UV-C IRRADIATION AND LANTIBIOTIC NISIN APPLICATIONS FOR FOOD BIOPRESERVATION

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ABSTRACT

UV-C IRRADIATION AND LANTIBIOTIC NISIN APPLICATIONS FOR FOOD BIOPRESERVATION

The antibacterial and antifungal activity of Lantibiotic Nisin and Essential oil Carvacrol against pathogenic and spoilage-forming microorganisms was investigated in this study. The antimicrobial activities of these compounds were observed *in vitro* on one Gram-positive (*Listeria monocytogenes*), two Gram-negative (*Cronobacter sakazakii* and *S.* Typhimurium) bacterial strains, and one fungus (*Candida albicans*). The study investigated the effect of combined treatments involving UV-C irradiation along with Nisin and carvacrol as antimicrobial agents on microbial growth, both at 4°C refrigerator temperature storage for two weeks, and on artificially inoculated chicken drumsticks with *L. monocytogenes* and *S.* Typhimurium. Total Aerobic Mesophilic, Yeast & Mold, and Enterobacter/Coliform populations were examined using plate counting on PCA, PDA, and VRBA media, for *L. monocytogenes*, and *S.* Typhimurium. PALCAM, OXFORD, and Bismuth Sulphite Agar, respectively.

The results indicate that samples, which were subject to a combination of UV-C irradiation dose and concentration of antimicrobial agents (2 × MIC), showed a significant decrease in pathogen count ($p \leq 0.05$) in comparison to the samples that received only a higher quantity of antimicrobial agents (2 × MIC). *C. albicans* was the most resistant microorganism to all treatments. In vitro experiments on cell growth indicated that the tested compounds exhibited varying toxicity levels on all tested bacterial species. The study found both synergistic and additive effects in UV-C combinations but did not observe any antagonistic effects. These findings present an optimistic outlook for future research into the antimicrobial mechanisms of Nisin and Carvacrol with UV-C against pathogens.

ÖZET

GIDA BİYOKORUMASI İÇİN UV-C IŞINLAMASI VE LANTİBİYOTİK NİSİN UYGULAMALARI

Bu çalışma, Nisin ve Karvakrolün, gıdalarda patojenik ve bozulma oluşturan mikroorganizmalara karşı antibakteriyel ve antifungal aktivitesini araştırmıştır. Bu bileşiklerin antimikrobiyal aktiviteleri in vitro olarak bir Gram-pozitif (*L. monocytogenes*), iki Gram-negatif (*C. sakazakii* ve *S.* Typhimurium) bakteri ve bir mantar (*C. albicans*) suşu üzerinde gözlendi. Ayrıca, antimikrobiyal ajanlar olarak Nisin ve Karvakrol ile UV-C ışınlamayı içeren kombine işlemlerin, buzdolabında saklama sırasında genel mikrobiyal büyüme üzerindeki etkisi, 4°C'de 2 hafta boyunca depolamadaki etkisi ve tavuk butuna yapay olarak ekimi yapılan *L. monocytogenes* ve *S.* Typhimurium üzerindeki etkisi incelendi. Toplam aerobik mezofilik, maya ve küf ve koliform popülasyonu, sırasıyla PCA, PDA ve VRBA ortamlarında, *L. monocytogenes* and *S.* Typhimurium. sayımları ise PALCAM, OXFORD ve Bismuth Sulphite Agar kullanılarak incelenmiştir.

Sonuçlar, UV-C ışınlama dozu ve antimikrobiyal ajan konsantrasyonu (2 x MİK) kombinasyonu ile işlenen numunelerin, yalnızca daha yüksek miktarda antimikrobiyal madde ile muamele edilen numunelere kıyasla patojen sayısında önemli bir azalmaya ($p \leq 0.05$) yol açtığını gösterdi. İn vitro hücre büyümesi deneyleri, test edilen bileşiklerin farklı etki seviyeleri ile tüm bakteri türleri üzerinde toksik etkilere sahip olduğunu göstermiştir. Sinerjistik ve aditif etkiler gözlenmiştir, ancak UV-C kombinasyonlarında antagonistik etkiler bulunmamıştır. Bu çalışmanın sonuçları, Lantibiyotik ve EO bileşenlerinin antimikrobiyal aktivite mekanizmaları hakkında daha fazla araştırma yapılması için umut vericidir.

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CHAPTER 1

INTRODUCTION

Animal-derived foods have a high susceptibility to spoilage because of their neutral pH, moisture, and rich nutrient content. In order to maintain the quality and safety of these foods, it is necessary to preserve them properly. If this is not done, human illness and disease outbreaks are possible. Food-borne illnesses are a significant public health concern worldwide, and they can lead to high costs. The food industry generally adopts various measures to guarantee food quality and safety, such as good manufacturing and hygiene practices. Therefore, numerous measures are typically taken to uphold food preservation. Preserving food is essential for ensuring food safety and quality. Various preservation techniques are available, including low temperature methods such as chilling and freezing, as well as thermal methods such as pasteurising and sterilising. Preservation using specific chemicals is also an option.

Nowadays, advanced preservation techniques such as biopreservation, irradiation and hurdle technologies are widely used. Traditional food preservation techniques change the state of the food and cause some nutrients to be lost. Therefore, modern techniques are more suitable for achieving food quality and safety. Today, it is also popular because of the globalization of the food market. Biopreservation is more reliable than the 'farm to fork' approach of all the preservation techniques used today. It can extend shelf-life with high quality and hygienic status, minimizing nutritional losses.

Biopreservation is a technique for extending the shelf life of food by using natural or controlled microbiota or antimicrobial agents. Due to their thermo-resistant, non-toxic, and broad-spectrum bactericidal effects, LAB's bacteriocins are considered to be a highly potent biopreservative. LAB bacteriocin, nisin, which is widely used in the meat industries and has been approved by the Food and Drug Administration (FDA).

Phytochemicals include carotenoids, vitamins, terpenoids, nitrogenous components, and phenolic acids, which are responsible for the antibacterial activity of plants. Phenolic compounds possess antioxidant properties linked to the presence of

hydroxyl groups within their molecular structures. (Demirel et al., 2019; Ziaková & Brandšteterová, 2011). On the other hand, flavonoids make up the predominant portion of plant-derived phenolic compounds, with a total of over 8000 identified compounds. (Tungmunnithum et al., 2018).

Bioactive compounds have been isolated from various plant components, serving as a valuable reservoir of medicinal agents (Jouda et al., 2016). Owing to their abundance in plants, their inherent antibacterial attributes render them highly valuable. Traditionally, antimicrobial agents are employed to manage diseases stemming from microorganisms. Yet, the emergence of microbial resistance hampers the efficacy and significance of such agents (Marini-Bettòlo, 1980). Plant extracts provide a natural reservoir of antimicrobial compounds (Tepe et al., 2004). These secondary metabolites, exhibiting antimicrobial properties, impede the growth of microorganisms in food, thereby extending the shelf life of edibles (Burt, 2004).

