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Simulation study to assess the effectiveness of gamma radiation for inactivation of viruses on food packaging material

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\textbf{ABSTRACT}

The recent COVID-19 pandemic spread across the globe has raised the concern about the possible transmission of viruses through food packaging material during domestic and international trade. Therefore, mitigation strategies are needed to address these safety issues. Preliminary in-silico study showed that interactions between food packaging material and viral surface proteins were possibly hydrophobic in nature with most favourable interaction having a binding free energy of $-5.24$ kcal/mol. Since these interactions can cause viruses to adsorb on the food packets and get transmitted during supply chain, it is necessary to inactivate the viruses. In this context, efficacy of gamma irradiation in inactivating the viruses on the food packaging material was assessed. For this simulation study P1 (virulent) bacteriophage of \textit{E. coli} was used as a model system. Gamma irradiation of food packets at an absorbed dose >8 kGy was found to completely inactivate the infectivity of P1(virulent) bacteriophage when co-cultured with \textit{E. coli} host and assayed for viral plaque formation. Reduction in infectivity of P1(vir) phage was more prominent at ambient temperature ($25 \pm 2 \degree C$) as compared to cold temperature ($6 \pm 2 \degree C$) when assayed after storage (one week). Gamma irradiation (2 kGy) completely inactivated the virus particles on food packets when stored for 1 week at both the above temperatures. It is thus proposed that gamma irradiation (2 kGy) can possibly be integrated as a final treatment of the packaged food products to rule out the possibility of viral transmission. However, the efficacy of radiation processing against different pathogenic viruses needs to be determined prior to actual commercial deployment.

\textbf{1. Introduction}

The COVID-19 pandemic caused by SARS-CoV-2, a novel coronavirus, has spread worldwide in almost every country. This has adversely impacted the different socio-economic domains of the life across the nations negatively affecting their economies. The food supply sector has also been significantly affected during this pandemic. As per the World Health Organization (WHO) and Centre for Disease Control (CDC), the primary source of disease chain propagation is human-to-human contact through respiratory droplets. However, the likelihood of virus transmission through other infected material, including food and packaging surfaces cannot be completely ruled out (Han et al., 2021). Also, there are limited scientific reports on the duration for which SARS-CoV-2 can remain viable on food-packaging surfaces.

Occurrence and detection of SARS-CoV-2 in frozen foods apparently indicate that viral contamination and food borne transmission may present a systemic risk in the ongoing pandemic. Since the beginning of July 2020, at least nine incidences of food contamination have been reported across China where occurrence of SARS-CoV-2 was documented on imported foods, predominantly on the packaging materials (Han et al., 2021). Furthermore, Chinese Centre for Disease Control and Prevention also pointed that imported sea-foods contaminated with the virus were the more probable cause of the re-emerged outbreak (China Daily, 2020; Global Times, 2020). New Zealand had also revealed viral transmission through an import receiving facility. Besides, during April–May 2020, the CDC identified significant (16,233) cases of COVID-19, which includes 86 deaths, among the workers at meat and poultry processing facilities located in 23 states (CDC 2020).

The possibility of virus transmission through frozen food is possible as the virus has been reported to be retained in frozen flesh foods for up to 21 days (Chin et al., 2020). Due to the limitation of proper scientific evidences, the WHO has recommended further investigation of frozen foods and packaging as a potential source of transmission. Though different quality checks are implemented in all the stages of the food...
processing and supply chain, utmost safety considerations are required at the consumer level as this serves as the prominent source of infection.

There are different processing modalities that are implemented during food processing. However, gamma irradiation being an eco-friendly, cold physical process is being used in more than 60 countries across the world for various socio-economic applications such as assurance of food safety, security, to overcome quarantine barrier of international trade, sterilization of medical devices, inactivation of bacteria and viruses in animal sera and bio-therapeutics (Jinia et al., 2020). Therefore, in the present simulation study, P1 (virulent) phage was employed as a model system for assessing the effectiveness of gamma radiation technology for disinfecting food packaging material, as all the viruses have a similar structure comprising of viral capsid protein encapsulating the nucleic acid. Moreover, an in-silico study to understand the nature of interactions between polyethylene (commonly used as food packets) and viral surface proteins was also performed.

