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REVIEW ARTICLE

Risk Assessment of Foodborne and Waterborne Viruses, Detection Methods, and Possibilities of Eradication during Non-thermal Food Processing

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Abstract

In recent years, an increase in epidemic outbreaks has been observed worldwide, the etiological factors of which are foodborne and waterborne viruses. Viruses are stable in food matrices and can survive in the product environment without losing their replication capacity. Moreover, most of these pathogens have a low infectious dose. This article reviews the currently available information on food- and waterborne viruses. The main groups of eukaryotic viruses associated with foodborne infections and the possibilities of implementing effective detection and control methods for viral contamination in the food industry were characterized. The threats to the dairy industry resulting from the presence and possibility of infection of starter cultures by prokaryotic viruses – bacteriophages were also discussed. The presented information may support decision-making regarding ensuring food safety and provide guidelines that will allow the food industry to implement effective detection and thus eradicating methods for virus control during food production.

KEYWORDS
foodborne and waterborne viruses, risk assessment, virus detection, non-thermal processing technologies, bacteriophages, food industry

Introduction

Foodborne and waterborne microorganisms significantly contribute to the global increase in the risk of the occurrence of diseases [Di Cola et al. 2021]. Food poisoning outbreaks pose a threat to consumer health not only by causing diseases but also due to the costs associated with actions to limit the spread of foodborne diseases in the population [Bosch et al. 2018]. Guidelines for maintaining hygiene during food production processes are optimized mainly to prevent bacterial infections, and their effectiveness in eradicating viruses is only partially effective. An additional problem is that most food- and waterborne viruses are difficult to cultivate in cell cultures, making it difficult to conduct reliable studies on the inactivation of these pathogens [Koopmans, Duizer 2004].

Viruses are small particles (on average size ranging from 20 to 1000 nm) that cause various diseases in plants, humans, and animals [Roos 2020]. Each group of viruses exhibits tropism and has its typical host range and cellular preferences. Viruses can be transmitted through various routes: droplet transmission (from the coughing of an infected person), during sexual intercourse, or through contact with the feces of a person infected with a gastrointestinal virus, with the blood of a person infected with bloodborne viruses, or with animals infected with zoonotic viruses. Virions can also be transmitted by organisms that act as vectors for virus transmission (such as mosquitoes and ticks). In the context of food- and waterborne infections, the most important are viruses that infect cells lining the gastrointestinal tract, spreading through excretion in feces or as a result of vomiting [Koopmans, Duizer 2004].

Unlike microorganisms cultured on microbiological media, food- and waterborne viruses, except bacteriophages (i.e. viruses that replicate only in bacterial cells), do not replicate in food and require specific living eukaryotic cells to replicate their particles [Pexara, Govaris 2020]. The presence of just a few virus particles is enough to cause disease, as they replicate in the host cells at a high rate and are then transmitted to the environment through excreted feces [Koopmans, Duizer 2004]. Viruses are usually stable outside the host, exhibit resistance to external factors (e.g. acids), and can survive food processing and storage [O'Brien et al. 2021; Pexara, Govaris 2020].

The pathogenic properties of viruses have been known for a long time, but it was only at the end of the 1970s that it was observed that food can be a vector for the transmission of viruses pathogenic to animals, including humans [O'Brien et al. 2021; Roos 2020]. The latest data indicate that there has been an increase in viral epidemics among food- and waterborne disease outbreaks in recent years [Pexara, Govaris 2020]. In the report of risk assessment experts ('Final report on 'the identification of food safety priorities using the Delphi technique'), it was recognized that monitoring foodborne viruses is one of the most important priorities in food safety [Rowe, Bolger 2016]. According to the report of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), in 2017, viruses accounted for 7.8% of all food- and waterborne disease outbreaks [EFSA, ECDC 2018]. In Poland, according to the Sanitary Condition of the Country for 2022 prepared by the Chief Sanitary Inspectorate, there were 57,111 cases of foodborne viral infections reported, indicating a consistently high number of cases (for comparison, during the COVID-19 period: in 2021 – 23,365 cases of infections; in 2020 – 14,450 cases; in the pre-COVID-19 period: in 2019 – 62,333 cases; in 2018 – 48,577 cases; in 2017 – 55,563 cases). The most frequently reported viral infections are rotavirus (34,027 cases in 2022), norovirus (5,893 cases in 2022), and hepatitis A virus-related cases (232 cases in 2022) [GIS 2019; GIS 2023]. The issue of foodborne viruses primarily affects minimally processed and ready-to-eat products, therefore new alternative methods are needed to ensure the safety of such an assortment [Bosch et al. 2018; Di Cola et al. 2021; Pexara, Govaris 2020].

