Cold Plasma Technology Controlling Listeria and Extending Shelf-Life in Cheese

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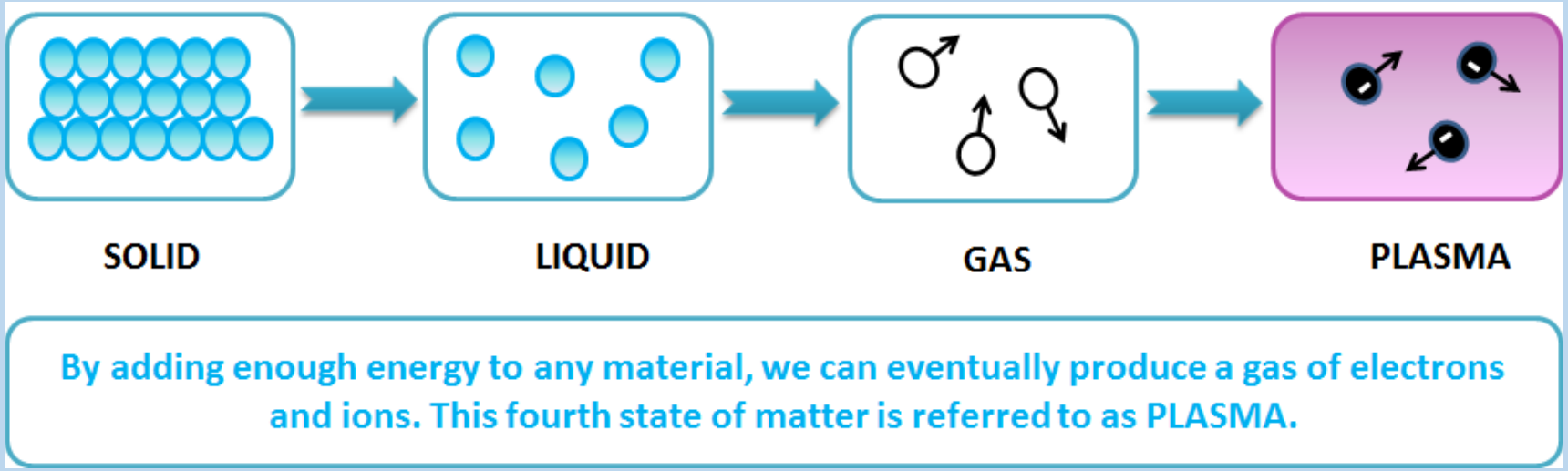
What is Plasma?



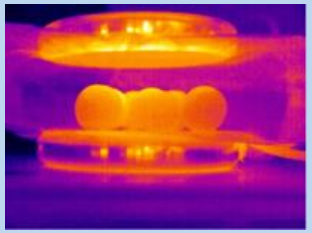
Left: Aurora Australis (non-thermal; Courtesy of Joseph M. Acaba, Astronaut, NASA);
Right: Cloud to ground lightning strike (thermal).

Left: Fluorescent Tube lights (Flickr user: OimaxThis);
Right: Swimming pool ozonation system (SaviorO3)

In-package Cold Plasma Technology

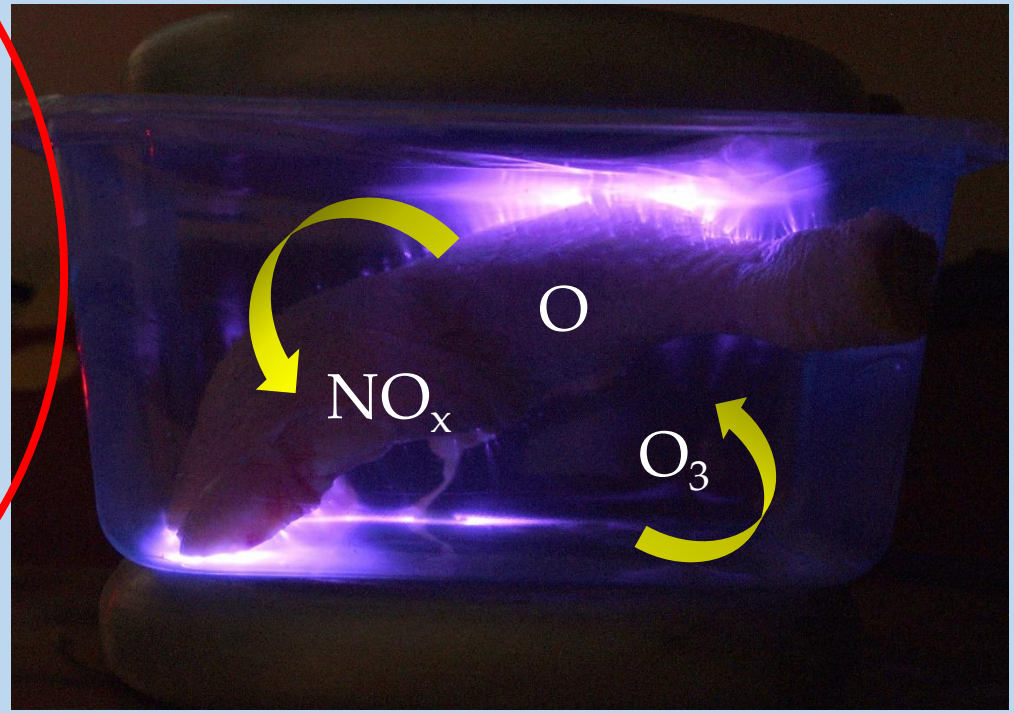
High Voltage (> 30 kV)

Microbes/Pathogens



T_f - T_i ~ 10 ˚C

NO ENTRY



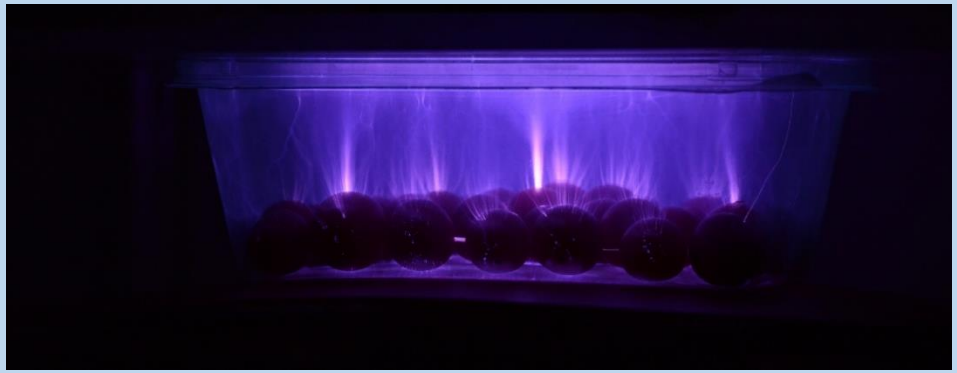
In-package treatment of chicken leg with He/Air Mixture at 60 kV p-p

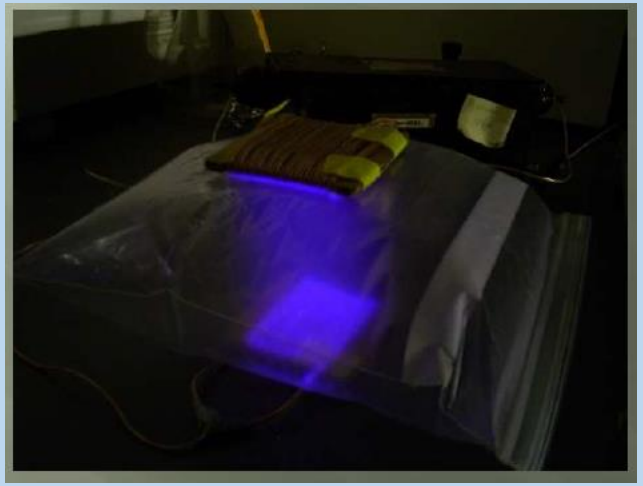
- Nonthermal process
- Dry Process
  - Sustainability
- Fairly rapid
- Minimal Effluent
- No toxic residuals known
- Low energy consumption
- Low operating costs (with Air or known MAP gases)
- Applicable for solid as well as liquid foods
- Simple and scalable to industrial demand
- Effective against biofilms and bacterial spores

Image courtesy, N.N. Misra

High Voltage Atmospheric Cold Plasma

- High Voltage Atmospheric Cold Plasma (HVACP) technology
  - is capable of producing both reactive oxygen species (singlet oxygen $^1O_2$, ozone, etc.) and reactive nitrogen species (N_2O, NO, NO_2 etc.)
  - has shown the ability in controlling pathogen (*E. coli O157:H7, Salmonella spp., Listeria monocytogenes, etc.)* in various products(strawberry, cherry tomato, etc.) without affecting the quality
  - can be applied into extending shelf-life of different products (pork, strawberry, cherry tomato etc.)





