Journal of Applied Microbiology, 2023, 134, 1–13
https://doi.org/10.1093/jambio/lxad133
Advance access publication date: 4 July 2023
Review Article

JOURNAL OF APPLIED MICROBIOLOGY
OXFORD UNIVERSITY PRESS

Antimicrobial efficiency of chlorine dioxide and its potential use as anti-SARS-CoV-2 agent: mechanisms of action and interactions with gut microbiota

Audry Peredo-Lovillo [1], Haydee Eliza Romero-Luna [2], Naida Juárez-Trujillo [3], Maribel Jiménez-Fernández [3,*]

[1]Facultad de Ciencias Químicas, Universidad Veracruzana, C.P. 94340, Orizaba, Veracruz, Mexico
[2]Subdirección de Investigación y Posgrado, Instituto Tecnológico Superior de Xalapa, C.P. 91096, Xalapa-Enríquez, Veracruz, Mexico
[3]Centro de Investigación y Desarrollo en Alimentos, Universidad Veracruzana, C.P. 91190, Xalapa-Enríquez, Veracruz, Mexico

*Corresponding author: Centro de Investigación y Desarrollo en Alimentos, Universidad Veracruzana, Dr. Luis Castelazo Ayala s/n, Industrial Ánimas, C.P. 91190, Xalapa-Enríquez, Veracruz, Mexico; E-mail: maribjimenez@uv.mx

Abstract

Chlorine dioxide (ClO_2) is a disinfectant gas with strong antifungal, antibacterial, and antiviral activities. Applied on hard, non-porous surfaces as an aqueous solution or gas, the ClO_2 exerts antimicrobial activity through its interaction and destabilization of cell membrane proteins, as well as through DNA/RNA oxidation, triggering cell death. As for viruses, the ClO_2 promotes protein denaturalization mechanisms, preventing the union between the human cells and the viral envelope. Currently, ClO_2 has been pointed out as a potential anti-SARS-CoV-2 clinical treatment for use in humans with the ability to oxidize the cysteine residues in the spike protein of SARS-CoV-2, inhibiting the subsequent binding with the Angiotensin-converting enzyme type 2 receptor, located in the alveolar cells. Orally administered ClO_2 reaches the gut tract and exacerbates the symptoms of COVID-19, generating a dysbiosis with gut inflammation and diarrhea as side effects, and once absorbed, produces toxic effects including methemoglobinemia and hemoglobinuria, which can trigger respiratory diseases. These effects are dose-dependent and may not be entirely consistent between individuals since the gut microbiota composition is highly heterogeneous. However, to support the use of ClO_2 as an anti-SARS-CoV-2 agent, further studies focused on its effectiveness and safety both in healthy and immunocompromised individuals, are needed.

Keywords: antimicrobials, disinfection, probiotics, intestinal microbiology, viruses

Introduction

Chemically active antimicrobials and disinfectants are used to control the spread of diseases caused mainly by the presence of pathogenic microorganisms and viruses. The effectiveness of disinfectants varies according to their chemical nature, which makes them effective in inactivating all kinds of viruses in addition to vegetative bacteria, mycobacteria, and bacterial spores. On the contrary, other disinfectants such as sodium hypochlorite and peroximonosulfate are only capable of eliminating vegetative bacteria and most enveloped viruses but are ineffective against nonenveloped viruses, mycobacteria, some fungi, and most bacteria spores (Campagna et al. 2016). Similarly, biofilm-forming bacterial cells have been reported to be between 10 and 100 times more resistant to chemical sanitizers than their planktonic cells, a fact that has represented an enormous challenge for the food industry due to the wide variety of food pathogenic bacteria that have the ability to generate biofilms (Yuan et al. 2021). In this sense, chlorine dioxide (ClO_2) is a strong oxidant agent recognized as an efficient disinfectant at low concentrations (0.1 mg mL^{-1}) and a wide pH range (3–8). The applications of ClO_2 as a biocide include mainly the disinfection of drinking water, although its use in ensuring the microbiological safety of agricultural products has gained importance since these products are highly perishable due to the poor pre-harvest management and subsequent contamination by phytopathogenic and human pathogenic microorganisms, conditions that allow them to be identified as potential vehicles for disease outbreaks (Ran et al. 2019, Sun et al. 2019). The ClO_2 is used in different steps of food production, such as processing, transportation, and packaging, reducing the incidence of cholera, dysentery, and typhoid fever, being also used as an effective antiparasitic and antiviral reagent (Ofori et al. 2018, Ran et al. 2019). In post-harvest preservation of foods, the ClO_2 is used to control the presence of undesirable bacteria such as *Escherichia coli* and *Listeria monocytogenes*, spore-forming organisms, and toxigenic molds such as *Aspergillus flavus* and *Nosema bombycis* (Wang et al. 2010). These effects delay the senescence of strawberries, tomatoes, longan fruit, and other harvested fruits and vegetables (Liu et al. 2020). For instance, it has been demonstrated an efficient reduction in green mold disease produced by *Botrytis cinerea* in green pepper and winter jujube, positively correlated with the ClO_2 concentration, finding that 150 μmol L^{-1} of ClO_2 inhibited the spore germination and changed the mycelial morphology (Fu et al. 2019). Similarly, these effects have been also reported in bacterial cultures of *E. coli*, where the mechanisms encompass inhibition of protein synthesis and oxidation of amino acids (Park et al. 2018).

Historically, ClO_2 achieved more popularity in the 1970s and 1980s; however, during the recent pandemic gener-

Received: January 18, 2023. **Revised:** June 27, 2023. **Accepted:** July 3, 2023

ated by Coronavirus Disease 2019 (COVID-19), its use has regained importance since it has been considered an effective option for the prevention of severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) transmission, once it is applied as an oral treatment to reduce infections in humans (Arellano-Gutiérrez et al. 2021, Insignares-Carrione et al. 2021, Medina-Avitia et al. 2021). In March 2020, the World Health Organization (WHO 2023) declared COVID-19 disease a pandemic with 118 000 confirmed cases, with 4291 deaths in 114 counties. Currently, the number of confirmed cases has reached 767 364 883, with 6 938 353 deaths worldwide until 5 June, 2023 (Burela et al. 2020, Jafari et al. 2022, WHO 2023). Due to the urgent need for COVID-19 treatment, humanity has turned to the development of new treatments to prevent the SARS-CoV-2 infection, including antiviral agents, repurposed drugs, and protease inhibitors to convalescent plasma, and monoclonal antibodies studied in clinical trials. Nevertheless, no licensed but potentially effective disinfectants, such as ClO_2, are used as a drug or medication able to avoid SARS-CoV-2 infection, even when there is no convincing scientific support about its mechanism of action, dose, administration protocol, and possible side effects (Burela et al. 2020, Jafari et al. 2022). The development of dysbiosis (an imbalance in the intestinal microbiota communities) has been one of the side effects reported as a consequence of the oral administration of ClO_2 in animal models and humans (Sultan et al. 2014, Lardieri et al. 2021, Sciurba et al. 2021). In this sense, it is important to consider that the uncontrolled intake of ClO_2 as an anti-SARS-CoV-2 agent can also trigger a malfunction of the gastrointestinal system (characterized by the presence of diarrhea), a decrease in the immune response, and, therefore, a greater severity of the symptoms of COVID-19 disease and a longer recovery time (Zuo et al. 2020). Hence, the present work aims to offer an extensive review of the mechanisms involved in the antifungal, antibacterial, and antiviral activities of ClO_2, as well as discuss the possible effects on host generated by its interactions with the gut microbiota after its oral consumption as a potential anti-SARS-CoV-2 agent.

Chemical and disinfectant properties of chlorine dioxide

ClO_2 is a water-soluble yellow gas with a short life in the air, but is stable as a free radical. ClO_2 is recognized as an attractive biocidal due to its strong oxidizing activity 2.5 times higher than that of diatomic chlorine, over a wide range of pH 3–8 and temperature, against proteins from viruses and bacteria (Kingsley and Annous 2019, Sun et al. 2019, Rubio-Casillas and Campra-Madrid 2021). The use of ClO_2 in the gas form is more effective for the disinfection of fruits and vegetables since it has the potential to reach more areas than liquid disinfectants due to its hydrophobic fruit coatings and possible surface air pockets (Kingsley and Annous 2019).

For drinking water disinfection, a ClO_2 solution is used since its application is more tolerable because it does not generate taste and odors (Medina-Avitia et al. 2021). In drinking water, safe and permissible concentrations of ClO_2 and its by-product, the chlorite ion (ClO_2^-), have been regulated by different agencies. For example, in the United States of America (USA), the EPA (Environmental Protection Agency) has established maximum concentrations of 0.8 and 1.0 mg L^{-1} for ClO_2 and ClO_2^- ions, respectively (Peltzer et al. 2022). Nevertheless, the FDA (Food and Drug Agency) in the USA, and the COFEPRIS (Federal Commission for Protection against Sanitary Risks) in Mexico, have pointed out that the prolongated administration (>90 days) of ClO_2 oral solution at concentrations >40 ppm (~40 mg L^{-1}), can cause kidney and liver failure as well as red blood cells disruption (Rubio-Casillas and Campra-Madrid 2021, Cao et al. 2022). In addition to drinking water, the ClO_2 solutions have been successfully used as a disinfectant in fruits and vegetables since the gaseous ClO_2, dispersed in the aqueous solution, can exert higher disinfectant properties due to its ease of penetrating bacterial cells in comparison to sodium hypochlorite (Lee et al. 2018, Hatanaka et al. 2021). Lately, ClO_2 had been recommended for SARS-CoV-2 inactivation when it is used at concentrations significantly higher than those used in drinking water disinfection. For example, disinfection of domestic areas can be achieved applying a ClO_2 concentration of 20 mg L^{-1}, whereas solutions over 500 mg L^{-1} are useful for disinfection of hospital and health care units (Malka and Park 2022, Peltzer et al. 2022).