2. Material and methods

2.1. In-silico interaction of viral surface proteins with polyethylene

The protein structure file (.pdb file) of some of the viral surface proteins like SARS-CoV-2 spike glycoprotein (PDB ID: 6VXX), major capsid protein P1 of the phage phi6 (PDB ID: 4K7H), capsid protein P1 of the phage phi8 (PDB ID: 4BTP), were obtained from protein data bank. The 3D structure data file (.sdf file) of polyethylene was obtained from https://www.chemtube3d.com. The interaction of the protein with polyethylene was predicted using Autodock tools version 1.5.6 (Morris et al., 1998), with the Lamarckian genetic algorithm (Morris et al., 1998). For preparation of protein input files, all water molecules were removed, polar hydrogen were added and molecule’s energy was minimised. The grid box covering whole protein structure for docking was centered at x = 197.505, y = 223.533, z = 207.441 points for SARS-CoV-2 spike glycoprotein, x = 87.416, y = 17.821, z = 28.335 points for major capsid protein P1 of the phage phi6 and x = -27.158, y = 371.387, z = 417.904 points for capsid protein P1 of the phage phi8. Docking for 50 number of runs was carried out using Lamarckian Genetic Algorithm (LGA), and all other parameters set to default. The results were evaluated using inter molecular interactions and binding free energy (ΔGbind) in the lowest energy cluster with maximum cluster size. The interaction was visualized using Pymol (Seeliger and de Groot, 2010) and were calculated using Ligplot (Laskowski and Swindells, 2011).

2.2. Phage lysate preparation and enumeration of plaque forming unit

The model system used for the study was P1 bacteriophage which infects Escherichia coli (E. coli MG1655: F' iuvG rfb-50 rph-1) bacteria and cause cell lysis forming plaques. The phage lysate was prepared as per the method described earlier. Briefly, exponentially (O.D.600nm ~ 0.3) growing E. coli MG1655 culture (5 ml), was co-incubated with P1 phage stock (200 μl), in Luria broth medium supplemented with 0.2% glucose and 25 mM of calcium chloride. The incubation was performed under shaking condition at 37°C. After 6 h, the culture lysate was centrifuged and the cell free supernatant containing viral particles was collected. The plaque forming units (PFU) were determined using phage titration, where different dilutions of the lysate were added to 100 μl culture of E. coli cells and mixed with Top-agar (0.8% agar in nutrient broth supplemented with 0.2% glucose and 25 mM calcium chloride) and poured onto the pre-set bottom agar plates. These plates were kept at 37 °C overnight and the PFU enumeration was performed the following day.

2.3. Gamma radiation treatment

An aliquot (100 μl) of the viral lysate was spread onto 4 cm² area on the packaging material (low density polyethylene; 200 gauge) under sterile conditions and dried for around 3-4 h at room temperature. This was later subjected to gamma radiation treatment in a cobalt-60 gamma chamber at Food Technology Division, Bhabha Atomic Research Centre, Mumbai, India. The dosimetry was performed using standard ceric-cerous sulphate dosimeter where the absorbed dose was measured by potentiometry (American Society for Testing and Materials, 1993). The ceric-cerous sulphate dosimeters (9 nos.) were affixed on a plastic plate in three rows and columns at equal spacing and three such plates were placed across the chamber. The absorbed dose was determined from potential difference using the dose calibration data. The ratio of the maximum (Dmax) to the minimum dose (Dmin) in the irradiation chamber was calculated as the dose uniformity ratio (Dmax/Dmin), which was found to be 1.25. The dose of irradiation was used in a range of 2-10 kGy. After the radiation treatment individual samples were reconstituted and dilution plating was performed as described above.

