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Biofilm Formation of Foodborne Pathogens and Strategies of Its Prevention and Biocontrol: A Review

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Abstract

Background and Objective: Foodborne pathogens and cross-contamination of food products pose a serious risk to the food industry as many outbreaks are associated with biofilm formation, which increases post-processing contaminations and risks to public health. This review aimed to study the biofilm formation of spoilage and pathogenic bacteria in foods and on food contact surfaces, which subsequently represent serious challenges to the food industry and may decrease shelf life and increase transmission of diseases.

Results and Conclusion: Chemical and physical methods (e.g. sanitizing with chemicals and heat treatment) are not sufficiently applicable for biofilm removal in food sectors due to the increase of bacterial resistances, ingredient damages and possible residues in food matrix. During meat processing, the environment is filled with complex multispecies communities of microorganisms, majorly connected to the surface forming biofilms that are difficult to treat. Furthermore, bacterial cell relationships between various genera and species play a key role in the attachment process and formation of strong biofilms, as well as in the resistance of the biofilm community members against antimicrobial treatments. Thus, control of these biofilms are difficult in food industries since the biofilm cells secrete exopolymeteric substances that include preventing barrier or lessening contact with environmental stresses such as antimicrobial agents as well as the host immune system. Biofilms are highly resistant to conventional antimicrobial therapies and lead to persistent infections. Hence, there is a high need for novel strategies other than conventional antibiotic therapies to control biofilm-based infections. Bacterial biofilm formation and its problems in the food industry were discussed in this study in addition to various safety strategies aiming to provide novel insights into biofilm control in the food industry for improving food quality and safety.

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1. Introduction

A biofilm is a complex community of microorganisms that adhere to a surface, forming multiple layers that protect their growth, proliferation and survival [1]. It can lead to antibiotic resistances, nosocomial infections and food-borne illnesses. However, biofilms benefit the microbes by helping them in adhesion, metabolite exchange, quorum sensing and drug resistance [2]. Microbial biofilms are composed of diverse bacteria surrounded by their exopolysaccharides and typically attached to biotic and abiotic surfaces, resulting in food poisoning with diarrhea, vomiting, enteritis, stomach discomfort and headaches in humans [3, 4]. Presence of biofilms in food-processing environments, food contact surfaces, processing equipment such as stainless steel, rubber, plastic and Teflon and completed products increases danger of spoiling, diminishes shelf life and increases possibilities of infectious disease outbreaks associated with foods [5]. Search for efficient ways to control microbes and their biofilms still needs further efforts [6]. This review covered topics associated with particular microorganisms that create biofilms in the food sector, illustrating the biofilm formation process, stages of development, interactions between

microorganisms and various novel methods and strategies of biocontrol.

2. Results and Discussion

1. Biofilm Formation

Several factors affect biofilm formation, including metabolism, signaling molecules, culture media, matrix and variations in cellular and genetic makeup [7]. Generally, biofilm formation consists of four common steps (Figure 1), initially produced on biotic or abiotic surfaces through reversible and irreversible adhesion, using adhesive proteins, lipopolysaccharides, flagella and pili [8]. Furthermore, biofilm maturation occurs in two stages of cell-to-cell communication and production of auto-inducer molecules. These molecules primarily consist of proteins, exopolysaccharides, DNA, RNA, enzymes, microbial cells and water with water being the major component responsible for nutrient movement within the biofilm matrix. Exopolysaccharides serve as a protective shield, enhancing microbial adhesion within biofilms, ensuring their structural integrity and facilitating nutrient acquisition [9,10,11].

