



# Hurdle Technology using Combined Ozone and Chemical Treatments to Reduce Coronavirus Viral Load Contaminated on Export Durian Fruit

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## Abstract

The objective of this research was to develop a hurdle technology with a synergistic effect between gaseous ozone and chemical treatment (OZ-CT) to reduce the coronavirus viral load contaminated in durian fruit. The Porcine Epidemic Diarrhea Virus (PEDV), a swine coronavirus used as the surrogated virus for viral contaminant test to SARS-CoV-2, was treated in a combination of a sodium hypochlorite solution of 3,000 ppm and ozone gas fumigation, ranging from 900 - 3,500 ppm. The remaining virus was evaluated by realtime-PCR. The result shows that applying sodium hypochlorite spray, followed by ozone fumigation at 900 ppm with holding time for 12 hours could reduce the PEDV viral load below the cut-off of realtime-PCR at Ct>36 = negative, which is considered a fully viral breakdown. Treated durian slightly delayed the ripening without affecting fruit quality. The OZ-CT hurdle technology is of great potential to minimize the coronavirus for the fruit export industry, not only for durian but also other commodity fruits.

## Novelty of Research

- The OZ-CT hurdle technology, which combines ozone fumigation with chemical treatment was developed to reduce coronavirus viral load on durian fruit. No previous research had reported the use of this method before.
- The effectiveness of OZ-CT technology in reducing viral load ranges up to 100%, depending on factors such as ozone concentration, holding time, surface morphology of the material, and loading size.
- This technology has the potential to support the Zero-COVID policy for fruits exported to China.

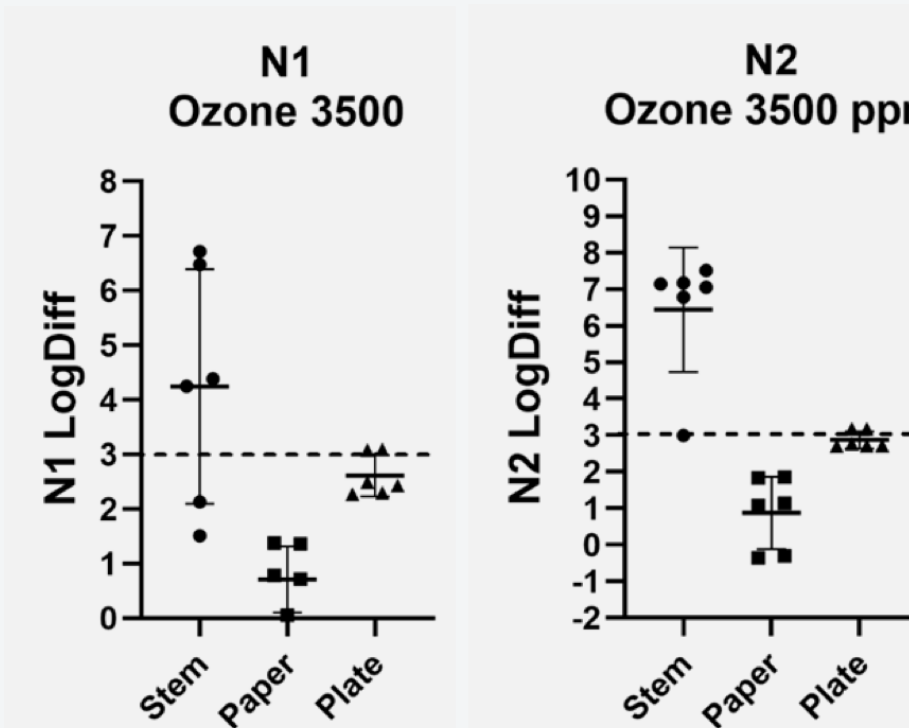
## Rationales

The Zero-COVID policy is crucial for Thai durian fruit exports to China. In 2022, 734,501 tons of durian were exported valued at USD 3,598 Million. Chinese officials use RT-PCR to detect SAR-CoV2 residue on sampling fruit boxes from refrigerating containers. If the virus is detected, the entire lot of durian may be rejected, causing significant economic damage to the Thai export industry.