2.4. Simulation for phage inactivation from the food packet by irradiation

The simulation was performed wherein a food commodity (~150 g turmeric) was packed in LDPE packets. The P1 phage lysate stock was spread onto the package and dried as described above. These packets were stored at ambient (25 ± 2 °C) as well as cold temperature (6 ± 2 °C) for one week, simulating the time period required during transport. At the end of one week, the food packets were subjected to different doses (2-10 kGy) of gamma radiation. At the end, infectivity of the reconstituted phage was evaluated as per the method described above. All the experiments were conducted in three independent sets having three replicates each.

3. Result and discussion

Countries like USA, China, Germany, Brazil, Japan, France, India, are among the largest importers as well as exporters of food and agricultural commodities. The import-export flows of food commodities could pose threat of viral transmission during international/domestic trade along with the food commodities.

A preliminary study was performed to understand the kind of interactions between viral surface protein and food packaging material. Since polyethylene is among the most commonly used food packaging material, its interaction with surface proteins of some of the RNA viruses was analysed through in-silico studies. The binding energy (ΔGbind) obtained after docking the interacting molecule with the protein, provides the binding affinity between the two molecules. The degree of binding between the protein and interacting molecule is termed as binding affinity. Since the free energy of a favourable system is always negative as it is the energy released when two molecules interact, therefore a more negative binding energy denotes high binding affinity. It was observed that the major interactions between the polyethylene (commonly used as food packets) and viral surface proteins are possibly hydrophobic in nature (Fig. 1). The interaction of the major capsid protein P1 of the bacteriophage phi6 with polyethylene showed a binding energy of ~5.24 kcal/mol, while that of SARS-CoV-2 spike protein with polyethylene was found to be ~3.43 kcal/mol. The capsid protein P1 of the bacteriophage phi8 showed a binding energy of ~2.78 kcal/mol. The interactions were found to be weak in nature but favourable to let the viruses adsorb on the food packets. Although these weak interactions can be disintegrated by soaps and detergents, but since the idea is inconceivable for food packets, alternate technological intervention like gamma radiation is necessary for effective control of viruses. Further, the efficacy of gamma irradiation was evaluated for phage inactivation from the surface of the food packaging material.
3.1. Determination of gamma irradiation dose required for inactivation of P1 phage

The complete loss of P1 phage infectivity was achieved by the application of gamma radiation at a dose of 8 kGy (Fig. 2). The phage lysate showed a titre of $7.08 \pm 0.02 \log_{10}$ PFU/ml. The phages upon adsorption onto the food packaging material showed a titre value of $6.08 \pm 0.02 \log_{10}$ PFU/ml. The dose required to reduce phage titre by one log ($D_{10}$ value) was estimated based upon phage infectivity inactivation kinetics. The $D_{10}$ value was found to be $\approx 1$ kGy after adsorption at the total titre of $7.08 \pm 0.02 \log_{10}$ PFU/ml in suspension (Fig. 3A). Most of the coronaviruses have been reported to have a $D_{10}$ value of less than 2 kGy. The virus sensitivity to irradiation also depends on the medium of sample suspension. Previous studies on independent samples of MS2 bacteriophages in water, autoclaved raw sewage, and a tryptone solution were exposed to gamma rays which showed that the dose required for a 1 log reduction was 0.5 kGy in water, 1.0 kGy in autoclaved raw sewage, and 1.2 kGy in 1% tryptone (Jebri et al., 2013). A more recent study by Leung et al. (2020), showed that radiation dose of 10 kGy was required to completely inactivate $10^{6.5}$ TCID$_{50}$/ml of SARS-CoV-2, where TCID$_{50}$ is the median tissue culture infectious dose and the value corresponds to 6.5 log$_{10}$units. The inactivation of viruses by gamma radiation can be attributed to the destruction of genetic material either directly by radiolytic cleavage or cross-linking, or indirectly leading to radiolysis of water molecules generating hydroxyl radicals (OH), which cause irreparable damage to the nucleic acids and proteins (Hume et al., 2016). In this study the P1(vir) phage particles were adsorbed onto the surface of the low density polyethylene packaging material, making them more sensitive to environmental conditions and radiation stress. However, if the phage particles remain suspended in Luria Broth medium (containing 0.2% glucose and 25 mM of calcium chloride), a much higher dose was required for its complete inactivation (Fig. 3B), indicating that viruses in protein rich medium have a protective mechanism which dampens the effects of gamma irradiation. Some studies have reported that proteins in solution have a negative impact on gamma radiation induced degradation, by acting as a scavenger (Hume et al., 2016). These scavenger molecules quench hydroxyl radicals thereby preventing the destruction of viral nucleic acids. Previous studies recommended 20 kGy radiation dose as safe for inactivation of viruses like coronaviruses. A more recent study has shown that even at a dose of 10 kGy the SARS-CoV was completely inactivated (Feldmann et al., 2019). These studies were performed with the virus suspended in cell culture medium (like Dulbecco’s modified Eagle’s medium [DMEM] with 10% fetal bovine serum [FBS]), which is rich in protein and therefore required a higher dose of inactivation.

