

Chapter

Natural Antimicrobials: The Anti-Biofilm Potential of Garlic and Thyme Essential Oils against *Salmonella typhimurium*

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Abstract

Salmonella typhimurium is a pathogenic bacterium that presents significant challenges in food processing environments, primarily due to its ability to form biofilms, which confer resistance to traditional cleaning protocols and antimicrobial treatments. With the increasing prevalence of antibiotic-resistant strains, the exploration of natural antimicrobials, particularly garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) essential oils, has become increasingly important. This chapter investigates the antibacterial and anti-biofilm properties of these essential oils, highlighting their ability to disrupt quorum sensing, inhibit biofilm formation, and suppress virulence factors. The potential applications of these oils as natural preservatives in food safety are discussed, presenting a promising natural approach to combating microbial contamination and enhancing food safety.

Keywords: *Salmonella typhimurium*, pathogenic bacterium, biofilm formation, antibiotic-resistant strains, garlic essential oil (*Allium sativum*), thyme essential oil (*Thymus vulgaris*), quorum sensing disruption, antibacterial properties, anti-biofilm activity, natural antimicrobials, food safety, natural preservatives

1. Introduction

In recent years, the issue of food safety has increasingly garnered attention from public health experts, consumers, and regulatory agencies, primarily due to the persistent threat posed by foodborne pathogens such as *Salmonella typhimurium* [1]. This particular strain of *Salmonella* is renowned for its ability to contaminate a wide array of food products, leading to significant health risks and economic losses [2]. The capacity of *S. typhimurium* to form robust biofilms enables it to adhere to surfaces within food processing environments, creating a protective matrix that facilitates its survival and resistance to cleaning and sanitizing efforts [3]. Consequently, biofilms not only complicate contamination control but also serve as reservoirs for persistent infections, ultimately leading to outbreaks with serious public health ramifications [4].

Compounding this issue is the alarming rise in antibiotic resistance among bacterial strains, including *S. typhimurium*, which reduces the efficacy of conventional antibiotic treatments and highlights the urgent need for alternative interventions [5]. As the limitations of traditional antimicrobial strategies become evident, there is growing interest in exploring natural and holistic approaches to food safety that can mitigate the risks associated with antibiotic-resistant pathogens [6]. Natural antimicrobials, particularly those derived from plants, have attracted significant interest due to their ability to offer effective antibacterial properties with fewer side effects compared to synthetic alternatives [7].

This chapter explores the potential of garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) essential oils as promising natural agents in the fight against biofilm formation and microbial contamination [8]. The unique phytochemical composition of these essential oils contributes to their multifaceted mechanisms of action, which include the suppression of virulence gene expression, disruption of bacterial communication pathways through quorum sensing, and interference with adherence mechanisms used by bacteria to colonize surfaces [9]. By investigating the efficacy and safety of these natural compounds, this chapter aims to shed light on their role as viable alternatives to traditional preservatives and antimicrobials in the food industry [10]. Ultimately, the exploration of garlic and thyme essential oils underscores the potential for sustainable and innovative strategies to enhance food safety, combat antibiotic-resistant pathogens, and promote a healthier food supply [11].

Salmonella typhimurium is a significant pathogen that poses serious challenges to public health, particularly in food safety [12]. Understanding the characteristics of *S. typhimurium* is crucial to addressing its impact on health and hygiene practices in food processing environments [13]. This bacterium is notorious for its ability to form biofilms, which are structured communities of bacteria enmeshed in a self-produced matrix of polymeric substances [14]. Biofilms not only enhance bacterial resistance to environmental stresses and disinfectants but also promote persistence on surfaces of food-processing equipment [15]. The formation of biofilms is a crucial factor that contributes to the survival and proliferation of *S. typhimurium* in various settings, facilitating its transmission through contaminated food products [16]. The presence of biofilms can lead to persistent contamination of food processing facilities, resulting in recurrent outbreaks of salmonellosis [17]. Moreover, the resilient nature of biofilms complicates eradication efforts, making routine cleaning and sanitization protocols less effective [18]. Understanding the biofilm-forming abilities of *S. typhimurium* is essential for developing targeted strategies to minimize its persistence in the food supply chain, thereby reducing the risk of salmonellosis outbreaks and addressing broader public health implications [19].