The limitations for the use of ClO_2 as a disinfectant in food products and domestic or clinical surfaces are mainly based on its reactive nature related to its oxidizing capacity, which can make it toxic for human health at higher concentrations (Malka and Park 2022). Conversely, in Latin American countries the use and commercialization of ClO_2 solutions as a treatment and preventing therapy against SARS-CoV-2 infection increased in the last three years. This context represents an opportunity to venture into a new research field related to the emerging anti-SARS-CoV-2 agents, which must be studied and evaluated according to their antiviral effectiveness, as well as their impact host health (Peltzer et al. 2022). Therefore, it is necessary to describe the antimicrobial mechanisms of ClO_2 (specifically those involved in the SARS-CoV-2 inactivation), as well as its interactions with the gastrointestinal tract and gut microbiota (recognized as a modulator of the host immune response) once it is orally consumed as a therapeutic agent against SARS-CoV-2. This information may contribute to understand the effectiveness of ClO_2 as an antiviral agent and identify its safety under specific physiological conditions of host. Latest information related to this topic is given below.

Antimicrobial mechanisms of chlorine dioxide

Chemical disinfectants can be categorized into three classes according to their mechanisms of action, either as denaturants, reactants, and oxidants. Denaturing disinfectants include those substances that disrupt mainly protein and lipid structures from lipophilic enveloped viruses, such as, for example, quaternary ammonium salt compounds, phenolics, and alcohols. On the other hand, reactants (including aldehydes and ethylene dioxide) form and break covalent bonds, altering nucleic acids (DNA and RNA), and protein structure and synthesis. Finally, oxidants are the largest group used for disinfection and include peroxides, peroxymonosulfates, and halogens such as hypochlorite, iodine, and ClO_2. These disinfectants destabilize cell membranes, react with amino acids, interrupt protein synthesis, and oxidize proteins, enzymes, and possible DNA/RNA/proteins, making them useful for the elimination of a broad spectrum of pathogenic microorganisms (Campagna et al. 2016, Sun et al. 2019). The antimicrobial mechanisms of ClO_2 have not been deeply studied, but most of them are based on the generation of oxidative stress and the subsequent excessive production of reactive oxygen species

(ROS), which finally cause cell injury (Medina-Avitia et al. 2021).

As mentioned, the mechanism by which ClO_2 inactivates microbial species encompasses selective oxidation, resulting in the formation of ClO_2^- and chlorate (ClO_3^-) ions as the main by-products, with chlorite constituting ~70% of the total by-products of the reaction (Ofori et al. 2018). Moreover, ClO_2 releases oxygen when it encounters acidic environments (generated by lactic acid or acidity of pathogens), becoming alkaline due to the elimination by oxidation of acidifying unicellular organisms, which are unable to tolerate and dissipate the electromagnetic overload (Insignares-Carrione et al. 2020). One of the main advantages of using ClO_2 as a possible therapeutic agent against different kinds of infections is the impossibility of resistance by bacteria and viruses, due to the oxidative stress it generates, which selectively affects compounds such as phenols and thiols, the latter present in amino acids such as cysteine, tryptophan, tyrosine, proline, and hydroxyproline, essential for viability and activity of the undesirable microorganisms (Loginova et al. 2008, Stewart et al. 2008, Insignares-Carrione et al. 2020). Also, in comparison to chlorine, ClO_2 does not chlorinate organic compounds to produce carcinogenic trihalomethanes and does not react with ammonia to form chloramines. Both advantages make ClO_2 very attractive for its use as an antimicrobial agent in foods (Kingsley and Annous 2019).

The mechanisms by which oxidation of vital molecules occurs are not fully understood and are dependent on the species of microorganism and its biochemical characteristics, as well as its composition (Fig. 1). Generally, gaseous ClO_2 is less effective against Gram-positive than Gram-negative bacteria, while commonly molds and yeasts have an intermediated tolerance (Sun et al. 2019). Therefore, in the following lines, the antiviral, antifungal, and antibacterial activities of ClO_2 are listed, including their mechanisms of interaction and/or action.

Antibacterial properties

As reported by Singh et al. (2021), the gaseous form of ClO_2 has offered greater effectiveness for disinfection than the liquid form of ClO_2, since gaseous ClO_2 reaches and inactivates pathogenic bacterial cells because of its high diffusivity and penetrability, especially when it is used to obtain a safer food packaging. The antibacterial efficacy of ClO_2 is related to the destabilization of cell membranes, mainly by metabolism disruption after reactions between oxygenated compounds with ClO_2. Once ClO_2 penetrates the bacterial cell membranes, it can cause respiration inhibition and deterioration of the trans-membrane ionic gradient. In addition, gaseous ClO_2 can increase the permeability of the cell membrane by changing the spatial formation of lipids and proteins in the intermembrane space. Once inside the bacterial cell, ClO_2 oxidizes organic compounds, initiating cell metabolism interruptions, mainly bacterial protein synthesis derived from the oxidation of amino acids. Subsequently, in oxidated amino acids (cysteine, tyrosine, tryptophan, histidine, and proline), the ClO_2 forms disulfide analogs after oxidation of mercapto groups of enzymes, and finally a malfunction is observed in the physiological functions in those enzymes (Sun et al. 2019, Al-Sa'ady et al. 2020, Singh et al. 2021). For sporulated bacteria, such as *Bacillus subtilis*, the exposition to ClO_2 resulted in either the inactivation of spores or spore formation inhibition, both related to membrane damage (Sun et al. 2019).

Although its main application consists of drinking water disinfection, gaseous ClO_2 dissolved in water, has been successfully used against pathogenic bacteria of clinical origin. According to this, the use of ClO_2 has shown a higher antibacterial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* (isolated from sputum), *Enterobacter aerogenes* (isolated from urine), and methicillin-resistant *Staphylococcus aureus* (isolated from wound infection), in comparison with pure chlorine (Cl_2), which was 4–7 times less effective than ClO_2, against these same bacterial species (Al-Sa'ady et al. 2020).

The low resistance of Gram-negative bacteria to gaseous ClO_2 is boosted by the presence of a thin layer of peptidoglycan that is more easily penetrated by gaseous ClO_2 (Sun et al. 2019). In the food industry, the use of ClO_2 has evolved beyond its application as a sanitizing gas. For example, a custom-made antimicrobial film coated with sustained-release ClO_2 microcapsules in fresh mangos packaging (Zhang et al. 2019). The authors of the previous report observed that control mangos (without antibacterial ClO_2 film), showed a loss of edible and commercial value after 21 days of storage, while the mangos packaged with the antimicrobial ClO_2 film still retained edible and commercial value at the same storage time. These findings exhibit a potential use of solid ClO_2 for fresh food (mainly fruits and vegetables) preservation, based on its antimicrobial characteristics. In the same way, it has been reported that ClO_2 gas can reduce the bacterial load of *L. monocytogenes* (ATCC 19111, ATCC 19115, and ATCC 15313), *E. coli* O157:H7 (ATCC 35150, ATCC 43889, and ATCC 43890), and *Salmonella* Typhimurium (ATCC 19585, ATCC 43971, and DT 104), inoculated on fresh tomatoes surface, observing that when the ClO_2 gas concentration, relative humidity (RH), and treatment time increase, inactivation levels also reduced the bacterial load below the detection limit (0.48 log CFU per cm^2), either within 15 min of exposition to 30 ppmv of ClO_2 gas at 70% RH and within 10 min at 90% RH at the same ClO_2 gas concentration (Park et al. 2018). In this sense, it is possible to alter the effectiveness of ClO_2 by modifying intrinsic factors such as sample surface characteristics or food product characteristics, and extrinsic factors including gas concentration, RH, and treatment time (Park and Kang 2018).

Secondly, the ClO_2 gas has been also used to sanitize food contact surfaces by the inactivation of the biofilm cells of foodborne pathogens. For example, an exposure time of 20 min to 50 ppmv of ClO_2 gas at 60% of RH, resulted in log reductions from 2.08 to 4.62 and 2.08 to 4.41 of the biofilm cells of three pathogenic bacterial strains (*E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes*), on stainless steel and high-density polyethylene surfaces. While, with 50 ppmv of ClO_2 gas at 90% of RH, the levels of biofilm cells of all mentioned pathogens were reduced to below the detection limit [0.48 log CFU $(cm^2)^{-1}$], after 20 min exposure (Kim and Park 2022). Therefore, and based on the previous results, to establish an optimum ClO_2 gas treatment for the disinfection of both fresh fruits and vegetables as well as food processing facilities, environmental conditions such as RH, must be considered to maximize the antibacterial efficacy of ClO_2 (Kim and Park 2022).