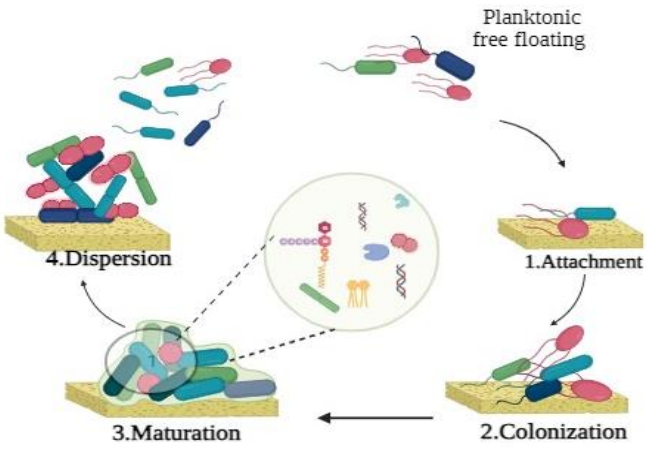

Figure 1. Stages of bacterial development that lead to the production of bacterial biofilm

1.1 Formation of Biofilm in Food Industry

In the food industry, surfaces and equipment that come into contact with foods are often occupied by microorganisms that can form biofilms [12,13]. Bacterial biofilms in foods pose severe hazards to human health, leading to systemic diseases, food intoxication and gastroenteritis and presence of bacterial biofilms on tables, staff gloves, animal carcasses, water, milk and other liquid pipelines has been documented [14].

1.2 Biofilm Resistance to Antimicrobial Agents

Bacteria living in biofilms show 10 to 1000-fold increases in drug resistance, compared to their planktonic stages. Various multidrug-resistant (MDR) bacteria such as *Salmonella* spp., methicillin-resistant *Staphylococcus aureus* (MRSA), *Listeria monocytogenes, Campylobacter jejuni, Escherichia coli* O157:H7 and vancomycin-resistant enterococci (VRE) have been linked to foodborne outbreaks, presenting a significant public health threat [15]. Based on the estimates, biofilm matrix and EPS prevent bacteria from antibiotic exposure, providing them an adaptive advantage by preventing chemical stressors from penetrating deeper biofilm regions [16]. In biofilms, quorum sensing and horizontal gene transfer are the most commonly observed mechanisms [17]. Biofilm awareness in fight against MDR bacteria needs further discussion and persistence of biofilms in foods creates an ideal environment for resistance mechanism exchange; hence, greater awareness of these dangers is necessary.

1.3 Quorum Sensing System

Quorum sensing system is a communication system between the cells that allows them to send chemical signals, enabling cooperative gene expression, which leads to increased population density, enhanced biofilm formation and increased production of extracellular polymeric substances [18]. Recent studies have shown that Gram-negative bacteria produce acylated homoserin lactones (AHLs) as autoinducers, while Gram-positive bacteria use peptides (AIPs) [19].

1.4 Horizontal Gene Transfer

Horizontal gene transfer is a widely recognized mechanism; through which, bacteria adapt and spread resistance to antimicrobial agents using mobile genetic elements (MGEs), which pose a significant threat to global public health [20]. Release and transfer of bacterial DNA play a role in biofilm synthesis and contribute to the spread of antibiotic resistance. Resistance plasmids can spread through the conjugation process, promoting development of resistant biofilms within the densely populated structure of biofilms. Over time, drug resistance leads to the preferential expression of certain genes, resulting in increased productions of proteins associated with virulence and antibiotic resistance, which can alter characteristics of biofilm resistance.

1.5 Common Foodborne Pathogens Forming Biofilms

Bacterial pathogens can contaminate meats because meats are rich in vitamins, minerals and proteins and include high water contents (75%) and acceptable pH ranges [21, 22]. The greatest dangers to food safety worldwide are environmentally hazardous microorganisms that can infect cattle during various processing procedures and foodborne illnesses associated with uncooked meats. These pollutants are challenging to clean off and disinfect, putting customers' health at major risks. A variety of bacterial pathogens can cause meat-borne diseases, by infecting animals or

