Bacteriophage applications as biocontrol agent in food packaging materials

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Bacteriophages or phages are the most prevalent organisms in the world and spread widely in different species of food. The most promising system is the use of bacteriophages against pathogenic bacteria in food for biocontrol. In order to provide biocontrol in an effective manner, it is necessary to determine the host phage by control of bacteriophages for each pathogen species. Nowadays, researchers are focusing on foodborne pathogenic bacteria such as *Campylobacter jejuni*, *E. coli* O157: H7, *Listeria monocytogenes* and *Salmonella* spp. In recent years, several studies have been carried out with the aim of developing new packaging films with antimicrobial properties and the use of bacteriophages with antimicrobial properties in active packaging formulations is an outstanding research topic. To expand the use of phages beyond direct spraying of phages on food products, there is a need to develop material formulations that can encapsulate phages for improved stability and release at target sites for delivery. Thus, phages can have significant applications as additives to packaging material formulations. In this chapter, the antimicrobial properties of bacteriophages and their potential for use in food packaging materials will be discussed with potential applications of their advantages and disadvantages.

Keywords Bacteriophage; Antimicrobial; Biocontrol; Food packaging

1. Introduction

Foodborne illnesses are recognized worldwide as one of the most serious problems that concern public health. Despite safety concepts such as modern technologies, GMP (Good Manufacturing Practices), quality control and hygiene, HACCP (Hazard Analysis Critical Control Point) and risk assessment, the number of foodborne illnesses and poisonings has increased in the past decade. The most common foodborne infections in the European Union are caused by *Campylobacter* spp., *Salmonella* spp. and *Listeria* spp. and some viruses [1]. There is a need for a longer complex food chain that can reduce the risks of microbiological contamination due to the globalization of the food market, the production of functional foods for new demand, new production techniques and the increased demand for minimally processed ready-made products. Direct application of the chemical substances used for the purpose of reducing the pathogenic contamination in the food industry on fresh fruits, vegetables and ready-to-eat foods are inconvenient.

Therefore, new control strategies are needed to reduce microbial load on the raw material, prevent bacterial pathogens from being placed in the food chain, and to meet consumer needs that require "minimally processed food containing minimal chemical preservatives" [2,3,4]. Biocontrol as an alternative food preservation technology increases the shelf life and hygienic quality of the product and minimizes the effect on the sensory and nutritional properties of easily degradable food products.

Bacteriophages, defined as bacterial viruses, are the most prevalent organisms in the world and multiply only in living bacteria as obligatory parasites of bacteria [5, 6]. Bacteriophages were first discovered in 1896 by Ernest Hankin and the antimicrobial activity of bacteriophages was described by Frederick Twort in 1915. However, in 1919 Felix d'Herelle was known as the first scientist to use bacteriophages for the treatment of dysentery for therapeutic purposes [7,8]. The classification of bacteriophages can be grouped into 13 groups based on their shape, size, nucleic acid shape and presence or absence of lipids. Another classification is based on the morphological characteristics of the tail of bacteriophages. Accordingly, bacteriophages are separated three groups as *Myoviridae* (shortenable tail), *Siphoviridae* (long, not shortenable tail) and *Podoviridae* (very short tail) [9]. Bacteriophages are multiplied by lytic or lysogenic cycle of life. In the lytic cycle, seen in lethal phages such as the T4 phage, the host cell is broken and dies immediately after virion (out of the cell virus) multiply. The lysogenic cycle does not cause to break down of the host cell. In the lysogenic cycle, phage is infected, but it continues to live. As long as the host cell health is in place, the virus will continue to exist, but if the nutrient sources are exhausted, the endogenous phages will be activated. A proliferation process is initiated and the host cell is not fragmented. Because the lysogenic cycle allows the host cell to multiply, the virus continues to exist in the offspring of the cell. Occasionally, endogenous phages will bring new functions to the host bacterial genome during periods of inactivity. This situation is beneficial to host bacteria, and this phenomenon is called lysogenic transformation [5].