The antibacterial properties of garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) essential oils have been extensively researched, revealing their effectiveness as natural antimicrobial agents against a variety of pathogens, including *Salmonella typhimurium* [20]. Garlic essential oil is particularly renowned for its rich composition of bioactive compounds, among which allicin is the most prominent [21]. Allicin, a sulfur-containing compound released when garlic is crushed or chopped, has been shown to possess potent antimicrobial activity [22]. Mechanistically, allicin exerts its antibacterial effects primarily by disrupting the integrity of bacterial cell membranes [23]. This disruption leads to increased permeability, allowing for uncontrolled influx and efflux of ions and other small molecules essential for bacterial survival, ultimately culminating in cell lysis [24]. Furthermore, garlic oil has been implicated in the suppression of virulence gene expression within *Salmonella* [25], interfering with

regulatory pathways that control the expression of factors crucial for virulence, such as fimbriae and secreted toxins (**Figure 1**) [26].

In addition to garlic, thyme essential oil emerges as another significant natural antimicrobial with a distinct mechanism of action [27]. Rich in phenolic compounds such as thymol and carvacrol, thyme essential oil is recognized for its broad-spectrum antibacterial properties [28]. Thymol acts by disrupting bacterial cell membranes similarly to allicin but also targets the respiratory chain, leading to a decline in ATP production and overall energy disruption within bacterial cells [29]. Carvacrol complements this effect by interfering with biosynthetic pathways necessary for cell wall synthesis and functioning [30]. Importantly, thyme oil has been demonstrated to disrupt quorum sensing, a cell-to-cell communication process that bacteria use to coordinate behaviors such as biofilm formation and virulence factor expression [31]. By inhibiting quorum sensing, thyme essential oil effectively diminishes the ability of *Salmonella* to establish biofilms, crucial for its persistence in host environments and food processing settings [32].

Moreover, the combination of garlic and thyme essential oils provides a synergistic effect, enhancing their overall antimicrobial activity [33]. Research suggests that when used in conjunction, these essential oils may potentiate each other's effects, leading to reduced minimum inhibitory concentrations and enhanced efficacy against biofilm-forming pathogens [34]. This synergism is particularly valuable in food safety applications, where preventing biofilm formation is essential in reducing the risk of contamination and ensuring the safety of food products [35]. Additionally, both garlic and thyme essential oils possess anti-inflammatory properties, which can further mitigate the immune response triggered by foodborne pathogens [36], offering potential therapeutic benefits in managing gastrointestinal infections caused by *Salmonella* [37]. Overall, a comprehensive understanding of the antibacterial properties, mechanisms of action, and potential synergistic effects of garlic and thyme essential oils highlights their role as promising natural alternatives in combating antibiotic-resistant pathogens like *Salmonella typhimurium*, paving the way for innovative applications in the food industry [38].

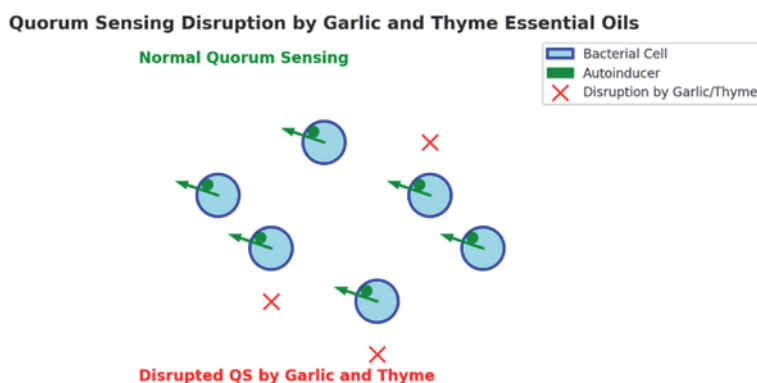


Figure 1. Quorum sensing and its disruption by garlic and thyme: illustrates the quorum sensing (QS) mechanism in *Salmonella typhimurium* and how garlic (allicin) and thyme (thymol, carvacrol) essential oils disrupt this process. On the left, bacterial cells communicate by releasing signaling molecules called autoinducers. As these accumulate, they activate biofilm formation and virulence factors. On the right, garlic and thyme essential oils interfere with this communication, disrupting membrane integrity, inhibiting quorum sensing, and reducing biofilm formation, offering a natural approach to combating bacterial contamination [26].