The pharmaceutical industry, which gained prominence through the synthetic production of active compounds from plants during the 1800s, considerably veered away from traditional approaches. Nonetheless, over the past 25-30 years, there has been a resurgence of interest in alternative medicine. This resurgence is prompted by the shortcomings of synthetic pharmaceuticals employed in modern medical practices, which often fail to achieve desired outcomes, lead to numerous unfavorable side effects, produce singular effects, and share similar limitations. Natural plant-based remedies hold greater appeal compared to synthetic treatments, primarily due to their reduced adverse effects and potential for multiple benefits. Consequently, research in botanical medicine, with its substantial historical medical influence, has emerged as an intriguing and compelling field of study

Increasingly, consumer preferences are gravitating toward foods characterized by superior quality, minimal processing, elevated nutritional content, and freshness. Consequently, the ongoing emphasis remains on ensuring the microbiological safety of food items while simultaneously preserving their nutritional and sensory attributes (Pattanayaiying et al., 2015). To retain desirable characteristics like texture, flavor, color, and nutrient content, prepared dishes often undergo gentle heat treatment. Although this approach effectively targets vegetative bacterial forms within the food, it falls short in eradicating heat-resistant spores. As a result, an appropriate disinfection strategy becomes imperative to thoroughly eliminate these persistent spore contaminants.

Consequently, both scientists and the food industry are actively exploring inventive and emerging approaches capable of eradicating undesirable microorganisms while minimizing their impact on food quality. This pursuit has driven substantial endeavors in the development of non-thermal technologies, aimed at circumventing the adverse effects of thermal treatments and ensuring the production of safe food products (Birmpa et al., 2013; Severino et al., 2014). A burgeoning interest in non-thermal preservation methods, particularly the integration of non-thermal techniques with antimicrobial interventions (Luu-Thi et al., 2015; Masana et al., 2015), has the potential to amplify the lethal impact of non-thermal processing. This integration enables the preservation of a food product's physicochemical attributes while safeguarding its nutritional value (Raso & Barbosa-Canovas, 2003; Ross et al., 2003).

CHAPTER 2

LITERATURE REVIEW

2.1. Biopreservation in Food Industry

Biopreservation represents a food preservation strategy that upholds both food safety and quality, while also elongating the shelf life of edibles. This method harnesses the inherent antimicrobial capabilities of naturally existing microorganisms and their antimicrobial byproducts. The natural antimicrobial agents employed in food biopreservation are outlined in Table 1.

Biopreservation methods, tailored to meet the essential safety requirements of diverse food items, predominantly hinge upon the efficacy of biological antimicrobial mechanisms. This often involves the utilization of components like lactic acid bacteria (LAB), their bacteriocins, bacteriophages, and enzymes encoded by bacteriophages. Moreover, biopreservation aligns with the growing consumer demand for "healthier" and more "natural" food options, while also facilitating reductions in the usage of salt, sugar, and synthetic additives. This is achieved through the application of microorganisms or their metabolic byproducts (Mani-López et al., 2018; Borges et al., 2022).

Table 1: Natural antimicrobials for food biopreservation (V. P. Singh, 2018).

	Antimicrobials	Source	Food biopreservation
Antimicrobial substances derived from bacterial cell metabolism	CO_2	Heterofermentative LAB produces CO_2 as a byproduct of sugar fermentation.	Creation of an anaerobic environment and antagonistic effects specifically against aerobic bacteria and produce carbonic acid.
	Organic acids	Main end products of fermentation.	Decrease the pH of the surrounding environment, creating a selective barrier against non-acidophiles Lactic acid exerts an antimicrobial effect by disruption of the cytoplasmic membrane and interference with membrane potential
	Diacetyl (2,3-butanedione)	LAB as a by-product of metabolic activity	Antibacterial activity against *Listeria*, *Salmonella*, *Escherichia coli*, *Yersinia*, and *Aeromonas*.
	Hydrogen peroxide	Produces by LAB in the presence of oxygen and action of flavoprotein oxidases or NADH peroxidase.	Antibacterial effect through oxidative damage of proteins and increase of membrane permeability.
	Reuterin	Low molecular - weight antimicrobial compound produced by *Lactobacillus reuteri* and some other LAB.	Antimicrobial activity against bacteria, yeasts, and molds by inhibiting DNA synthesis.
	Reutericyclin	Reutericyclin-producing strains of LAB.	Reutericyclin acts as a proton ionophore and dissipation of the proton motive force against gram-positive bacteria including *Lactobacillus* spp., *Bacillus subtilis*, *Bacillus cereus*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Listeria innocua*.
Antifungal compounds	Natamycin (pimaricin)	Produced from *Streptomyces natalensis*	Broad-spectrum antifungal biopreservative for foods and beverages by binding irreversibly to the cell membrane of fungi and causes membrane hyperpermeability leading to rapid leakage of essential ions and peptides and ultimately cell lysis.
Antimicrobials from Plant Sources	Essential oils such as saponins, flavonoids, **carvacrol**, thymol, citral, eugenol, linalool, terpenes, and their precursors	Plant material like flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots.	The concentration of 0.05–0.1% of essential oils has demonstrated activity against pathogens, such as *Salmonella* typhimurium, *E. coli* O157:H7, *L. monocytogenes*, *B. cereus* and *S. aureus* in food systems.

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Table 1: (Cont.)

Antimicrobials from Animal Sources	Lysozyme	Naturally found as part of defense system of living organisms.	Generally recognized as safe (GRAS) for direct addition to foods.
	Lactoferrin	Natural protein found in milk and other secretions.	Antimicrobial activity due to its iron-binding capacity and polycationic nature against a wide range of bacteria including foodborne pathogens like *Carnobacterium*, *Listeria monocytogenes*, *Escherichia coli*, *Klebsiella* and viruses (Lönnerdal, 2011; Gyawali and Ibrahim, 2014).
	Lactoperoxidase	Antimicrobial system that originated from milk.	Effective against gram-negative bacteria.
	Ovotransferrin	Produced by hydrolysis of natural proteins	Inhibits bacterial growth due to iron deprivation
	Protamine	Naturally present in spermatic cells of fish, birds and mammals.	Broad antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and fungi. Used as preservative in wide variety of foods ranging from confection items to fruits and rice
	Pleurocidin	Present in myeloid cells and mucosal tissues of many Vertebrates and Invertebrates.	Antimicrobial activity against several foodborne bacteria such as *L. monocytogenes* and *E. coli* O157:H7, and pathogenic fungi.
	Chitosan	Polycationic biopolymer naturally present in exoskeletons of crustaceans and arthropods.	Considered as safe food additive and has antibacterial activity against both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *E. coli*, *Shigella dysenteria*, and *Salmonella* Typhimurium (Gyawali and Ibrahim, 2014). Used in biodegradable edible coatings, singly or dosed with other antimicrobial substances (Elsabee et al., 2013).