Contrary to what was expected, there are currently no reliable reports about the impact of the use of ClO_2, either as

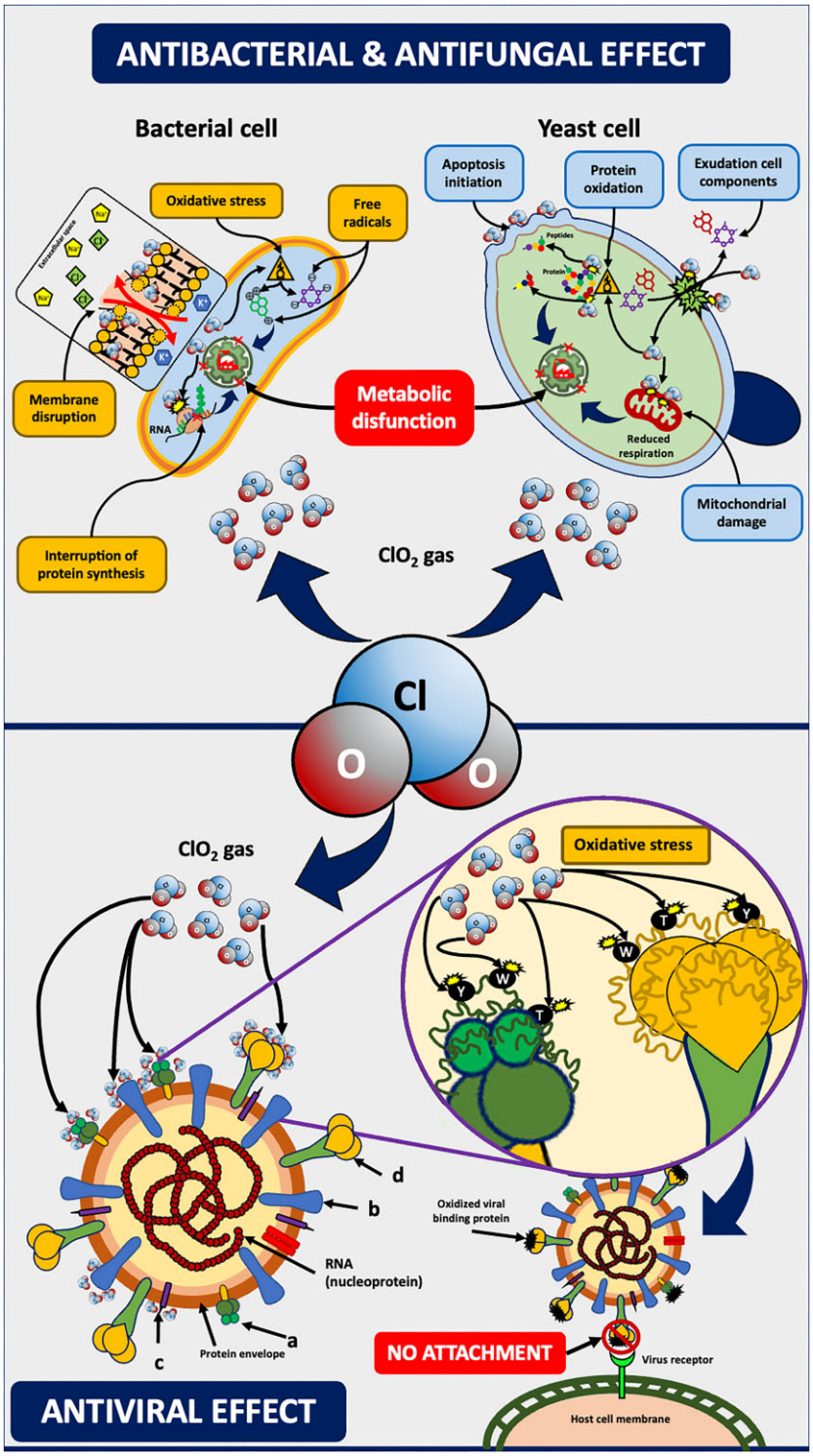

Figure 1. Antimicrobial mechanisms of chlorine dioxide (CO_2). In bacterial cells, CO_2 acts as a membrane disruptor, triggering the deterioration of the trans-membrane ionic gradient by a leakage of ions with physiological importance. Once inside the cell, CO_2 promotes the generation of free radicals and reactive oxygen species involved in oxidative stress. This oxidative effect contributes to the inhibition of protein synthesis, specifically at ribosomal level, where thiol (-SH) groups from amino acids are also oxidized to less stable compounds due to the loss of their disulfide bounds. In yeast cells, the CO_2 can induce apoptosis because of membrane destabilization, loss of integrity, and oxidation of membrane peptides. This membrane destabilization includes the exudation of cell components from the intra- to the extracellular space due to changes in permeability and lipid peroxidation. Once inside the yeast cell, the ClO_2 oxidizes proteins and enzymes to small peptides and inactive proteins, respectively. In the mitochondria, the presence of ClO_2 generates an abnormal membrane potential, this effect decreases the respiratory activity and therefore the cell viability. Finally, in viruses the ClO_2 acts as an oxidizing agent of viral binding proteins such as hemagglutinin (**a**), neuraminidase (**b**), membrane glycoproteins (**c**), and spike proteins (**d**). In these proteins, the ClO_2 oxidizes the thiol (-SH) groups in the amino acid residues such as tyrosine (**Y**), tryptophan (**W**), and threonine (**T**). The oxidation of viral binding proteins limits their interactions with the host receptors, preventing their attachment and subsequent infection. This effect has been observed specifically in the spike protein from SARS-CoV-2 and the angiotensin-converting enzyme 2 receptor in humans, after using ClO_2 gas as an anti-SARS-CoV-2 agent.

an aqueous solution or as a gas, on those desirable bacteria in food, such as lactic acid bacteria and/or probiotics (e.g. *Lactobacillus sakei* subsp. *carnosus* and *Leuconostoc mesenteroides* subsp. *mesenteroides*), which, according to the constitution of their cell wall, they could suffer less damage than Gram-negative bacteria once they interact with this disinfectant. Differences in tolerance between Gram-positive and Gram-negative bacteria to ClO_2, in a gaseous form, are based on the structural constitution of their cell wall. While Gram-negative bacteria have a thinner peptidoglycan layer, easily penetrated by gaseous ClO_2, Gram-positive bacteria show greater resistance since their peptidoglycan layer is thicker and more difficult to penetrate by the same gaseous ClO_2 (Vandekinderen et al. 2009, Sun et al. 2019). It should be noted that in the last two years, the intake of ClO_2 has become popular due to its potential activity as an anti-SARS-CoV-2 agent at a concentration of 2.19 mg L^{-1} (Peltzer et al. 2022). This activity and the recurrent consumption of ClO_2 suggest the presence of interactions of the sanitizing agent with the intestinal microbiota, including probiotics, potentially probiotic lactic acid bacteria, and other beneficial Gram-positive bacteria. However, the outcome of these interactions and their possible health effects are not fully understood or are still under analysis.

Antifungal properties

As part of its antifungal activity, the ClO_2 can inhibit the growth of *Penicillium digitatum*, using as a mechanism of action the destruction of membrane integrity of fungal cells (Liu et al. 2020). However, other alterations such as the promotion of apoptosis and changes in the membrane potential were observed as a response to the diffusive transport of ClO_2 into the cell. Once inside the fungal cell, the ClO_2 promotes significant levels of protein release, lipid peroxidation, changes in cell permeability, and hence the exudation of cell components. Also, in this same study, ClO_2 was related to different negative effects on mitochondrial functions, including abnormal membrane potential, compromising microbial respiration, and promoting the loss of cell viability. Treatments consisting of short exposure times and high concentrations of ClO_2 have been investigated to determine the optimum conditions for fungal inhibition through *in vivo* assays. An example of this is the complete inhibition of mycelial growth of *Alternaria alternata* and *Stemphylium vesicarium* after 3 min of treatment with 10 mg mL^{-1} of gaseous ClO_2, observed on the fresh tomato peel surface (Trinetta et al. 2013). Meanwhile, this same ClO_2 concentration was used in an *in vivo* study in which complete inhibition of *A. alternata* and *S. vesicarium*, previously inoculated on the fresh Roma tomatoes peel surface, was achieved after 7 and 5 min, respectively. As can be seen, the efficacy of ClO_2 can be reduced in *in vivo* studies since, in some cases, its application is carried out in low RH environments (RH $\leq$ 70%), which decreases its diffusion and subsequent penetration into the microbial cell (Park and Kang 2018, Park et al. 2018). In comparison, *in vivo* reduction of fungal populations by ClO_2 can be more efficient at RH of 90% than 70%, because at high humidity spore swelling occurs and, thus, the size of openings available to ClO_2 molecules increases, resulting in a greater antimicrobial efficacy (Sun et al. 2019). Further, the use of ClO_2 is not limited to fresh fruits, but also it has been used as an antifungal agent for fruit and vegetable packing house surfaces, being effective in the reduction of *Botrytis cinerea*, *Penicillium expansum*, *Mucor piriformis*, and *Cryptosporiopsis perennas* by spore inactivation after 1 min exposure to 3–5 μg L^{-1} ClO_2 aqueous solution, while effective control of *Stachybotrys chartatum*, *Chaetomium globosum*, *Penicillium chrysogenum*, and *Cladosporium cladosporioides* was observed at 1.4–1.8 μg L^{-1} ClO_2 solution after 24 h treatment (Trinetta et al. 2013).

As for the yeasts, the mechanism of action of ClO_2 is like that exerted in bacteria cells. An alteration in cell structure, as well as an inhibition of key enzyme activities of metabolic pathways, was observed in *Saccharomyces cerevisiae* strain 13 482 after exposure to 100 mg L^{-1} of ClO_2. Moreover, ion leakages of K^+, Ca^+, and Mg^+ were detected and related to the inactivation rate. Nonetheless, protein and DNA leakage were not observed during and after exposure to ClO_2 (Zhu et al. 2013).

Antiviral properties

The antiviral activity of ClO_2 has been well documented, specifically against echoviruses, enteroviruses, polioviruses, noroviruses, caliciviruses, mumps virus, and coronaviruses (in which SARS-CoV-2 is included) (Wang et al. 2005, Jin et al. 2013, Montazeri et al. 2017, Zhong et al. 2017). The antiviral activity of ClO_2 has been tested *in vitro* against human and animal viruses; for example, human influenza was inactivated by 99.99% at 15 s using 1 ppm, while with 10 ppm, the inactivation of Measles and Herpes viruses was achieved at 30 and 15 s, respectively (Sanekata et al. 2010). Similarly, ClO_2 concentrations from 0.2 to 10 ppm were used to inactivate or destroy poliomyelitis and hepatitis A viruses, rotavirus, human immunodeficiency virus type 1 (HIV-1), and coronavirus that causes SARS (Rubio-Casillas and Campra-Madrid 2021). In foods, it has been demonstrated that constant low levels of ClO_2 (0.63 ppm-h g^{-1}) can inactivate foodborne viruses such as Tulane virus, a human norovirus surrogate, on the surface of different berries (blueberries, raspberries, blackberries, and strawberries). Interestingly, the use of a direct constant level of ClO_2 allows equal treatments regardless of the size of the berry, since all the samples treated with ClO_2 showed the same reduction of 2.9 PFU (plaque-forming units) g^{-1} of virus load in all the samples (Kingsley and Annous 2019).