The application of garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) essential oils as natural preservatives in food safety is not only innovative but also represents a significant advancement in addressing contemporary challenges faced by the food industry [39]. In response to rising consumer demand for transparency in food labeling and increasing awareness of the potential adverse effects associated with synthetic preservatives, manufacturers are progressively exploring natural alternatives [40]. Garlic essential oil, rich in bioactive compounds such as allicin and diallyl sulfones, has garnered attention for its remarkable antimicrobial properties effective against a spectrum of foodborne pathogens, including *Salmonella typhimurium* [41]. Allicin, in particular, exhibits powerful antibacterial activity by disrupting bacterial cell membranes and inhibiting enzyme function, thereby minimizing the risk of infection associated with contaminated food products [42]. Conversely, thyme essential oil is characterized by high concentrations of thymol and carvacrol, which not only demonstrate strong antibacterial activity but also possess antioxidant properties that help preserve the flavor, color, and nutritional value of food items [43]. Integrating these essential oils into food products holds significant promise for inhibiting bacterial growth and biofilm formation, critical in extending the shelf life of perishable goods such as meats, dairy products, and fresh produce [44].

Furthermore, the application of garlic and thyme essential oils as natural preservatives aligns with the emerging trend of clean-label products sought by consumers increasingly wary of artificial additives [45]. These essential oils can play a dual role by acting as both antimicrobial and natural flavor enhancers, ensuring that food remains safe for consumption and appealing to consumers' palates [46]. Incorporating these essential oils into processing protocols can critically enhance current food safety practices [47]. For instance, they can be employed in coating technologies or incorporated into packaging materials, creating a barrier against microbial contamination and providing a proactive solution to the issue of foodborne pathogens [48].

Moreover, ongoing research continues to uncover the synergistic effects when garlic and thyme essential oils are combined with other natural preservatives or food processing techniques, suggesting a compounding effect that can further elevate their antimicrobial efficacy [49]. Such findings imply that using these essential oils could lead to more effective strategies in minimizing microbial loads not only during production but also throughout the supply chain, including storage and distribution [50]. The utilization of garlic and thyme essential oils positions the food industry to effectively combat the rising tide of antibiotic-resistant microorganisms, which pose severe public health challenges [51]. By embracing these natural antimicrobial agents, food producers can better align with broader sustainability goals while enhancing public health outcomes [52]. Overall, incorporating these essential oils into food safety not only addresses immediate safety concerns but also contributes to a long-term vision of promoting more sustainable, health-conscious practices in the food industry [53].

2. *Salmonella typhimurium* biofilm formation

Biofilms are structured bacterial communities that provide protection to bacteria from environmental stressors, including disinfectants and antibiotics [19]. *S. typhimurium* forms biofilms on various surfaces, making it difficult to remove from food processing environments [20, 21]. The extracellular polymeric substance (EPS)

matrix that forms biofilms acts as a barrier that reduces the effectiveness of antimicrobial agents, allowing bacteria to survive [22–24].

Quorum sensing (QS) plays a critical role in biofilm formation by regulating gene expression in response to population density [25, 26]. Disruption of QS signaling has been shown to inhibit biofilm formation, making it a promising target for controlling *S. typhimurium* [27–29].

Essential oils, including those derived from garlic and thyme, have been shown to interfere with quorum sensing, thereby inhibiting biofilm formation and reducing bacterial virulence [30–32]. These findings suggest that natural antimicrobials could be an effective alternative to synthetic antimicrobials in controlling microbial contamination in food processing environments [33, 34].

3. Mechanisms of action of garlic essential oil

Garlic essential oil is known for its potent antimicrobial properties, primarily due to the presence of allicin, a sulfur-containing compound released when garlic is crushed [35–37]. Allicin disrupts bacterial cell membranes, increasing their permeability and leading to cell death [38–40]. In addition, allicin interferes with quorum sensing, which is essential for biofilm formation and bacterial virulence [41].

By inhibiting QS, garlic essential oil reduces the expression of biofilm-associated genes, such as those responsible for fimbriae and flagella production, which are necessary for bacterial adhesion and invasion [42, 43]. One study showed that garlic essential oil reduced biofilm formation by over 80% in *S. typhimurium* [44].

Other compounds in garlic essential oil, such as diallyl sulfide and diallyl disulfide, contribute to its broad-spectrum antimicrobial activity. These compounds have been shown to inhibit the growth of various foodborne pathogens, including *Escherichia coli* and *Staphylococcus aureus* [45–48]. The primary antimicrobial compounds in essential garlic oil are listed in **Table 1**.