Biopreservatives suitable for food applications must comply with generally accepted safety criteria and not have any harmful effects on food regarding pathogenicity or toxicity. In the biological agents used in food production, two different classifications can be distinguished: starter and protective cultures. The former encompasses the utilization of microorganisms to initiate fermentation processes, facilitating the creation of distinct compounds that confer the unique texture and flavor attributes found in fermented products. In contrast, protective cultures predominantly function to control antimicrobial activity, proficiently curbing the propagation and survival of detrimental

microorganisms within foods. However, combining starter and preventer cultures is often preferable when considering their integration into the food industry process facilitated by natural microbial growth or deliberate inoculation produces a variety of beneficial products due to the presence of bacteria. These bacteria have a pivotal function in minimizing food spoilage and eradicating pathogens and their associated byproducts. Lactic acid bacteria (LAB) are predominantly used as primary organisms. In addition, metabolic byproducts such as organic acids have antimicrobial properties and contribute to the distinctive taste and texture characteristics observed in foods (Ganguly, 2013; Lucera et al., 2012). Specifically, lactic acid bacteria (LAB) are recognized for generating bacteriocins, which are proteins or peptides that has natural antimicrobial properties. In addition, organic acids and hydrogen peroxide are among the important naturally occurring compounds with antimicrobial properties produced by LAB.

Because of their qualities like being non-toxic, non-immunogenic, heat-resistant, and possessing wide-ranging bactericidal attributes, LAB bacteriocins are noteworthy as biopreservatives. They demonstrate remarkable effectiveness, particularly against Gram-positive bacteria and certain compromised Gram-negative bacteria, encompassing various pathogenic strains. The frequently encountered LAB bacteriocin is Nisin, notable for its extensive utility in sectors such as vegetable products, dairy, and meat industries. Nisin holds the distinction of being approved by the Food and Drug Administration (FDA). Beyond LAB metabolites, other factors like bacteriophages and endolysins also hold promising potential in the realms of food processing, preservation, and safety.

Bacteriocins are categorized into the following four main classes according to their properties, molecular weight, structure, and post-translational modification (Table 2):

Table 2: Classification of bacteriocins (V. P. Singh, 2018).

Class	Nomenclature	Qualities	Examples	Reference
Class-I	Post-translationally modified peptides called **Lantibiotics**	Characterized by distinctive thioether-based intramolecular rings of lanthionine and βmethyl-lanthionine	**Nisin**, discovered in 1928, lacticin *L. lactis*, citolysin of *E. faecalis*, and lacticin of *L. lactis*	Klaenhammer, 1993 Twomey et al., 2002 Xie & van der Donk, 2004 Gillor et al., 2008
Class-II	Thermostable, nonmodified non-lantibiotic linear peptides of	Characterized by short cationic peptides with high isoelectric points. It contains potential *Listeria* activity	Pediocin PA1/AcH produced by *Pediococcus*, Enterocin EJ97 by *E. faecalis*.	Breukink et. al., 1999 Drider et al., 2006 Gillor et al., 2008
Class-III	Comprises large (> 30 KDa) heat labile proteins like colicin- V and microcins.	Bacteriocins are Gram-negative circular peptides characterized by a peptide bond between the C- and N-terminus. It possesses bacteriolytic extracellular enzymes like hemolysins and muramidases which can mimic the physiological activities of bacteriocins.	Helveticin J of *L. helveticus* and bacteriocin Bc-48 of *E. faecalis*.	Joerger & Klaenhammer, 1990 Wiedemann et al., 2001 Gillor et al., 2008
Class-IV	Circular peptides posses intriguing and novel type of antimicrobial substances produced not only by bacteria but also by plants and mammalian cells.	Characterized by a peptide bond between the C- and N-terminus are clustered. They are existed in form of head-to-tail peptide chain ligation, which makes thermo molecules with neither an origin nor an end.	Enterocin AS-48	Heng et al., 2007 Gillor et al., 2008 Martínez et al., 2008

Class I: Known as heat-stable Lantibiotics, these are modified peptide chains featuring distinctive thioether amino acids like Lanthionine (Lan) and Methyllanthionine (MeLan). These modifications occur via post-translational processes, resulting in covalent bonds between specific amino acids. Within Class I, two subclasses exist: Subclass Ia and Class Ib. Subclass Ia comprises elongated, flexible peptides with positive charges, while Class Ib encompasses globular, rigid peptides with either negative charges or no net charge.

Class II: These bacteriocins are non-lantibiotic, relatively small, minimally modified, and heat-stable. Class II has further subdivisions: Subclasses IIa, IIb, and IIc, which include various peptide bacteriocins, including circular bacteriocins. Subclass IIa incorporates antilisterial pediocin-type bacteriocins.

Class III: Comprising bacteriocins with molecular weights surpassing 30 kDa, this category includes larger, heat-labile molecules.

Class IV: These molecules are characterized by heat stability and are associated with lipid and carbohydrate complexes.

Bacteriocins are odorless, tasteless, and colorless substances and and are capable of integrating into the composition of food products. They are considered safe for human consumption, compared to chemical preservatives. However, thorough research on the chemicals must be done before they can be employed as bio-preserving agents on an industrial scale, and they must also be given obtain legal authorization for use as dietary supplements. Common applications of bacteriocins in food biopreservation encompass several approaches: the inclusion of purified bacteriocins directly into food items, the introduction of bacteriocin-producing LAB into food products, and the utilization of ingredients during food processing that have undergone fermentation with bacterial strains capable of producing bacteriocins.

Biopreservatives are additionally recognized as environmentally friendly agents, derived from either extracts or secondary metabolites of natural plant origins. These substances possess antimicrobial characteristics effective against a wide spectrum of foodborne microorganisms, which make them applicable to maintain food quality and safety (Mani-López et al., 2018; Pisoschi et al., 2018; Villalobos-Delgado et al., 2019). Combining several natural additives can successfully achieve biopreservation, which preserves food quality and nutritional characteristics while limiting bacteria development and achieving food safety. (Gómez-Sala et al., 2016; Han et al., 2023)

For food preservation, a variety of supplementary metabolites, terpenoids, and alcoholic compounds of aromatic and therapeutic plants can be used (Figure 1).

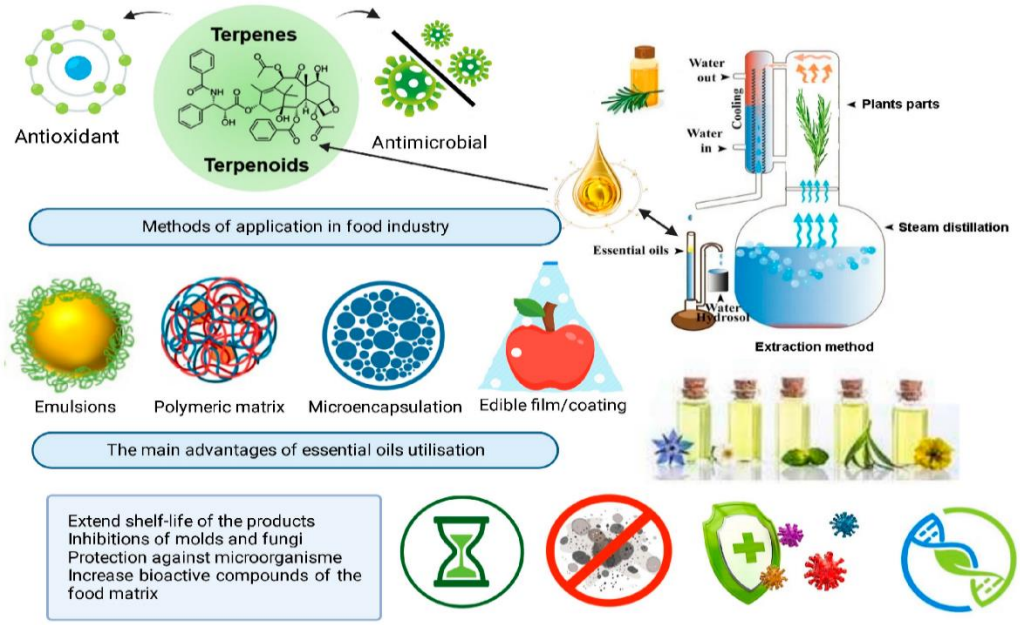

Figure 1: Essential Oils (EOs) application in the food industry (Salanță & Cropotova, 2022).