In addition to the doses used for virus inactivation, other factors such as temperature and pH have an important role in the viricidal activity of ClO_2. For example, Jin et al. (2013), reported the inactivation of enterovirus 71 (EV71) in drinking water samples, using a 4.24–6.62 mg L^{-1} min^{-1} of ClO_2. However, the inactivation of EV71 was temperature- and pH-dependent, with a maximum EV71 reduction of 4-log when the water samples were treated with the mentioned ClO_2 concentrations at 36°C and the pH adjusted to 8.2. Furthermore, damage in the EV71 genome was observed because of ClO_2 exposure. Through an RT-PCR analysis, it was possible to identify damage on the 5′ non-coding region (1–118 nt length), which is closely related to the viral infectivity. EV71 is an enteric human virus that spreads via the fecal–oral route and can be excreted from an infected individual to aquatic environments and spread to drinking water. In infected individuals, the EV71 is associated with hand, foot, and mouth disease, aseptic meningitis, encephalitis, and poliomyelitis-like paralysis (Solomon et al. 2010, Plevka et al. 2012). Thus, the EV71 inactivation in drinking water is an important feature to consider in the development of new antiviral agents.

ClO_2 inactivates virus by the denaturation of envelope proteins responsible for cell entry, a mechanism based on the oxidation of amino acids, mainly thiol (-SH) residues of tyrosine, threonine, and tryptophan. So, these covalent oxidative modifications trigger the denaturation of proteins from the viral envelope decreasing the viral infectiveness (Al-Sa'ady et al. 2020, Rubio-Casillas and Campra-Madrid 2021). An example of this is the inactivation of influenza virus by ClO_2 through oxidation of tryptophan residue (W153) in hemagglutinin (a spike protein of the viruses), thereby suppressing its receptor-binding capacity (Káli-Kullai et al. 2020). In addition to the antiviral effect previously described, ClO_2 has also demonstrated control and modulation over the symptomatic response generated by a viral infection. For example, an *in vitro* study carried out by Zhu et al. (2019), showed that ClO_2 can inhibit the synthesis of pro-inflammatory molecules (IL-1, IL-6, and TNF-α), derived from the exposition of pig alveolar macrophages and MARC-145 cells to porcine reproductive and respiratory syndrome virus (PRRSV1). In this study, the authors concluded that ClO_2 can reduce the viral replication of PRRSV1 by interrupting the synthesis of RNA and proteins, and thus, the pathogenesis of this disease is diminished. These findings suggest that the ClO_2 can not only be used as a useful agent to prevent viral infections, but also as an adjuvant in the control of the disease generated once the infection is present in the host.

Nowadays, the humanity is adapting to the "new normality" once cases of SARS-CoV-2 infections worldwide have been brought under control; however, during the period of confinement, alternative treatments using emergent anti-infective agents, including ClO_2, were explored. Specifically, ClO_2 gained popularity by being considered an anti-SARS-CoV-2 agent administered orally as an aqueous solution, even though its effectiveness and its possible side effects were not fully identified or verified. In this context, in the following lines, current information about the anti-SARS-CoV-2 efficiency of ClO_2, as well as its possible side effects derived from its interaction with the gut microbiota, once it is orally consumed, are described.

Effect of chlorine dioxide on SARS-CoV-2

As mentioned above, the main mechanism by which ClO_2 exerts its antiviral properties is related to its ability to denature the viral envelop proteins responsible for the cell attachment, preventing the subsequent replication, and host infection. In relation to SARS-CoV-2, previous computational studies using the Chemistry at HARvard Macromolecular Mechanics (CHARMM) (C36) force field (FF), have demonstrated, through molecular optimization and dynamics, a potentially anti-SARS-CoV-2 effect of ClO_2 based on its denaturing effect specifically on the disulfide bonds, formed between the spike protein of SARS-CoV-2 and ACE-2 (Angiotensin-converting enzyme type 2) receptors, located in the human alveolar cells (Hati and Bhattacharyya 2020, WHO 2020a). Such simulations, based on molecular structure, carried out to study protein structure–dynamics–function relationships, allowed to predict possible interactions of ClO_2 with the amino acids responsible for the formation of disulfide bonds. Based on the 54 tyrosine, 12 tryptophan, and 40 cysteine residues present in the spike protein of the SARS-CoV-2. Computational 3D simulations based on the data obtained from electron cryomicroscopy and manipulated with the ChimeraX (UCSF) augmented reality software, allowed to identify to the cysteine residues (Cys480–Cys488) as the target residues to be oxidize by ClO_2 to prevent the binding between SARS-CoV-2 spike protein and the ACE-2 receptor (Insignares-Carrione et al. 2020). Thus, the oxidative behavior of ClO_2 emerges as a therapeutic mechanism by which the formation of disulfide bonds and binding between the ACE-2 and spike protein of SARS-CoV-2 could be prevented, attenuating the incidence of COVID-19 (Insignares-Carione et al. 2021). In this regard, it was confirmed that the binding capacity of purified spike proteins to ACE-2 human receptors was inhibited by 50% in a concentration-dependent manner with concentrations of 7.6 and 5.8 μmol L^{-1} of ClO_2 for the SARS-CoV-2 type variants from England (variant B.1.1.7) and South Africa (variant B.1.351), respectively, indicating that ClO_2 can be a useful strategy in blocking the transmission of not just a specific SARS-CoV-2 variant (Ogata and Miura 2021). Commonly, aqueous solutions of sodium hypochlorite are used as an anti-SARS-CoV-2 agent; however, the use of hypochlorite for human administration is not approved and is very controversial. So, it has been reported that ClO_2 gas dissolved in water can be a up to 10 times stronger antiviral agent against SARS-CoV-2 than sodium hypochlorite. This fact was observed by Hatanaka et al. (2021), who used both disinfectant agents in the treatment of VeroE6/TMPRSS2 cells infected with SARS-CoV-2, observing inhibition of 99.99% of the virus within 10 s of exposure to a solution of 24 ppm of ClO_2. Conversely, a solution of 24 ppm of sodium chlorite only inactivated from 90 to 99% of the virus after 3 min of exposure to ClO_2 under similar conditions. Additionally, the antiviral activity of ClO_2 for the treatment of COVID-19 disease has been already evaluated in a clinical study carried out by Insignares-Carrione et al. (2021). In this research, the antiviral activity of ClO_2 was evaluated in 20 patients with active infection of SARS-CoV-2, who received a daily dose of a ClO_2 solution at 30 mg L^1 for 21 days. The results obtained were compared to those of a control group (20 patients who no received the oral ClO_2 solution). At the end of the 14th day of the study, symptoms in infected patients such as fever and chills decreased in their entirety, while cough and dyspnea decreased by 60 and 75%, respectively, in comparison with the control group. In summary, the studies previously discussed highlight the use of aqueous solutions of ClO_2, at controlled concentrations, as an alternative for the treatment of COVID-19; although, to offer more information about its effectiveness and safety, deeper clinical studies are still necessary.

Responses of gut microbiota to SARS-CoV-2 infection and chlorine dioxide oral administration

Concerning the foregoing, the following lines discuss the most recent findings related to the gut microbiota responses during SARS-CoV-2 infection and after oral administration of ClO_2.

Gut microbiota response to SARS-CoV-2 infection

Gut microbiota can indirectly mediate both the colonization of non-indigenous microorganisms and viral infections by stimulating host responses such as mucosal immune defenses (Xiang et al. 2021). Therefore, the gut microbiota is closely related to the proper functioning of immune system and can be altered by many immunologic diseases, including COVID-19.

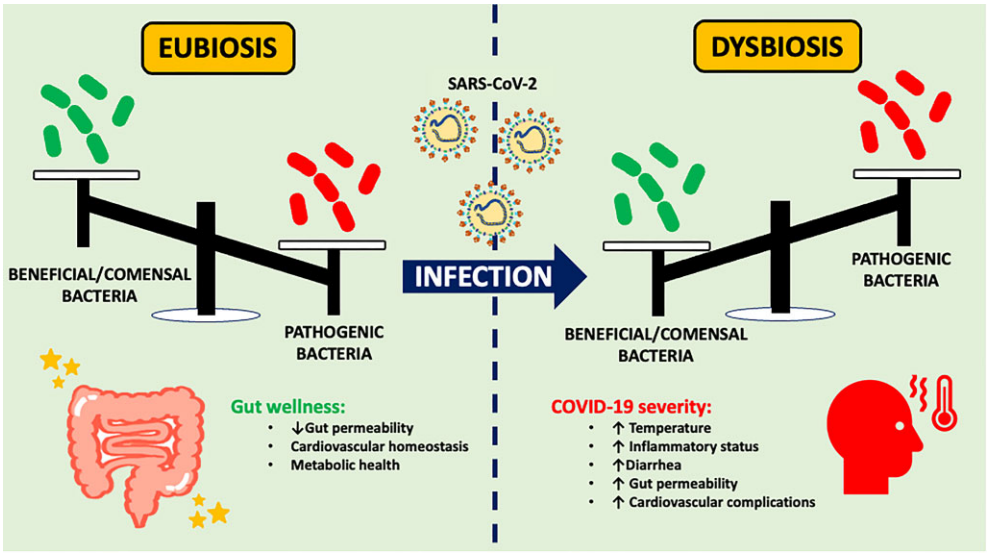

Figure 2. Gut microbiota response during SARS-CoV-2 infection. SARS-CoV-2 infection causes an alteration in the richness and diversity of human gut microbiota, generating a dysbiotic state that allows the increase in the concentration of gut pathogenic promoting the severity of COVID-19 symptoms such as high temperature, gut permeability, and inflammation, as well as cardiovascular complications and diarrhea.

However, it is important to mention that gut microbiota composition is highly heterogeneous between individuals of different ages, eating habits, and geographies, thus the following reported findings may not entirely consistent across the different *in vitro* and clinical studies (Patel and Roper 2021).