contaminating meat during meat processing such as *Salmonella* spp., *E. coli*, *Campylobacter* spp., *L. monocytogenes*, *Yersinia enterocolitica*, *Brucella* spp., *Mycobacterium bovis*, *Bacillus anthracis* and toxin-producing *Staphylococcus aureus*, *Clostridium* spp. and *B. cereus* [23]. Meat-borne diseases can be categorized into infections, intoxications, allergies, metabolic food disorders and idiosyncratic illnesses [24]. Harmful bacteria can build up on various equipment and biotic and abiotic surfaces and eventually create biofilms whereas over 90% of bacteria live. They create biofilms on gloves and surfaces of silicon, rubber plastic, glass and stainless steel [25]. Significance and effects of biofilms on the food industry have been demonstrated in several studies, where a variety of pathogens such as *L. monocytogenes*, *Y. enterocolitica*, *C. jejuni*, *B. cereus* and *E. coli* O157:H7 frequently cross-contaminate these food products [26]. Available studies have shown that the coexistence of multiple bacterial species could increase biofilm development and enhance pathogen persistence by promoting EPS production. Examples of these relevant biofilm-forming pathogens in the food industry are briefly described.

1.5.1 Gram-negative Bacteria

Approximately 80% of the available foods in Saudi Arabian markets are imported with 15.71% of these imports are meat-based. *Escherichia coli*, *Salmonella* spp. and *Pseudomonas aeruginosa* include several important virulence factors, form biofilms and easily contaminate meats. However, handling and consuming animal-derived products contaminated with *E. coli* biofilms can pose health risks while Shiga toxin-producing *E. coli* and Enterohaemorrhagic *E. coli* are important enteric pathogens linked to outbreaks and severe gastroenteritis. Verotoxigenic *E. coli* produce verotoxins while *E. coli* O157:H7 is a human pathogen responsible for outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) and can be transmitted through raw milks, drinking waters, fresh meats and vegetables. A major element affecting production of *E. coli* biofilms is temperature. For example, after 7 d of incubation at 15 °C, quantity of adhering and planktonic cells increased on beef surfaces, which included a serious issue for meat processing plants [27]. Isolates with a higher capacity for mature biofilms showed resistance to sanitization [28].

Salmonella spp. propagate at 35–37 °C and includes two species of *S. bongori* and *S. enterica*, the most prevalent pathogens in the food industry and the causative agent in several foodborne outbreaks [29, 30]. *Salmonella enterica* is commonly associated with refrigerated poultry products stored on shelves during food processing or in supermarkets. Three various types of *Salmonella* are important for human health, including non-typhoid *Salmonella*, *S. typhi* and *S. paratyphi*. Fresh poultry and meat are highly prone due to their nutrient-rich content, high water activity and near-neutral pH (5.5–6.5), creating optimal environmental conditions for *Salmonella* spp., which are not spore-formers and can easily be destroyed by heat at 60 °C for 15–20 min. Furthermore, growth of most isolates was inhibited below 7 °C and pH 4.5, while nontyphoidal salmonellosis in the US is nearly 1.35 million illnesses per year [31]. Because many people reside in Saudi Arabia during haj and umrah seasons, a significant prevalence of *Salmonella* infection occurs [32]. *Pseudomonas aeruginosa* is wide spread on meat surfaces and in low-acid dairy products and affecting more than 2 million individuals and killing roughly 90,000 of them annually [33]. Because of its adaptability, *P. aeruginosa*
may grow at temperatures lower than 7°C and contaminate fresh meat sold in stores, causing its spoilage via lipolytic, saccharolytic and proteolytic processes [34]. In addition, the microorganism secretes extracellular enzymes that cause breakdown of foods and includes a high degree of medication resistance, which can result in serious acute and chronic infections in immunocompromised people. Human infections usually affect the respiratory tract (RT), soft tissues, blood vessels, urinary tract (UT) and wounds [35]. Carbapenem-resistant strains of *P. aeruginosa* pose a hazard to public health [36]. Due to the abundance of EPS, cells can adhere to stainless-steel surfaces and create biofilms alone or with other pathogens and produce multispecies biofilms, increasing their stability and resistance [37].