Satureja essential oil (EO) can be encapsulated with chitosan to effectively hinder the growth of *Pseudomonas* spp., molds, and yeasts in refrigerated meat (Noori et al., 2018). Encapsulation of Oregano EO with whey protein isolate prevents mold and yeast growth, ensuring the preservation of grated cheese. Furthermore, the application of nano-emulsified ginger essential oil with sodium caseinate proves valuable in safeguarding chicken fillets against a spectrum of harmful microorganisms, including *Listeria monocytogenes*, *Salmonella* Typhimurium, psychrophilic bacteria, molds, and yeasts (Kim et al., 2020).

2.2. Current Lantibiotic Applications in Food Industry

Within the food industry, bacteriocins, bacteriocin-producing LAB, and lantibiotics find applications in various sectors, including the production of fermented foods, as well as the biopreservation of dairy products, fruits, vegetables, beverages, seafood, and meat (Table 3) (Verma et al., 2022).

Table 3: Prominent bacteriocins and their potential applications within the food sector (V. P. Singh 2018).

	Bacteriocin	**Food application**
Dairy industry	**Nisin**	*Clostridium butulinum* in cheese, *L. monocytogenes* in cheeses such as Camembert, Ricotta and Manchego
	Pediocin AcH	Effective against milk and Cheddar and Munster cheeses against *L. monocytogenes*, *S. aureus* and *E. coli* O157:H7 lacticin against undesirable LAB. *L. monocytogenes* and *B. cereus* in Cheddar, Cottage cheese and yogurt and enterocin AS-48 against *B. cereus*, *S. aureus* and *L. monocytogenes* in milk and Manchego cheese.
Meat industry	**Nisin**, Enterocin AS-48, Enterocins A and B, Sakacin, Leucocin A and especially Pediocin PA-l/AcH alone or in combination with several physicochemical treatments like modified atmosphere packaging, high hydrostatic pressure (HHP), heat and chemical preservatives	Quite effective against *L. monocytogenes* and other pathogens.
	Bacteriocinogenic LAB	Bio-protective cultures to protect pathogens in food processing.
	Pediocin PA-l/AcH	It is more suitable for use in meat and meat products than nisin but *P. acidilactici* is not an indigenous meat strain.

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Table 3: (Cont.)

Vegetable products	**Nisin**	In tinned vegetables and fruit juices.
	Pediocin PA-1/AcH	In salad and fruit juices.
	Enterocin AS-48	Effective against *B. cereus* in rice and vegetables and against pathogens such as *E. coli* O157:H7, *S. aureus* and the spoilage bacterium *Alicyclobacillus acidoterrestris.*
Fish products	Combination of nisin and Microgard	Gram-negative microorganisms generally encountered in fresh chilled salmon and *L. monocytogenes* in frozen thawed salmon.
	Bacteriocins culture containing *Carnobacterium divergens* culture in combination with lactic acid, sodium chloride, and/or nisin	In inhibition of *L. monocytogenes* in rainbow trout.

Numerous LAB have acquired Generally Recognized as Safe (GRAS) designation due to their traditional roles in food fermentation and extensive history as bacteria safe for food use. Numerous bacteriocins have demonstrated heightened stability and efficacy in conditions of acidic pH, elevated temperatures beyond the standard range (significant for instances of temperature abuse), and temperatures below the standard range (pertinent for refrigerated foods)

Bacteriocins offer the benefit of effectively targeting both opportunistic and pathogenic bacteria, including those resistant to antibiotics. Moreover, numerous bacteriocins exhibit synergistic interactions with antibiotics, aiding in the mitigation of side effects and required dosage. They also display synergistic efficacy alongside other biomolecules such as citric acid and nisin in countering pathogens like *L. monocytogenes* and *S. aureus* (Soltani et al., 2021) (Figure 2).

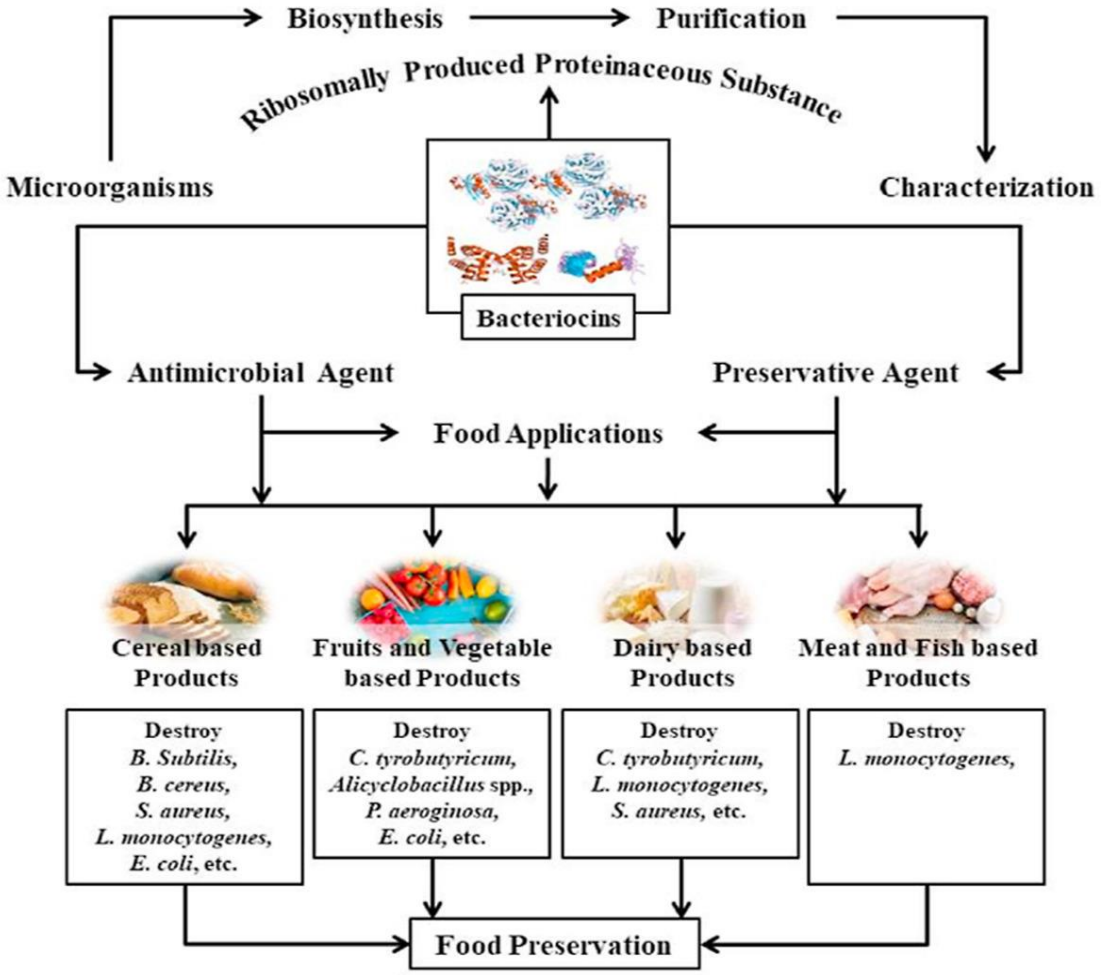

Figure 2: Utilization of Bacteriocins as Antimicrobial and Preservative Agents in the Food Industry (Verma D. K. et al., 2022).