Ozone gas is a highly effective disinfectant for microbes and has potent oxidizing properties. When combined with chemical treatments, it can provide a synergistic effect on target viruses such as SAR-CoV2 or PEDV. Limited research, such as Tizaoui et al. (2022), has reported on the effectiveness of ozone gas in inactivating SAR-CoV2 on various inorganic surfaces. However, there is no report on the use of ozone gas to disinfect coronavirus contaminated on the whole durian fruit. It is also interesting to explore the synergistic effect of chemical treatments and ozone fumigation on the disinfection efficacy of the coronavirus on fruit.

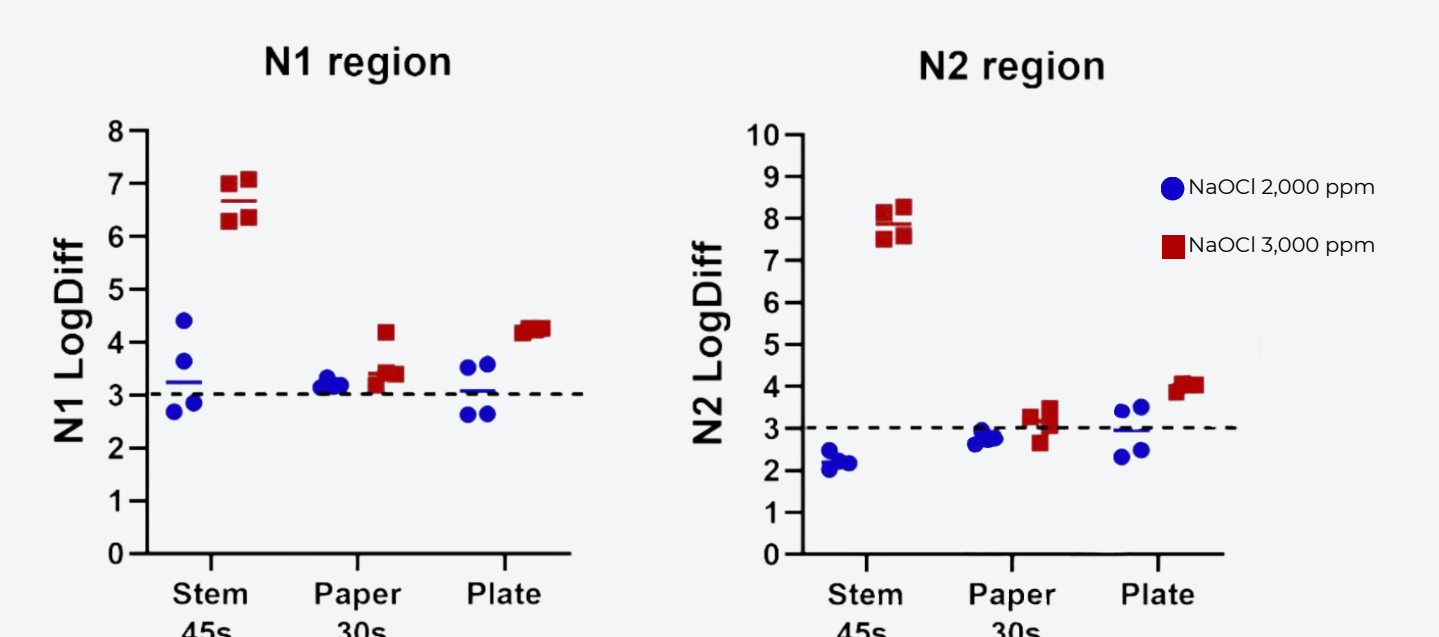
## Highlighted Results

Effect of ozone gas on SAR-CoV2



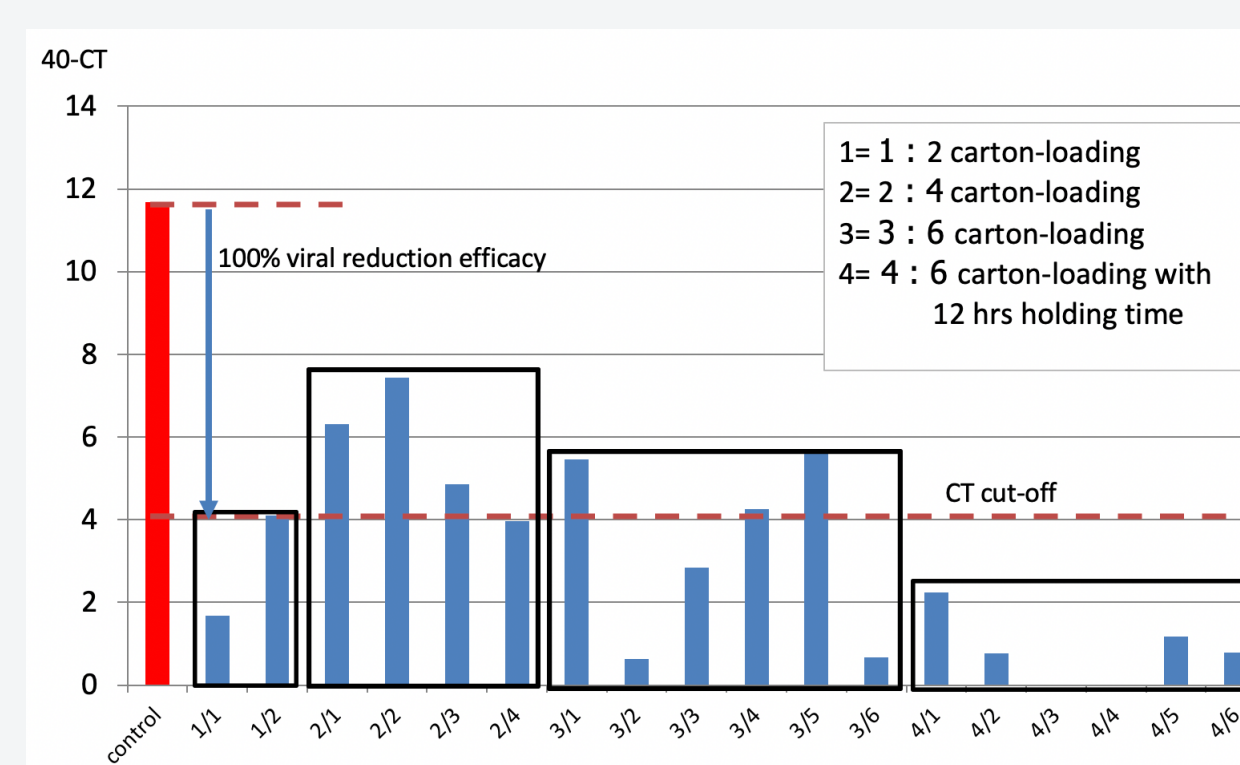
- Ozone gas at 3,500 ppm with a holding time of 2 hrs can reduce SAR CoV2 at a maximum of 7 LogDiff.
- Stem was the most effective material for SAR CoV2 inactivation by ozone.