4. Anti-biofilm activity of thyme essential oil

Thyme essential oil is another natural antimicrobial with significant antibacterial properties due to the presence of phenolic compounds such as thymol and carvacrol [49–51]. Thymol and carvacrol disrupt bacterial membranes, increasing permeability and leading to cell lysis [52, 53, 57]. These compounds also interfere with bacterial energy production and the synthesis of cell walls, further inhibiting bacterial growth [58].

Essential oil	Active compounds	Antimicrobial activity	Target pathogens
Garlic (<i>Allium sativum</i>)	Allicin, Diallyl sulfide, Diallyl disulfide	Disrupts bacterial cell membranes, inhibits quorum sensing	<i>Salmonella typhimurium</i> , <i>E. coli</i> , <i>S. aureus</i>
Thyme (<i>Thymus vulgaris</i>)	Thymol, Carvacrol, p-Cymene	Disrupts membrane integrity, inhibits cell wall synthesis	<i>S. typhimurium</i> , <i>Listeria monocytogenes</i> , <i>Bacillus cereus</i>

Table 1.
Antimicrobial compounds in garlic and thyme essential oils synergistic effects of garlic and thyme essential oils [54–56].

Combination	Minimum Inhibitory Concentration (MIC)	Reduction in biofilm formation	Mechanisms
Garlic + Thyme Essential Oils	Lower MIC compared to individual oils	>95%	Membrane disruption, quorum sensing inhibition, biofilm breakdown

Table 2.
Synergistic effects of garlic and thyme essential oils against Salmonella typhimurium [70–72].

Research has shown that thyme essential oil is effective at inhibiting biofilm formation in *S. typhimurium* by disrupting quorum sensing [59–61]. By reducing QS signaling, thyme essential oil inhibits the expression of biofilm-related genes, making it difficult for bacteria to form stable biofilms on food processing surfaces [62]. A study reported that thyme essential oil reduced biofilm formation by 70% in *S. typhimurium* [63].

Thyme essential oil has also been shown to inhibit the growth of other food-borne pathogens, such as *Listeria monocytogenes*, *Bacillus cereus*, and *Campylobacter jejuni* [54–56]. The main bioactive compounds in the thyme essential oil are summarized in **Table 1**.

When used together, garlic and thyme essential oils demonstrate a synergistic effect that enhances their antimicrobial efficacy [64, 65]. Studies have shown that combining these essential oils reduces the minimum inhibitory concentration (MIC) required to inhibit bacterial growth, making them more effective at lower concentrations [66, 67]. This is especially important in food safety applications, where minimizing the concentration of antimicrobials is crucial for maintaining food quality [68].

In one study, the combination of garlic and thyme essential oils reduced biofilm formation by over 95% in *S. typhimurium* [69]. The synergistic effect is attributed to the complementary mechanisms of allicin, thymol, and carvacrol. Allicin disrupts bacterial membranes and inhibits quorum sensing, while thymol and carvacrol weaken the bacterial cell wall, further enhancing the antimicrobial effect [70–72]. These results are summarized in **Table 2**.

5. Applications in food safety

Garlic and thyme essential oils offer significant potential as natural preservatives and surface decontaminants in food safety applications [73, 74]. These oils can be incorporated into food packaging materials or applied directly to food products to prevent microbial contamination and extend the shelf life of perishable goods [75]. Their natural antimicrobial properties make them attractive alternatives to synthetic preservatives, especially as consumers demand clean-label products [76, 77].

In food processing environments, garlic and thyme essential oils can be used to disinfect surfaces and equipment, thereby reducing the risk of contamination by *S. typhimurium* and other pathogens [78, 79]. The ability of these oils to reduce biofilm formation on food processing surfaces makes them particularly effective for preventing contamination in packaged and ready-to-eat foods [80]. These applications are summarized in **Table 3**.

Application	Essential oil used	Mode of action	Examples
Food Packaging	Garlic, Thyme	Antimicrobial coatings, encapsulation in packaging	Meat, dairy, fresh produce
Surface Decontamination	Garlic, Thyme	Direct application on surfaces to inhibit biofilm formation	Food processing equipment
Food Preservation	Garlic, Thyme	Reduces microbial contamination, extends shelf life	Packaged foods, ready-to-eat products

Table 3.
Summary of applications of essential oils in food preservation [80].

