 4 days of storage in controlled conditions.

Ramazzina et al., 2015



Bioactive compounds in rocket treated with PAW

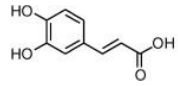


Laurita, R., Gozzi, G., Tappi, S., Capelli, F., Bisag, A., Laghi, G., ... & Vannini, L. (2021).

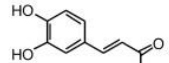


Polyphenols in lamb's lettuce

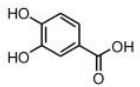
Grzegorzewski, et al. (2010)



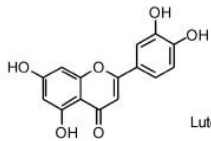
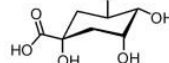
Caffeic acid



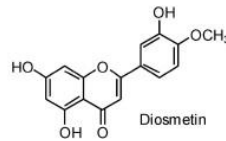
Chlorogenic acid



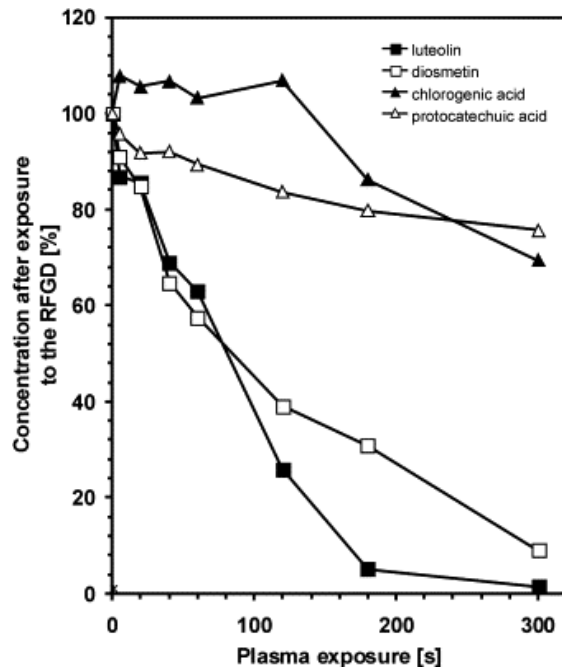
Protocatechuic acid



Luteolin

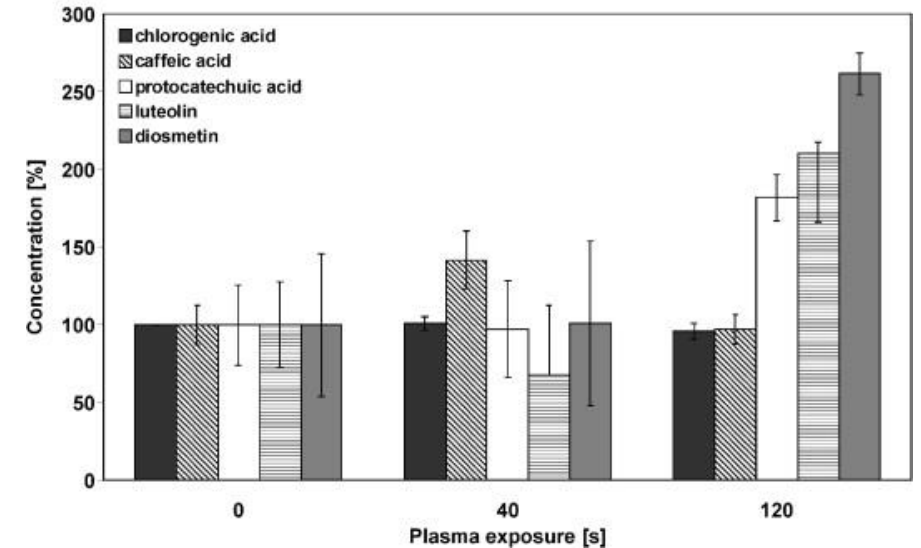
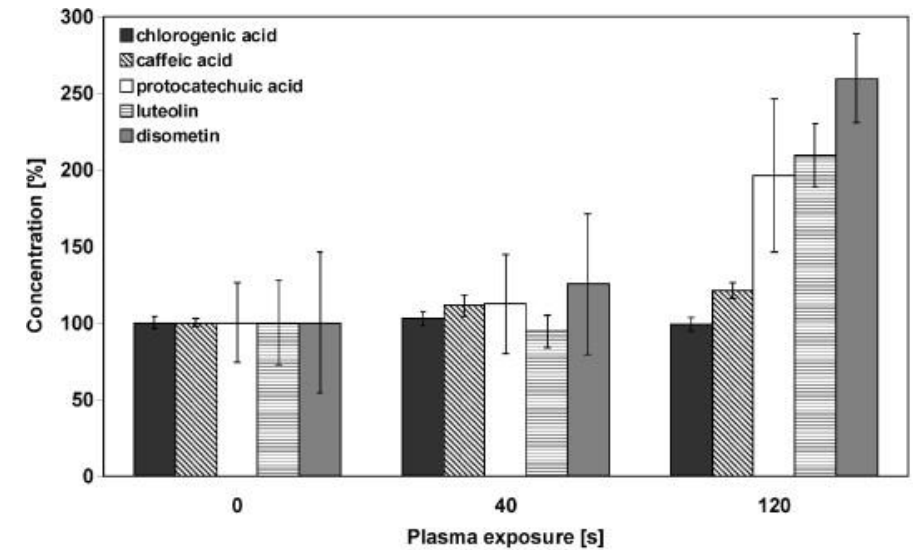