With the discovery of the phages, the use of them for preventive and therapeutic purposes against bacterial infections has also been on the agenda. Phage therapy has been used for the treatment of diseases such as typhoid, cholera, urinary tract infections when phages were first discovered. However, it has been observed that have not received positive results from any of the treatments performed for various reasons such as poor quality control and insufficient purification. In 1928, the antibiotic era began with Alexander Fleming's discovery of penicillin and interest in phages lost significance

[6, 10]. Nowadays, inappropriate use of antibiotics seems to cause the rapid spread of resistant bacteria in the society [11]. The application areas of phages include water and food safety, agriculture and animal health issues. In recent years, research on phage molecular biology has made it possible to find use in biotechnological applications of different fields such as nanotechnology, vaccine development, drug applications, bacterial detection systems, development of new antimicrobials against antibiotic resistant bacteria. Another promising application area of phages is their use as natural antimicrobials with the aim of inhibiting undesirable bacteria in the foods that are reported to be accepted by the consumer [3,12]. The reason for this use is that bacteriophages have a very large environmental distribution and are found in high numbers, including the digestive system of humans, as well as groundwater, rivers, irrigation waters, wastewater, oceans, bioaerosols and greenhouses [3,7].

Since bacteriophages can be used as antimicrobial agents, phage-based antimicrobial agents have begun to be produced by many companies worldwide. Pathogenic bacteria can also be contaminated during animal cutting, milking, storage, packaging, or applied technological processes. Especially, phages that are commercially produced in the name of food additive and developed against the most important pathogens such as *E. coli*, *Salmonella*, *Listeria* and *Campylobacter* in farm animals approved to use in ready-to-eat foods, animal feeding and therapy by the US Food and Drug Administration and the Ministry of Agriculture [13, 14].

In all previous biocontrol studies with the use of bacteriophages, microorganisms were inoculated on the food, surface and subsequently phage applied to the inoculated surface, typically by inhalation of the phages to the inoculated surface. It is known that in many cases the target pathogens may be distributed and localized on the surface and may also be present on one or both surfaces of the food surface. This situation reduces the effect of phage therapy. In order to expand the phage use except that the phages are sprayed directly onto the surface of food products, there is a need to develop material formulations with ingredients such as encapsulated phages that will enhance, improve phage stability, and release into the target area. Thus, the phage will increase in importance as an additive in the formulations of packaging materials [15].

2. Biocontrol of some pathogenic bacteria with bacteriophages

Bacteriophages have a suitable structure for reducing or preventing disease and colony formation in livestock, decontaminating raw products such as fresh fruits and vegetables from harmful substances, extending the shelf life of easily disturbed foods as natural protectors. The use of bacteriophages as biocontrol agents is noteworthy as an alternative approach that can be used in the control of both human and food pathogens due to the increased specificity of antimicrobial resistance increases and phage detection of common pathogenic microorganisms [3, 4, 16, 17]. In order to provide biocontrol in an effective manner, it is necessary to determine the host phage with the bacteriophages control for each pathogens. Researchers are focusing on foodborne pathogenic bacteria such as *Campylobacter*, *E. coli* O157: H7, *Listeria* and *Salmonella*. As a result of these studies, the first commercial product based on phage to be used in foodstuffs was manufactured in the Netherlands. However, when compared to other preservatives, the phage use is a cost-effective alternative [18].