3.2. Low dose of gamma irradiation required for inactivation of phage from food packets

On the infected food packets, the viral infectivity reduced on its own at cold temperature from $6.08 \pm 0.02 \log_{10}$ PFU/ml to $2.57 \pm 0.05 \log_{10}$ PFU/ml after one week. Reduction in infectivity of P1 phage was more prominent at ambient temperature and this reduced to $1.7 \pm 0.1 \log_{10}$ PFU/ml during one-week time period. Furthermore, upon gamma irradiation (2 kGy), no plaques were observed indicating that dose of 2 kGy or above is able to reduce the infectivity of the P1(vir) phage completely (Fig. 4).

An earlier report too has indicated that human coronaviruses can remain infectious on inanimate surfaces like metal, glass or plastic under room temperature for up to 9 days (Kampf et al., 2020). Besides, previous studies have shown that at relatively higher temperatures including 30°C or more the duration of persistence of virus is shorter.
(Kampf et al., 2020). This is in accordance with our observation, and therefore only 2 kGy gamma radiation dose was found to be effective in inactivating the P1 phage at the initial titre level of 7.08 ± 0.02 log10 PFU/ml. Benefits of the lower dose of irradiation required to inactivate phage is that it can be applied to a variety of food commodities including cereals, fresh fruits and vegetables, seafood and meat (fresh and frozen), the major food commodities traded internationally.

4. Conclusion

The current simulation study ascertained the effectiveness of gamma irradiation (2 kGy) in inactivating P1(vir) phage from the food packets after storage of one week. This irradiation dose offers a practical feasibility for irradiation of broad range food commodities and may provide a solution to the concern of viral contamination through trade during the current scenario.

Fig. 2. Figure shows the plaque forming ability of P1 phage on (E. coli), (A) Control (non-irradiated samples): E. coli cells infected with P1 phage showing plaques (marked by arrows) at dilution 10^-1, (B) Irradiated at 8 kGy: No plaques observed even in undiluted samples.

Fig. 3. Figure showing the loss in infectivity of the P1 phage virus as observed by reduction in phage titre upon irradiation (A) Viral phage particles dried onto the surface of food package material, (B) Viral particles suspended in liquid medium. Different letters (a-d) indicate significant differences in the mean value at p < 0.05.

(Kampf et al., 2020).
Author Statement

Jyoti Tripathi: Conceptualization, Writing - original draft, Writing-review & editing, Investigation, Formal analysis, Validation, Data curation. Sudhanshu Saxena: Conceptualization, Writing - original draft, Writing-review & editing, Investigation, Formal analysis. Satyendra Gautam: Conceptualization, Writing - original draft, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

American Society for Testing and Materials, 1993. Standard practice for use of a ceric cerous dosimetry system. E1205-93. In: Annual Book of Standards, vol. 12. American Society for Testing and Materials, West Conshohoken, PA 02.