The occurrence of viruses in the food production environment and in food itself is a problem for both the food industry and regulatory authorities. Routine monitoring of foodborne viral infections in surveillance systems is only conducted by some highly developed countries (e.g. USA) [Bosch et al. 2018]. The development of standardized detection methods, such as the ISO standard for detecting norovirus or hepatitis A virus using real-time reverse transcription–quantitative polymerase chain reaction (RT-qPCR), allows for linking the increasing number of infections with these viruses to the consumption of contaminated food [ISO 2017]. Unfortunately, there is a lack of sufficient studies linking the presence of viral genetic material in samples with the infectivity of these virions [Bosch et al. 2018].

1. Characteristics of foodborne and waterborne viruses

Viruses are obligatory intracellular parasites that require susceptible host cells to replicate their particles [Roos 2020]. The human virome consists of eukaryotic viruses that infect body cells and bacteriophages, which target the strain- or species-specific bacteria of the gut microbiota [Liang, Bushman 2021]. Various viral particles are present in the human gastrointestinal tract (mainly in the intestine), but only some of them are commonly considered pathogenic [Jagirdhar et al. 2023]. Depending on the type of diseases they cause, viruses can be divided into a) viruses causing gastroenteritis; b) foodborne hepatitis viruses; and c) viruses replicating in the human intestine, which may lead to diseases as a result of migration to other organs (e.g. the central nervous system). Food can serve as a vector for the transmission of many viruses from different families, which can be an etiological factor for various diseases, including diarrhoea, severe neurological diseases, polio, myocarditis, respiratory diseases, or haemorrhagic fever. The most commonly reported symptoms of food- and waterborne viral infections are gastroenteritis, and hepatitis (liver inflammation) [Bosch et al. 2016]. Table 1 below presents the classification of food- and waterborne viruses based on the target tissue of these pathogens.

Among the viruses transmitted through food and water, enterotropic viruses dominate, showing affinity to the tissues of the gastrointestinal tract, where they can further migrate and infect internal organs. Hepatotropic viruses, such as HAV and HEV, trigger cytopathic and immunological mechanisms, resulting in inflammatory changes in the liver and hepatocyte necrosis. Food can also serve as a vector for neurotropic and pneumotropic viruses, responsible for infections of the central nervous system and respiratory tract, respectively. An example of pantropic (multitropic) viruses is the Ebola virus, which can cause infections throughout the body, although it shows a tendency to an affinity for dendritic cells, monocytes, endothelial cells, and hepatocytes. Among viruses containing the ssRNA genome, the majority have positive polarity and during their replication cycle, their genome acts as mRNA. In the case of viruses with negative polarity ssRNA, these particles contain an RNA-dependent RNA polymerase, allowing the transcription of the virus RNA into mRNA and translation of proteins within the cell's ribosomes.

2. Assessment of the risk of foodborne and waterborne viruses and methods for detecting viral infections in the food environment

To identify the potential risks related to the transmission of viruses and other biological factors in the food chain and implement effective monitoring measures, international food safety control organizations suggest conducting a risk assessment [Dong et al. 2015].

There are two fundamental approaches to risk assessment: epidemiological (top-down), starting from the analysis of disease-related data and aiming to eliminate the risk posed by a food product, and a food chain-based approach (bottom-up), starting from the analysis of the potential risk posed by a food product towards estimating the probability of disease occurrence caused by this factor [Bosch et al. 2018].

Risk assessment based on an epidemiological approach can provide information on the frequency of the occurrence of specific viruses in certain food products [Kirk et al. 2015]. The collected data can then be used to develop an integrated monitoring system and risk management strategy related to virus transmission in the supply chain [Bradshaw, Jaykus 2016].