Effect of NaOCl on SAR CoV2



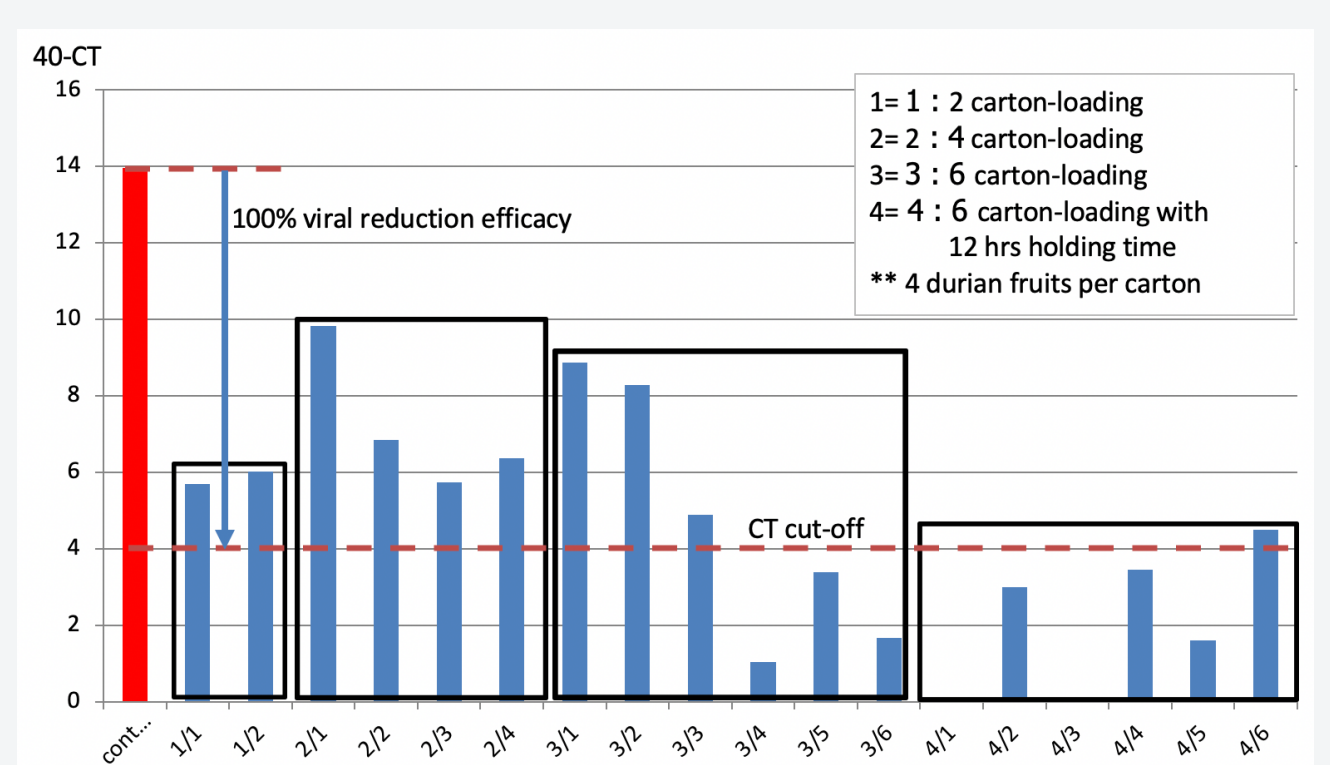
- NaOCl 3,000 ppm with holding time of 45 sec was more effective to reduce SAR CoV2 than that of 2,000 ppm with maximum of 6-8 LogDiff.
- Stem was the most effective material for SAR CoV2 inactivation by NaOCl 3,000 ppm.