Because of their protein composition, thermal stability, non-toxicity to human organisms, and antagonistic impact on the majority of Gram-positive bacteria, bacteriocins are a class of chemicals that the food sector finds to be very desirable (Ayivi et al., 2020; Zapanik et al., 2022). The food industry currently only uses bacteriocins made by LAB to a limited extent. In the food supply chain, only the lantibiotics nisin (E234) and pediocin PA-1/Ac H are commercially available as preservatives (Pérez-Ramos et al., 2021).

Due to competition for nutrients or the production of antagonistic substances like bacteriocins, as well as other compounds such as organic acids, free fatty acids, hydrogen peroxide, carbon dioxide, ethanol, and enzymes (Shah et al., 2016; Vieco-Saiz et al.,

2019), LAB exhibit the capability of bioprotection or the inhibition of growth in numerous other microorganisms linked to food. Furthermore, bacteriocins have demonstrated effectiveness in addressing various human and animal illnesses and infections, including mastitis. In a study comparing healthy and malignant cell membranes, Meade et al. (2020) proposed the potential application of bacteriocins against cancer cells.

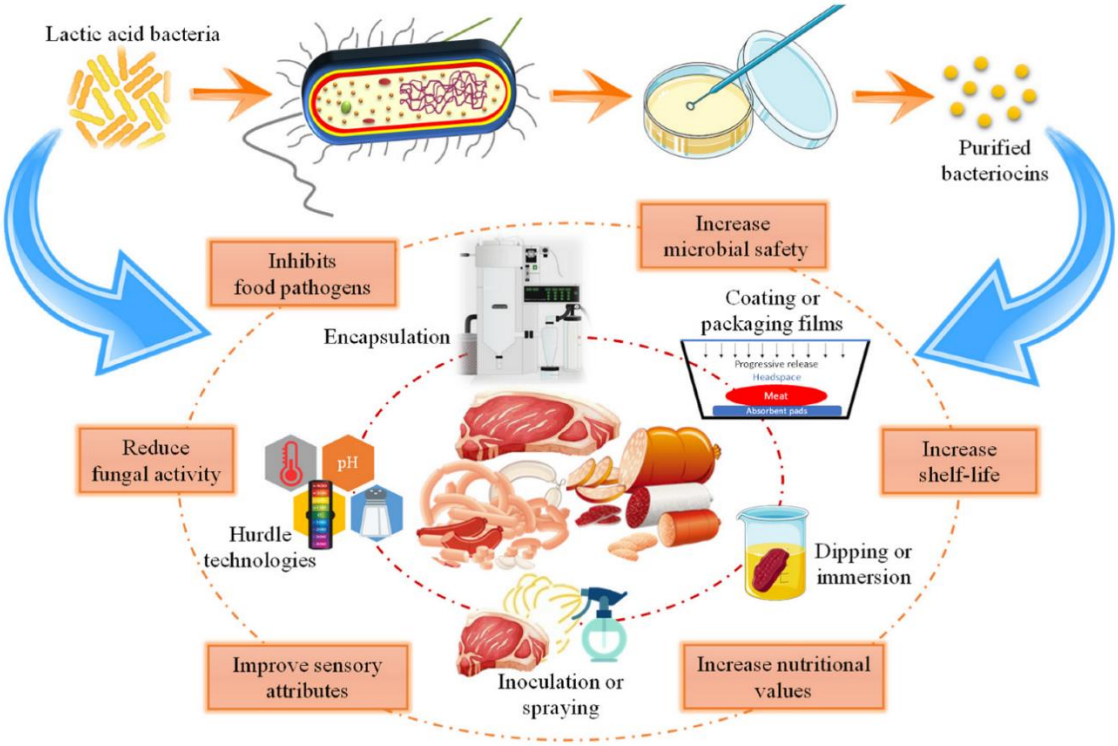

Figure 3: Impact of Lactic Acid Bacteria and Bacteriocins on Quality and Safety Enhancement in Meat and Meat Products (Bhattacharya, D. Et al., 2022).

2.2.1. Structure, Mode of Action and Applications of Lantibiotic Nisin

Nisin, a member of the Lantibiotics group, represents bacterial-derived antimicrobial peptides that have been integral to food preservation for the past four decades. There exists considerable intrigue and potential in broadening its scope to encompass biomedicine, aiming to formulate innovative antibiotic alternatives. The

primary challenge lies in its inherent limited spectrum of antimicrobial activity, which primarily targets Gram-positive bacteria. Vukomanovic and colleagues present a significant advancement in nano-engineering that widens the efficacy of nisin to encompass Gram-negative bacteria. Their approach involves the creation of a nanocomposite, characterized by a notable density of positively charged groups. This is achieved by attaching nisin molecules onto the surface of gold nano-features, which are uniformly arrayed on spherical carbon templates. Before the assembly process, none of the individual components within the nanocomposite impacted bacterial growth. However, this scenario shifted significantly following the successful formation of the nanocomposite. They demonstrated for the first time how interactions enabled by this kind of structure are capable of destroying the Gram-negative bacteria's cell wall. The developed approach offers new opportunities for using lantibiotics in creating post-antibiotic medications, as supported by the nisin model (Vukomanović, M. et al., 2017).

Antibacterial action against other comparable or nearly related bacterial strains is exhibited by lantibiotics, proteinaceous toxins produced by specific types of bacteria by ribosome synthesis (Nissen-Meyer & Nes 1997). Nisin, a member of these peptides and a potent and secure food additive, has been authorized for use as a food preservative in more than 50 countries for almost 40 years (Asaduzzaman & Sonomoto, 2009) and is listed on the positive lists of the FDA and the EU (E234 food additive). It is widely acknowledged that these bacterial antimicrobial peptides have the potential to pave the way for a novel generation of natural antimicrobials, driven by their improved stability and heightened efficacy (Clardy et al., 2006; Hancock & Sahl 2006). According to Breukink et al. (1999) and Hasper et al. (2006), interactions with bacterial membranes are a key component of the process they employ. Lantibiotics employ diverse mechanisms to disrupt bacterial cells, which encompass binding to lipid II, influencing the synthesis of bacterial cell walls, inhibiting bacterial growth, and initiating the creation of pores within the cellular membrane, ultimately leading to cellular demise (Ross & Vederas, 2011; Wiedemann et al., 2004; Kramer et al., 2008). Nisin, for instance, intricately forms a complex with the lipid-II wall precursor, thus influencing cell wall construction and serving as a docking molecule for the generation of stable, 2-2.5 nm-sized pores that accommodate the lipid-II molecule for brief durations (Breukink et al., 2004; Wiedemann et al., 2004; Hsu et al., 2004). The ability of antimicrobial peptides to counter antibiotic-resistant species is attributed to their multifaceted mechanism and the utilization of dual modes of action (Ross & Vederas, 2011; Kramer et al., 2008).