1. *Ziuzina, D., et al. (2014). Food Microbiology,* ***42****: 109-116.*
2. *Misra, N.N., et. al. (2014). Journal of Food Engineering,* ***125****: 131-138*
3. *Misra, N.N., et. al. (2014). Journal of Bioscience and Bioengineering,* ***118*** *(2): 177-182*
4. *Kronn, T.G., et. al. (2015). Transactions of the ASABE* ***58****(2): 493-500*

Center for Disease Control and Prevention Facts

Listeriosis is a serious infection usually caused by eating food contaminated with the bacterium *Listeria monocytogenes*. An estimated 1,600 people get listeriosis each year, and about 260 die. The infection is most likely to sicken pregnant women and their newborns, adults aged 65 or older, and people with weakened immune systems.

Queso fresco and other soft cheeses

Soft cheeses made with unpasteurized milk (also called raw milk) are estimated to be 50 to 160 times more likely to cause *Listeria* infection than when they are made with pasteurized milk.

Although pasteurization of milk kills *Listeria*, products made from pasteurized milk can still become contaminated if they are produced in facilities with unsanitary conditions.

2017

- Vulto Creamery Soft Raw Milk Cheese – Listeriosis

2016

- Frozen Vegetables – Listeriosis
- Raw Milk – Listeriosis
- Packaged Salads – Listeriosis

2015

- Soft Cheeses – Listeriosis
- Ice Cream – Listeriosis

2014

- Commercially Produced, Prepackaged Caramel Apples – Listeriosis
- Bean Sprouts – Listeriosis
- Cheese – Listeriosis
- Dairy Products – Listeriosis

2013

- Cheese – Listeriosis

2012

- Ricotta Salata Cheese – Listeriosis

2011

- Cantaloupes – Listeriosis

https://www.cdc.gov/listeria/prevention.html

Queso Fresco

Photo credit: gourmesleuth

- Queso fresco cheese (QFC)
  - one of the popular fresh soft Mexican-style cheeses
  - risk of *Listeria monocytogenes* contamination
  - high moisture (45 – 55%), low salt content and near neutral pH (6.0 – 6.5)
  - no effective commercial technology to reduce pathogens in Queso Fresco and other soft cheeses

https://ethnicspoon.com/refried-beans-avocado-tomato-queso-fresco-tostada/

James Ransom for the Wall Street Journal

Cold Plasma Activities in Cheese

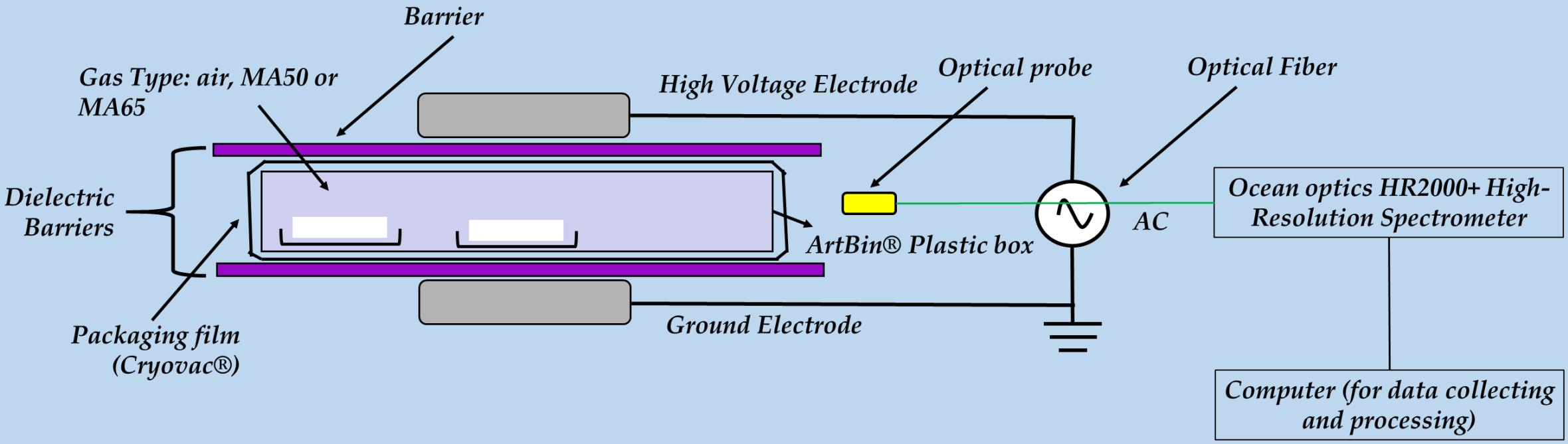
1. **Funded - Evaluate the effect of direct HVACP treatment on *Listeria innocua* inactivation, quality and background microorganisms of 10 g QFC crumbles followed by 28 days refrigeration storage – Completed in 2019**
2. **Funded - Evaluate the effect of direct HVACP treatment on Listeria *monocytogenes* in 100 gram QFC crumbles – underway (to be completed by March 2021)**
3. **Pending - Treatment of shredded cheeses using cold plasma technology for quality improvement: USDA-SBIR Phase I project pending. (if funded) completed by Dec 2021.**

Evaluate the effect of direct HVACP treatment on Listeria innocua inactivation, quality and background microorganisms of QFC crumbles followed by 28 days refrigeration storage