A lot of researches have been performed against *Salmonella* and *Campylobacter* in chickens treated phages and the pathogenic *E.coli* in ruminant animals treated with phages [19, 20]. In one study, In one study, when the combination of KH1 and SH1 phages were applied to cattle, the number of *E. coli* was reduced by keeping phages around 10^6 PFU/ml in the water that cattle drink [21]. Very little work has been done in relation to the use of phages in *E. Coli* O157: H7 biocontrol in food, researches usually concentrate on live animals. In another study, phages were applied to the surface of the meat to prevent pathogen development. The mixture consisting of three different phages was applied to beef cattle contaminated with 10^3 CFU/g of *E. coli* O157: H7. *E. coli* O157:H7 were not detected in most samples after storing at 37 °C. The antimicrobial activity of phages is limited during the processing and storage of food, but the results are promising. In the production of cheddar cheese, there was a decrease in the number of viable cells after storage by the addition of pages to milk contaminated with *Salmonella* [22]. Similarly, development of *S. aureus* was inhibited by phages during milk and clot production and the inhibition continues during the storage and ripening of acid coagulated and semi-hard cheeses [23, 24]. Salmonella page was also analyzed in fruits. There was a significant decrease in the target bacteria while the number of phages was stable. On the other hand, rapid degradation of phages was observed because of the low pH of the apples [25]. Bacteriophages are also effective in reducing the number of *Campylobacter*. Hence the risk of cross-contamination throughout the animal's body is eliminated [26, 27]. In another study, many bacteriophages effects were observed in contaminated chicken skin. At 4 °C, antibacterial phage activity was detected and 95% of the target cells were determined be inhibited [28]. In a study was about L. monocytogenes, a commercial product called Listex P100, approved by the FDA (Food and Drug Administration), was used as a biocontrol agent. This product is based on the lethal P100 phage and the complete destruction of the target cells [29]. The preparate of 6 bacteriophages isolated from the environment (LMP 102) are being developed as a contribution to ready food [30]. Other examples of phage-based bioprocessing approaches are inhibition of *Enterobacter sakazakii* in infant milk and *Salmonella* Typhimurium in chicken sausages [31, 32]. The other contribution of phages to food safety is that they can be used for the identification of foodborne pathogens. Many phage-based methods have been developed to detect bacteria in food while phages are used for a long time in determining bacterial strains [4]. The studies in which the antimicrobial effects of bacteriophages are determined shown in Table 1.

Table 1. Antimicrobial effects of bacteriophages against some foodborne pathogenic bacteria

Pathogen	Bacteriophage	Assay conditions	Sample	Reference
Salmonella Enteritidis	SJ2	Storage at 8 °C for 99 days	Cheddar cheese	[4]
Salmonella Enteritidis	Litic *Salmonella* phage cocktail	Storage at 5, 10 and 20 °C for 48 hours	Melon and apple slices	[25]
Salmonella Javiana	*Enterobacter asburiae* JX1 and combination of 5 different litic bacteriophage	Post-harvest storage	Tomatoes	[33]
Salmonella spp.	*Enterobacter asburiae* JX1 and combination of 6 different litic bacteriophage	Storage during 4 days	Mung bean and alfalfa seeds	[34]
Salmonella Typhimurium	*Salmonella* Typhimurium phage P7	Incubation at 5 and 24 °C for 24 hours	Raw and cooked beef	[35]
Salmonella Enteritidis	*Salmonella* phage 12	Incubation at 4 °C for 24 hours	Chicken skin	[28]
Campylobacter jejuni	*C. jejuni* phage 12673	Incubation at 4 °C for 24 hours	Chicken skin	[29]
Campylobacter jejuni	*C. jejuni* phage Φ2	Incubation at 4 and -20 °C for 10 days	Chicken skin	[36]
Listeria monocytogenes	Alone and combined effect of phage LH7 and Nisin	Storage at 4 for 4 weeks	Liquid medium and vacuum packed raw beef	[37]
Listeria monocytogenes	Specific LM-103 and LMP-102 litic phages	Storage at 10 °C for 7 days	Melon and apple slices	[25]
Listeria monocytogenes	P100 litic phage	Storage at 6 °C	Soft cheese	[29]
Listeria monocytogenes	P100 litic phage	Storage at 4, 10 and 22 °C for 15 m, 30, 1 h and 10 days	Catfish fillets	[38]
Listeria monocytogenes	P100 litic phage	Storage at 4, 10 and 30 °C for 10 days	Raw salmon fillets	[39]
Listeria monocytogenes	Virulent A511 and P100 litic phage	Storage at 6 °C for 6 days	Ready to eat foods	[40]
Staphylococcus aureus	Staphylococcal bacteriophage K	Storage at 4 °C for 2 h after pasteurization	Raw bovine milk	[41]
Staphylococcus aureus	Nisin and Endolicin LysH5	Incubation at 37 °C for 4 and 6 hours	Pasteurized milk	[42]
Staphylococcus aureus	Phage cocktail formed 8 different Staphylococcal bacteriophage	Incubation at 4, 20 and 37 °C	UHT and pasteurized milk	[24]
Enterobacter sakazakii	ESP 1-3 and ESP 732-1 phages	Incubation at 12, 24 and 37 °C	Reconstitute newborn formulation	[31]