1.5.2 Gram-positive bacteria

Listeria monocytogenes is a rod-shaped, non-spore-forming, facultative-anaerobic Gram-positive bacterium. It causes human infections of listeriosis, a serious illness that includes septicemia and meningitis, particularly in immunocompromised individuals and is capable of growing at temperatures ranging 3–45 °C with the optimal temperature of 30–37 °C [38]. *Listeria monocytogenes* is a harmful foodborne microorganism that is killed by pasteurization. Consumption of dairy products, meats, fishes, fruits, soft cheeses, ice creams and poultries has been linked to listeriosis epidemics [39]. It can form biofilms on surfaces commonly detected in the food industry and is resistant to treatments with heat up to 60 °C [40]. It can thrive in a broad range of conditions, including high salinities (10%), cold temperatures (4 °C), low water activities (< 0.9) and wide pH ranges (4.1–9.6) [41]. Post-processing contamination with *Listeria* spp. may be resulted from inadequate cleaning and poor separation techniques between ready-to-eat and raw foods [42]. In addition, *L. monocytogenes* is one of the most significant pathogenic microorganisms due to its high mortality rates (15.6%) and one of the major causes of hospitalizations and deaths in the US [43].

Staphylococcus aureus is responsible for staphylococcal food poisoning (SFP) and produces enterotoxins within the temperature range of 10–46 °C. *Staphylococcus* genus includes more than 50 recognized species; of which, *S. aureus* is commonly detected in food products and reaches foods through raw materials and grows best on meat, poultry and egg products. In food production chain, it may develop biofilms on living and non-living surfaces, resist desiccation and thrive on a variety of surfaces. Furthermore, strains of *S. aureus* that produce enterotoxins have been identified in a variety of food samples. [44]. Other Gram-positive bacteria such as *Brochothrix thermosphacta* and *Carnobacterium* spp. can form biofilms in the meat-processing environment [45].

1.6 Strategies for Controlling Biofilm Formation in the Food Industry

Pathogenic bacteria that form biofilms create strong defenses against antibiotics and are difficult to treat. Removing these biofilms is a critical challenge due to the severe effects on public health [46, 47]. Chemical and physical methods have been used to inhibit bacterial biofilms in the food industry. Chemical treatments can help; however, mechanical treatments such as clean-in-place are not effective. The most reliable way to prevent bacterial biofilm growth is through aseptic processing, routine disinfection and equipment sterilization. Various disinfectants and novel biofilm elimination methods of the food industry are briefly summarized (Figure 2).