The primary challenge hindering the clinical application of lantibiotics, including nisin, lies in their short half-life within the bloodstream. Furthermore, their effectiveness is restricted by a limited scope of antibacterial activity. While Gram-negative bacteria possess an outer lipopolysaccharide membrane shielding their cytoplasmic membrane, nisin demonstrates potent antimicrobial effects against a diverse array of Gram-positive bacteria, encompassing *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus*, among others (Asaduzzaman & Sonomoto, 2009). Notably, considerable effort has been invested in modifying the antibacterial activity spectrum of lantibiotics (Riley & Gillor, 2007; Field et al., 2015).

By binding to lipid II, nisin inhibits the production of cell walls and forms pores in the cytoplasmic membrane as a double mode of antimicrobial action. Physiological conditions such as pH, ionic strength, temperature, and target cell growth phase can alter lysine's activity. Several proteins, polysaccharides, and DNA synthesis disturbances were observed in sensitive cells after exposure to nisin.

The solubility and stability of nisin, as a pivotal food preservative, constitute critical considerations. Nisin exhibits solubility in aqueous conditions, with optimal stability achieved in acidic environments. Its solubility has been noted as 12% at pH 2.5 and 4% at pH 5.0, while remaining considerably lower at neutral pH. Notably, at a pH of 2.0, it displayed complete stability when subjected to heating at 115.6°C. However, at pH 5.0, its activity decreased by 40%, and at pH 6.8, the reduction exceeded 90%. When heated, nisin benefits from the protection offered by large protein molecules, such as milk proteins. Food pasteurization or Tyndallization has no effect on nisin, but they significantly increase the susceptibility of bacterial spores to the antimicrobial effects of nisin. As a preservative, it finds wide application area in products such as:

-Nisin prevents *Clostridium tyrobutyricum* spores from growing in maturing cheeses. It prevents the growth of thermophilic bacteria spores that are able to survive prolonged pasteurization in milk.

-The lysine in canned food kills first of all thermophilic bacteria such as *B. stearothermophilus* and *Clostridium thermosaccharolyticum* species.

-The use of nisin allows the reduction of nitrate contents in meat. Also, when used in conjunction with other antibacterial agents, such as pediocin, or with appropriate processing technologies, nisin ffectively inhibits the growth of *L. monocytogenes* species.

Nisin supplementation additionally enables the regulation of alcohol fermentation processes and the mitigation of infections caused by lactic fermentation bacteria, such as

Lactobacillus and *Pediococcus*. The existing utilization of nisin in the domain of food preservation is detailed in Table 4.

Table 4: Current application of nisin for the purpose of food preservation (Karpiński, T. M., & Szkaradkiewicz, A., 2016).

Food type	**Typical target organisms**	**Level of nisin (mg kg^{-1} / mg l^{-1})**
Dairy products (milk and cheese)	*Clostridium* spp. *Bacillus* spp. *Listeria monocytogenes*	0.25–15
Meat (ham, pork, beef, chicken)	*Salmonella* Typhimurium *Escherichia coli* O157:H7 *Brochothrix thermosphacta* *L. monocytogenes* Lactic acid bacteria	1–25
Seafood (fishes, crabs, lobsters)	*L. monocytogenes* *C. botulinum*	1–25
Pasteurized soups	*B. cereus* *C. pasteurianum*	2.5–6.25
Canned foods	*C. botulinum* *C. thermosaccharolyticum*	2.5–5
Dipping sauces & salad dressings	Lactic acid bacteria	1.25–6.25
Beer, wine, alcohol	Lactic acid bacteria	0.25–37.5

Nisin has been approved for food preservation in more than 40 countries and has been utilized for this purpose for over half a century. The primary commercially available form of nisin used as a food preservative is Nisaplin™, produced by Danisco (DuPont), which consists of 2.5% nisin active ingredient, 77.5% sodium chloride (salt), and nonfat

dry milk with 12% protein and 6% carbohydrate content. Within the European Union, the authorization for nisin (E 234) as a food preservative is established through Directive 95/2/EC, which regulates food additives, excluding colors and sweeteners. Currently, nisin is sanctioned for specific food items as delineated in Directive 95/2/EC. This includes semolina and tapioca puddings, as well as similar products (at a level of 3 mg/kg), ripened and processed cheese (at 12.5 mg/kg), and mascarpone cheese (at 10 mg/kg). Comprehensive studies have verified the safety of nisin for human consumption, leading to an established acceptable daily intake (ADI) of 2.9 mg/person/day. The AFC Panel, entrusted with evaluating food additives, flavorings, processing aids, and materials in contact with food, issued an opinion on January 26, 2006, regarding nisin's use as a food additive. This opinion confirmed the ADI of 0.13 mg/kg body weight, which was initially set by the Scientific Committee on Food in 1990. Moreover, the Joint Expert Committee on Food Additives of the UN Food and Agriculture Organization and the World Health Organization recommended a daily intake limit of 60 mg of pure nisin for an individual weighing 70 kg. Notably, no maximum limits are stipulated for nisin usage in processed cheese in Australia, France, or Great Britain. In the United States, the maximum dosage limits for nisin application are 200 mg/kg in canned and plant protein foods and 500 mg/kg in dairy and meat products, with typical doses ranging from 100 to 200 mg/kg. Australia and New Zealand permit nisin in cream products at a maximum level of 10 mg/kg, hot plate flour products (like crumpets, flapjacks, and pikelets) at a maximum level of 250 mg/kg, and several other food products, encompassing cheese, dairy desserts, tomato products, liquid egg products, beer, dips, sauces, mayonnaises, and salad dressings, at levels in accordance with good manufacturing practices (Karpiński et al., 2016).

Many studies have also been done on the use of bacteriocins in food packaging as antibacterial agents (Balasubramanian et al., 2001). Studies have revealed that the utilization of cellulose and polyethylene-polyamide bags infused with nisin and lacticin 3147 effectively curtails the proliferation of LAB, *L. innocua*, and *Staphylococcus aureus* in sliced cheese and ham. Additionally, nisin-coated low-density polyethylene foil was observed to hinder the growth of *Micrococcus luteus* ATTC 1240 in both raw and pasteurized milk. Food packets made of paper, cardboard, and edible covers were also included in the trials. (Mauriello et al. 2005). The presence of *M. luteus* in TSB, along with the bacterial composition in milk, was effectively suppressed through the utilization

of nisin-coated films. The release of nisin exhibited a dependence on pH and temperature factors.

Nisin, a cationic peptide categorized as a bacteriocin, is produced by specific strains of *Lactococcus lactis*. It stands as the sole bacteriocin endorsed as safe for application in the food industry by the World Health Organization. Displaying antibacterial properties, nisin notably targets numerous Gram-positive bacteria, encompassing pathogens responsible for foodborne illnesses such as *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* (Brewer et al., 2002; Lopez-Pedemonte et al., 2003; Sobrino-Lopez and Martin-Belloso, 2006). Nevertheless, its impact against Gram-negative bacteria, yeasts, and molds is comparatively limited.