Table 1. Classification of foodborne and waterborne viruses depending on their tissue tropism. The table is based on: [Abreu et al. 2020, Bosch et al. 2016, Bosch et al. 2018, Buczek et al. 2022, Han et al. 2021, Huang et al. 2021, Mazur-Panasiuk et al. 2021, Pexara, Govaris 2020, Pyrć 2015, Sharma et al. 2019, Stobnicka-Kupiec et al. 2019, Stobnicka-Kubiec et al. 2022, Wróblewska 2023].

Primary tissue tropism	Virus common name (abbreviation)	Particle / Genome	Taxonomy		Clinical symptoms	Transmission (infection dose)	Associated foods
			Genus	Family			
Enterotropic viruses	Human norovirus (HuNoV)	non-enveloped / ssRNA(+)	*Norovirus*	*Caliciviridae*	acute gastroenteritis, nausea, violent vomiting, watery diarrhoea, loss of appetite, fever	fecal-oral route (100 particles in 1 ml)	oysters, shellfish, fish, vegetables
	Human sapovirus (HSaV)	non-enveloped / ssRNA(+)	*Sapovirus*	*Caliciviridae*	gastroenteritis, nausea, vomiting, severe abdominal cramps and diarrhoea	fecal-oral route	shellfish (mainly oysters and mussels)
	Aichi virus (AiV)	non-enveloped / ssRNA(+)	*Kobuvirus*	*Picornaviridae*	gastroenteritis, nausea, vomiting, diarrhoea	fecal-oral route	raw shellfish
	Human astrovirus (HAtV)	non-enveloped / ssRNA(+)	*Mamastrovirus*	*Astroviridae*	gastroenteritis, diarrhoea, vomiting, fever	fecal-oral route	mussels, fruits and vegetables
	Human rotavirus (HRoV)	non-enveloped / segmented dsRNA	*Rotavirus*	*Sedoreoviridae*	gastroenteritis in children, diarrhoea in adults	fecal-oral or droplet routes (10-100 particles in 1 ml)	mussels, oysters, fruit, vegetables, water
	Human orthoreovirus (HOrV)	non-enveloped / segmented dsRNA	*Orthoreovirus*	*Spinareoviridae*	gastroenteritis, fever	fecal-oral or droplet routes	raw milk, meat
	Human adenovirus (HAdV)	non-enveloped / dsDNA	*Mastadenovirus*	*Adenoviridae*	gastroenteritis, watery diarrhoea with mucus, fever, vomiting, respiratory disease	fecal-oral or droplet routes	seafood (mainly shellfish)
	Parvovirus (PV)	non-enveloped / ssDNA	*Erythroparvovirus* (B19) *Protoparvovirus* (CPV, FPV)	*Parvoviridae*	gastroenteritis, vomiting, and fever in dogs (PV CPV) and cats (PV FPV), in humans only PV B19 is a pathogen and causes erythema infectiosum	fecal-oral route in animals, droplet route in humans	raw milk
Hepatotropic viruses	Hepatitis A virus (HAV)	non-enveloped / ssRNA(+)	*Hepatovirus*	*Picornaviridae*	hepatitis	fecal-oral route (10-100 particles)	fruits, vegetables, milk, shellfish
	Hepatitis E virus (HEV)	non-enveloped / ssRNA(+)	*Paslahepevirus*	*Hepeviridae*	hepatitis	fecal-oral route	raw or undercooked wild boar, deer and pork meat, liver and liver sausages
Neurotropic viruses	Poliovirus (WPV)	non-enveloped / ssRNA(+)	*Enterovirus*	*Picornaviridae*	flaccid paralysis, meningitis (very rare), fever or asymptomatic	fecal-oral or droplet routes	food contaminated with throat secretions and the patient's feces
	Non-polio enteroviruses (EVs)	non-enveloped / ssRNA(+)	*Enterovirus*	*Picornaviridae*	meningitis, herpes, flaccid paralysis, cranial nerve dysfunction, myocarditis, heart defects, respiratory diseases	fecal-oral or droplet routes (1-10 particles)	shellfish (mainly oysters), mollusks
	Human parechovirus (HPeV)	non-enveloped / ssRNA(+)	*Parechovirus*	*Picornaviridae*	meningitis, respiratory diseases, gastroenteritis	fecal-oral or droplet routes	shellfish
	Nipah virus (NiV)	enveloped / ssRNA(−)	*Henipavirus*	*Paramyxoviridae*	encephalitis, respiratory diseases	oral or droplet routes	pork
	Polyomaviruses (PyVs)	non-enveloped / circular dsDNA	*Alphapolyomavirus* and *Betapolyomavirus*	*Polyomaviridae*	chronic infections, progressive multifocal leukoencephalopathy, urinary tract diseases	fecal-oral or droplet routes	oysters, contaminated water
	Tick-borne encephalitis virus (TBEV)	non-enveloped / ssRNA(+)	*Orthoflavivirus*	*Flaviviridae*	encephalitis, meningitis	oral route	unpasteurized dairy products and milk from infected animals
Pneumotropic viruses	Human coronavirus (HCoV)	enveloped / ssRNA(+)	*Betacoronavirus*	*Coronaviridae*	respiratory diseases, SARS, MERS, gastroenteritis	oral or droplet routes	unpasteurized dairy products, fruit, vegetables, meat after insufficient heat treatment, frozen and chilled food
	Avian influenza virus (AIV)	enveloped / segmented ssRNA(−)	*Alphainfluenzavirus*	*Orthomyxoviridae*	flu, respiratory diseases	fecal-oral route	mollusks, poultry, water contaminated with feces of infected birds
Pantropic viruses	Ebola virus (EBOV)	enveloped / ssRNA(−)	*Orthoebolavirus*	*Filoviridae*	fever, chills, severe headaches and muscle pain, sore throat, vomiting blood and diarrhoea, inflammation of the stomach, intestines and liver	oral route	contaminated wild animal meat and contaminated water