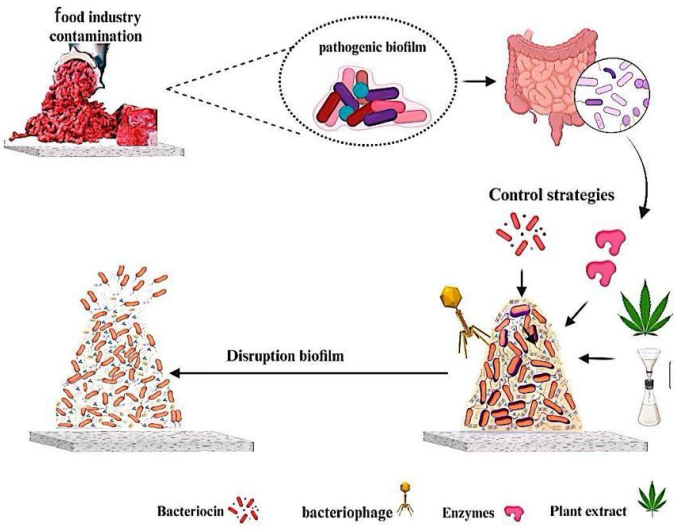

Figure 2. Suggested strategies for controlling biofilms in the food industry

1.6.1 Chemical and Physical Treatments

Biofilms can be treated with concentration and time-dependent chemical sanitizers. Decreasing bacterial populations to human-safe levels is the goal of sanitation. Sanitizing food-processing equipment is necessary to avoid cross-contamination between batches of foods. Stages of general cleaning methods for places that handle and manufacture foods include physical pre-cleaning, detergent washing, rinsing, sanitation, final rinsing and drying. Spraying detergents in form of foam or aerosol spray is possible as long as the right doses and time are used for surface contact. Alkaline and acidic chemicals are widely used as detergents in the food industry. A majority of disinfectants are safe to use on non-food-contact surfaces; nevertheless, food-contact and occasionally non-contact surfaces should be rinsed with high-quality water. The most popular sanitizer in the food industry is aqueous ClO_2, which acts well against *B. cereus* endospores in biofilms on steel surfaces [48]. In the food industry, chlorine-based sanitizers are most frequently used; nevertheless, several microorganisms have developed resistance to chlorine treatments. Food factories frequently use sodium hypochlorite or NaOCl [49]. Moreover, hydrogen peroxide (H_2O_2) and NaClO were successful in removing biofilms of *S. aureus* and *P. aeruginosa*; however, aqueous ClO_2 was more effective than NaOCl in eliminating *E. coli* O_{157}:H_7 biofilms [50]. In the food industry, H_2O_2 is a powerful oxidizing disinfectant that is often used. When it is exposed to biofilms, H_2O_2 produces free radicals that kill the bacteria at concentrations of 0.08–5%, without harmful side effects. Quaternary ammonium compounds are frequently used as sanitizers, removing biofilms and leading to bacterial lysis [51]. Steam heat treatment is a method used to decrease number of harmful bacteria and biofilm populations in production areas [52]. Non-thermal plasma is a partially ionized gas with low temperature and promising antibacterial characteristics. It can destroy bacterial biofilms of *Pseudomonas* spp., *S. enterica* and *Bacillus* spp. Ozone breaks down the cellular envelopes of a variety of microorganisms, including viruses, bacterial biofilms and protozoans.

1.6.2 Elimination of Biofilms Using Biological Strategies

In recent years, a more efficient and ecologically friendly control method for the elimination of or managing growth of dangerous biofilms is use of enzymes, bacteriophages, bacteriocin and plant extracts, which have been discussed based on safe and green approaches to control pathogen biofilm formation.

1.6.2.1 Enzyme against bacterial Biofilm

Enzymes or proteins are biologically active macromolecules against biofilm formation since proteases or other degrading enzymes have shown the ability to inhibit

biofilm formation [53]. Enzymes are detected to include therapeutic functions in removal of pathogenic biofilms and can widely be used in detergents of food industries. In recent times, a variety of enzymes enriched products have been commercialized that include tablets, rinsing solutions, chewing gums for dental treatments and denitrifies containing enzymes such as lysins, dextranase, mutants that can serve to play an effective role in disintegration of the biofilm matrix [54]. The most often used enzyme types vary depending on the makeup of the biofilm as proteases, cellulases, polysaccharide depolymerases, alginate lyases and dispersin B [55]. Proteinase K and lysozyme have been verified to include promising antibiofilm activities [56]. The α-amylase enzyme includes potential to operate as an antibiofilm agent against bacterial species that produce biofilms, including *S. aureus* and *P. aeruginosa* [57]. Protease formulations were effective in eliminating *S. aureus* biofilms from polystyrene surfaces; however, combinations of protease, amylase and cellulase were needed to eradicate biofilms of *P. aeruginosa*. Cellulase effectively inhibited biomass and microcolony formation by *P. aeruginosa* on glass surfaces in partial [58].