Diosmetin



Degradation of **pure compounds** upon exposure to a RFGD plasma (75 W, $p(O_2) = 0.5$ mbar).

**MATRIX EFFECT!
PRODUCTION OF NEW
POLYPHENOLS/HIGHER
EXTRACTION?**

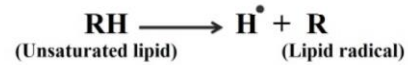


Contents of phenolic acids and flavonoids in freeze-dried **lettuce leaves** after exposure to the RFGD plasma ($p(O_2) = 0.5$ mbar) at 75 W (top), at 150 W (bottom)

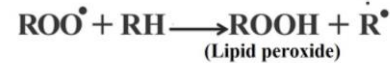
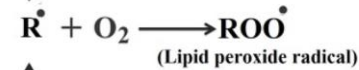
Effect on Lipid oxidation



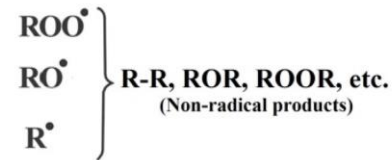
Initiation



Propagation

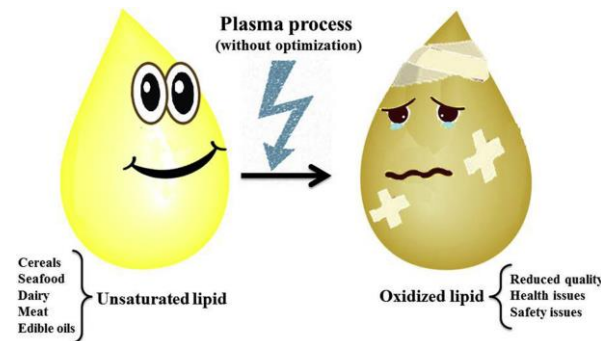


Termination

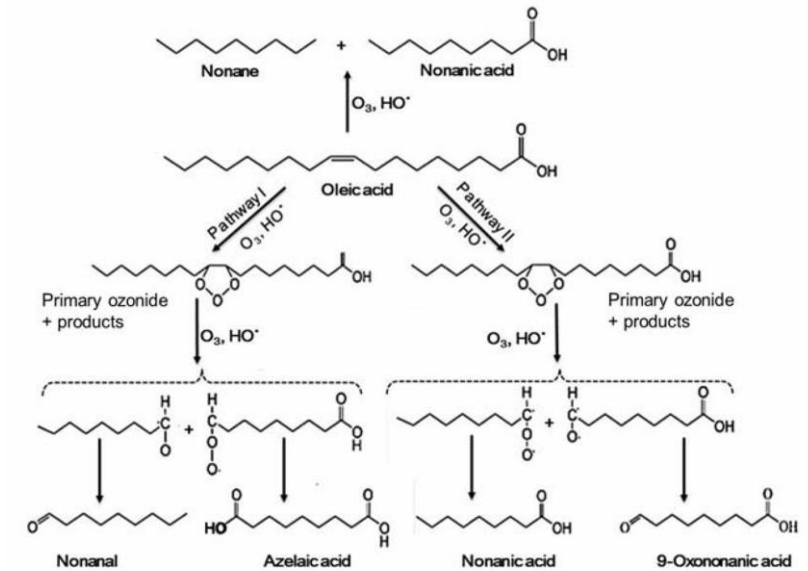


Effects of lipid oxidation in foods:

- Modification of colour
- Development of off-odours (rancid)
- Decrease in nutritional value
- Formation of toxic compounds



COLD PLASMA IS COMPOSED BY HIGHLY OXIDATIVE SPECIES → INDUCTION OF LIPID OXIDATION IS EXPECTED

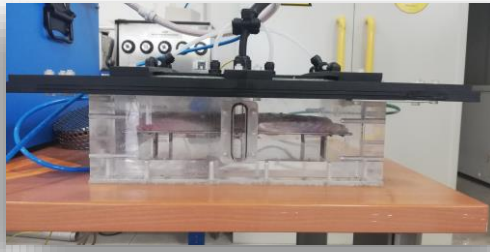


The mechanisms of plasma induced oxidation for oleic acid (Sarangapani et al., 2017).



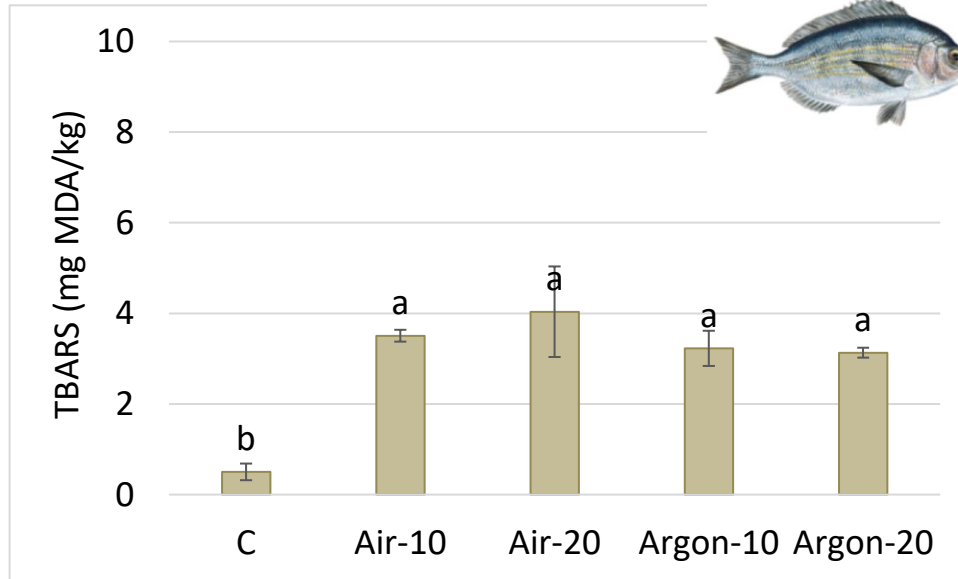
Effect on lipid oxidation in food matrices

Seabreams from Kefalonia



Current 20mA
Voltage 18 kV
Frequency 5 kHz
Time 20 min
 2 Atmospheres:
 Air: 20% O₂/80% N₂
 Argon: 20% O₂/80% Argon