Abbreviations: CPV – canine parvovirus; dsDNA – double-stranded deoxyribonucleic acid; dsRNA – double-stranded ribonucleic acid; FPV – feline panleukopenia virus; MERS – Middle East respiratory syndrome; ssDNA – single-stranded deoxyribonucleic acid; ssRNA – single-stranded ribonucleic acid; SARS – severe acute respiratory syndrome. The table uses the current taxonomy of viruses adopted by the International Committee on Taxonomy of Viruses (ICTV; as of June 29, 2024).

The majority of developed food chain-based risk assessments focus on enterotropic waterborne viruses (mainly rotaviruses) [Bosch et al. 2018]. When assessing the risk of virus transfer through food, a wide range of viruses and food products are taken into account. The main viruses analysed for risk assessment are HuNoV and HAV, as they are most commonly transmitted through food and water [De Keuckelaere et al. 2015]. Conducting such analyses can assist public health managers in prioritizing different illnesses in the population and identifying effective interventions to minimize the impact of food- and waterborne viral diseases on consumer health.

The result of the most important interventions aimed at controlling viruses in the food chain should be establishing microbiological criteria for these pathogens. An example of such activities may be the implementation of monitoring at every stage of the production process, such as harvesting shellfish from virus-free waters, establishing the permissible limit of HuNoV in seafood for consumption purposes, and controlling food products for compliance with allowed limits [Bosch et al. 2018]. Risk management should include the implementation of effective food safety assurance systems (such as Good Agriculture Practice (GAP), Good Hygiene Practice (GHP), and Good Manufacturing Practice (GMP)), implementing the "From Farm to Fork" Strategy, which is at the core of the European Green Deal, along with appropriate validation and verification procedures [Bosch et al. 2018; Crotta et al. 2018]. Risk management may also include the increased supervision of high-risk food products (including soft fruits, such as frozen strawberries) [Commission Implementing Regulation (EU) No 1235/2012], implementing education on hand hygiene compliance at every stage of the production process, and implementing a strategy for dealing with viral diseases in agricultural and food industry workers [Franck et al. 2015].

Most of the currently used methods for detecting food- and waterborne viruses rely on the use of molecular biology methods – the routinely used PCR technique and others, each of which has its advantages and disadvantages.