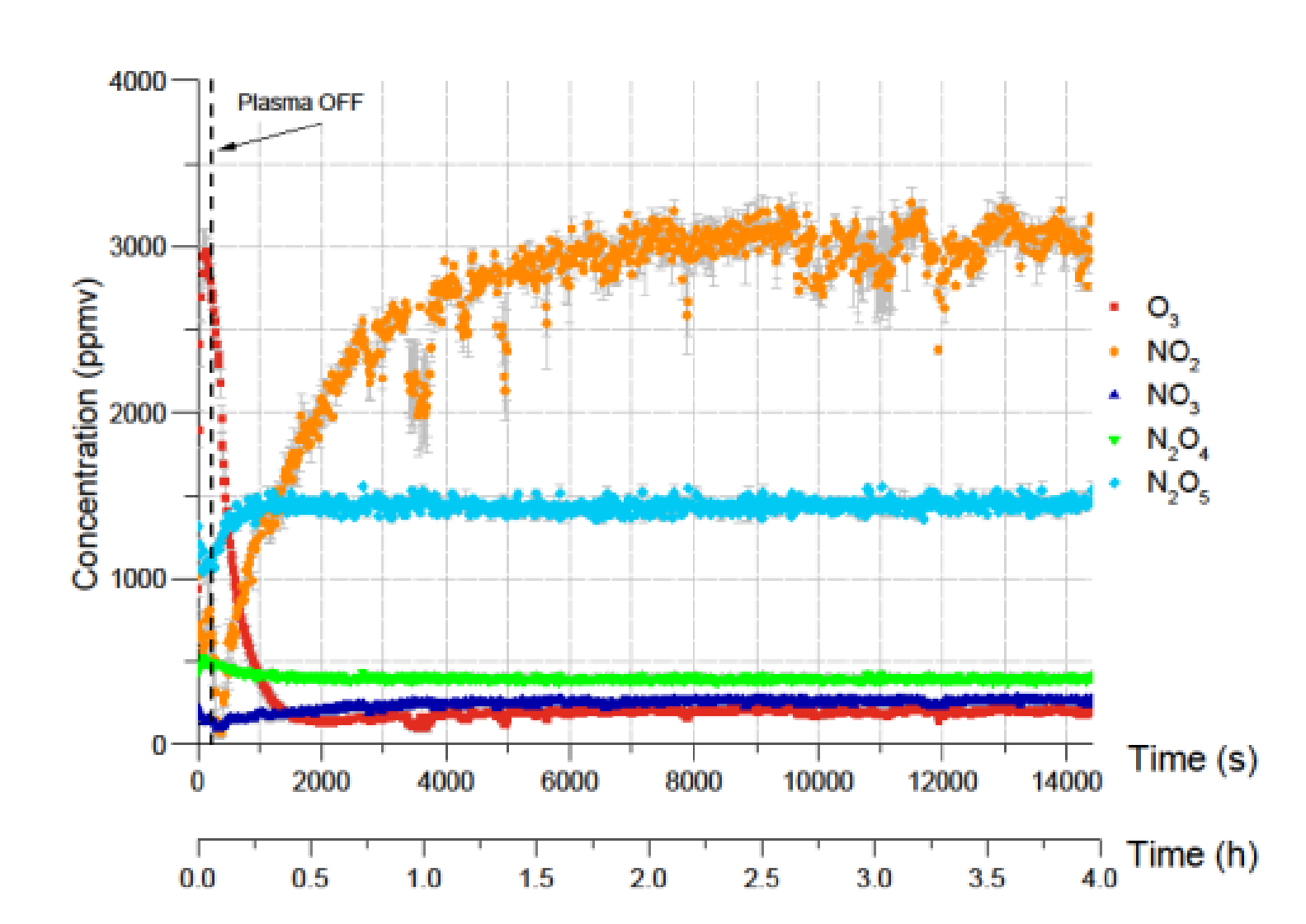
Materials and Methods

- 10 g crumbled Queso fresco was inoculated with *Listeria innocua* (LI) inoculum resulting 6.0 $\log_{10}$ CFU/g.
- Inoculated samples were treated with HVACP under direct mode of exposure in air for 4 or 6 min at 100 kV
- Inoculated treated or control samples were stored at 4°C for up to 28 days for microbial enumeration
- Non-inoculated treated and control samples were stored at 4°C for up to 28 days for
  - Background microflora enumeration
  - Lipid peroxidation (TBARS) , moisture, and pH
- Optical adsorption spectra were recorded by a computer controlled Ocean Optics spectrometer (Ocean Optics, Inc., FL, USA)