6. Conclusion

The persistent challenge posed by *Styphimurium*, particularly its capacity to form resilient biofilms within food processing environments, underscores a critical concern in modern food safety management [57]. This pathogenic bacterium not only jeopardizes public health through potential contamination of various food products but has also demonstrated an alarming ability to adapt and survive under harsh environmental conditions, complicating effective control measures and food safety protocols [58]. The rise of antibiotic-resistant strains has further exacerbated these challenges, rendering conventional treatment options less effective and raising urgent questions about mitigation strategies [59]. Therefore, exploring and developing alternative strategies that can effectively complement or replace antibiotic treatments is essential, focusing on naturally derived solutions that are safe, effective, and widely acceptable to consumers [60].

In light of these pressing concerns, this chapter highlights the substantial promise of natural antimicrobials, such as garlic (*Allium sativum*) and thyme (*Thymus vulgaris*) essential oils, in the ongoing battle against *Salmonella* biofilms [61]. These essential oils, long celebrated for their traditional medicinal properties, are increasingly recognized by the scientific community for their potent antibacterial activities against a range of pathogens, including multidrug-resistant strains [62]. The unique phytochemical compositions of garlic and thyme reveal sophisticated mechanisms of action that not only inhibit bacterial growth but also target and disrupt critical cellular processes essential for pathogenicity [63]. For instance, the ability of these essential oils to suppress virulence gene expression represents a promising avenue for reducing the pathogenic potential of *Salmonella*, while their interference with quorum sensing pathways serves to disrupt communication systems that bacteria rely on for biofilm formation and maturation [54]. Such multifaceted modes of action elevate garlic and thyme essential oils to significant relevance in natural food preservation research [55].

Moreover, the implications of incorporating these essential oils into food safety interventions extend beyond simple antibacterial effects; they pave the way for a more holistic approach to food safety that prioritizes natural solutions over synthetic alternatives, often met with increasing consumer skepticism and regulatory scrutiny [56]. As consumers today become more health-conscious and demand cleaner and safer food products, the strategic integration of garlic and thyme essential oils into food safety protocols can enhance the safety of food products and potentially revolutionize current industry practices by providing effective and sustainable alternatives to conventional preservatives [64]. However, harnessing the full potential of garlic and thyme essential oils requires further comprehensive research to elucidate their precise mechanisms of action, optimal application methods, and potential interactions with various food

components or processing conditions [65]. Understanding these factors will provide a more nuanced comprehension of how these natural agents can be effectively utilized in food processing systems while ensuring consumer safety and maximizing efficacy [66].

To that end, continued investigation into the bioactive compounds present in garlic and thyme, as well as their synergistic effects with other natural preservatives, is essential in developing innovative food safety strategies that address both consumer expectations and foodborne pathogen challenges [67]. Ultimately, the strategic employment of garlic and thyme essential oils signifies not only a proactive and innovative approach to safeguarding public health but also represents a paradigm shift toward environmentally sustainable practices in food production [68]. By embracing such innovative methods, the food industry can proactively contribute to creating healthier food systems, safeguard consumer health, and play an important role in the broader global effort to combat antibiotic resistance in foodborne pathogens [69]. This multidimensional strategy holds the potential for improving food safety outcomes and reflects a profound commitment to nurturing the health of both consumers and the environment, ensuring that future generations can enjoy safe, wholesome food produced through sustainable means [70].

The persistence of *S. typhimurium* in food processing environments, particularly through biofilm formation, presents a major challenge to food safety. Garlic and thyme essential oils, with their potent antimicrobial and anti-biofilm properties, offer a natural and effective alternative to synthetic antimicrobials [81–83]. By disrupting quorum sensing, inhibiting biofilm formation, and reducing bacterial virulence, these essential oils have the potential to revolutionize food safety practices and promote cleaner, more sustainable food preservation methods [84, 85].

Further research is needed to optimize the application methods and concentrations of these essential oils to fully harness their potential in combating foodborne pathogens and ensuring the safety of food products [86–88].

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Conflict of interest

The author declares no conflict of interest.

Abbreviations

QS	quorum sensing
MIC	minimum inhibitory concentration
EPS	extracellular polymeric substances
EO	essential oils
<i>S. typhimurium</i>	<i>Salmonella typhimurium</i>

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