Sea bream

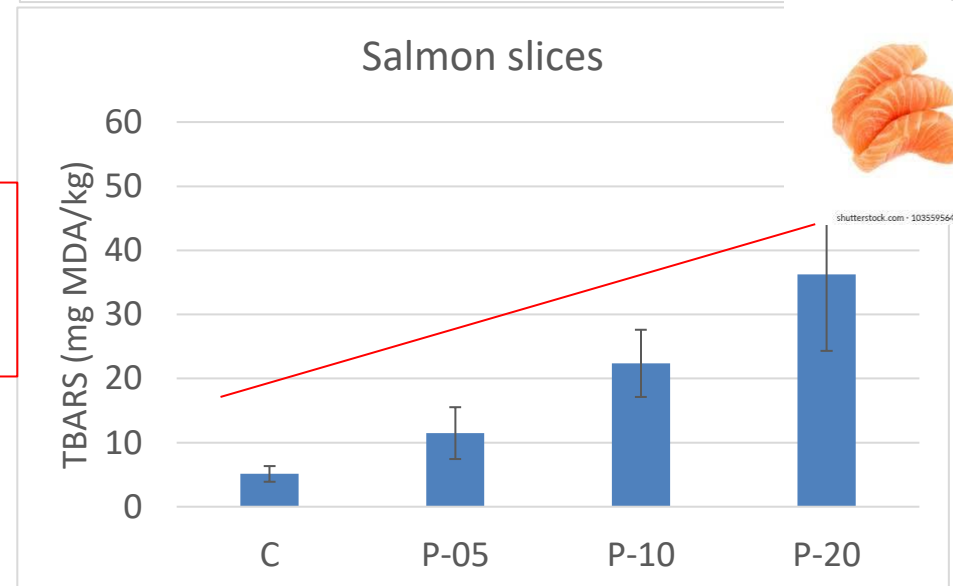


Increase of lipid oxidation in PUFA rich matrix

Cheese slices

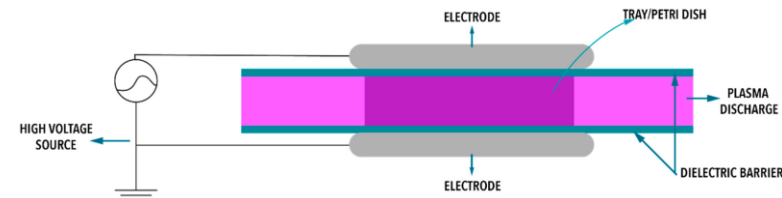
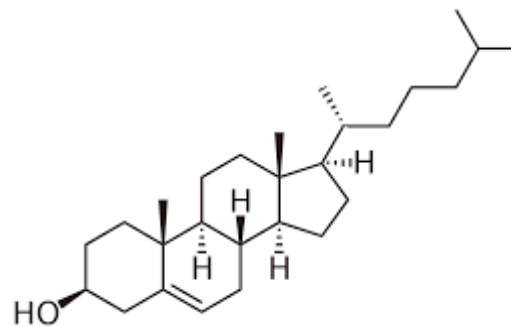
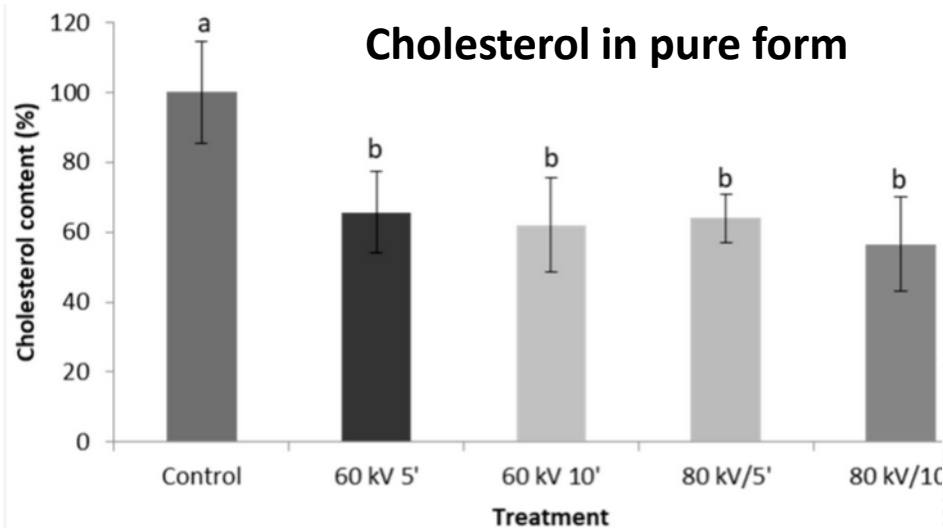


