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To cite this article: B Velebit *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012101

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Surface adsorption and survival of SARS-CoV-2 on frozen meat

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Abstract. The first case of a severe acute respiratory syndrome caused by coronavirus-2 was reported in December 2019 in China. The disease spread globally quickly, causing the 2019–2021 COVID-19 pandemic. The meat industry became concerned over the possibility of transmitting the virus in the slaughterhouse environment. The level of air exchange strongly affects the distribution of SARS-CoV-2 aerosols within the slaughterhouses. The adsorption of the SARS-CoV-2 virus on the surface of the frozen meat is dictated mainly by the interplay of electrostatic forces between the virion and tissue (pH) and environmental conditions (temperature and humidity) in the vicinity of adsorption micro-location. Suppose the virus contaminates the meat surface, whereby pH is 5.5 or less. In that case, it firmly adsorbs due to bonds established by protonated amine group and a hydrogen bond between the COOH group of the viral protein and oxygen in hydroxyl groups present on meat surfaces. The meat surface, coated with a thin water film, interacts with the SARS-CoV-2 virions by establishing strong hydrogen bonds. Although there is no proof of COVID-19 contraction by food consumption, the strong surface adsorption and ability of SARS-CoV-2 to survive meat freezing indicate a potential risk of virus transmission by meat.

1. Introduction

One of the most severe varieties belonging to the β -CoV genus of coronavirus – the SARS-CoV-2 – has emerged in late November 2019 in Wuhan, China causing a global pandemic. The World Health Organization (WHO) officially announced this fact on March 11, 2020 [1]. Over the following months, the infection rate raised progressively, so the disease became a severe global threat to public health, the international economy, and the eudaemonia of individuals.

The SARS-CoV-2 virion particle measures 125 nm in diameter. The particle itself consists of the envelope (composed of lipids and proteins) and a single-stranded RNA molecule (29,903 nucleotides). Viral RNA contains a couple of genes encoding structural proteins and enzymes responsible for its reproduction and spreading.

To control a pandemic, one of the risk assessment factors was understanding the virus's spreading mechanism. The majority of scientific reports have claimed that the principal transmission route of the SARS-CoV-2 was to be from person to person, primarily through aerosols or droplets released from the respiratory tract during coughing or sneezing and then inhaled [2]. Also, several reports indicated plausible transmission of the virus via the gastrointestinal tract based on the successful isolation and



identification of SARS-CoV-2 RNA from feces [3]. Although respiratory RNA viruses possess a lipid envelope, their general structure is not robust. Single-stranded RNA is a chemically unstable molecule and readily undergoes hydrolysis. In particular, ribonucleases that are ubiquitous in the environment (mainly originating from the human skin) tend to denature viral RNA quickly. Outside of the human body, SARS-CoV-2 has a short life. Virions tend to degrade in dry conditions and during irradiation by ultraviolet light from sunlight or artificial sources. The harmful effect of UV reflects in cross-linking of the purine and pyrimidine bases in the viral RNA. With these facts in mind, it is clear that the only chance for the SARS-CoV-2 virus to survive is to nest itself in places where it is protected and appears in huge numbers.

Following the reports on COVID-19 outbreaks in major U.S. and German meat processing facilities [4,5], the meat industry became concerned over the possibility of transmitting the virus in the slaughterhouse environment. Working conditions in slaughterhouses and meat processing plants are aimed to maintain a low ambient temperature for food safety reasons. However, these conditions improve the survivability of SARS-CoV-2 at lower temperatures in comparison to room temperature [6]. Next, the coughing or sneezing of the infected workers in this environment is followed by airborne transmission of the aerosolized virus from the lungs. Duration of the aerosol infectivity is mainly dependent upon droplet size and air circulation intensity. Tiny virus-laden aerosol droplets evaporate quickly on the surfaces, and if the intensity of air circulation in a closed environment is high, one could consider that the virus does not vanish at all. The majority of slaughterhouses are equipped with a forced ventilation system. The air exchange inside these facilities consists of forceful moving of outdoor air and recirculating inside airflow. In some meat industries, operating procedures such as meat processing and packaging involve minimal and close contact between the workers during all-day shifts, so the level of air exchange strongly affects the distribution of SARS-CoV-2 aerosols indoors, presenting significant risk.

2. Meat surface adsorption of the SARS-CoV-2

The WHO report declared the possible transmission of SARS-CoV-2 by frozen food packages in February 2021 [7]. This report raised concerns if COVID-19 could be contracted by consumers coming into contact with contaminated frozen foods and packaging. Of course, this virus is not a foodborne pathogen, transmitted by a fecal-oral route such as Norovirus or Hepatitis A virus, diminishing the risk of COVID-19 contraction by oral consumption to almost zero. However, β -CoV genus coronaviruses such as SARS-CoV and MERS-CoV substantially differ in transmissibility potential, which is reflected in a violent spreading rate, particularly noted in crowded environments [8]. The deposition of virus-contaminated droplets onto frozen meat and fomites significantly increases the risk of people being infected through daily contact with contaminated surfaces or objects (fomites).

The adsorption of the SARS-CoV-2 virus on the surface of the frozen meat is dictated mainly by the interplay of electrostatic forces' interactions between the virion and tissue (pH) and environmental conditions (temperature and humidity) in the vicinity of adsorption micro-location.