Gastrointestinal tract is considered an extrapulmonary site for SARS-CoV-2 infection since receptor ACE-2 is highly expressed in the intestinal enterocyte. So, once the SARS-CoV-2 infects and actively replicates in the intestinal enterocytes, symptomatic gastrointestinal COVID-19 is triggered in the host (Zuo et al. 2021). These facts suggest the possibility that SARS-CoV-2 interacts (directly or indirectly) with the gut microbiota adhered to the intestinal walls, resulting in dysbiotic communities that may persist even after recovery from COVID-19 (Kaźmierczak-Siedlecka et al. 2020, Patel and Roper 2021, Xiang et al. 2021). Thus, SARS-CoV-2 infection modifies the composition of the gut microbiota, which could predispose patients not only to greater severity and mortality of COVID-19, but also to prolonging such symptoms weeks or months after SARS-CoV-2 infection, a phenomenon known as long COVID or post-COVID syndrome (Raveendran et al. 2021). Similarly, the microbial imbalance of gut microbiota can cause that COVID-19 patients have a greater probability of suffering co-infections and super-infections with other respiratory or systemic viruses (Fig. 2; Cox et al. 2020).

Meta-analysis indicated that up to 20% of COVID-19 patients had gastrointestinal symptoms, including nausea, vomiting, abdominal pain, and diarrhea (Vodnar et al. 2020, Zuo et al. 2021). Additionally, fecal calprotectin was founded elevated in COVID-19 patients, indicating gut inflammation. These effects are related to gut microbiota modulation during and after SARS-CoV-2 infection; however, it is unknown about the activity of SARS-CoV-2 in the gut or gut microbiota of COVID-19 patients (Zuo et al. 2021). In the gut, the SARS-CoV-2 can reduce the expression of ACE-2 and the number of circulating angiogenic cells, thus endangering the gut endothelium, promoting a dysbiosis and effecting the hots immune response and metabolism (Romani et al. 2022). In this sense, in previous report, Yeoh et al. (2021), compared the microbiota composition of hospitalized COVID-19 and non-COVID-19 subjects, finding a more abundance of phylum *Bacteroidetes* (23.9%) in COVID-19 patients, while in non-COVID-19 individuals, *Actinobacteria* (26.1%) were more abundant phylum. It is important to note that these changes in the diversity of the intestinal microbiota can persist even when the symptomatic and hospitalization periods of COVID-19 patients has ended. However, this prolonged imbalance of microbial diversity is not entirely negative, since it has been observed that some species of *Bacteroides* (such as *Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis*, and *Bacteroides ovatus*), downregulated the ACE-2 expression in colon and thus, play a potential role by blocking host entry, preventing future reinfections by SARS-CoV-2 (Donati Zeppa et al. 2020, Zuo et al. 2020). Nevertheless, during this period of dysbiosis, concentrations of pro-inflammatory cytokines and blood biomarkers (C protein reactive, lactate dehydrogenase, aspartate aminotransferase, and γ-glutamyl transferase, TNF-α, CXCL10, CCL10, and IL10) were elevated, thereby increasing the COVID-19 severity (Vabret et al. 2020). In accordance with the above, significant alterations in fecal microbiomes of COVID-19 patients, in which the abundance of *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* have been correlated with COVID-19 severity (Zuo et al. 2020). Additionally, several gut commensal bacteria with known immunomodulatory potential, such as *Eubacterium rectale*, *Faecalibacterium prausnitzii*, and bifidobacteria, underrepresented in COVID-19 patients, remaining at low concentrations up to 30 days after disease clearance (Zuo et al. 2020). Therefore, gut microbiota can be involved in the magnitude of COVID-19 severity, either modulating the immune response or increasing the symptoms during and after disease resolution. Based on the previous studies, it is possible to establish that the gut microbiota plays an important role in the pathogenesis process of the SARS-CoV-2 and the eventual illness of COVID-19.

Currently, the existence of a "quiescent" intestinal infection of SARS-CoV-2 has been reported in patients with COVID-19, being characterized by a persisting viral infection and replica-

tion at intestinal level and by a higher abundance of opportunistic pathogens such as *Collinsella aerofaciens*, *Collinsella tanakaei*, *Streptococcus infantis*, and *Morganella morganii* in fecal samples. Based on these findings, it has been postulated that SARS-CoV-2 may be transmitted through the fecal-oral route, even from those individuals with asymptomatic COVID-19 or with a negative respiratory tract but positive fecal SARS-CoV-2 tests (Zuo et al. 2021). However, future research involved in the infectivity and pathogenesis of SARS-CoV-2 in the gastrointestinal tract, as well as measures gut infection level and interactions with gut microbiota, are needed.

Gut microbiota response to oral administration of dioxide during SARS-CoV-2 infection

Nutrition, diet, environmental factors, genetics, medication, and comorbidities play an important role in shaping the diversity of gut microbiota. The low microbial diversity of gut microbiota during dysbiosis or derived from uncontrolled medication are associated with higher rates of serious bacterial and viral infections, including influenza, pneumonia, and SARS-CoV-2. So, improving the profile of gut microbiota can be considered as a prophylactic measure to minimize the impact of COVID-19, mainly in elderly and immunodeficient patients (Vodnar et al. 2020). Mostly, COVID-19 clinical conditions and fatalities occur in elderly people, mainly due to aging-related factors that render the elderly more prone to this disease. Comorbidities such as hypertension, hyperlipidemias, cardiovascular diseases, diabetes, and cancer are commonly related to the consumption of more than five medicines per day, which not only implies an increase in drug side effects but also affects the gut microbiota and gradually decreases the antiviral capacity of the host (Donati Zeppa et al. 2020, Lithander et al. 2020). Research studies have demonstrated alterations in gut microbiota composition at increasing the number of co-administered drugs. For example, the maintenance of relative abundance of pathogenic *Helicobacter* and reduction of *Lachnospiraceae* and *Succinivibrionaceae* (aids in the maintenance of host cardiorespiratory health and regulation of inflammation), were observed in patients using additional drugs during viral infection. So, an increase in mortality in COVID-19 patients may be related to the lowest gut microbiota diversity index (Ticinesi and Milani 2017, Donati Zeppa et al. 2020). Accordingly, the uncontrolled use of anti-SARS-CoV-2 medications not yet authorized, such as ClO_2, can represent a health risk since this disinfectant, rather than eliminate the SARS-CoV-2, alter the diversity of the gut microbiota, ameliorating the immune response of the host, and generating a state of dysbiosis that can increase the severity of the disease (Xiang et al. 2021).

The different surges of COVID-19 have triggered the use of drugs with not enough scientific support and lack of sanitary authorization as alternatives for the effective treatment of COVID-19 worldwide (Burela et al. 2020, WHO 2020b, Lardieri et al. 2021). In this sense, the use of ClO_2 has generated controversy due to its toxic reactions reported in humans exposed to different concentrations (Liester 2021). In animal studies, adverse reactions including oral and digestive irritation, anemia and methemoglobinemia, altered thyroid function, and neurotoxicity have been observed (Rubio-Casillas and Campra-Madrid 2021). Regarding this, the FDA has published a warning about the use of ClO_2 as an anti-SARS-CoV-2 agent, due to its oxidative behavior and the adverse effects after consumption, including vomiting, diarrhea, dehydration, hypotension, abdominal pain, methemoglobinemia, and systemic failures. Nevertheless, several studies have demonstrated that all these symptoms are completely reversible after ClO_2 consumption, highlighting that its toxicity is strongly dose-dependent (Bathina et al. 2013, Romanovsky et al. 2013, Loh and Shafi 2014, Ma et al. 2017, FDA 2020, Lardieri et al. 2021).

Nowadays, gastrointestinal damage symptoms have been reported because of an administration of a liquid solution ClO_2 (FDA 2020, Lardieri et al. 2021). It has been observed that drinking water with concentrations from 1 to 1000 mg mL^{-1} of ClO_2, decreased red blood cell counts, half-life erythrocytes hemoglobin (HEB) concentration, and packed cell volume in animal and *in vitro* models at 30–60 days of exposure. Likewise, the consumption of ClO_2 has been related to the presence of oxidative stress, since the ClO_2^- can oxidize HEB to methemoglobin (mHEB), which suppresses glutathione concentration (an antioxidant mechanism), increasing the levels of the oxidative hydrogen peroxide. At the gastrointestinal level, exposure to ClO_2 affects the gastric mucosa, promoting symptoms such as nausea, vomiting, and abdominal pain (Medina-Avitia et al. 2021).

As mentioned, the SARS-CoV-2 infection causes a prolongated dysbiosis in gut microbiota, which can be postponed until after COVID-19 recovery. This imbalance of the diversity of the gut microbiota can be increased with the consumption of uncontrolled medications, which can mean additional damage to the host's health. An example of this is the indiscriminate consumption of ClO_2 as a possible anti-SARS-CoV-2 agent and the lack of information about its possible impacts on the intestinal microbiota and host health both during and after COVID-19. There are few studies related to the consumption of ClO_2 and its impact on the composition of the gut microbiota. For instance, a murine study showed that the daily administration of 100 ppm ClO_2 tablet to C57BL/6 mice for 28 days generated a greater overall effect on gut microbiota than a 70% ethanol solution, but a lesser effect than the hydrogen peroxide and potassium peroxymonosulfate. In addition, the taxon-by-taxon analysis showed no differences in Firmicutes, Bacteroidetes, or any of the lower abundance phyla, between the cecum microbiota of mice treated with ClO_2 and that of the water control mice. On the contrary, in fecal microbiota, significant differences in lower abundance phyla (*Verrucomicrobia*, *Actinobacteria*, and *Proteobacteria*) were observed between ClO_2 and water-control mice at 14, 21, and 28 days (Sciurba et al. 2021). These results indicate that ClO_2 can be considered a potential anti-SARS-CoV-2 agent with minimal effects on the gut microbiota; however, it is necessary to clarify the maximum concentration allowed to avoid possible side effects that compromise the health of the host.