Salmon slices



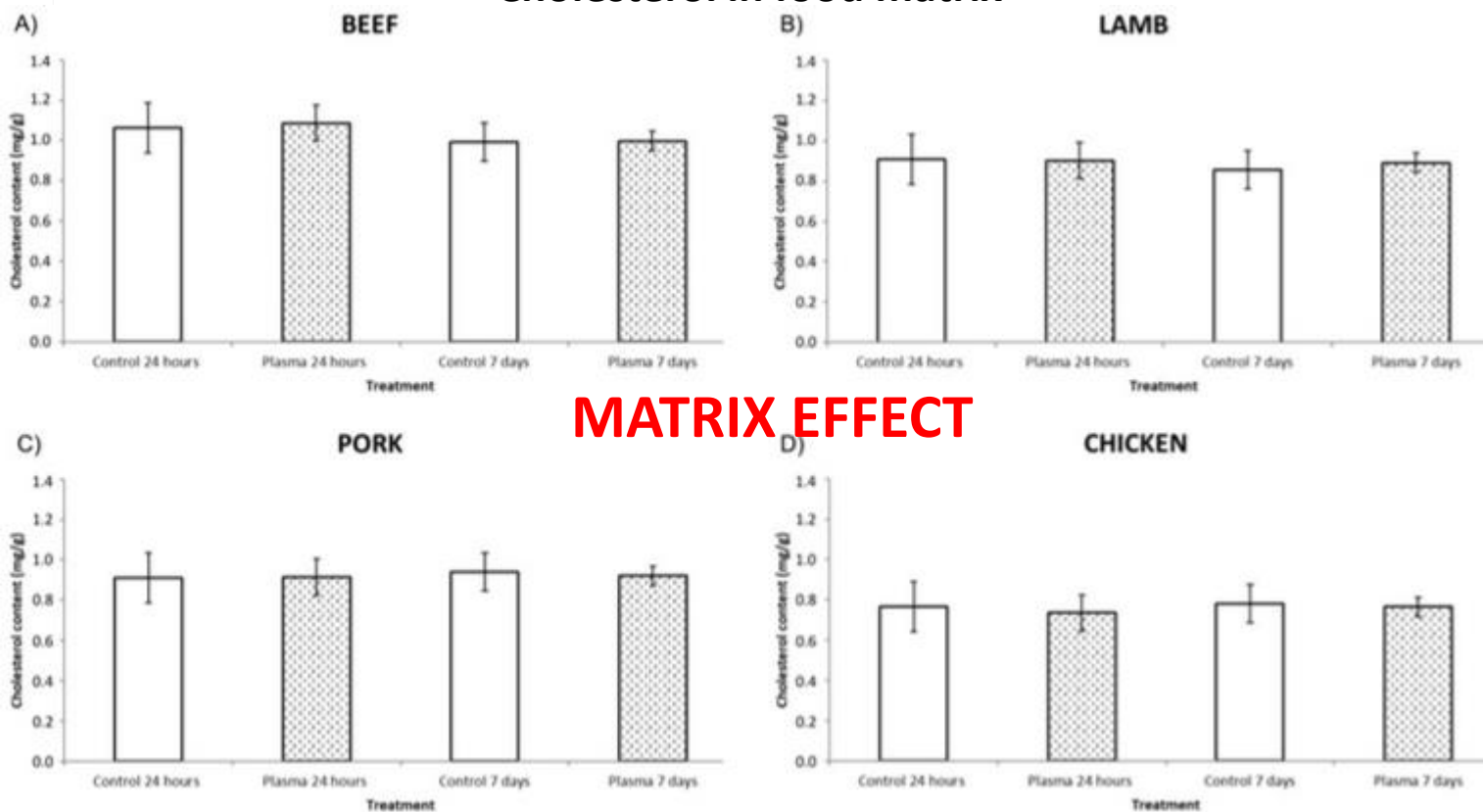
Effect on cholesterol

Cholesterol in pure form



Two polypropylene (PP) dielectric layers (2 mm thickness)
60 kV and 80 kV
5 and 10 min

Cholesterol in food matrix



MATRIX EFFECT

Cholesterol oxidation products (COPs)
?
Toxic compounds!!