The ISO detection method [ISO 2017] for the quantitative and qualitative detection of HuNoV and HAV using RT-qPCR in food matrices (such as bivalve mollusks, green leafy vegetables, berry fruits, and bottled water) describes the protocol for virus extraction and their genetic material, and then RNA adsorption on silica. The standardized virus detection method allows diagnostic laboratories to conduct interlaboratory studies and verify results obtained in different units. Detailed control and the interpretation of results facilitate laboratories in obtaining accreditation certificates. The RT-qPCR method [ISO 2017] is characterized by the complexity of the procedure and is not suitable for highly processed food matrices, where low levels of virus contamination may lead to their undetection. This technique is costly due to the need for a large number of reaction purity controls and the purchase of commercial positive controls. Moreover, it does not allow for the differentiation of infectious and non-infectious virions [Bosch et al. 2018].

Another method of detection of food- and waterborne viruses is quantification and confirmation. The quantification cycle is the moment when the reaction enters the logarithmic phase of a product increase, and the fluorescence level allows for a reliable readout. Therefore, the quantification of viruses in epidemiological studies and routine monitoring of the levels of these pathogens in food matrices can serve as the basis for determining their acceptable level in food products and developing a quantitative risk assessment [Pinto et al. 2009]. Confirming positive RT-qPCR results through sequencing provides valuable information on the epidemiology of virus strains, however, due to low sensitivity, confirmation is a complicated procedure. Quantification by RT-qPCR is sensitive to reaction inhibitors and, as previously mentioned, unreliable at low levels of food contamination by viruses. This technique, combined with the confirmation of positive RT-qPCR results, increases the cost of studies and is a time-consuming procedure [Bosch et al. 2018].

Viruses with intact capsid structures can only infect cells. Detection of the genetic material of these particles by RT-qPCR does not necessarily mean that in every case we are dealing with infectious particles. The molecular detection of viruses with intact capsids allows for the detection of only virulent particles, thus limiting the overestimation of the number of infectious particles. The costs of this procedure, due to the need for the preparation of numerous reagents, the use of infectious and non-infectious controls each time, and the detailed analysis of protocols depending on the type of virus and food matrix, are significantly higher compared to the standard PCR method [Moreno et al. 2015; Sánchez et al. 2012].

To detect certain enterotropic infectious viruses, methods based on cell cultures can be used. This involves sequentially concentrating and purifying viruses extracted from a food matrix. The use of an integrated system based on the initial amplification of viral genetic material and removal of inhibitors in cell cultures before detection by RT-qPCR (or qPCR) shortens the time of detecting infectious virus particles. The method of an integrated cell culture reverse transcriptase quantitative polymerase chain reaction (ICC-RT-qPCR) is much more sensitive compared to cell culture alone, reduces the analysis time compared to cell culture, and allows for the detection of infectious viruses that do not exhibit cytopathic effects. The application of this technique for wild-type enteric viruses is complicated due to difficulties in their cultivation. The ICC-RT-qPCR method is not quantitative if it is not used as a test for the most probable number [Bosch et al. 2018; Yoe-Jin 2006].

New methods for quantitative virus detection are constantly being developed. Digital PCR (dPCR) technology, compared to RT-qPCR, is less sensitive to the presence of reaction inhibitors in food matrices and provides more reliable quantitative assessment results. Moreover, unlike RT-qPCR, the dPCR method does not require the preparation of standard curves. On the other hand, the use of next generation sequencing (NGS) technology can provide completely new data useful for designing primers and probes for PCR reactions. The NGS method also allows for the detection of previously unknown viruses. The introduction of new methods, like any unconventional technology, is associated with high costs, including sample preparation for analysis (isolation of genetic material, preparation of sequencing libraries, and costs of system reagent kits for the sequencer) [Bosch et al. 2018; Kishida et al. 2014; Moore et al. 2015].

3. The impact of innovative non-thermal processing technologies on the eradication of foodborne and waterborne viruses

To control and/or inactivate viruses transmitted through food and water, external and internal factors related to the food matrix, innovative processing technologies or chemical food preservation methods can be used [Bosch et al. 2018]. Increasing consumer awareness regarding the impact of consuming minimally processed products, preserved with non-thermal techniques to preserve bioactive compounds and reduce chemical food preservatives, has led to a growing demand for minimally processed food in recent years [Ragaert et al. 2004]. Therefore, methods for inactivating food- and waterborne viruses using innovative non-thermal food processing technologies, including high hydrostatic pressure, ultraviolet radiation, radiation sterilization (irradiation), pulsed electric field, and cold plasma, are discussed.