Effect of OZ-CT (900 / 3,000 ppm) treatment for PEDV reduction on carton outer



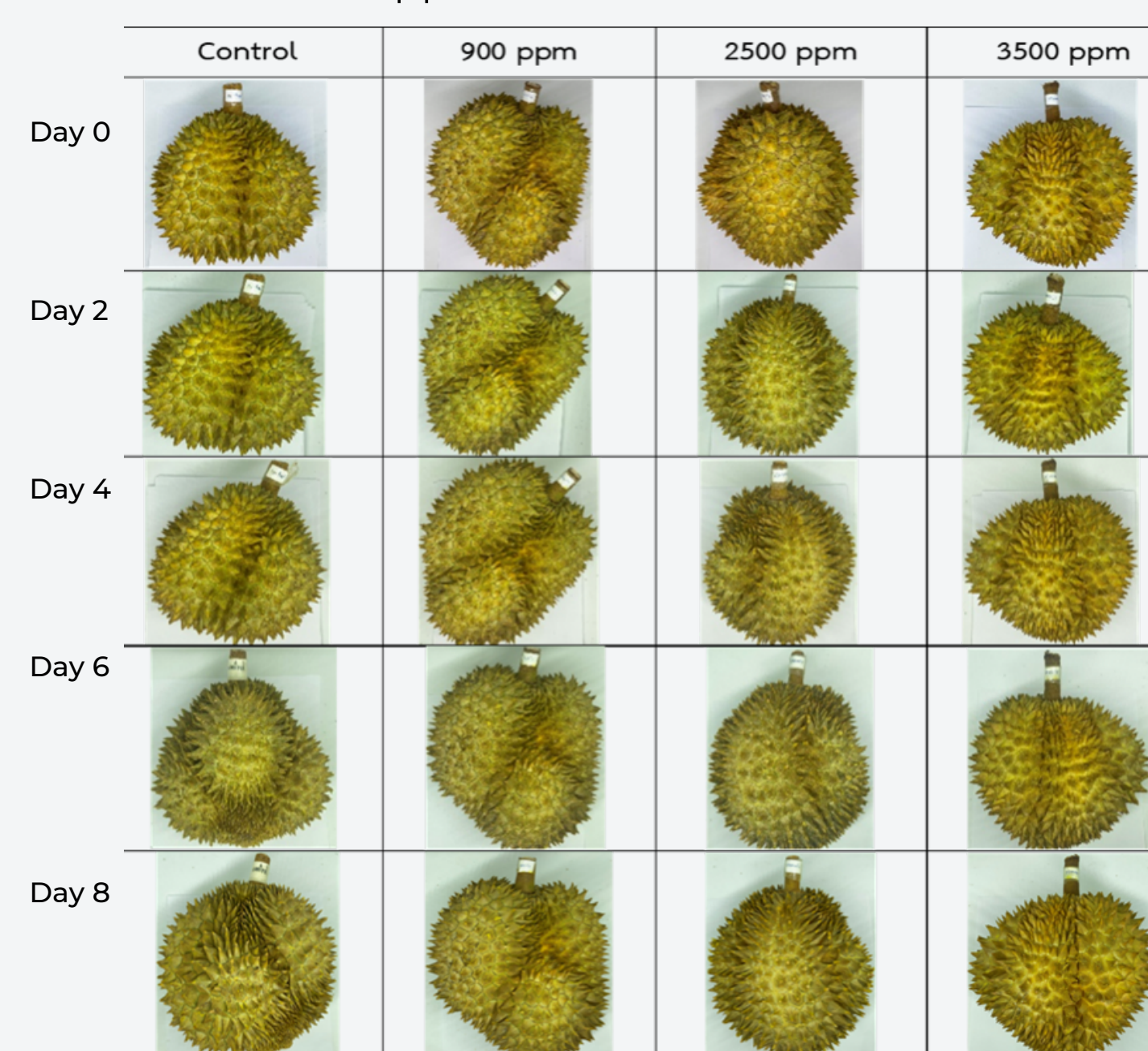
- The OZ-CT treatment without holding time was less effective on the carton outer for the 4 and 6 carton-loadings than that of 2 cartons for the viral reduction.
- OZ-CT treatment was 100% effective to reduce PEDV viral load on the carton outer when increase the holding time up to 12 hrs.

Effect of OZ-CT (900 / 3,000 ppm) treatment for PEDV reduction on durian stem inside carton