The use of bacteriophage has many advantages. Phages are natural control agents for bacteria and they do not affect the smell, taste, texture and color of foods. Phages are host-specific and only affect the target bacterium. Bacteriophages are found everywhere in natural environmental conditions and are friendly to the environment. The total number of phages on Earth is estimated at 10^{30}-10^{32} and there are more than one hundred million types of bacteriophages. For this reason, phage therapy is much cheaper than developing new antimicrobials. On average, there are more than 10 bacteriophages per bacterium. Poultry products, fruit and vegetable products, and retail cheeses contain 10^8 PFU/g bacteriophages [43, 44]. Bacteriophages are considered safe for a variety of reasons. Phages are host-specific and do not infect animals and plants, including humans. No evidence has been obtained on the negative effects of studies on phage animal or phage human interactions in more than 80 years. In addition, phages act as competitive inhibitors of pathogens and do not affect the normal saprophyte microflora. From the perspective of food safety, the use of lytic phages alone is important, as lysogenic phages can transfer bacterial genes such as virulence factor and antibiotic resistance [43]. However, there are still some concerns about the use of bacteriophages. These worries are;

(1) Rapid cell disruption of bacteria can lead to the release of large amounts of endotoxins bound to the bacterial membrane.

(2) Some phages can encode toxins.

(3) There is a lack of pharmacokinetic data.

(4) Neutralization of phages by the host immune system may cause phage therapy failure.

(5) The conversion of lytic phages to lysogenic phages may lead to bacterial immunity and at the same time alter the virulence of bacteria [44].

As a result, experimental evidence of antimicrobial activity of phages at during food processing and storage is still not sufficient, but the results are quite encouraging. Bacteriophages are known as the new antimicrobial agents of the food industry and studies have focused on expanding their use in food products.

3. Bacteriophages for potential antimicrobial use in food packaging

Active packaging has been developed with the aim of extending the shelf life or enhancing its sensory properties and safety with package, product and environmental interactions, maintaining the quality of the product [45]. Some examples of active packaging methods include oxygen, carbon dioxide, ethylene and moisture absorbers, antimicrobial containing films and coatings, and flavor emitters / emulsifying systems [46]. The most important development in active packaging systems is the antimicrobial packaging of controlled release of antimicrobials from the packaging material. Organic acids, bacteriocins, antibiotics, fungicides, chelating agents and parabens can be found in food packaging materials and exhibit antimicrobial activity [47]. Antimicrobial packaging is a suitable protection method, especially for fresh red meat, poultry meat, aquaculture, processed meat products and dairy products [45, 48]. In these systems, where the release of antimicrobial compounds is controlled, only the initial microorganisms are not inhibited and the microbial growth that is present during storage and transport of the product may also be inhibited. These systems are particularly important in terms of ensuring food safety [49]. Today, many of the antimicrobial active packaging materials exhibit broad spectrum antimicrobial properties, not specifically targeting a single pathogenic species. There is a significant need to develop new antimicrobial packaging materials that exhibit high specificity only to the pathogenic organism when the commensal flora is being protected. The specificity of antimicrobial activity specific to pathogens, which are a small part of the total microorganism concentration of food, is important. [50]. Thus, the developing pathogenic specificity of antimicrobial active packaging materials may increase antimicrobial activity by reducing interactions with non-target microorganisms. Moreover, many non-pathogenic microorganisms are important and necessary for the production of certain dairy products and fermented foods. In addition, commensal bacteria, such as probiotics are useful for human health, and even these probiotics can control the development of pathogenic bacteria [51]. Due to all reasons, the use of bacteriophages to achieve inactivation of the target bacterium with only targeted pathogen-directed biomolecation, while maintaining normal microflora, is one of the popular research subjects in recent years, unlike packaging materials that exhibit broad spectrum antimicrobial activity.