The high hydrostatic pressure (HHP) technique involves applying ultra-high pressure (200-800 MPa) to hermetically sealed food products in a thermally insulated vessel [Koutsoumanis et al. 2022]. The finished product is placed in a special pressure chamber filled with a pressure transmitting fluid. It is transported through a liquid medium (typically water, but also propylene glycol, silicone oil, or castor oil), ensuring uniform pasteurization and the immediate achievement of high-pressure parameters [Huang et al. 2020]. High-pressure processing, also known as pascalization, can extend the shelf life of food by inactivating microorganisms and enzymes with minimal impact on the sensory, physical, and nutritional properties of the food [Abera 2019]. Currently, the food industry uses HHP for the preservation, among others, of fresh shellfish, vegetable products, juices, beverages, jams, ready-to-eat meat products, and drinkable yogurts [Lou et al. 2015]. HHP is used to eliminate bacteria, protozoa, and fungi transmitted through food and water. In terms of pressure sensitivity, microorganisms are divided into three groups: Gram-negative bacteria, which are inactivated at pressures of 300 MPa and above, fungi (yeasts and molds) at 400 MPa, and Gram-positive bacteria at 600 MPa and above. Viruses, like bacterial spores, can only be effectively inactivated at very high pressures and simultaneous heating [Dumay et al. 2010]. The HHP method denatures the capsid proteins of viruses, preventing infectious virions from attaching and penetrating host cells. In the case of enveloped viruses, it has been shown that HHP also affects their denaturation. Studies indicate a heterogeneous response of foodborne viruses to HHP preservation. The effectiveness of this method, even for the same type of virus, depends on the strain [Pexara, Govaris 2020]. Apart from the parameters of the HHP process itself, the acidity of the food matrix also affects virus inactivation - low pH significantly increases particle inactivation. Another important factor is the composition of the matrix, as the presence of lipids, carbohydrates, salt compounds, or proteins limits the effectiveness of HHP against viruses, and the same virus may exhibit different pressure resistance in different food products [Emmoth et al. 2017].

Ultraviolet light (UV) is a form of electromagnetic radiation with a wavelength ranging from 10 to 400 nm, which can damage organisms [Pexara, Govaris 2020]. UV is a cost-effective, non-toxic, and easy-to-apply method for food preservation. UV light can be used for both food preservation and disinfection purposes, such as for eggshells, ready-to-eat meals, meat, vegetables, food packaging surfaces, and production lines [Hirneisen et al. 2010]. UV radiation inactivates microorganisms by disrupting DNA replication and transcription processes. The effectiveness of UV light in eliminating viruses depends on the food composition. UV light neutralizes the nucleic acids of the virus, and high doses interact with the capsid proteins, leading to genome destabilization by the RNases present in the food environment. The effectiveness of virus inactivation by UV light is influenced by the type of virus genetic material, capsid protein structure, host cell type, and virus morphology and aggregation. It has been shown that viruses with a single-stranded genome are 10 times more sensitive to UV light compared to viruses with a double-stranded genome [Hirneisen et al. 2010; Pexara, Govaris 2020].

Ionizing radiation (irradiation) is considered to be one of the best methods for reducing microorganisms in food while preserving its nutritional value and sensory quality [Nowicka et al. 2014]. In the EU, radiation is used in France, the Netherlands, and Belgium for preserving fruits and vegetables, cereals, meat products, and shellfish, while in Poland it is used for preserving dried vegetables, mushrooms, and spices [Jędrzejczyk et al. 2010]. According to the recommendations of the Food and Agriculture Organization of the United Nations (FAO), the total maximum absorbed dose by food should not exceed 10 kGy [Indiarto, Qonit 2020, Jędrzejczyk et al. 2010]. Free radicals generated by radiation can induce stress reactions in vegetables, leading to the increased synthesis of antioxidant compounds [Ayhan 2017]. Depolymerization of cell wall components, such as pectins, cellulose, and hemicelluloses, caused by gamma radiation, may result in decreased firmness and the softening of plant tissues [Prakash et al. 2003]. The effectiveness of radiation against viruses depends on the size of their particles, suspending medium, food product, and process temperature [Huang et al. 2019]. Most viruses are much more resistant to radiation than bacteria in vegetative form, parasites, and fungi, which may be due to their smaller size and even shorter genome (especially in the case of ssRNA viruses). Furthermore, the dose of radiation allowed by the US Food and Drug Administration (USFDA) at 4 kGy enables a reduction in virus levels by a maximum of one logarithmic unit and higher doses are required for the complete eradication of virions in most food products [Bosch et al. 2018].