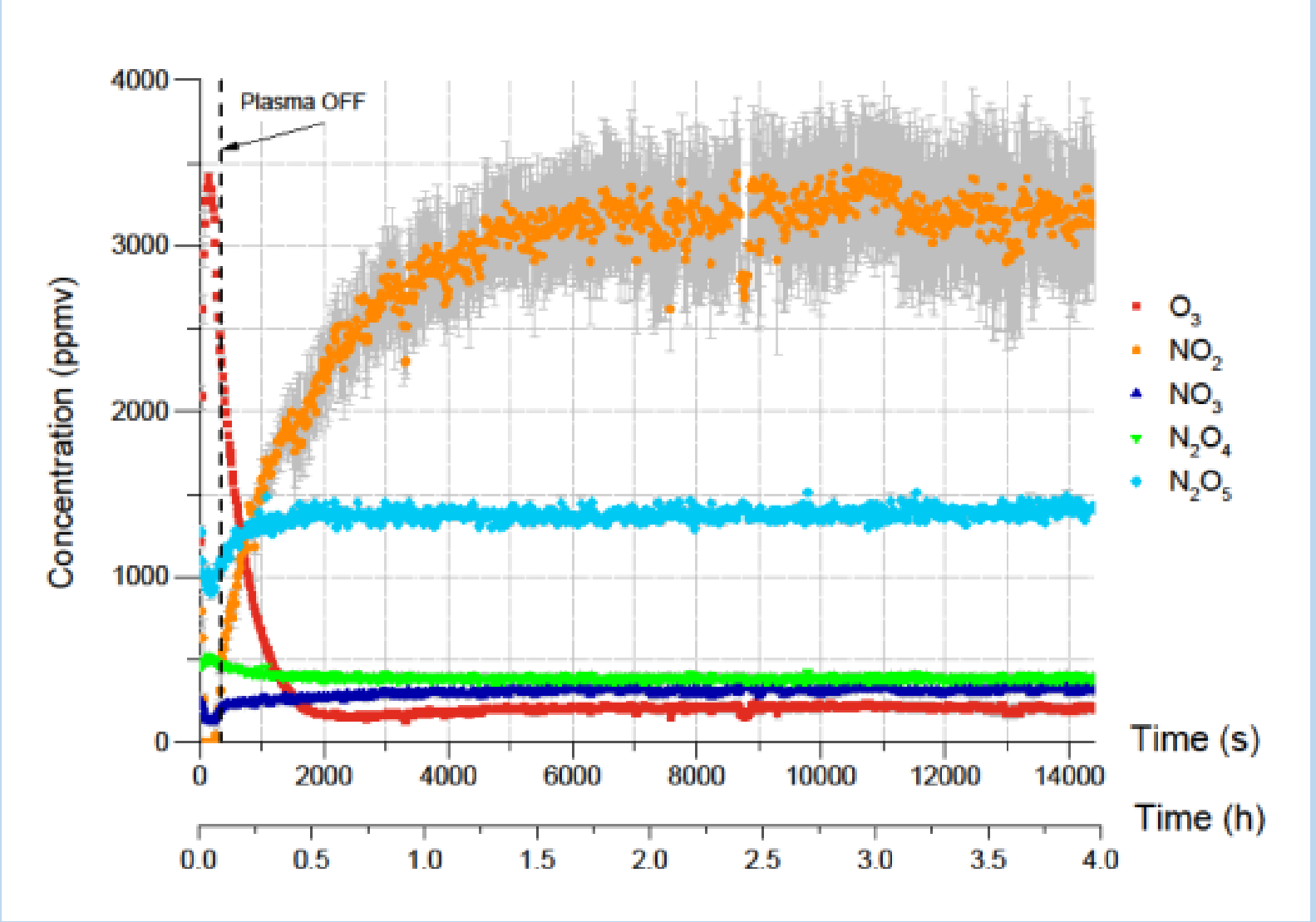
Plasma Set-up Schematic

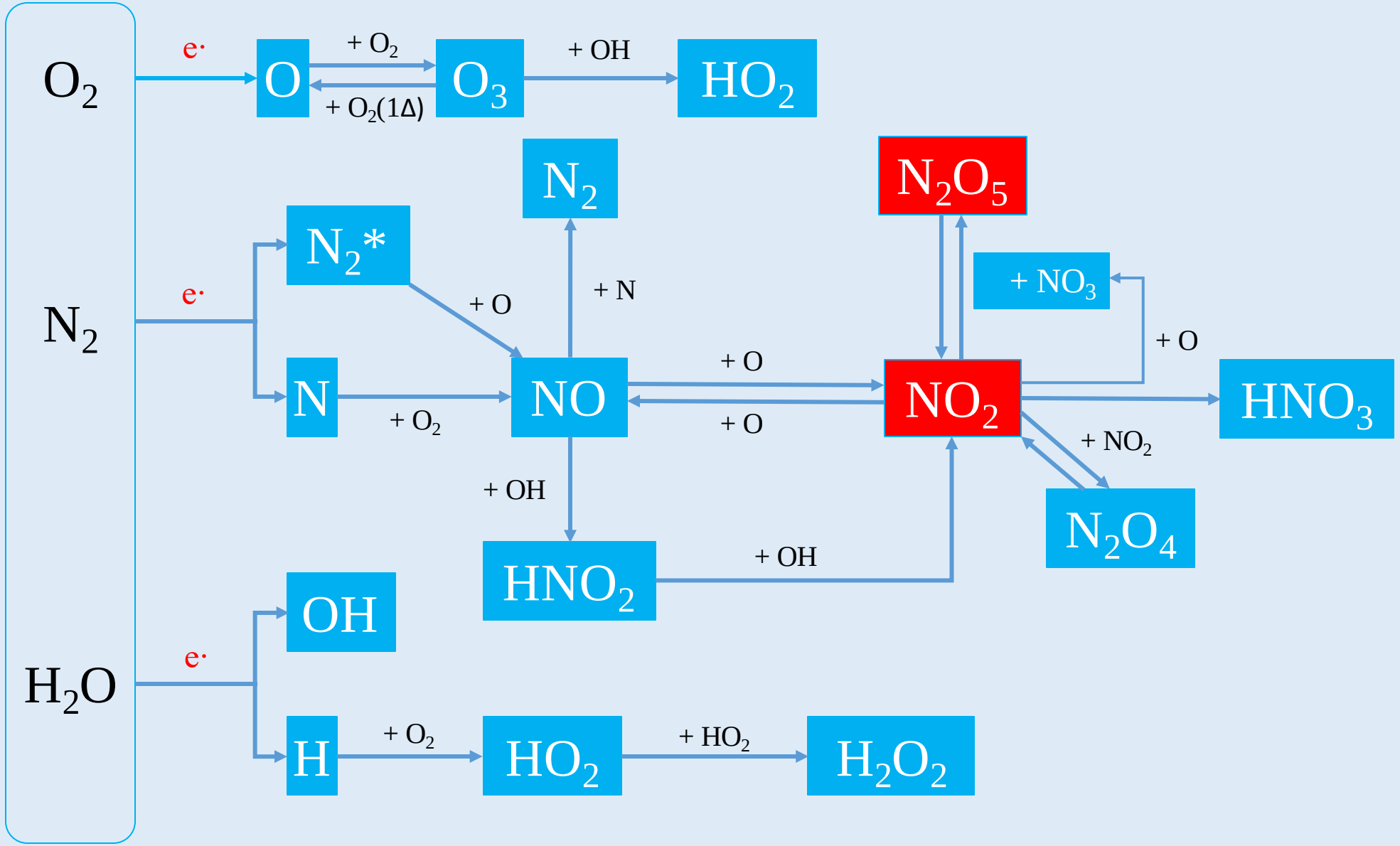


Results – Post-discharge composition after 4 min treatment

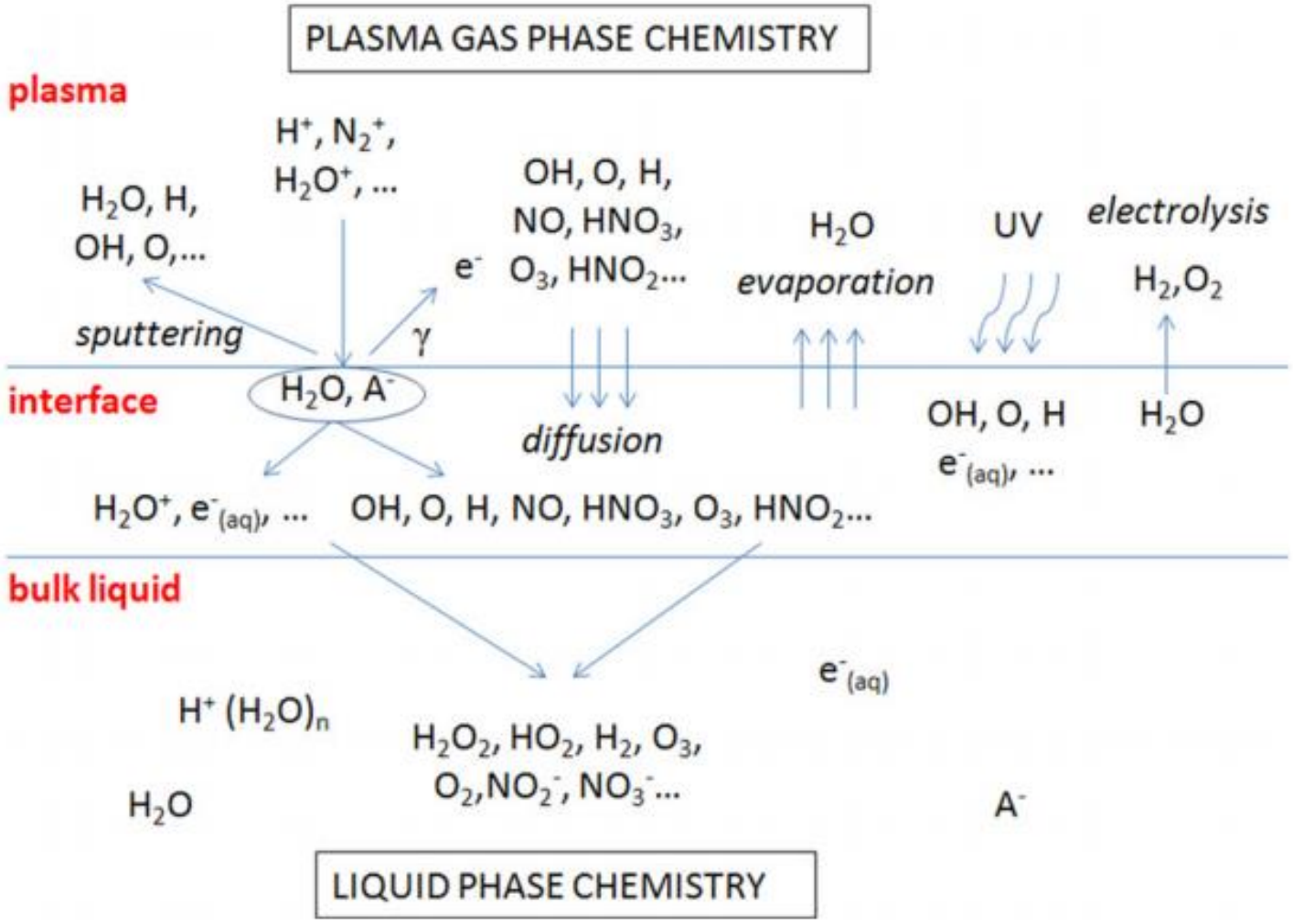


Results – Post-discharge composition after 6 min treatment





Adapted from R.A. Wolf, (2013) Atmospheric pressure plasma for surface modification



Samukawa et al. (2012), *Journal of Physics D: Applied Physics* 45.25, 253001.