Toxic and safety aspects of oral administration of chlorine dioxide

Derived from the indiscriminate oral consumption of ClO_2 during COVID-19 pandemic, different regulatory agencies and scientific societies have postulated statements warning about the lack of solid evidence for the efficiency of ClO_2 in SARS-CoV-2 inactivation and modulation of COVID-19 disease symptoms (Andrés et al. 2022). Studies have reported toxic effects of ClO_2 exposition or consumption, either by inhalation, parenteral, or oral routes. Specifically, oral

administration of ClO_2 facilitates its absorption at the gastrointestinal level, and once absorbed, maximum peaks levels in blood can be observed within one hour after a single dose is administered. Once in blood, most of the ClO_2 and its derivatives (chlorite, chlorate, and chloride) remain in plasma, followed by kidneys, lungs, stomach, intestines, liver, and spleen, to be finally excreted through urine and feces within 72 h after oral consumption (Burela et al. 2020, Soriano-Moreno et al. 2021).

Separately to oral administration, some adverse reactions, such as acute liver failure, arterial hypotension, hydroelectrolytic imbalance, nausea, vomiting, diarrhea, digestive mucosa irritation, respiratory failure, arrythmias, headaches, dizziness, tremors, anxiety, drowsiness, and mainly methemoglobinemia or hemoglobinuria, have been identified as a response to direct exposition to ClO_2 or to its derivatives (Andrés et al. 2022). Methemoglobinemia is characterized by an oxidation of hemoglobin generated by ClO_2. This oxidation turns hemoglobin into methemoglobin, which is uncapable to bind oxygen molecules and thus hinders oxygen transport in the body, resulting in a hypoxia and gradual respiratory failure. Previous reports indicate that hypoxia and methemoglobinemia can be developed either by accidentally ingestion or low doses of ClO_2 (<100 mL of 28% ClO_2 solution). However, in both cases, to save the lives of the patients, endotracheal intubation, and kidney transplant, were required, respectively. The endotracheal intubation was required to counteract a severe hypoxia with no response to oxygen therapy, while the kidney transplant, accompanied by blood transfusions, were necessary to maintain constant blood oxygen levels, which dropped due to the development of methemoglobinemia (Romanovsky et al. 2013, Hagiwara and Inoue 2015). Nevertheless, as mentioned previously, the toxic effects of ClO_2 are dependent of several factors, including the physiological state of host, the concentration of ClO_2, and the time of exposition to the agent. Therefore, the possibility of ClO_2 being cytotoxic to humans is low; however, this cytotoxicity can be increased either by mixing with other disinfectants, e.g. chlorine or derived from the presence of its released by-products, including chlorite (ClO_2^-), chlorate (ClO_3^-), and chloride (Cl^-) (Jefri et al. 2022). In animal models, it has been shown that the cytotoxicity of ClO_2 is size- or weight-dependent. For example, a study reported by Ma et al. (2017), demonstrated that ClO_2 concentrations up to 40 ppm in drinking water did not show toxicity in mouse lung fibroblasts L929 cells, but antimicrobial and antifungal effects were observed at ClO_2 concentrations of 5 and 20 ppm, respectively. Meanwhile, a similar test carried out in African green monkeys (*Cercopithecus aethiops*) demonstrated that a ClO_2 concentration of 200 ppm in drinking water caused erythema and ulceration of the oral mucosa after one week exposure (Bercz et al. 1982). These reports corroborate the fact that, based on dimensions of the subjects, the ClO_2 may not be considered a toxic agent; however, the concentration and exposure time are factors to consider for future controlled applications in humans, mainly as a potentially anti-SARS-CoV-2 agent.

In the past decades, ClO_2 was widely used as a drinking water sanitizing agent, which can produce a variety of oxidized by-products once it reacts with organic material. Even so, the EPA had listed the ClO_2 in group D (no classifiable for human carcinogenicity), due to the controversial data related to the genotoxicity in human and animal models (Buschini et al. 2004). In this sense, recent studies related to the genotoxicity of ClO_2 have reported that, before rapidly degrading into its by-products, ClO_2 can damage DNA and RNA structures by inducing DNA mutations and hindering its ability to act as a template for RNA replication (Zhang et al. 2023). However, these effects are dependent on the concentration of ClO_2 used and, in some cases, exceed the limits established by the normativity. For example, Buschini et al. (2004), reported that ClO_2 showed a weakly genotoxic damage on human leukocytes with a dose of 0.2 ppm, while a genotoxic effect was only observed in cells of *S. cerevisiae* strain D7 exposed to doses higher (5- to 10-fold) than the concentration used for water disinfection. In this context, Monarca et al. (2000), observed, through an Ames test, that wastewater treated with 1.5 ppm of ClO_2 exhibited mutagenicity in *Vibrio fischeri*, a marine bacterium commonly founded in wastewater effluents. Moreover, a genotoxicity test using human HepG2 cells revealed that the genotoxicity of ClO_2 is triggered by the presence of its derivative compounds, such as ClO_3^-, which led to an increase of DNA damage in cells after exposure to 0.001 ppm for 24 h at 37°C with 5% CO_2 (Feretti et al. 2008).

Regarding its genotoxic effects on viral species, studies have revealed that ClO_2 decreased the viral infectivity of EV71 by altering the 1–118 nt region located at 5′-non-coding region of the viral genome (Jin et al. 2013). Similarly, ClO_2 was able to produce RNA damage and decrease the complete infectivity of Poliovirus 1 after 3 min of exposure to 5 ppm of ClO_2 (Simonet and Gantzer 2006). Thus, the ClO_2 exerts genotoxic effects as antiviral mechanisms, either preventing the viral infection or replication inside the infected cell (Ge et al. 2021).

As mentioned above, during the disinfection process, the ClO_2 encounters naturally occurring organic or inorganic matter and then, rapidly degrades to its different by-products (ClO_2^-, ClO_3^-, and Cl^-). There is evidence supporting that ClO_2 by-products, specifically chlorite, have genotoxic effects. For instance, a study conducted on *E. coli* strains deficient in oxygen-scavenging enzymes, demonstrated that chlorite was highly cytotoxic due to the formation of reactive oxygen species that promote oxidative damage in cells (Ueno et al. 2000, Richardson et al. 2007). On the contrary, there is information indicating relative lower genotoxic effects and non-carcinogenic risks of ClO_2 used for drinking water disinfection (Kimura et al. 2019, Du et al. 2021). Based on these findings, it is possible to infer that the genotoxic properties of the ClO_2 are more related to the release and presence of its by-products than to the original molecule. However, studies focused on clarifying the mechanisms by which the ClO_2 or its by-products (ClO_2^-, ClO_3^-, and Cl^-) exert their genotoxic effects are still necessary.

Despite the above information, there are currently no published scientific evidence nor competent authorities that supports the positive benefits of ClO_2 on human health. Likewise, studies focused on the oral administration of ClO_2 as an anti-SARS-CoV-2 agent and its safety for human health are scarce, and in some of them, the results are controversial or not conclusive. So, more studies in the field are still required.

Conclusions and future perspectives

In the industry, ClO_2 is a well-researched and widely used disinfectant agent with strong antifungal, antibacterial, and antiviral activities reported both in *in vitro* and *in vivo* studies. As an antimicrobial agent, the ClO_2 gas is recognized for its

greater penetration abilities and minimal impact on the operators. Conversely, aqueous solutions of ClO_2 present several undesirable effects, including low reactivity and penetration capacity, as well as bleaching effects in food products after their application.

Regarding its toxicity, it has been observed that controlled administration of ClO_2, at low concentrations (≤40 ppm), does not represent a risk to human health, since the possible effects are latent while the exposure continues. On the contrary, high concentrations (>40 ppm) of ClO_2 can cause adverse effects, such as hematologic and renal complications, which disappear once the exposure to ClO_2 is suspended and when the host health damage is not serious. In the same way, the ClO_2 cytotoxicity is low for humans; however, it can be increased either by mixing with other disinfectants or derived from the presence of its released by-products (e.g. ClO_2^-, ClO_3^-, and Cl^-). This information potentializes the use of ClO_2 as a possible treatment for COVID-19. Nevertheless, randomized clinical controlled and double-blind studies related to the use of ClO_2 for prevention and treatment of COVID-19 are still needed to explore the efficiency in the treatment and the possible side effects, either in healthy individuals as well as in immunosuppressed patients or in those with comorbidities such as chronic degenerative diseases. Likewise, the potential use of ClO_2 as an anti-SARS-CoV-2 agent demands more in-depth studies related to the impact that this disinfectant can generate on the gut microbiota composition since an imbalance of microbial species or dysbiosis could increase the severity of symptoms in COVID-19 patients, such as inflammation or diarrhea.

Conflict of interest

None of the authors have conflict of interest to be declared.

Author contributions

Audry Peredo-Lovillo (Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing), Haydee Eliza Romero-Luna (Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – review & editing), Naida Juárez-Trujillo (Investigation, Methodology, Supervision, Writing – review & editing) and Maribel Jiménez-Fernández (Conceptualization, Formal analysis, Investigation, Project administration, Validation, Visualization, Writing – review & editing)

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

References

Al-Sa'ady AT, Nahar HS, Saffah FF. Antibacterial activities of chlorine gas and chlorine dioxide gas against some pathogenic bacteria. *Eurasian J Biosci* 2020;**14**:3875–82.