Another non-thermal method of food preservation is the pulsed electric field (PEF). During the preservation process with PEF, food products placed between two electrodes are exposed to a high-voltage electric field ranging from 20 to 70 kV/cm for several microseconds [Gomez et al. 2019]. This process does not heat the food but instead causes local changes in the structure and degradation of cell membranes [Nowicka et al. 2014]. PEF is a better technology than traditional thermal food processing because it guarantees the preservation of sensory and physical characteristics of products, as well as a higher content of nutrients and phytochemicals while simultaneously inactivating biological contaminants [Gómez et al. 2019; Salehi 2020]. The stress caused by momentary changes in membrane potential can lead to the loss of tissue turgor and increased extraction of valuable components from cells, limiting the application of this technique to the preservation of fresh plant-based products [Zhao et al. 2014]. Among animal-derived products, PEF is used for preserving milk,

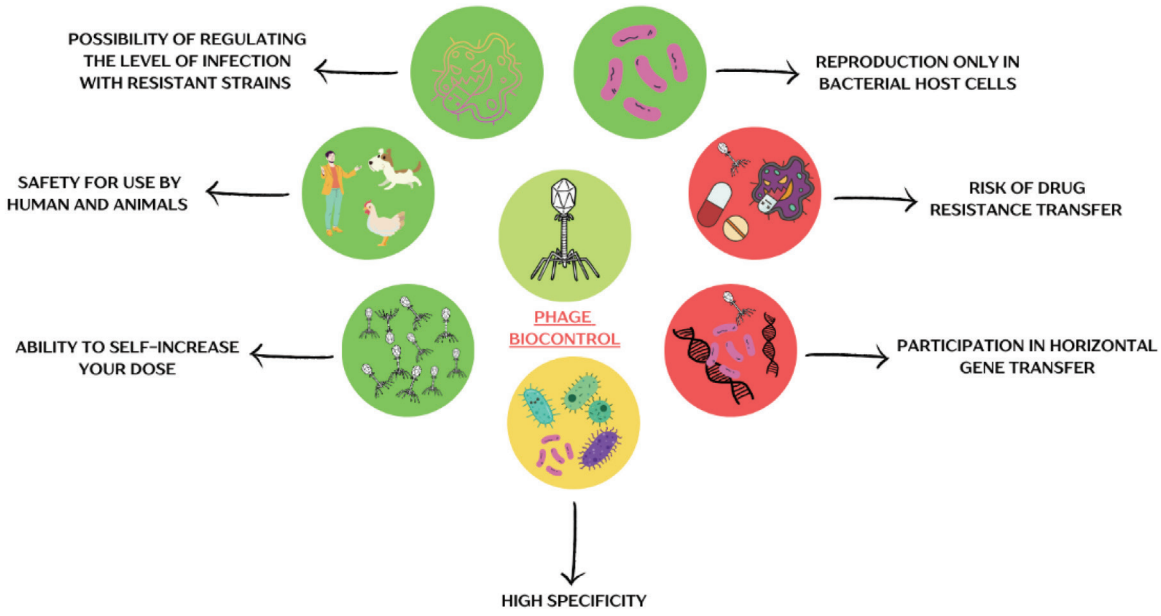

Figure 1. Benefits and threats related to using bacteriophages as food biocontrol agents (own study). The figure was prepared in Canva.

yogurt drinks, and liquid egg products. PEF successfully inactivates vegetative bacteria and molds, while spores are difficult to destroy using this method. The effectiveness of PEF in microbial inactivation depends on process factors (electric field intensity, pulse width, temperature, and process time), microbiological factors (number, type, and growth stage of microorganism), and factors related to the food matrix (acidity, antimicrobial agents, presence of ions, and ionic conductivity). The level of microbial inactivation increases with higher electric field intensity, process time, and temperature [Pexara, Govaris 2020]. Studies indicate that viruses are not effectively inactivated by PEF treatment [Khadre, Yousef 2002].