Table 12. Peroxide (O_2^{2-}), Nitrite (NO_2^-) and Nitrate (NO_3^-) of QFC treated with HVACP for four minutes in dry air for up to 28 days storage at 4 °C. Same small letter beside each measurement indicated no significant (p>0.05) different within the same compound measured

Day	Average peroxide	Average nitrite	Average nitrate
0	100^{a}	12.5^{a}	250^{ab}
1	30^{b}	10^{a}	250^{ab}
5	30^{b}	10^{a}	262^{ab}
10	28^{b}	10^{a}	238^{b}
15	30^{b}	10^{a}	250^{ab}
20	30^{b}	10^{a}	250^{ab}
23	12^{c}	10^{a}	250^{ab}
25	15^{c}	10^{a}	275^{ab}
28	18^{c}	10^{a}	250^{ab}

Table 13. Peroxide (O_2^{2-}), Nitrite (NO_2^-) and Nitrate (NO_3^-) of QFC treated with HVACP for six minutes in dry air for up to 28 days storage at 4 °C. Same small letter beside each measurement indicated no significant (p>0.05) different within the same compound measured

Day	Average peroxide	Average nitrite	Average nitrate
0	100^{a}	20^{a}	300^{c}
1	80^{b}	20^{a}	275^{c}
5	58^{c}	18^{a}	263^{c}
10	30^{e}	20^{a}	275^{c}
15	30^{e}	15^{ab}	500^{a}
20	50^{cd}	10^{b}	500^{a}
23	33^{e}	10^{b}	425^{b}
25	40^{de}	10^{b}	463^{ab}
28	40^{de}	10^{b}	475^{ab}

Results – Listeria innocua recovery

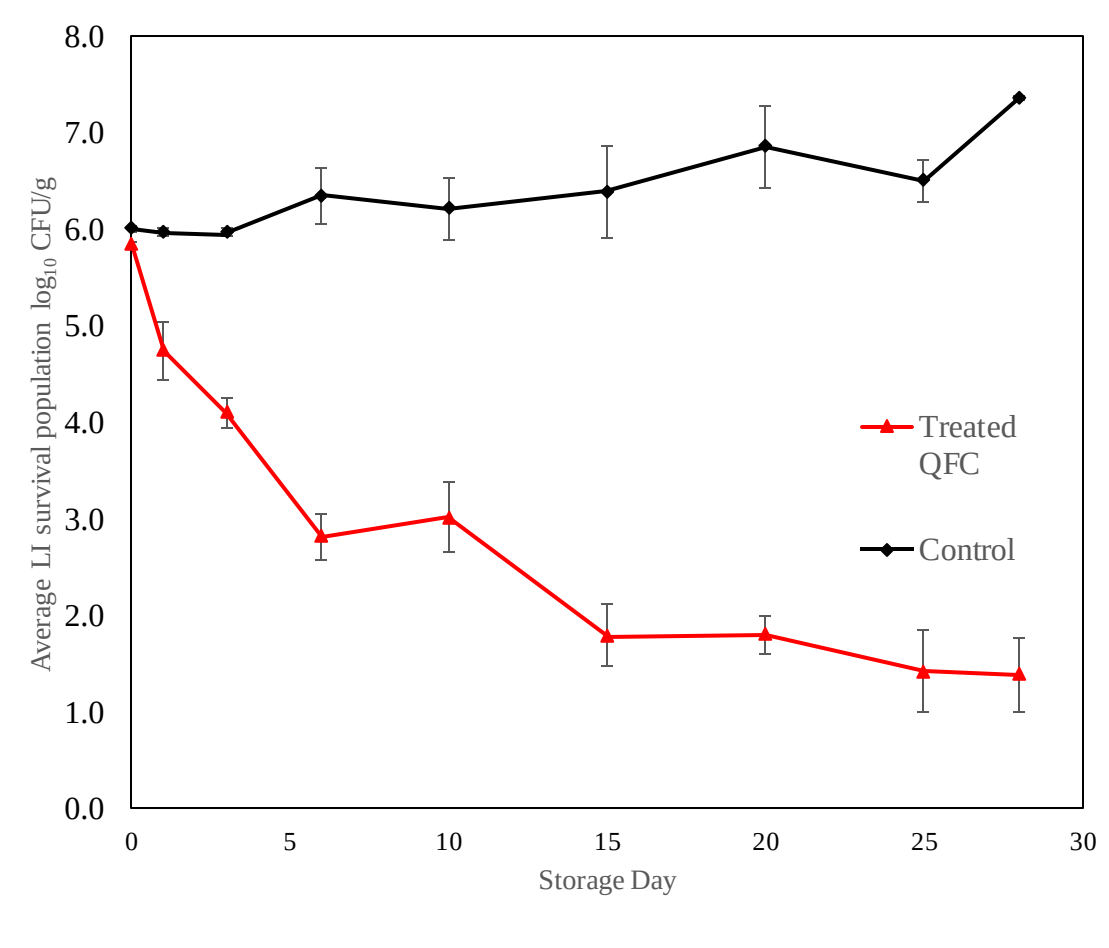


Figure 18. *Listeria innocua* population (log_{10} CFU/g) in QFC after HVACP (Direct) treatment in dry air at 100 kV (228 watts) for <u>four minutes</u> within 28 days storage at 4°C. Detection limit is 1.0 log_{10} CFU/g.

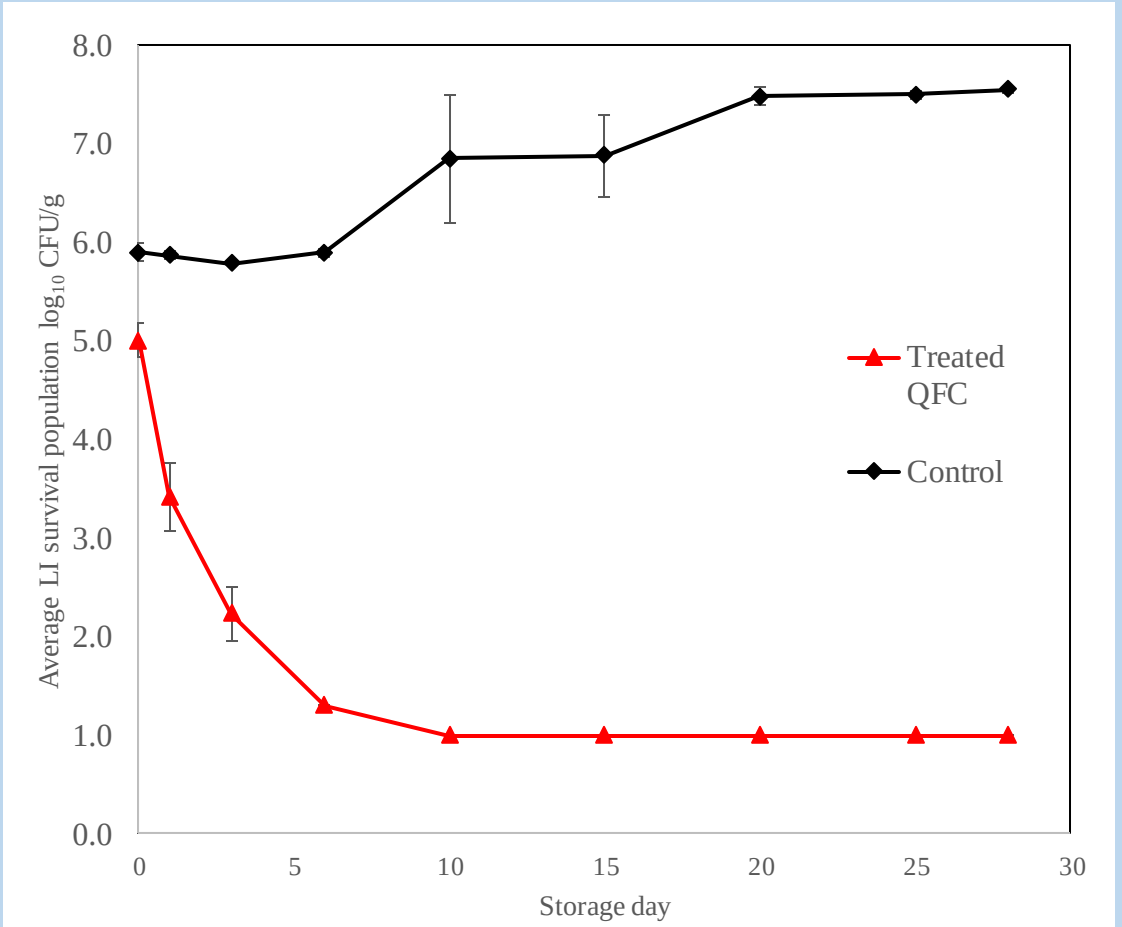


Figure 19. *Listeria innocua* population (log_{10} CFU/g) in QFC after HVACP (Direct) treatment in dry air at 100 kV (228 watts) for <u>six minutes</u> within 28 days storage at 4°C. Detection limit is 1.0 log_{10} CFU/g.