2.1. The influence of pH on SARS-CoV-2 adsorption to the meat surface

In a normal living muscle, the pH is approximately 7.2. At this pH, myofibrillar proteins have a net negative charge, otherwise responsible for water-holding capacity. Several hours after the slaughtering, during the *post mortem* changes, the pH value of the muscle tissue drops to pH=5.4-5.8 (pork), i.e., pH=5.4-5.7 (beef). Once the isoelectric point of myofibrillar proteins is reached, they possess a net neutral charge, decreasing their water-holding capacity.

The majority of SARS-CoV-2 virions carry a net negative charge at neutral pH since their isoelectric point is below pI [9]. However, the size of SARS-CoV-2 particles is relatively large (compared to other RNA viruses). This results in extreme heterogeneity of its outer surface proteins that contain multiple "patches" of positive and negative charge in the pH range rendering viruses stable [10, 11]. The isoelectric point of the SARS-CoV-2 "spike" glycoprotein is pI=6.2 [12]. Suppose a SARS-CoV-2 virus is transmitted on the surface of the muscular tissue when the meat pH is approximately 5.5. In that case,

the net charge of the SARS-CoV-2 particle will become positive due to the protonation of both the carboxylate and amine groups. Protonated amine group (NH_3^+) will electrostatically bind to the electron-rich meat matrix. At the same time, a hydrogen bond will be established between the COOH group of the viral protein and oxygen in hydroxyl (OH) groups present on meat surfaces. Both processes will promote strong virus adsorption.

2.2. The effect of humidity and temperature molecules on SARS-CoV-2 adsorption to the meat surface

The capacity of SARS-CoV-2 to survive and continue to be infectious is also governed by the humidity and temperature [13]. The complex interaction between the virus and meat surfaces is primarily governed by the surface energy of the water molecules [12].

In the liquid phase, present water molecules tend to shrink from their vapor phase on a surface between the outer edge of the viral envelope and meat matrix. Subsequently, the shrinking of water molecules forms liquid "bonds" of a curved shape [14]. The hydrophilic meat surface, coated with a thin capillary water film, interacts with the SARS-CoV-2 virions by establishing strong hydrogen bonds between water molecules and proteins protruding through the virus envelope. Furthermore, suppose there is a gap between two adjacent virus particles whose distance is smaller than the distance between the virus and meat surface. In that case, the water molecules can quickly fill that gap, creating an active centre for further aggregation of SARS-CoV-2 particles.

Once the temperature in the environment rises higher than 12°C , the thin water engulfing virions into complexes tends to vaporize, leading to molecular instability, significantly lesser water bridge-linking, and a lower quantity of SARS-CoV-2 particles that potentially could adsorb onto frozen meat surface. The fact indicates that in a slaughterhouse environment, at temperatures of $4\text{--}7^\circ\text{C}$, water molecules (meat matrix, condensation, washing) play an essential role in keeping virus-laden droplets infectious long enough.

3. SARS-CoV-2 survival in frozen meat

Currently, there is no scientific proof to support the hypothesis that handling or consumption of food is associated with the contraction of COVID-19. Current scientific opinion is that the probability of exposure of consumers to SARS-CoV-2 via food is very low with high uncertainty [15]. However, the uncertainty associated with this estimate is high as there is still no evidence to confirm or refute the hypothesis that people can be infected by ingesting SARS-CoV-2 in food.

Several published papers have dealt with the topic. A research study by Han et al. [16] investigated a series of findings involving frozen food and storage environment as carriers of SARS-CoV-2, discussing the likelihood of contamination in "cold chain." In the same study, the authors hypothesized that low temperatures could generate a favorable condition for SARS-CoV-2 to maintain its viability during more extended exposure. Next, Dhakal et al. [17] assessed for survivability of herpes simplex virus 1 and SARS-CoV-2 in chicken and seafood. In this study, these two viruses were held at 4°C and at 0 h, 1 h, and 24 h after inoculation. At all three time points, recovery of SARS-CoV-2 was similar from chicken, salmon, shrimp, and spinach, ranging from 3.4 to 4.3 log PFU/mL. However, the rate of virus recovery from apples and mushrooms at T0 was significantly lower compared to poultry and seafood. In the end, they discovered that direct comparison of infectious virus titers with viral genome copies using the common RT-qPCR method could (at best) indicate only the presence of SARS-CoV-2 RNA. The result, in no way, correlates with the number of infectious viruses. Moreover, Harbourt et al. [18] reported that SARS-CoV-2 remains stable on porcine skin for 96 h at 22°C , 8 h at 37°C , and 14 days at 4°C . In their opinion, these findings indicate a substantial risk of infection and virus shedding by meat handling. However, no published data exist on the long-term survival and infectivity of SARS-CoV-2 in essential commodities such as beef and pork.

4. Conclusions

In meat processing facilities where close-proximity working procedures are often encountered, the aerosols from the respiratory tract of the infected workers act as a primary source for meat contamination. The poor filtration of recirculated air, high relative humidity, and a slightly acidic pH favour electrostatic adsorption of SARS-CoV-2 to the surface of frozen meat. Once adsorbed, SARS-CoV-2 is capable of surviving chilling and most probably freezing temperatures. Although there is no proof of COVID-19 contraction by food consumption, the food business operators should be aware of potential risks of virus transmission by meat. They should also implement more stringent antimicrobial measures during this pandemic since the SARS-CoV-2 is way more resistant than bacterial flora commonly found in food processing facilities.

Acknowledgment

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, according to the provisions of the Contract on research financing in 2021 (No 451-03-9/2021-14/200050 dated 05.02.2021).

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