A new approach to the use of bacteriophages as biocontrol is to add bacteriophages to an absorbent material and use these absorbent pads directly on the food surface. By means of these pads, bacteriophages are diffused into the substrate, thus offering new ways of keeping the vegetables fresh for a long time both retailers and consumers. Meireles et al. [52] inoculated *Salmonella* Typhimurium bacteriophages isolated from chicken and pig excrements into absorbent pads. The presence of bacterial growth was determined at 6, 12, 24, 36 and 48 hours of working with bacteriophages at different concentrations such as 10^6, 10^8 and 10^9 PFU/mg. The metabolic activity of the host cell is very important in the absorption of bacteriophage to the host cell surface. Storage of food in refrigerated conditions is important for bacteria control, but studies have shown that these conditions are the limiting factors for bacteriophage activity. Lone et al. [53] developed a new protocol for biocontrol of *L. monocytogenes* and *E. coli* in ready-to-eat foods. In this study, phage cocktails were immolized and applied to cellulose membranes, pads or direct papers encapsulated in alginate. The number of *L. monocytogenes* decreased by 2 logarithm units at 4 and 12 ^oC after 24 hours in direct application. A reduction of 1 logarithmic unit of bacterial cell count has been achieved by applying immobilized bacteriophage on cellulose membranes. In addition, cultivated *E. coli* in the alfalfa seeds showed a decrease of 1 logarithmic unit within 1

hour. Chai et al. [54] have developed a new technique. In the study, glass coupons coated with the bacteriophage exopolymerase enzyme were used to reduce health risks from the accumulation of bacterial biofilms. The researchers have shown that the thermal stability of the bacteriophage enzyme preparation can be seen to be reduced by a minimum amount of 10 minutes at 75 °C. After 4 hours of application to the enzyme preparation, 80% of the biofilm-forming bacteria were eliminated. This ratio increased to 92% when it was supplemented with chlorine dioxide application for 30 minutes. Researchers have indicated that the use of the depolymerase enzyme can reduce the number of platonic bacteria in the liquid medium as well as in biofilms. This situation may possibly increase the enzyme spread in the glass container. The enzyme is determined to break down sugar residues from the exopolymeric matrix of *Klebsiella aerogenes*, as confirmed by HPLC analysis. This suggests that enzyme and bacteriophage activity can be measured by detecting sugar residues in solution. In addition, the enzyme's activity against sugar residues also suggests that the spectrum of cleavage activity can be extended to the capsule layer surrounding these Gram negative cells [53] In a study conducted by Korehei and Kadla [55], they developed a new method for attaching T4 bacteriophages on Poly Ethylene Oxide (PEO) fibers. These T4 bacteriophages, which are active against *E. coli*, provide an initial release by the proposed method, as well as an effective release of bacteriophage into the buffer medium. Transmission Electron Microscopy (TEM) data shown that bacteriophages are randomly distributed on fibers and are distributed more deeply in the fiber core. While TEM images of fibers at different stages of the dissolution process reveal that randomly dispersed bacteriophages are rapidly separated, bacteriophages found in the depths of the fibers have more slowly dissociated in the buffer solution. Analysis for T4 bacteriophage revealed that 100% of the particles were released within 30 minutes during the immersion of the PEO fibers into the aqueous buffer. The researchers suggest that this is probably due to the hydrophobic nature of the dissolution of PEO fibers in aqueous media. The experiments have shown that bacteriophages can be modulated by varying the thickness of the fibers where the change in molecular weight is between 100 and 600 k. This change in thickness resulted in a 1 logarithmic decrease in the bacteriophage release over a 10 minute test period. These results support slow release bacteriophage release following a high initial onset. In addition, researchers report that PEO fibers reduce the release rate at the same time that they are combined with cellulose diacetate fibers. These results show that the inclusion of bacteriophages with the materials used makes food packaging as much as possible, but the materials used greatly affect bacteriophage release.