1.6.2.2. Bacteriophages against Biofilm

Bacteriophages (phages) are bacterial viruses, acknowledged as the most diverse and abundant entities. Bacteriophages are mostly used in primary production to ensure food safety, biosanitization and biopreservation [59]. Phages can break down biofilms spread through developed biofilms and then show their antimicrobial characteristics inside them. Phage treatments are injected directly into food products during the biopreservation processes to extend the food shelf life and used in biosanitization to avoid biofilms on equipment surfaces [60]. Bacteriophages can create enzymes that break down the biofilm structure and presence of phage receptor sites such as endolysins and depolymerases. Use of phages as biocontrol agents in foods is affected by various factors, including the food matrix, surface area and structure, bacterial species, inhibitory compound and phage dose [61]. A commercial product, LISTEXTM, has been developed from the bacteriophage P100, which uses an enzymatic process to cause cell lysis and EPS breakdown. The US Department of Agriculture (USDA) has approved use of this natural, non-toxic phage product. It is effective against *L. monocytogenes*. Additionally, it seems that *L. monocytogenes* biofilms are susceptible to phage biocontrol. Phage Guard Listex, which uses phage P100, effectively removes biofilms from stainless steel surfaces. A user of *Listeria* phage P100 (under the commercial name of Listex P100) is a biological agent, formed to remove the biofilms in processed meat products [62]. In addition, a phage cocktail was used for 1 h to destroy and decrease pathogen populations of *E. coli* O_{157}:H_7 on stainless steels, ceramic tiles and high-density polyethylene coupons [63]. Endolysin is the second kind of enzyme produced by phages that include potential uses for sanitization. During the final stage of their lytic cycle, they release progeny of virions through the breakdown of the cell wall, which were active against Gram-positive bacteria [64]. Depolymerases are types of enzyme that may prevent production of biofilms and break down capsular polysaccharides in Gram-negative bacteria [65].

1.6.2.3 Bacteriocins against biofilms

Lactic acid bacteria (LAB) are used to produce fermented foods and the most important genera in controlling spoilage and pathogenic microbes are *Lactobacillus* and *Bifidobacterium* due to the production of bacteriocins and acids. Bacteriocins from LAB are used as alternatives to chemical food preservatives. They can spread through cell membranes and release internal components such as K^+ and inorganic phosphate or they can prevent production of proteins, RNA and DNA [66]. Due to its safety in the gastrointestinal system, bacteriocin has extensively been used as a food preservative in the food sector for several years. Use of bacteriocin in biopreservation systems can meet consumers' demands and numerous compounds, including nisin, natamycin, subtilin, pediocin, tylosin and carnocyclin A, are used as food preservatives [67].

1.6.2.4. Plant Extracts against Bacterial Biofilm

Numerous substances from plants such as complex mixtures of monoterpenoids, plant-based essential oils, sesquiterpenoids and flavonoids have shown anti-biofilm characteristics [68, 69]. Previous materials can be used as an alternative to synthetic preservatives. Specifically, several flavonoids inhibited generation of bacterial toxins in various food products. In addition, bacterial cell adherence to stainless steel was strongly inhibited by essential oils and other plant components that are abundant in almost all plants such as phenolic chemicals, tannins, terpenoids, glucosinolates derivatives, alkaloids and thiols. Although the food industry uses a variety of plant-based extracts and essential oils in meat preservation, pomegranate and cranberry extracts are particularly popular due to their antibacterial and antifungal characteristics [70, 71, 72].

3. Conclusion

Controlling biofilm formation in the food industry is essential for food safety, quality and hygiene. Traditional methods such as cleaning and sanitizing with chemicals and heat treatment are critical; however, effectiveness was limited due to the increase of antimicrobial resistance and vitamin damage by heat. Moreover, biofilm cells secrete extracellular polymeric substances that include a barrier preventing or lessening contact with environmental stresses and decreasing effects of antimicrobial agents and the host immune system. Various methods have been investigated to

prevent and remove biofilms, each offering unique advantages. However effectiveness of traditional methods was limited, biological control methods such as bacteriophages and probiotics offer promising sustainable solutions and may be further effective in specific settings due to targeting harmful pathogens without affecting the environment. Recent advances in combination of physical, chemical and biological methods in food processing environments may be effective keys to biocontrol biofilms, ensuring safety and quality of food products.