Andrés CMC, Lastra JMPdI, Andrés Juan C *et al*. Chlorine dioxide: friend of foe for cell biomolecules? A chemical approach. *Int J Mol Sci* 2022;**23**:15660. https://doi.org/10.3390/ijms232415660

Arellano-Gutiérrez G, Aldana-Zaragoza EH, Pérez-Fabián A. Intestinal perforation associated with chlorine dioxide ingestion: and adult chronic consumer during COVID-19 pandemic. *Clin J Gastroenterol* 2021;**14**:1655–60. https://doi.org/10.1007/s12328-021-01527-y

Bathina G, Yadla M, Burri S *et al*. An unusual case of reversible acute kidney injury due to chlorine dioxide poisoning. *Ren Fail* 2013;**35**:1176–8. https://doi.org/10.3109/0886022X.2013.819711

Bercz J, Jones L, Garner. L *et al*. Subchronic toxicity of chlorine dioxide and related compounds in drinking water in the nonhuman primate. *Environ Health Perspect* 1982;**46**:47–55. https://doi.org/10.1289/ehp.824647

Burela A, Hernández-Vásquez A, Comandé D *et al*. Chlorine dioxide and chlorine derivatives for the prevention or treatment of COVID-19: a systematic review. *Rev Peru Med Exp Salud Publica* 2020;**37**:605–10. https://doi.org/10.17843/rpmesp.2020.374.6330

Buschini A, Carboni O, Furlini M *et al*. Sodium hypochlorite-, chlorine dioxide-, and peracetic acid-induced genotoxicity detected by the Comet assay and *Saccharomyces cerevisiae* D7 tests. *Mutagenesis* 2004;**19**:157–62. https://doi.org/10.10193/mutage/geh012

Campagna MV, Faure-Kumar E, Treger JA *et al*. Factors in the selection of surface disinfectants for use in laboratory animal setting. *J Am Assoc Lab Anim Sci* 2016;**55**:175–88.

Cao J, Shi Y, Wen M *et al*. Can nasal irrigation with chlorine dioxide be considered as a potential alternative therapy for respiratory infectious disease? The example of COVID-19. *BST* 2022;**16**:447–50. https://doi.org/10.5582/bst.2022.01495

Cox M, Loman N, Bogaert D *et al*. Co-infections: potentially lethal and unexplored in COVID-19. *The Lancet Microbe* 2020;**1**:e11. https://doi.org/10.1016/S2666-5247(20)30009-4

Donati Zeppa S, Agostini D, Piccoli G *et al*. Gut microbiota status in COVID-19: an unrecognized player? *Front Cell Infect Microbiol* 2020;**10**:576551. https://doi.org/10.3389/fcimb.2020.576551

Du Y, Zhao L, Ban J *et al*. Cumulative health risk assessment of disinfection by-products in drinking water by different disinfection methods in typical regions of China. *Sci Total Environ* 2021;**770**:144662. https://doi.org/10.1016/j.scitotenv.2020.144662

Feretti D, Zerbini I, Ceretti E *et al*. Evaluation of chlorite and chlorate genotoxicity using plant bioassays and *in vitro* DNA damage test. *Water Res* 2008;**42**:4075–82. https://doi.org/10.1016/j.watres.2008.06.018

Food & Drug Administration (FDA). FDA new release coronavirus (COVID-19) update: FDA warns seller marketing dangerous chlorine dioxide products that claim to treat or prevent COVID-19. 2020 https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-warns-seller-marketing-dangerous-chlorine-dioxide-products-claim [Last accessed date March 14, 2022].

Fu M-r, Zhang X-m, Jin T *et al*. Inhibitory of grey mold on green pepper and jujube by chlorine dioxide (CO_2) fumigation and its mechanisms. *Lebensmittel-Wissenschaft und-Technologie* 2019;**100**:335–40. https://doi.org/10.1016/j.lwt.2018.10.092

Ge Y, Zhang X, Shu L *et al*. Kinetics and mechanisms of virus inactivation by chlorine dioxide in water treatment: a review. *Bull Environ Contam Toxicol* 2021;**106**:560–7. https://doi.org/10.1007/s00128-021-03137-3

Hagiwara Y, Inoue N. First case of methemoglobinemia caused by a ClO_2-based household product. *Pediatr Int* 2015;**57**:1182–3. https://doi.org/10.1111/ped.12708

Hatanaka N, Xu B, Yasugi M *et al*. Chlorine dioxide is a more potent antiviral agent against SARS-CoV-2 than sodium hypochlorite. *J Hosp Infect* 2021;**118**:20–26. https://doi.org/10.1016/j.jhin.2021.09.006

Hati S, Bhattacharyya S. Impact of thiol-disulfide balance on the binding of Covid-19 spike protein with Angiotensin-converting enzyme 2 receptor. *ACS Omega* 2020;**5**:16292–8. https://doi.org/10.1021/acsomega.0c02125

Insignares-Carrione E, Bolano Gómez B, Andrade Y *et al*. Determination of the effectiveness of chlorine dioxide in the treatment of COVID-19. *J Mol Genet Med* 2021;**15**:S2.

Insignares-Carrione E, Colano Gómez B, Ludwig Kalcker A. Chlorine dioxide in COVID-19: hypothesis about the possible mechanism of molecular action in SARS-CoV-2. *J Mol Genet Med* 2020;**14**:468.

Jafari A, Pouya FD, Nikman Z *et al*. Current advances and challenges in COVID-19 vaccine development: from conventional vaccines to next-generation vaccine platforms. *Mol Biol Rep* 2022;**49**:4943–57. https://doi.org/10.1007/s11033-022-07132-7

Jefri UHNM, Khan A, Lim YC *et al*. A systematic review on chlorine dioxide as a disinfectant. *J Med Life* 2022;**15**:313–8. https://doi.org/10.25122/jml-2021-0180

Jin M, Shan J, Chen Z *et al*. Chlorine dioxide inactivation of enterovirus 71 in water and its impact on genomic targets. *Environ Sci Technol* 2013;**47**:4590–7, https://doi.org/10.1021/es305282g

Káli-Kullai K, Wittmann M, Noszticzius Z *et al*. Can chlorine dioxide prevent the spreading of coronavirus or other viral infections? *Physiol Int* 2020;**107**:1–11. https://doi.org/10.1556/2060.2020.00015

Kaźmierczak-Siedlecka K, Vitale E, Makarewicz W. COVID-19—gastrointestinal and gut microbiota-related aspects. *Eur Rev Med Pharmacol Sci* 2020;**24**:10853–9. https://doi.org/10.266355/eurrev_202010_23448

Kim S, Park S. Chlorine dioxide gas mediated inactivation of the biofilm cells of. *J Food Sci Technol* 2022;**59**:4863–9. https://doi.org/10.1007/s13197-022-05574-2

Kimura SY, Cuthbertson AA, Byer JD *et al*. The DBP exposome: development of a new method to simultaneously quantify priority disinfection byproducts and comprehensively identify unknowns. *Water Res* 2019;**148**:324–33. https://doi.org/10.1016/j.watres.2018.10.057

Kingsley DH, Annous BA. Evaluation of steady-state gaseous chlorine dioxide treatment for the inactivation of Tulane virus on berry fruits. *Food Environ Virol* 2019;**11**:214–9. https://doi.org/10.1007/s12560-019-09382-4

Lardieri A, Cheng C, Jones SC *et al*. Harmful effects of chlorine dioxide exposure. *Clin Toxicol* 2021;**59**:448–9. https://doi.org/10.1080/15563650.1818767

Lee H, Beuchant LR, Ryu JH *et al*. Inactivation of *Salmonella* Typhimurium on red peppers by treatment with gaseous chlorine dioxide followed by drying. *Food Microbiol* 2018;**76**:78–82. https://doi.org/10.1016/j.fm.2018.04.016

Liester MB. The chlorine dioxide controversy: a deadly poison or a cure for COVID-19? *Int J Med Med Sci* 2021;**13**:13–21. https://doi.org/10.5897/IJMMS2021.1461

Lithander FE, Neumann S, Tenison E *et al*. COVID-19 in older people: a rapid clinical review. *Age Ageing* 2020;**49**:501–15. https://doi.org/10.1093/ageing/afaaa093

Liu X, Jiao W, Du Y *et al*. Chlorine dioxide control green mold caused by *Penicillium digitatum* in citrus fruits and the mechanisms involved. *J Agric Food Chem* 2020;**68**:13897–905. https://doi.org/10.1021/acs.jafc.0c0588

Loginova IV, Rubstova SA, Kuchin AV. Oxidation by chlorine dioxide of methionine and cysteine derivatives to sulfoxides. *Chem Nat Compd* 2008;**44**:752–4. https://doi.org/10.1007/s10600-009-9182-8

Loh JMR, Shafi H. Kikuchi-Fujimoto disease presenting after consumption of "miracle mineral solution" (sodium chlorite). *Case Reports* 2014;**2014**:bcr2014205832. https://doi.org/10.1136/bcr-2014-205832

Ma J-W, Huang B-S, Hsu C-W *et al*. Efficacy and safety evaluation of a chlorine dioxide solution. *Int J Environ Res Public Health* 2017;**14**:329. https://doi.org/10.3390/ijerph14030329

Malka SK, Park M-H. Fresh produce safety and quality: chlorine dioxide's role. *Front Plant Sci* 2022;**12**:775629. https://doi.org/10.3389/fpls.2021.775629

Medina-Avitia E, Tella-Vega P,C, García-Estrada C. Acute kidney injury secondary to chlorine dioxide use for COVID-19 prevention. *Hemodial Int* 2021;**25**:E40–3. https://doi.org/10.1111/hdi.12941

Monarca S, Feretti D, Collivingnarelli C *et al*. The influence of different disinfectants on mutagenicity and toxicity of urban wastewater. *Water Res* 2000;**34**:4216–69. https://doi.org/10.1016/S0043-1354(00)00192-5

Montazeri N, Manuel C, Moorman E *et al*. Virucidal activity of fogged chlorine dioxide- and hydrogen peroxide-based disinfectants against human norovirus and its surrogate, feline calicivirus, on hard-to-reach surfaces. *Front Microbiol* 2017;**8**:1031. https://doi.org/10.3389/fmicb.2017.01031

Ofori I, Maddila S, Lin J *et al*. Chlorine dioxide inactivation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in water: the kinetics and mechanism. *J Water Proc Eng* 2018;**26**:46–54. https://doi.org/10.1016/j.jwpe.2018.09.001

Ogata N, Miura T. Inhibition of the binding of variants of SARS-CoV-2 coronavirus spike protein to a human receptor by chlorine dioxide. *Ann Pharmacol Pharm* 2021;**6**:1199.