The efficacy of nisin in food products hinges on its diffusion within the food matrix, influenced by various factors such as food composition, physicochemical attributes, and storage temperature (Carnet Ripoche et al., 2006). Consequently, an investigation was conducted to assess the antibacterial impacts of nisin, a polycyclic antibacterial peptide produced by *Lactococcus lactis*. Nisin finds application as a food preservative in diverse products, including cheese, processed meats, beverages, and cold-smoked salmon (Gharsallaoui et al., 2016; Kang et al., 2014; Soni et al., 2014; Tang et al., 2013).

2.2.2. Applications of Nisin on Chicken

Here are some potential applications of nisin on chicken:

1. Marination: Nisin can be added to marinades to improve chicken's microbial safety and shelf life. Marinating chicken with nisin has been shown to reduce the growth of bacteria such as *Listeria monocytogenes* and *Salmonella enterica*.
2. Packaging: Nisin can be incorporated into packaging materials to create antimicrobial films that can help extend the chicken’s shelf life. These films can be used to wrap individual chicken pieces or to line the interior of packaging containers.
3. Processing: Nisin can be added to chicken during processing to reduce bacterial contamination. This can be done by adding nisin to the chicken wash or by injecting nisin into the chicken meat.

4. Coating: Nisin can be added to coatings used on chicken products to improve their antimicrobial properties. This can include breading or batter used on chicken nuggets or other breaded chicken products.
5. Feeding: Nisin can also be added to chicken feed to improve the overall health of the birds and reduce the risk of bacterial infections. This can be especially beneficial in intensive poultry production systems where the risk of disease transmission is high.

Overall, the use of nisin in the poultry industry can improve the safety and shelf life of chicken products while reducing the need for traditional chemical preservatives. Additional research is needed to refine the use of nisin in various chicken products and processing techniques. Since consumers have become more concerned about chemical preservatives' side effects and demand natural preservatives in food, the use of natural preservatives has become increasingly popular (Raeisi et al. 2016).

2.2.3. Effects of Lantibiotic Nisin on *L. monocytogenes, C. sakazakii* and *C. albicans*

According to the Food Safety and Inspection Service of the US Department of Agriculture, 300,424 pounds of RTE meat products were recalled in 2005 because of a potential *L. monocytogenes* infection. According to Jay J.M. et al., *L. monocytogenes* can endure harsh physicochemical circumstances that are typically prohibitive, such as chilling temperatures, low pH, high salt concentrations, and high temperatures. Utilizing natural antimicrobial combinations against foodborne infections is currently of great interest. The use of these organic antibacterial mixtures can produce pathogens with non-lethal strains, aiding in the reduction of recontaminating infections in food. This multi-barrier strategy allows the use of antimicrobials at lower doses while having tremendous potential for food preservation (T. Sivarrooban et al., 2007).

In the field of food microbiology, nisin is one of the most attractive cationic peptides, and its antibacterial effects have been studied on various gram-positive foodborne and spoilage microorganisms, including *S. aureus, L. innocua, L. monocytogenes*, and *B. subtilis*, whose MICs are 7.8, 1, 250, and 125 g/ml, respectively.

Nisin has been shown to have antimicrobial action against *L. monocytogenes* in meat products (Gharsallaoui, Oulahal, Joly, and Degraeve, 2016). However, Zimet et al., found that when Nisin is applied directly to meat products, it has low solubility, interactions with lipids and other components, and a NIS-glutathione adduct. Nisin is therefore frequently combined with other synergistic protective agents or technological obstacles (Zimet et al., 2018).

In another study, the antimicrobial efficacy of nisin against *Cronobacter* spp. was assessed in peptone water and reconstituted Powdered Infant Milk Formula at varying temperatures. The synergy between nisin and mild heat treatment in inhibiting the growth of *Cronobacter* spp. was observed to diminish at 37°C. To enhance the antibacterial effectiveness against Gram-negative bacteria like *Cronobacter* spp., techniques to destabilize and permeabilize the outer membrane are commonly employed in combination with nisin. These methods encompass physical approaches like mild heat and sonication, as well as chemical methods involving metal chelators such as EDTA, disodium pyrophosphate, sodium hydrogen orthophosphate, citric acid, and lactic acid (Al-Nabulsi et al., 2009).

In another study, involving nisin Z in contact with both the host tissue and infectious agents like *C. albicans,* Lay et al. conducted an investigation to assess the safety of nisin Z on oral mucosa. They utilized gingival fibroblast and epithelial cell cultures to evaluate its innocuity, and further explored its potential synergistic impact with gingival cells against *C. albicans* adhesion and the transition from blastospore to hyphal form (Lay et al., 2008).

Beuchat et al. (1997) and Wong et al. (1999) conducted testing on various bacteriocins to assess their effectiveness against *B. cereus*. Their findings revealed that among the tested bacteriocins, nisin exhibited the broadest and most significant efficacy. In bakery products characterized by elevated moisture content, such as crumpets and liquid eggs, Nisin effectively hindered the proliferation of *B. cereus* (Delves-Broughton et al., 1996). Investigated was the ability of carvacrol and nisin, two naturally occurring antimicrobial compounds, to prevent the growth of *B. cereus* and *B. circulans*. The researchers also investigated the growth characteristics of *B. cereus* and *B. circulans* strains and possible competition between the two *Bacillus* species when kept at cold temperatures.

2.3. Essential Oil Carvacrol Treatments in Food Industry

Alkaloids, glycosides, tannins, resins, steroids, phenols, flavonoids, and volatile oils are just a few examples of the many volatile, aromatic, and bioactive compounds found in plant Essential Oils (EOs) and Extracts (Figure 4). These substances play a crucial role in various industries such as pharmaceuticals, food, agriculture, cosmetics, and healthcare due to their inherent antioxidant and antimicrobial properties (Bolouri et al., 2022; Franz & Novak 2020). These components are natural alternatives to synthetic additives having adverse effects subsequently lead to various toxic effects as carcinogenesis on consumers, maintain and increase consumers' health.