Results – Psychrotrophic microorganisms recovery

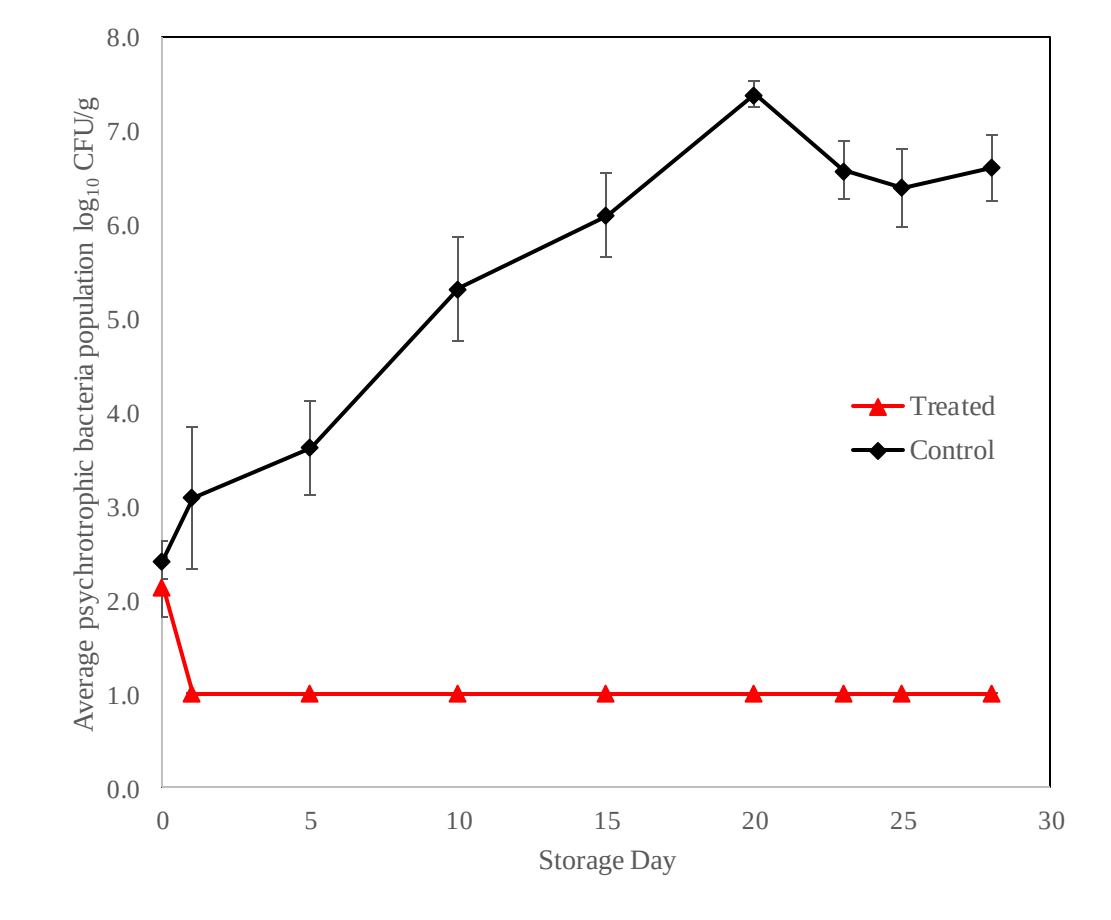


Figure 20. Psychrotrophic microorganisms population (log_{10} CFU/g) of QFC treated with HVACP (Direct) in dry air at 100 kV for <u>four minutes</u> within 28 days storage at 4°C. Detection limit is 1.0 log_{10} CFU/g.

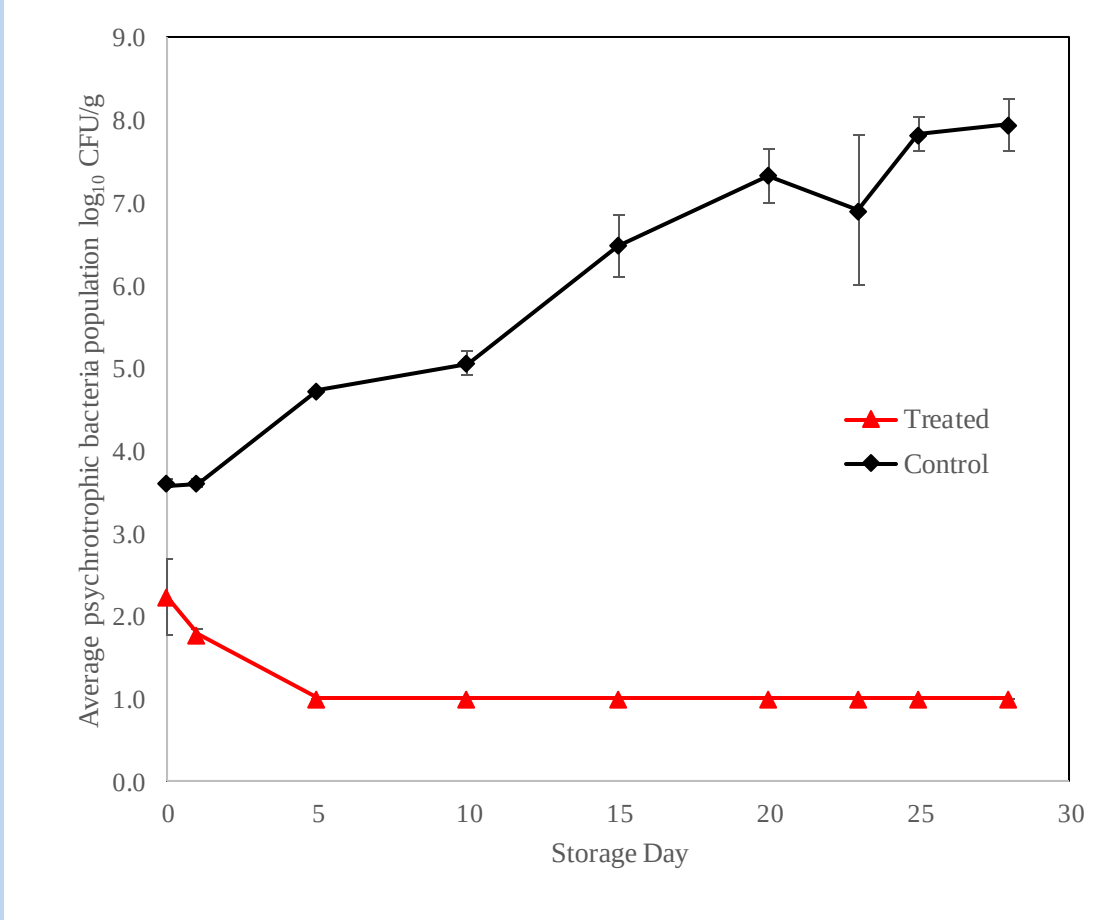


Figure 21. Psychrotrophic microorganisms population (log_{10} CFU/g) of QFC treated with HVACP (Direct) in dry air at 100 kV for <u>six minutes</u> within 28 days storage at 4°C. Detection limit is 1.0 log_{10} CFU/g.