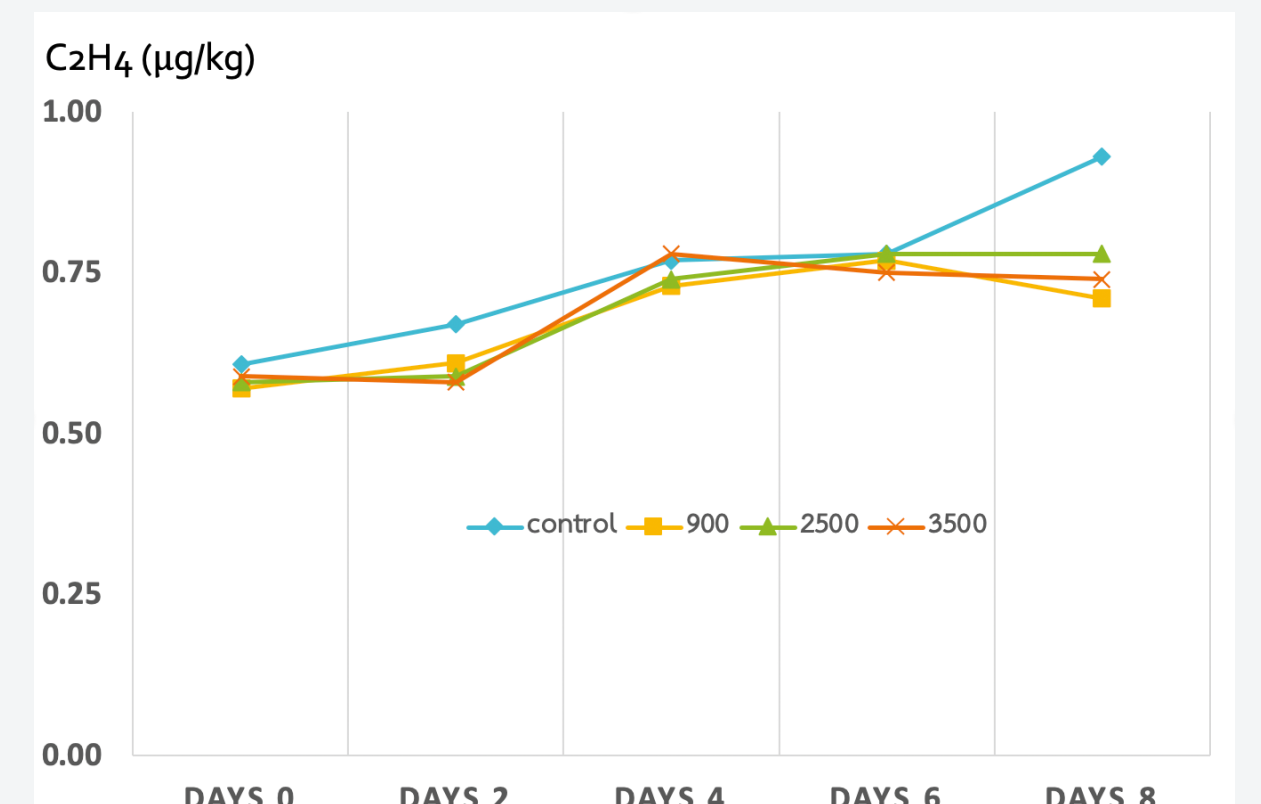


- The OZ-CT without holding time was less effective on the fruit stem inside the 4 and 6 carton-loadings than that of 2 cartons for the viral reduction.
- OZ-CT treatment was up to 100% effective to reduce PEDV viral load on fruit stem when increase the holding time up to 12 hrs.

Effect of OZ-CT (900 / 3,000 ppm) treatment on appearance of durian fruit



- The OZ-CT treatment did not cause adverse effect on the durian fruit during cold storage of 8 days at 13C.
- The OZ-CT fruits were found to delay the onset of ethylene peak, causing 3-4 day ripening shift possibly due to the effect of ozone as ethylene scrubber.

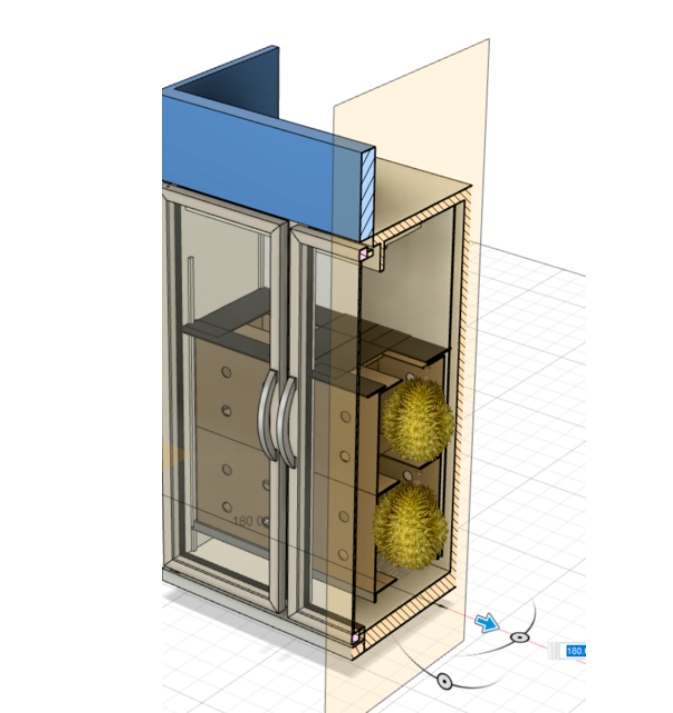
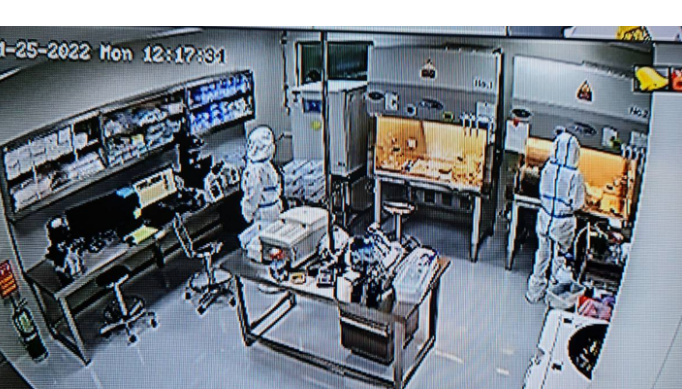