One of the major difficulties in developing bacteriophage based packaging materials is the stability of phages in packaging formulations. There are a limited number of studies on the stability of phages in packaging material formulations. The stability of encapsulated bacteriophages in active packaging material is a critical requirement for the successful integration of phage into packaging materials and application areas in food systems [15]. Microencapsulation studies have been carried out to increase the stability of bacteriophages affected by adverse environmental conditions in packaging materials. As a result of the microencapsulation process applied in the investigations, it has been stated that the bacteriophages maintain their viability for a longer time and their bioavailability is increased [56]. The most preferred microencapsulation method in the food industry is the spray drying method. This method, which is widely used in the food industry in order to remove water from the products, reduces the storage and transportation costs and protects the specific properties of the products, has been developed together with milk powder production. Despite the fact that spray drying is the most common and cheapest technique, the selection of material to be preferred for encapsulation is very important [57, 58]. In practice, both the material to be coated and the coating material are contained in the atomizer together. The major disadvantage of this method is the need for high temperatures and it has also been reported that it is difficult to maintain cell viability at the desired amount in the final product [59]. On the occasion of reports of working in high temperatures that cause certain problems in encapsulation practices [60, 61], some researchers should work on the development of encapsulation methods at lower temperatures [62]. Many researchers have attempted to encapsulate bacteriophages in different working conditions. Many researchers have attempted to encapsulate bacteriophages in different working conditions. A study was conducted to determine optimum industrial production conditions for microencapsulated phage production in the presence of lactose, trehalose, and dextran by spray drying in order to treat bacterial lung infection, commonly known to be caused by *Staphylococcus aureus*. The study has been reported as a 2.58 and 0.02 logarithmic unit with decreasing order during the microencapsulation process in *Myovirus romulus* and *Podovirus* LUZ 19 numbers. As a result of the study, it has been reported that *Myovirus romulus* number loss was higher due to its sensitive structure [63]. In the study, which is the continuation of this research, the effect of long-term storage on phages was examined. In this study of the change in storage of *P. aeruginosa* LUZ19 and *S. aureus* Romulus phage, the stability of microencapsulated phages obtained from trehalose and whey protein isolate stored in different temperature and relative humidity conditions was checked. While the optimal storage conditions in microencapsule application from trehalose powder were obtained at 0% relative humidity conditions at 4 °C, it has been reported that storage conditions at 25 °C and above adversely affect stability. It was reported that there was no decrease in the activation of the phages stored at 4 °C in the dark during the 1 month storage period in the microencapsulation process using whey protein isolate. As a result, it has reported that the storage conditions of the phages to be applied for therapeutic purposes were important parameters to be taken into account [15, 64]. In oral phage treatments, the activity of phages was reduced in acidic environmental conditions and in the presence of certain enzymes. Various coating materials should be developed by researchers to protect phage from acidic conditions and enzymes. While researchers reported that alginate and pectin-based polymers were protect phages from