Results – Mesophilic microorganisms recovery

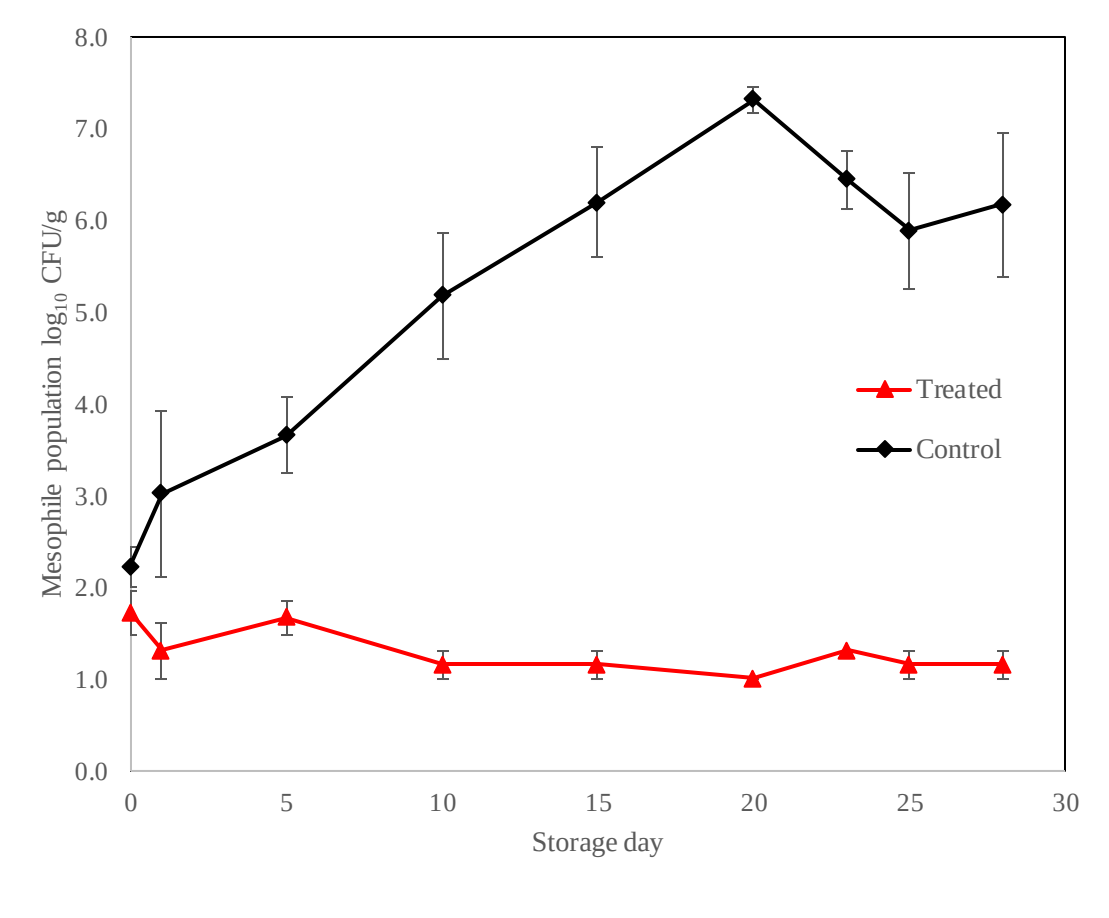


Figure 19. Mesophilic microorganisms population (log_{10} CFU/g) of QFC treated with HVACP (Direct) in dry air at 100 kV for <u>four minutes</u> within 28 days storage at 4°C. Detection limit is 1.0 log_{10} CFU/g.

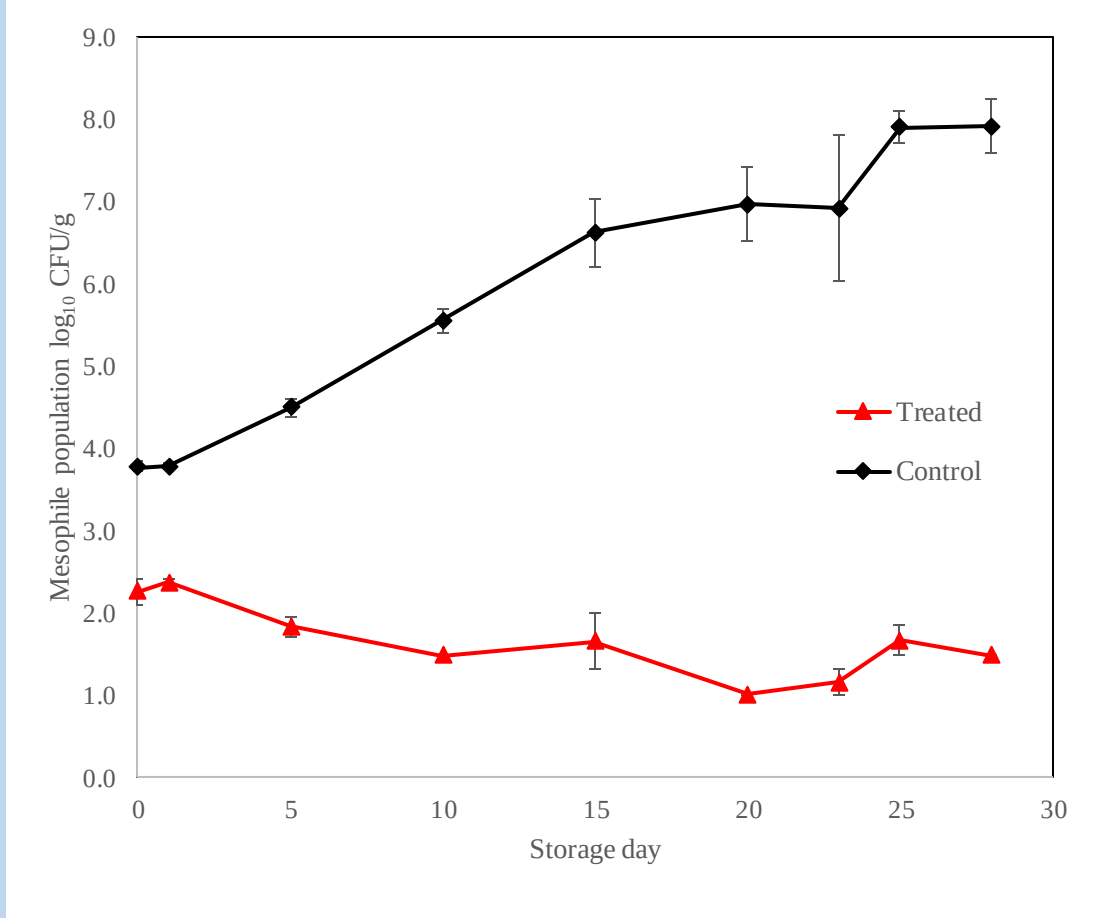


Figure 20. Mesophilic microorganisms population (log_{10} CFU/g) of QFC treated with HVACP (Direct) in dry air at 100 kV for <u>six minutes</u> within 28 days storage at 4°C. Detection limit is 1.0 log_{10} CFU/g.