## Research Methodology

Lab test : PEDV ozone fumigation



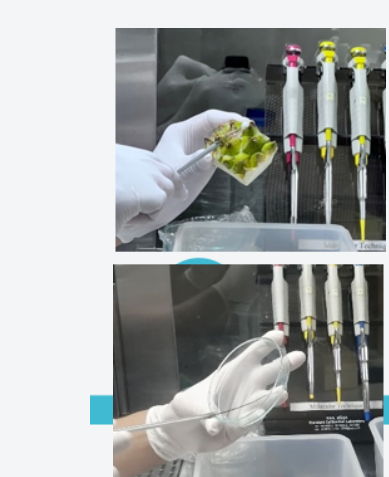
Lab test : SAR-CoV2 ozone fumigation (Biosafety level 3)



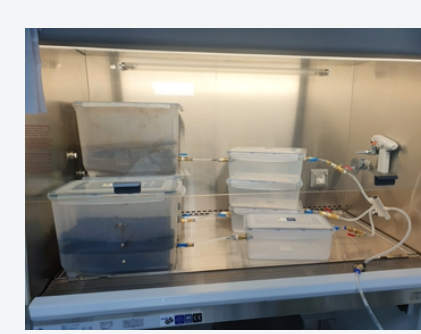
Semi-industrial scale test: PEDV ozone & chemical treatments



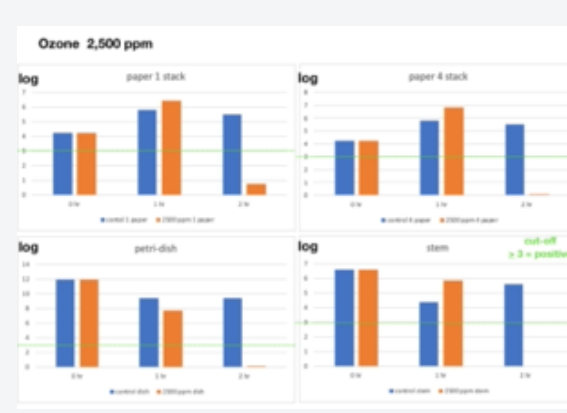
1/3 volume of 20' container test: PEDV ozone & chemical treatments



- Virus spiked on samples
1. Durian rind
  2. Durian stem
  3. Petri dish
  4. Carton paper



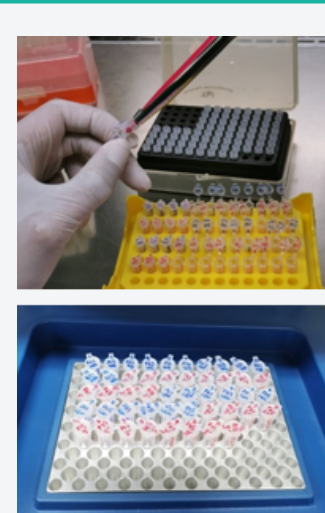
RT-PCR / RT-qPCR survival load analysis



Viral load preparation PEDV / SAR CoV2



- OZ-CT hurdle treatments
1. Ozone gas 900, 2500, and 3500 ppm
  2. NaOCl solution 3,000 ppm
  3. Ozone & NaOCl combination



Data analysis on disinfectant efficacy

## Conclusion

- The OZ-CT hurdle technology was successfully developed to reduce the coronavirus SAR CoV2 and PEDV contaminated on durian fruit in both lab and semi-industrial scale.
- The combination of NaOCl 3,000 ppm and Ozone gas 900 ppm was the optimal condition in this work that can reduce the viral load up to 100%.

## Acknowledgements

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