adverse environmental conditions as effective protective sheaths in phage applications, the use of whey protein isolates in microencapsule applications has been maintain phage viability and allow controlled release [65, 66]. Vanosek et al. [15], only a 1 logarithmic reduction was observed in films prepared with whey protein isolate incorporated T4 encapsulated bacteriophage during storage for 5 weeks at 22 °C and light. Gouvea et al. [67] conducted a study of the acetate cellulose films which prepared for the inactivation of *Salmonella Typhimurium* ATTC 14028, they determinated the stability of encapsulated bacteriophages in a 14 day storage period due to the average shelf life of a chilled product 3-10 days. Accordingly, the initial bacteriophage concentration in the study they performed was 10^{10} PFU/mL, but decreased to 10^{8} PFU/mL on the first day of storage. Along with a 2 logarithmic decrease in initial concentration, bacteriophage concentration was not detected after storage for 14 days [66]. In a different study by Bieganski et al. [68], stability of the *E. coli* target phage which Felix O1 was determined during 5 weeks storage period after being encapsulated in alginate microspheres. It has been determined that after air drying at 22 °C, only 12% of the phages were activity during storage and 6% of the cells have activity during storage at 22 °C. Despite the combination of trehalose (a stabilizing agent commonly used for viruses) in bacteriophage encapsulation, bacteriophage stability was not maintained [67] Salalha et al. [69] performed similar encapsulation of bacteriophages in synthetic PVA (polyvinyl alcohol) using the electrospinning process. After electrospinning, the live bacteriophage rate was found to be 1-6% [68]. As known, bacteriophages cannot survive for a long period of time without the presence of host cells because bacteriophages are host-specific intracellular parasites lacking their own metabolism [67]. The loss of bacteriophage viability in films probably was originated bacteriophages was not in contact with bacteria which means to film was not make contact with food. This is the main reason for decreased bacterial stabilization during refrigerator and ambient conditions throughout these studies. Previous studies have shown that the use of proteins, surfactants, and organic substances in solutions is important in achieving viral stabilization [70,71,72].