Centers for Disease Control and Prevention (CDC), 2020. Update: COVID-19 Among Workers in Meat and Poultry Processing Facilities-United States, April-May 2020. http://www.cdc.gov/mmwr/volumes/69/wr/mm6927e2.htm, (Accessed 2 September 2020).

Chin, A.W.H., Chu, J.T.S., Perera, M.R.A., Hui, K.P.Y., Yen, H., Chan, M.C.W., Peiris, M., Poon, L.L.M., 2020. Stability of SARS-CoV-2 in different environmental conditions. The Lancet Microb. 1 (1) https://doi.org/10.1016/S2666-5247(20)30003-3.

Cosconati, S., Forli, S., Perryman, A.L., Harris, R., Goodsell, D.S., Olson, A.J., 2010. Virtual screening with AutoDock: theory and practice. Expert Opin. Drug Discov. 5, 597–607.

China Daily, 2020. Experts see similarity in Beijing, Dalian outbreaks. https://www.chinadaily.com.cn/a/202007/30/WS6f228da3a31083481725d32c.html, (Accessed 2 September 2020).

Feldmann, F., Shupert, W.L., Haddock, E., Twardoski, B., Feldmann, H., 2019. Gamma irradiation as an effective method for inactivation of emerging viral pathogens. Am. J. Trop. Med. Hyg. 100 (5), 1275–1277. https://doi.org/10.4269/ajtmh.18-0937.

Global Times, 2020. COVID-19 Outbreaks in Wuhan, Beijing and Dalian Share Certain Similarities: China’s Top Epidemiologist. https://www.globaltimes.cn/content/1196130.shtml, (Accessed 2 September 2020).

Han, J., Zhang, X., He, S., Jia, P., 2021. Can the coronavirus disease be transmitted from food? A review of evidence, risks, policies and knowledge gaps. Environ. Chem. Lett. 19 (1), 5–16. https://doi.org/10.1007/s10311-020-01101-4.

Hume, A.J., Ames, J., Rennick, L.J., Duprex, W.P., Marzi, A., Tonkis, J., Mühlberger, E., 2016. Inactivation of RNA viruses by gamma irradiation: a study on mitigating factors. Viruses 8 (7), 204. https://doi.org/10.3390/v8070204.

Jebri, S., Hmaied, F., Jofre, J., Yahya, M., Mendez, J., Barkallah, I., Hamdi, M., 2013. Effect of gamma irradiation on bacteriophages used as viral indicators. Water Res. 47 (11), 3673–3678. https://doi.org/10.1016/j.watres.2013.04.036.

Jinia, A.J., Sunbul, N.B., Meert, C.A., Miller, C.A., Clarke, S.D., Kearfott, K.J., Matuszak, M.M., Pozzi, S.A., 2020. Review of sterilization techniques for medical and personal protective equipment contaminated with SARS-CoV-2. IEEE Access 8, 111347–111354. https://doi.org/10.1109/ACCESS.2020.3002886.

Kampf, G., Todt, D., Pfaender, S., Steinmann, E., 2020. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J. Hosp. Infect. 104 (3), 246–251. https://doi.org/10.1016/j.jhin.2020.01.022.

Laskowski, R.A., Swindells, M.B., 2011. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model. 51 (10), 2778–2786. https://doi.org/10.1021/ci200227u.Epub2011015.

Leung, A., Tran, K., Audet, J., Lavineway, S., Bastien, N., Krishnan, J., 2020. In vitro inactivation of SARS-CoV-2 using gamma radiation. Appl. Biosaf. 25 (3), 157–160.

Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A. J., 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J. Comput. Chem. 19 (14), 1639–1662.

Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., Olson, A.J., 2009. Autodock4 and AutoDockTools4: automated docking with selective receptor flexibility. J. Comput. Chem. 30, 2785–2791.

Seelig, D., de Groot, B.L., 2014. Lipid docking and binding site analysis with PyMOL and Autodock/Vina. J. Comput. Aided Mol. Des. 28, 417–422.