Pérez-Andrés et al. (2020)

Application of cold plasma in the food sector

Presentation structure:

- Research on Cold Plasma in the Food Sector
- Main applications:
 - Food decontamination
 - microorganisms
 - viruses
 - mycotoxins
 - pesticides
 - allergens
 - Modification of enzymatic activity
 - Functionalization of food components (proteins, starches)
- Secondary effects: effects on quality and nutritional properties, lipid oxidation
- **Legislative aspects**






Novel Foods Legislation


Established to **demonstrate** to the **consumer** that foods that had not been consumed to a significant degree within the European Union **prior to 1997**, had undergone a stringent and independent **safety assessment** (labelled to not mislead)

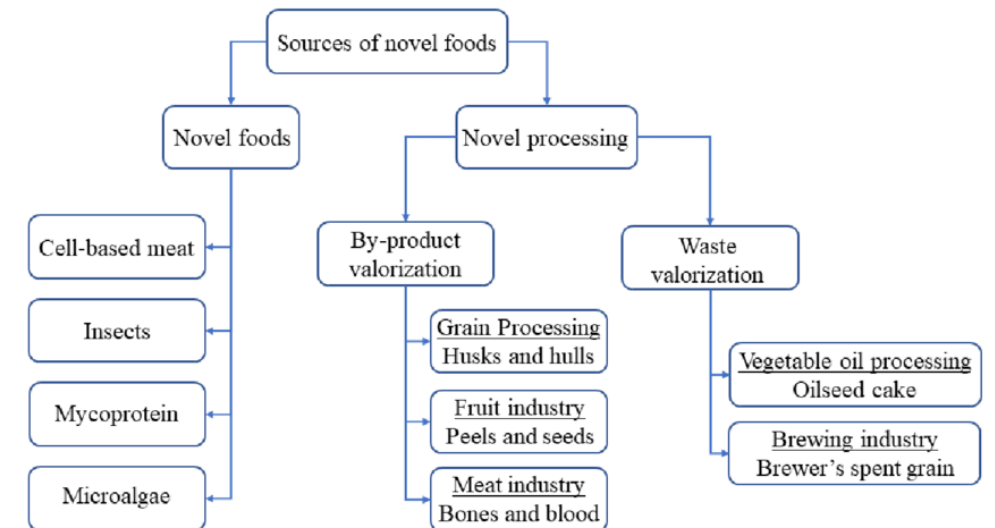
NEW EU RULES FOR NOVEL FOOD BECAME APPLICABLE ON 01 JANUARY 2018

Novel food is defined as food that has not been consumed to any significant degree in the EU before May 1997. This can refer to:

-  **Newly developed food**
-  **Food produced using new technologies/ processes**
-  **Food traditionally eaten outside of the EU**

Examples: chia seeds, oil from Buglossoides arvensis, rapeseed protein, coriander seed oil

 European Commission



Novel Foods Legislation

https://ec.europa.eu/food/safety/novel-food_en

'Novel Food' can be newly developed, innovative food, **food produced using new technologies** and production processes, as well as food which is or has been traditionally eaten outside of the EU.

The underlying principles underpinning Novel Food in the European Union are that Novel Foods must be:

- **Safe** for consumers
- Properly **labelled**, so as not to mislead consumers
- If novel food is intended to replace another food, it must not differ in a way that the consumption of the Novel Food would be **nutritionally disadvantageous** for the consumer.

Pre-market authorisation of Novel Foods on the basis of an evaluation in line with the above principles is necessary.

...Foods resulting from a production process not used for food production within the Union before 15 May 1997, which gives rise to significant changes in the composition or structure of a food, affecting its nutritional value, metabolism or level of undesirable substances



Novel Foods Legislation

First step: 'scientific assessment prior to authorisation to ensure their safety'.

Performed by the European Food Safety Authority (EFSA)

The EFSA convenes an **expert scientific panel**, collects **relevant information** and then develops an **expert report** on the benefits and risks of the technology resulting from a 'novel food' designation. Those novel food applications that receive 'authorisation' can then be sold in the EU.

The 'authorisation' sets out the **conditions for the novel food use, their designation as a food/food ingredient and labeling requirements.**

Among the **novel food ingredient approvals**, only a small percentage involve **new technology:**

UV (technology) treatment of mushrooms, bread, baker's yeast, and milk

HPP for fruit preparations



Where are we on Plasma Processing?

Currently, **uncertainty** remains in the EC regulatory approval process for cold plasma technologies due to the lack of definition within the evaluation criteria (e.g., 'risk to public health', 'nutritionally disadvantageous' and 'not misleading to the consumer') (Bourke et al., 2018).