Research results indicate that cold plasma (CP) effectively inactivates biological agents, including viruses, bacteria, spores, and fungi [Niedźwiedź et al. 2019]. The CP method is generated by applying an electromagnetic or electric field to a gas. The most important active forms generated by plasma discharges are neutral or excited particles and atoms, UV photons, negative and positive ions, free radicals, and electrons. The CP technique can be used as a method for disinfecting materials in direct contact with food, as well as for food sterilization and preservation. A significant disadvantage of CP is its impact on increased lipid oxidation, loss of vitamins, and deterioration of organoleptic properties during the processing and storage of food products [Chizoba Ekezie et al. 2017]. The application of cold plasma technology inactivates viruses through changes in the structure of their proteins, genetic material, and lipids found in their envelopes. The key virus-killing factor is the chemical interactions of active compounds, particularly reactive forms of oxygen and nitrogen, which damage nucleic acids, reducing gene expression [Pignata et al. 2017, Pradeep, Chulkyoon 2016].

4. Bacteriophages – friends or enemies in the food industry?

A completely separate group of viruses are bacteriophages (phages), which are particles capable of replicating only in bacterial host cells [Endersen, Coffey 2020]. Bacteriophages can be considered as an unconventional method of biocontrol for bacterial infections, however, on the other hand, they may pose a threat to the fermentation industry, such as in the dairy industry. Phage biopreparations can be used in three sectors of the agri-food industry. Biocontrol using bacteriophages can support primary production (including animal breeding and crop cultivation), biosanitization (mainly in production facilities, to prevent biofilm formation on equipment surfaces), and bioconservation (aimed at extending the microbiological shelf life of food by limiting the growth of saprophytic and pathogenic bacteria) [Gientka et al. 2021, Połaska, Sokołowska 2019]. In addition to the many advantages of using phages, there are also certain risks that should be taken into account when typing phages when developing biopreparations for effective biocontrol (Figure 1) [Wójcicki et al. 2021].

In the dairy industry, infections of starter cultures caused by contamination with bacteriophages remain the main cause of fermentation failures. Phage epidemics lead to significant economic losses associated with the wastage of raw materials, which ultimately leads to production delays and a decrease in the quality of the final product [Fernández et al. 2017, Ortiz Charneco et al. 2023]. On the other hand, bacteriophages in the dairy industry can be used as biocides to eliminate spoilage bacteria (such as psychrotrophic *Bacillus* and *Pseudomonas* genera, whose metabolic activity leads to the production of proteolytic and lipolytic enzymes that reduce the quality of milk) or pathogenic ones. Furthermore, bacteriophages in primary production can effectively treat mastitis, caused by *Staphylococcus aureus*. Finally, prophages of lactic acid bacteria can influence the acceleration of the metabolism of their bacterial hosts [O'Sullivan et al. 2020, Pujato et al. 2019].

Currently in the dairy industry, there is strict monitoring of entry points, fast and effective methods for detecting phages, and control measurements to reduce the risk of phage replication in

dairies. Effective disinfectants are used (including solutions of peroxyacetic acid or sodium hypochlorite) and the rotation of starters/milk strains is practiced. A promising solution for the future is the implementation of genetic engineering methods to construct lactic acid bacteria strains that are resistant to bacteriophages [Fernández et al. 2017, Panezai 2021, Pujato et al. 2019].

Phage detection in dairy production is based on monitoring the acidification process and conducting lysogen tests. Additionally, qPCR or PCR techniques are used (detection limit: 10^3 PFU ml^{-1}; PFU – plaque-forming unit) as well as the flow cytometry method (prior to analysis, lipid droplets need to be removed from the samples) [Fernández et al. 2017, Pujato et al. 2019].

Conclusion

In response to the increasing number of viral infections associated with food and water, it is important to implement a constant monitoring of the presence of major types of viruses in the food production area. This requires the development of effective (especially non-thermal) food processing methods and the implementation of modern, sensitive virus detection procedures. These strategies have the potential for the early identification of epidemic viral infections, and the data obtained can provide valuable information for assessing the risk associated with the presence of these pathogens in the food production environment.

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