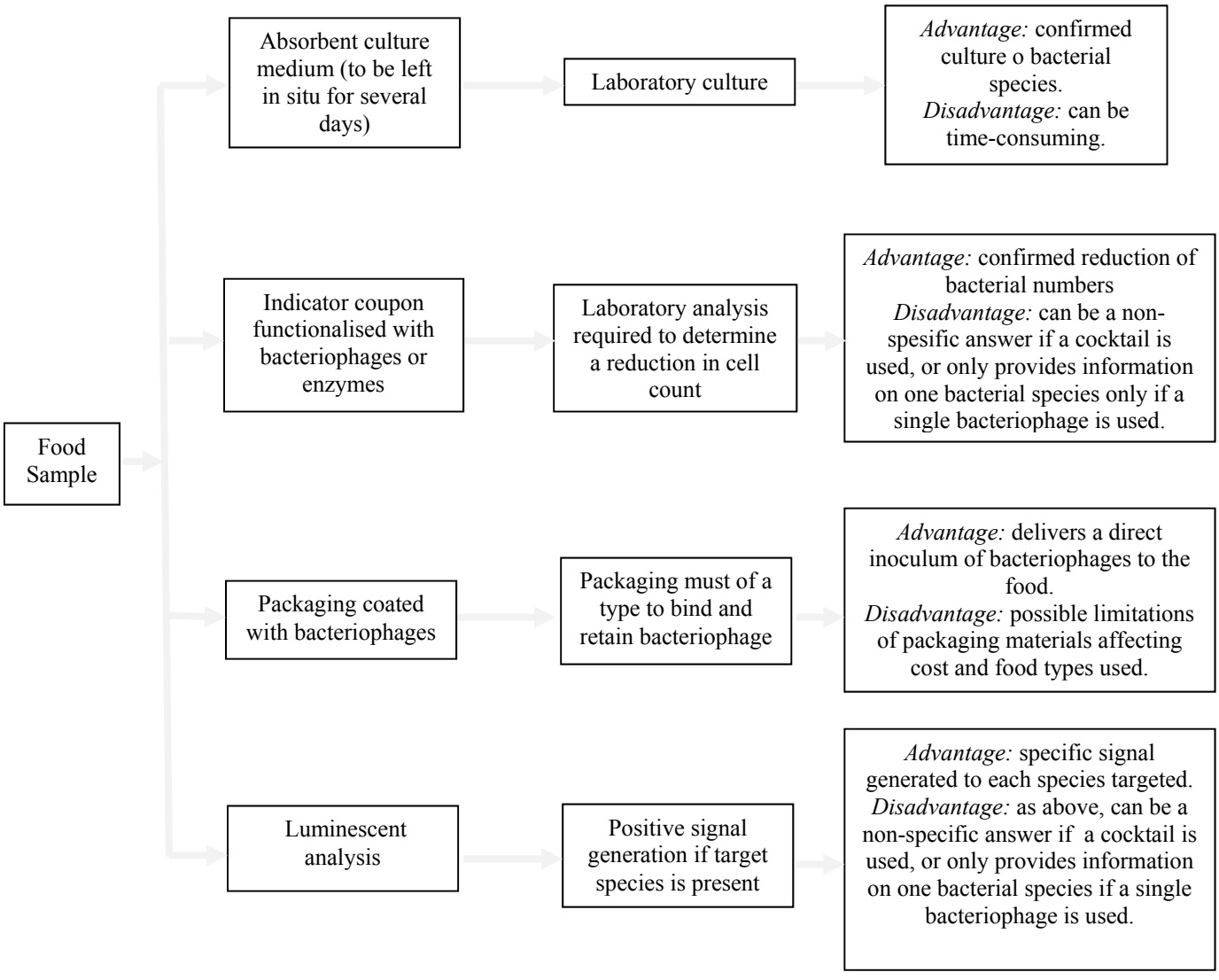

Fig. 1 Potential advantages and disadvantages associated with methods for the reduction of food-borne bacterial pathogens using packaging modified with bacteriophage [74]

One of the new approaches is to combine the bacteriophage activity with luminescence, which is remarkable for the rapid detection of biological agents [73] developed a new test for the detection of the presence of *Yersinia pestis* with the evolution of the bioluminescence signal. Detection of *Y. pestis* at a maximum level was achieved by a positive bioluminescence signal after incubation of 1x10 cells at 28 °C for 60 minutes in serum. This is an important part of the research. Firstly, complex biomolecules in the serum do not interfere with the infection cycle of bacteriophages. Secondly, it has shown that a bioluminescence signal can be observed rapidly. This event is very important for the safe consumption of food. If this technology can be transformed into an easy-to-use technology, and if it is used in many places in the food chain, it can gain importance to consumers and retailers and prevent retail trade of unsafe foods.

All these data suggest that bacteriophages retain their vitality when incorporated into packaging materials to provide antibacterial effects against bacterial pathogens, which are important human pathogens. The use of bacteriophages in packaging materials may further develop. It is an important step towards comforting people about the quality of food and therefore the safety of consumers and the safety of the use of packaging materials containing bacteriophages. The use of modified bacteriophages in packaging materials to reduce foodborne pathogens has a number of advantages and disadvantages, depending on the method chosen. These can be summarized as shown in Figure 1 [74].

4. Conclusion

The increase in the number of commercial firms working on this subject, as well as the increasing use of bacteriophages for treatment, biocontrol, biosanitration and biopreservation purposes in recent years is due to the fact that antimicrobial abilities of phages are found to be quite high. Currently, the uses of bacteriophage-derived protectors are more reliable and alternative for consumer profiles, which are trying to stay away from chemical preservatives. In order to obtain a positive result in the studies performed, phages should first be properly isolated from the environment they are in. Therefore, safer and technological working conditions are needed to ensure that bacteriophages are effectively used as alternative antimicrobial agents.

At present, many of the antimicrobial active packaging materials studies exhibit broad spectrum antimicrobial properties, not specifically targeting a single pathogenic species. Therefore, there is a significant need to develop new antimicrobial packaging materials that exhibit high specificity only to the pathogenic organism while maintaining commensal microflora. Model studies should be directed towards the development of materials that are capable of enhancing the antimicrobial effect on the pathogen, reducing the interaction with non-target microorganisms with developing pathogen specificity in active packaging materials. In most studies on biocontrol using bacteriophages, microorganisms were inoculated on the food surface and subsequently phage applied to the inoculated surface, typically by spraying. It is known that in many cases the target pathogens may be distributed and localized on the surface and may also be present on one or both surfaces of the food surface. This application technique can reduce the effect of phage therapy. With the new studies are aimed at expanding the use of phage by packaging food with phage-containing film, except that phages are directly sprayed onto the surface of food products.

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