Summary

- Both four and six min direct HVACP treatments in dry air were able to inactivate and inhibit the growth of *Listeria innocua,* as well as the background microflora
- Post-discharge plasma composition revealed the extended exposure of reactive gas species with the sample during storage.
- A slight decrease in moisture was observed with increasing storage period
  - No significant difference ($p<0.05$) between control and treated sample
- Minimal changes in pH and lipid oxidation were found over the 28 days storage

Results - Quality

Table 14. Lipid peroxidation (MDA), moisture content and pH of QFC treated with HVACP for four minutes in dry air for up to 28 days storage at 4 °C. Same small letter beside each measurement indicated no significant (p>0.05) different within the same treatment voltage

	Treated			Control		
Day	MDA(mg/kg)	MC%	pH	MDA(mg/kg)	MC%	pH
0	1.37^{a}	$50.25\%^{ab}$	5.69^{bc}	0.09^{d}	$50.26\%^{a}$	5.91^{ab}
1	1.29^{ab}	$50.76\%^{a}$	5.82^{a}	0.08^{d}	$50.65\%^{a}$	6.00^{a}
5	0.86^{ef}	$50.29\%^{ab}$	5.77^{ab}	0.08^{d}	$50.64\%^{a}$	5.97^{ab}
10	0.98^{de}	$50.04\%^{abc}$	5.67^{bc}	0.12^{cd}	$50.87\%^{a}$	5.85^{bc}
15	1.10^{cd}	$49.37\%^{abcd}$	5.59^{cd}	0.20^{bc}	$50.33\%^{a}$	5.77^{cd}
20	1.21^{bc}	$49.25\%^{abcd}$	5.53^{d}	0.20^{bc}	$49.32\%^{a}$	5.70^{de}
23	0.93^{e}	$47.69\%^{d}$	5.54^{d}	0.23^{ab}	$49.17\%^{a}$	5.50^{f}
25	0.94^{e}	$48.43\%^{bcd}$	5.57^{cd}	0.27^{ab}	$48.94\%^{a}$	5.57^{ef}
28	0.77^{f}	$47.76\%^{cd}$	5.57^{cd}	0.30^{a}	$49.86\%^{a}$	5.57^{ef}

Results - Quality

Table 15. Lipid peroxidation (MDA), moisture content and pH of QFC treated with HVACP for six minutes in dry air for up to 28 days storage at 4 °C. Same small letter beside each measurement indicated no significant (p>0.05) different within the same treatment voltage

	Treated			Control		
Day	MDA(mg/kg)	MC%	pH	MDA(mg/kg)	MC%	pH
0	1.56^{d}	$48.62\%^{a}$	5.52^{bc}	0.23^{d}	$49.48\%^{a}$	5.46^{c}
1	2.17^{b}	$48.43\%^{a}$	5.40^{c}	0.21^{d}	$49.51\%^{a}$	5.49^{c}
5	1.72^{cd}	$48.29\%^{a}$	5.26^{d}	0.24^{cd}	$49.17\%^{a}$	5.79^{b}
10	1.11^{f}	$47.42\%^{ab}$	5.56^{b}	0.26^{bcd}	$49.16\%^{a}$	5.90^{ab}
15	2.86^{a}	$44.42\%^{c}$	5.72^{a}	0.26^{bcd}	$45.73\%^{a}$	6.04^{a}
20	1.36^{e}	$44.41\%^{c}$	5.64^{ab}	0.35^{ab}	$42.76\%^{a}$	5.89^{ab}
23	1.11^{f}	$46.47\%^{abc}$	5.60^{ab}	0.38^{a}	$44.31\%^{a}$	5.95^{ab}
25	1.31^{e}	$45.05\%^{bc}$	5.57^{b}	0.29^{abcd}	$44.17\%^{a}$	5.91^{ab}
28	1.79^{c}	$41.43\%^{d}$	5.53^{bc}	0.33^{abc}	$44.87\%^{a}$	5.85^{ab}

Conclusions

- HVACP direct treatment has shown a great potential in microbial inactivation of *Listeria innocua,* as well as background microflora.
- Minimal effects on cheese quality were observed over 28 days after HVACP treatment.

Next Step

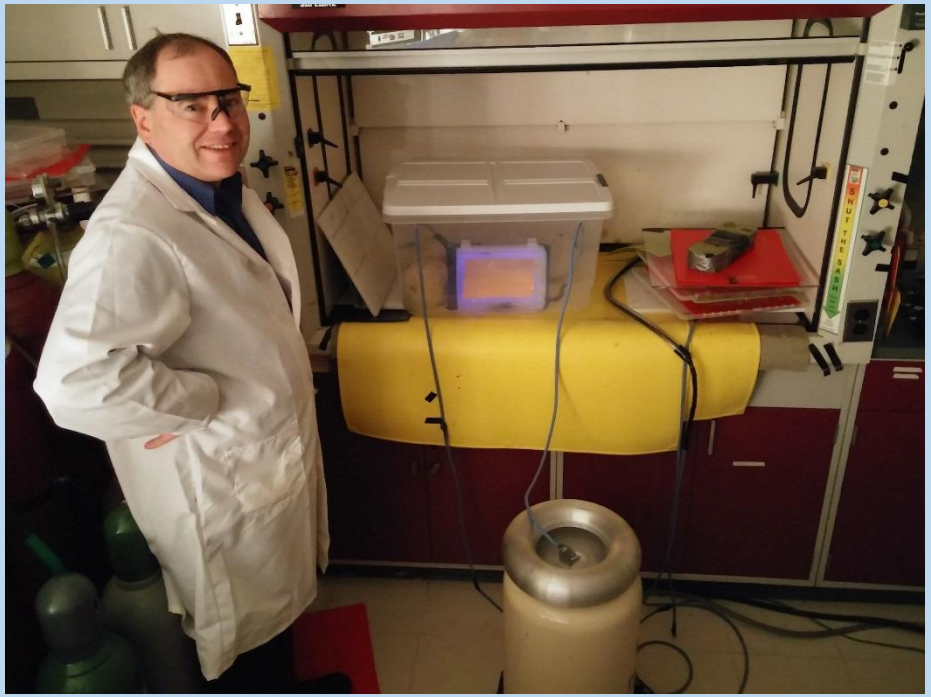
- Scale-up of the direct HVACP treatment to 100 gram samples and achieve greater bactericidal reductions through process optimization while maintaining minimal effects on cheese quality.

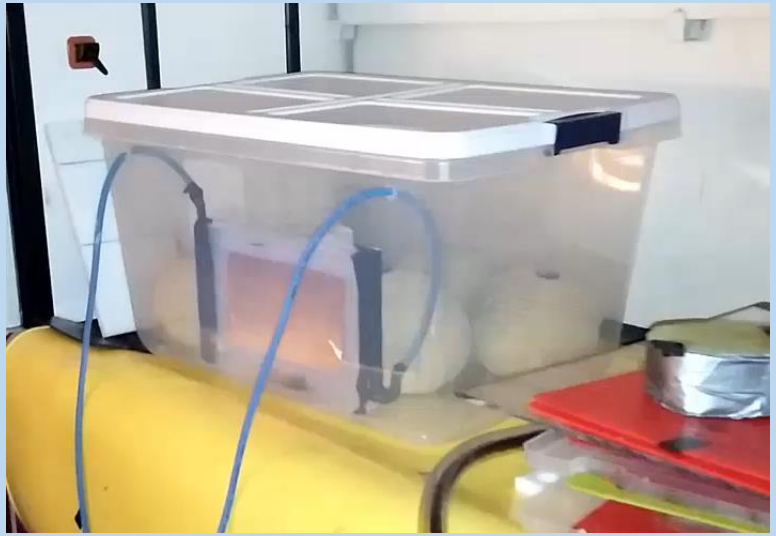
T=0h, no HVACP

Industrial Scale - Up
US (Cantaloupe Project)

T=24h, post HVACP

Prototype HVACP treatment chamber for whole cantaloupe (15-20).





Industrial HVACP Scale - Up

European research : Safebag, Safemeat, Watertreat, Campydecon

Acknowledgement: Research funding support provided by the National Dairy Council and Innovation Center for US Dairy

Acknowledgement: Currently working with Clean Crop Technologies to commercialize cold plasma for detoxification in peanuts.

QUESTIONS ??

Control (Dry air) 6 min (Dry air)

Dr. Zifan Wan – funded Ph.D. research