4. Conflict of Interest

The author reports no conflict of interest.

5. Authors Contributions

H.A, NZ and MMA conceptualized the idea and prepared the manuscript.

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تشکیل بیوفیلم (زی‌لایه) میکروب‌های بیماریزای غذایی و راهبردهای پیشگیری و کنترل زیستی: مقاله مروری

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واژگان کلیدی

- باکتریوفاژها (باکتری خوارها)
- کنترل زیستی
- میکروب‌های بیماری‌زا از طریق غذا
- مقاومت
- EPS

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چکیده

سابقه و هدف: میکروب‌های بیماری‌زای غذایی و آلودگی متقابل فرآورده‌های غذایی خطری جدی برای صنایع غذایی ایجاد می‌کنند، زیرا بسیاری از شیوع بیماری‌ها با تشکیل بیوفیلم یا زی‌لایه[۱] همراه است که موجب افزایش آلودگی‌های پس از فرآوری و خطرات سلامت عمومی می شود. این بررسی با هدف بررسی نحوه تشکیل زی‌لایه حاصل از فساد میکروبی و باکتری‌های بیماری‌زا در مواد غذایی و سطوح تماس با آنهاست، که خود می‌تواند بعدا چالش‌های جدی برای صنایع غذایی ایجادکند و باعث کاهش ماندگاری و افزایش امکان انتقال بیماری‌ها شود.

یافته‌ها و نتیجه‌گیری: روش‌های شیمیایی و فیزیکی (مانند ضدعفونی کردن با مواد شیمیایی و تیمار حرارتی) به‌دلیل افزایش مقاومت‌های باکتریایی، آسیب به مواد تشکیل دهنده و باقیمانده‌های احتمالی در ماتریس مواد غذایی[۲]، کارایی کافی برای حذف بیوفیلم در بخش‌های غذایی ندارند. هنگام فرآوری گوشت، چندین گونه میکروارگانیسم در محیط وجود دارند، که عمدتاً به سطح متصل می شوند و زی‌لایه هایی تشکیل می دهند که سالم‌سازی آنها دشوار است. علاوه بر این، روابط سلولی در بین جنس‌ها و گونه‌های گوناگون باکتریایی نقش کلیدی در فرآیند اتصال و تشکیل زی‌لایه‌های قوی و نیز مقاومت بخش‌های زی‌لایه در مقابل درمان‌های ضد میکروبی ایفا می‌کند. سلول‌های بیوفیلم مواد اگزوپلی‌متری ترشح می‌کنند که از ممانعت یا کاهش تماس با استرس‌های محیطی مانند عوامل ضدمیکروبی و همچنین سیستم ایمنی میزبان جلوگیری می‌کنند، از این‌رو، کنترل این زی‌لایه‌ها در صنایع غذایی دشوار است. زی‌لایه‌ها به درمان‌های ضد میکروبی معمولی بسیار مقاوم هستند و موجب بروز بیماری‌های عفونی مقاوم می‌شوند. از این رو، به استراتژی‌های جدید، به جز درمان‌های آنتی‌بیوتیکی متداول برای کنترل عفونت‌های ناشی از زی‌لایه، نیاز است. در این مطالعه، تشکیل زی‌لایه باکتریایی و مشکلات ناشی از آن در صنایع غذایی و همچنین استراتژی‌های ایمنی گوناگون با هدف ارائه راهکارهای جدید در مورد کنترل زی‌لایه در صنایع غذایی برای بهبود کیفیت و ایمنی مواد غذایی مورد بحث قرار گرفت.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

[۱] Biofilm لایهٔ حاصل از رشد میکرب‌ها بر روی سطوح مختلف
[۲] Food matrix