In depth investigations of the safety of plasma for food applications remain lacking.

Different results highlighted:

- the **complexity** resulting from the range of diverse plasma devices, treatment regimens, and target substances;
- the need for a better understanding of the effect of plasma-treated substances, studies focused more directly on **food-related toxicity** are needed;
- need for evaluations of the persistence of **cytotoxic effect** in food overtime, their concentration and their oral toxicity;
- Need to address and contextualize **potential of adverse effects** resulting from plasma-induced changes to food constituents

Does cold plasma treatment lead to residuals that are harmful to health

?

- Need to evaluate **consumer acceptance!!**



Take home message

Many promising applications for food stabilization and innovation

But

Processing optimization is necessary based on the characteristics of the raw material!!



Concluding remarks

Most studied applications:

- Microbial/viral/mycotoxins inactivation

Newer trends:

- Functionalization of components (foods and packaging)
- Enhancement of drying

Need for:

- In depth safety assessment → Novel food regulation



Plasma applications for smart and sustainable agriculture – PIAgri

<https://plagri.eu/>

The scientific community is making a multidisciplinary effort to make plasma applicable also for direct food processing!



Our researches on cold plasma

- **TECH4PATH – PRIN 2022** Non-thermal TECHNOLOGIES FOR the inactivation of emerging viral, bacterial and protozoan PATHOGENS on fruit and vegetable products

OBJECTIVE: Optimize non thermal processing for the inactivation of emerging pathogens and evaluate the effect on quality and environmental impact

- **PLASMAFOOD – PRIN 2017** Study and optimization of cold atmospheric plasma treatment for food safety and quality improvement

OBJECTIVE: Learn more about the effects of cold plasma on various aspects (microbiological, qualitative, chemical and nutritional)

- **PASS – EIT** “Plasma assisted sanitation systems” for the inactivation of SARS-CoV-2 on food contact materials

OBJECTIVE : use plasma as a strategy to inactivate the virus on food packaging materials

- **FuturEUAqua** Future growth in sustainable, resilient and climate friendly organic and conventional European aquaculture. Horizon 2020 Call: H2020-BG-2018-2020

OBJECTIVE : Apply cold plasma to increase the shelf life of seafood



Plasma Assisted Sanitation System



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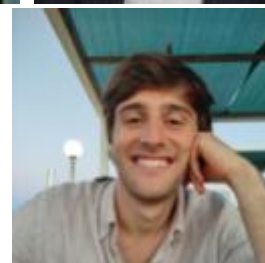
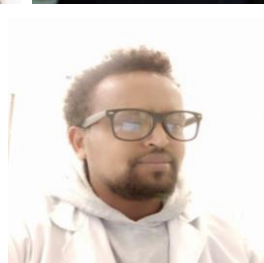
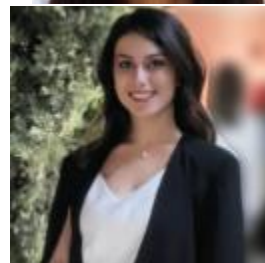
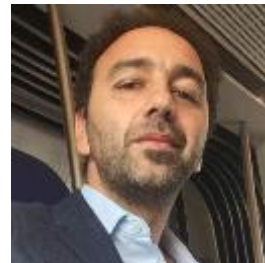
Research Group for Food Technology



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DEPARTMENT OF
AGRICULTURAL AND FOOD SCIENCES

CIRI AGROALIMENTARE



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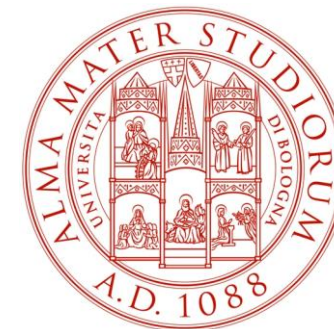
Research Group for Industrial Applications of Plasmas (IAP Group)

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for smart and
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Thank you!!

Questions?

Prof. Silvia Tappi

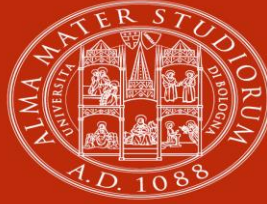
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Meet the presenter



Bibliographic references





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