Park SH, Kang DH. Effect of temperature on chlorine dioxide inactivation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on spinach, tomatoes, stainless steel, and glass surfaces. *Int J Food Microbiol* 2018;**275**:39–45. https://doi.org/10.1016/j.ijfoodmicro.2018.03.015

Park SH, Kim WJ, Kang DH. Effect of relative humidity on inactivation of foodborne pathogens using chlorine dioxide gas and its residues on tomatoes. *Lett Appl Microbiol* 2018;**67**:154–60. https://doi.org/10.1111/lam.13002

Patel P, Roper J. Gut microbiota composition is associated with COVID-19 disease severity. *Gastroenterology* 2021;**161**:722–4. https://doi.org/10.10153/j.gastro.2021.05.006

Peltzer PM, Boccioni APC, Attademo AM *et al*. Risk of dioxide as emerging contaminant during SARS-CoV-2 pandemic: enzyme, cardiac, and behavior effects on amphibian tadpoles. *Toxicol Environ Health Sci* 2022;**14**:47–57. https://doi.org/10.1007/s13530-021-00116-3

Plevka P, Perera R, Cardosa J *et al*. Crystal structure of human enterovirus 71. *Science* 2012;**336**:1274. https://doi.org/10.1126/science.1218713

Ran Y, Qingmin C, Maorun F. Chlorine dioxide generation method and its action mechanism for removing harmful substances and maintaining quality attributes of agricultural products. *Food Bioprocess Technol* 2019;**12**:1110–22. https://doi.org/10.1007/s11947-019-02279-x

Raveendran AV, Jayadevan R, Sashidharan S. Long COVID: an overview. *Diabetes Metab Syndr* 2021;**15**:869–75. https://doi.org/10.1016/j.dsx.2021.05.007

Richardson SD, Plewa MJ, Wagner ED *et al*. Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research. *Mutat Res* 2007;**636**:178–242. https://doi.org/10.1016/j.mrrev.2007.09.001

Romani L, Del Chierico F, Macari G *et al*. The relationship between pediatric gut microbiota and SARS-CoV-2 infection. *Front Cell Infect Microbiol* 2022;**12**:908492. https://doi.org/10.3389/fcimb.2022.908492

Romanovsky A, Djogovic D, Chin D. A case of sodium chlorite toxicity managed with concurrent renal replacement therapy and red cell exchange. *J Med Toxicol* 2013;**9**:67–70. https://doi.org/10.1007/s13181-012-0256-9

Rubio-Casillas A, Campra-Madrid P. Pharmacokinetics and pharmacodynamics of chlorine dioxide. *e-Cucba* 2021;**8**:21–35. https://doi.org/10.32870/ecucba.vi16.202

Sanekata T, Fukuda T, Miura T *et al*. Evaluation of the antiviral activity of chlorine dioxide and sodium hypochlorite against feline calicivirus, human influenza virus, measles virus, canine distemper virus, human herpesvirus, human adenovirus, canine adenovirus and canine parvovirus. *Biocontrol Sci* 2010;**15**:45–49. https://doi.org/10.4265/bio.15.45

Sciurba JD, Chlipala GE, Green SJ *et al*. Evaluation of effects of laboratory disinfectants on mouse gut microbiota. *Comp Med* 2021;**71**:492–501. https://doi.org/10.30802/AALAS-CM-21-000051

Simonet J, Gantzer C. Degradation of the Poliovirus 1 genome by chlorine dioxide. *J Appl Microbiol* 2006;**100**:862–70. https://doi.org/10.1111/j.1365-2672.2005.02850.x

Singh S, Maji PK, Lee YS *et al*. Applications of gaseous chlorine dioxide for antimicrobial food packaging. *Environ Chem Lett* 2021;**19**:253–70. https://doi.org/10.1007/s10311-020-01085-8

Solomon T, Lewthwaite P, Perera D *et al*. Virology, epidemiology, pathogenesis, and control of enterovirus 71. *Lancet Infect Dis* 2010;**10**:778–90. https://doi.org/10.1016/S1473-3099(10)70194-8

Soriano-Moreno DR, Fernandez-Guzman D, Ccami-Bernal F *et al*. Factor associated with the consumption of chlorine dioxide to prevent and treat COVID-19 in the Peruvian population: a cross-sectional study. *BMC Public Health* 2021;**21**:2109. https://doi.org/10.1186/s12889-021-12191-9

Stewart DJ, Napolitano MJ, Bakhmutova-Albert EV *et al*. Kinetics and mechanisms of chlorine dioxide of tryptophan. *Inorg Chem* 2008;**47**:1639–47. https://doi.org/10.1021/ic701761p

Sultan A, Ullah I, Khan S *et al*. Impact of chlorine dioxide as water acidifying agent on the performance, ileal microflora and intestinal histology in quails. *Arch Anim Breed* 2014;**57**:1–9. https://doi.org/10.7482/0003-9438-57-031

Sun X, Baldwin E, Bai J. Applications of gaseous chlorine dioxide on postharvest handling and storage of fruits and vegetables—a review. *Food Control* 2019;**95**:18–26. https://doi.org/10.1016/j.foodcont.2018.07

Ticinesi A, Milani C. Gut microbiota composition is associated with polypharmacy in elderly hospitalized patients. *Sci Rep* 2017;**7**:11102. https://doi.org/10.1038/s41598-017-10734-y

Trinetta V, Linton RH, Morgan MT. Use of chlorine dioxide gas for the postharvest control of *Alternaria alternata* and *Stemphylium vesicarium* on Roma tomatoes. *J Sci Food Agric* 2013;**93**:3330–3. https://doi.org/10.1002/jsfa.6180

Ueno H, Oishi K, Sayato Y *et al*. Oxidative cell damage in kat-sod assay of oxyhalides as inorganic disinfection by-products and their occurrence by ozonation. *Arch Environ Contam Toxicol* 2000;**38**:1–6. https://doi.org/10.1007/s002449910001

Vabret N, Britton GJ, Gruber C *et al*. The Sinai immunology review project, immunology of COVID-19: current state of the science. *Immunity* 2020;**52**:910–41. https://doi.org/10.1016/j.immuni.2020.05.002

Vandekinderen I, Devlieghere F, Van Camp J *et al*. Effects of food composition on the inactivation of foodborne microorganisms by chlorine dioxide. *Int J Food Microbiol* 2009;**131**:138–44. https://doi.org/10.1016/j.ijfoodmicro.2009.02.004

Vodnar D-C, Mitrea L, Teleky B-E *et al*. Coronavirus disease (COVID-19) caused by (SARS-CoV-2) infections: a real challenge for human gut microbiota. *Front Cell Infect Microbiol* 2020;**10**:575559. https://doi.org/10.3389/fcimb.2020.575559

Wang X-W, Li J-S, Jin M *et al*. Study on the resistance of severe acute respiratory syndrome-associated to coronavirus. *J Virol Methods* 2005;**126**:171–7. https://doi.org/10.1016/j.jviroment.2005.02.005

Wang Z, Liao F, Lin J *et al*. Inactivation and mechanisms of chlorine dioxide on *Nosema bombycis*. *J Invertebr Pathol* 2010;**104**:134–9. https://doi.org/10.1016/j.jip.2009.11.007

World Health Organization (WHO). COVID-19 homepage. 2023 https://covid19.who.int/ [Last accessed date June 5, 2023].

World Health Organization (WHO). Naming the coronavirus disease (COVID-19) and the virus that causes it. 2020a https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it [Last accessed date June 3, 2023].

World Health Organization (WHO). Corticosteroids for COVID-19. 2020b https://www.who.int/publications/i/items/WHO-2019-nCoV-Corticosteroids.2020.1 [Last accessed date September 20, 2022].

Xiang Z, Koo H, Chen Q *et al*. Potential implications of SARS-CoV-2 oral infection in the host microbiota. *J Oral Microbiol* 2021;**13**:1853451. https://doi.org/10.1080/20002297.2020.1853451

Yeoh YK, Zuo T, Liu GC-Y *et al*. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut* 2021;**70**:698–706. https://doi.org/10.1136/gutjnl-2020-323020

Yuan L, Sadiq FA, Wang N *et al*. Recent advances in understanding the control of disinfectants-resistant biofilms by hurdle technology in the food industry. *Crit Rev Food Sci Nutr* 2021;**61**:3876–91. https://doi.org/10.1080/10408398.2020.18093453

Zhang B, Huang C, Zhang L *et al*. Application of chlorine dioxide microcapsules sustained-release antibacterial films for preservation of mangos. *J Food Sci Technol* 2019;**56**:1095–103. https://doi.org/10.1007/s13197-019-03636-6

Zhang Y, Qiu J, Yang K *et al*. Generation, mechanisms, kinetics, and effects of gaseous chlorine dioxide in food preservation. *Comp Rev Food Sci Food Safe* 2023;**2023**:1–5. https://doi.org/10.1111/1541-4337.13177

Zhong Q, Carratalà Q, Ossola R *et al*. Cross-resistance of UV- or chlorine dioxide-resistant echovirus 11 to other disinfectants. *Front Microbiol* 2017;**8**:1928. https://doi.org/10.3389/fmicb.2017.01928

Zhu C, Chen Z, Yu G. Fungicidal mechanism of chlorine dioxide on *Saccharomyces cerevisiae*. *Ann Microbiol* 2013;**63**:495–502. https://doi.org/10.1007/s13213-012-0494-8

Zhu Z, Guo Y, Yu. P *et al*. Chlorine dioxide inhibits the replication of porcine reproductive and respiratory syndrome virus by blocking viral attachment. *Infect Genet Evol* 2019;**67**:78–87. https://doi.org/10.1016/j.meegid.2018.11.002

Zuo T, Liu Q, Zhang F *et al*. Depicting SARS-CoV-2 faecal viral activity in association with the gut microbiota composition in patients with COVID-19. *Gut* 2021;**70**:276–84. https://doi.org/10.1136/gutjnl-2020-322294

Zuo T, Zhang F, Lui GC *et al*. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. *Gastroenterology* 2020;**159**:944–55. https://doi.org/10.1053/j.gastro.2020.05.048

Received: January 18, 2023. **Revised:** June 27, 2023. **Accepted:** July 3, 2023