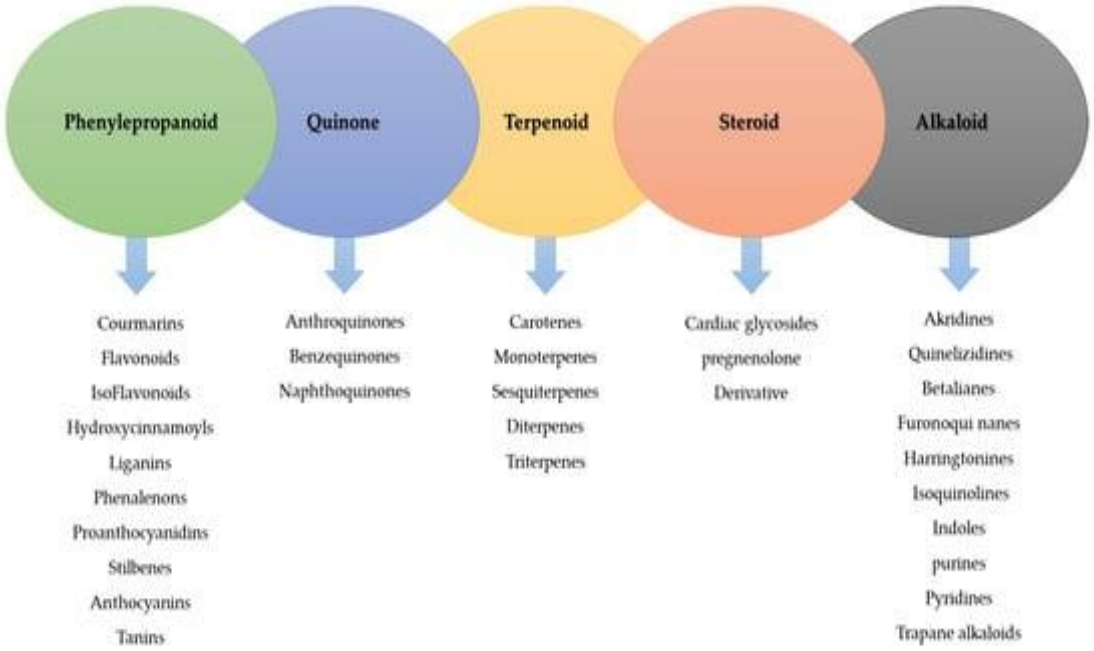

Figure 4: Main active compounds constituting plant essential oils and extracts (Bolorui P. et al., 2022).

Several notable plant constituents, such as eugenol, citral, thymol, carvacrol, cinnamic acid, cinnamaldehyde, and geraniol, hold significant significance across diverse industries owing to their antioxidative and antimicrobial properties (Burt 2004; Bolouri

et al., 2022; Franz & Novak 2020). The antimicrobial effects of these components on different microorganisms are strongly related to the type of the main compounds (Bhavaniramya et al., 2019; Conner, 1993; Ultee et al., 1998 Juven et al., 1994; Sivropoulou et al., 1996; Kim et al., 1995).

Phenolic compounds like carvacrol, citral, eugenol, cinnamaldehyde, and thymol exhibit higher antibacterial efficacy compared to terpenes. The three principal active substances that can effectively suppress the growth of microorganisms are carvacrol, thymol, and eugenol. The cell membrane's integrity can be compromised, which can then alter electron flow, proton gradients, active transport systems, and even cause cellular contents to coagulate. For their application as antibacterial agents in fresh meat, ground meat, seafood, and related packaging materials or edible films, essential oils derived from rosemary, oregano, clove, coriander, lemon leaf, basilica, balm, ginger, and basil have demonstrated notable potential (Alfonzo et al., 2017; El-Sayed et al., 2017; Khaleque et al., 2016; Moradi et al., 2016; Raeisi et al., 2016; Rodrigues et al., 2017).

Carvacrol demonstrates significant antimicrobial activity when compared to other chemical components found in essential oils. It serves as the primary constituent in the essential oil composition of oregano (containing 60–74% carvacrol) and thyme (with 45% carvacrol) (Lagouri et al., 1993; Arrebola et al., 1994). Its hydrophobic nature is believed to influence bacterial cell membranes, resulting in its biological effects (Ultee et al., 1999).

Ultee et al. (1998) demonstrated the impact of carvacrol on the development and production of diarrheal toxins by *B. cereus* in soup and brain heart infusion (BHI). The impact in soup required a carvacrol concentration that was 50 times higher.

The bactericidal, virucidal, and fungicidal activities of essential oils, also known as volatile oils, are well-known (Burt, 2004). It is important to carefully assess them to make sure that consumers will find them sensory-acceptable when added to foods before using them as "natural preservatives" (Nychas, 1995). Given the information presented earlier, further investigation is needed to explore the utilization of natural additives or alternative methods to enhance food safety and extend the shelf life of food products.

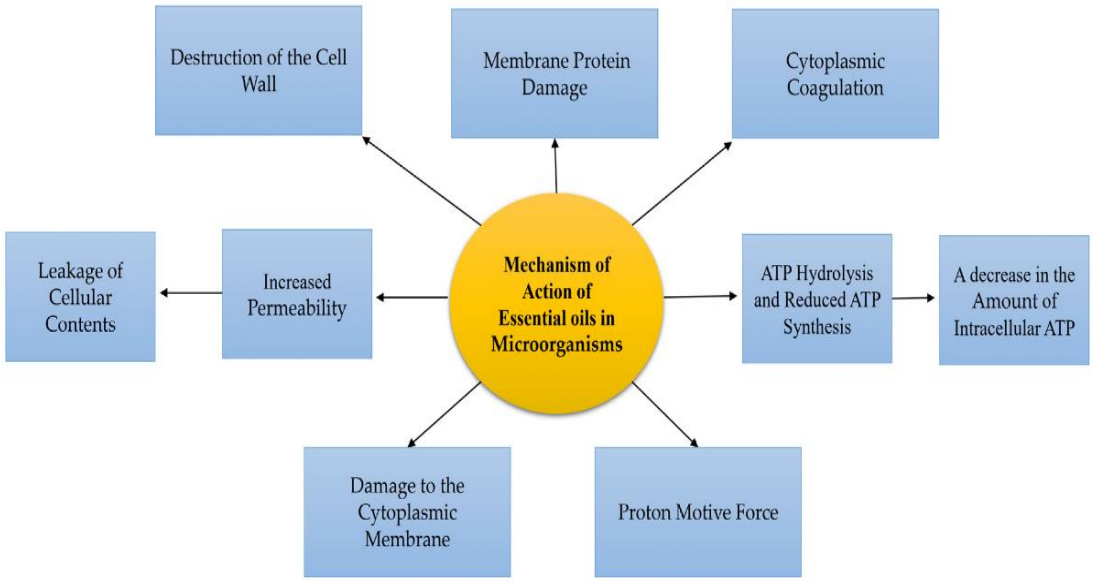

Figure 5: Various types of mechanisms of the activities of EOs on microorganisms (Bolorui P. et al., 2022)

Essential oils and their constituents have recently attracted much attention among various natural chemicals for active packaging applications (Rehman et al., 2020). Since phenolic compounds such as carvacrol (5-isopropyl-2-methylphenol) are particularly potent antimicrobial and antioxidant agents, they have received much attention in food preservation research (Figure 6; Burt 2004; Hosseini et al., 2009; Nieto 2020; Shahidi & Ambigaipalan 2015). *Escherichia coli* and *Botrytis cinerea* are just two examples of the many common food bacteria against which Carvacrol is known to be effective. (Ben Arfa et al., 2007; Du et al., 2008; Mascheroni et al., 2011; Tunç & Duman 2011), Other microorganisms are *Staphylococcus aureus* (Tunç & Duman 2011; Ramos et al., 2012), *Candida albicans* (Lambert et al., 2001), *Aspergillus niger*, *Saccharomyces cerevisiae***,** and *Listeria innocua* (Guarda et al., 2011). Aside from that, carvacrol's antioxidant capabilities have long been known (Aeschbach et al., 1994; Kulisic et al., 2004; Ramos et al., 2014). For these reasons, carvacrol has been incorporated into various substrates, including chitosan (López-Mata et al., 2013), starch (Fonseca et al., 2019), PLA (Qin et al., 2017), among others (Klinmalai et al., 2021). The very hydrophobic nature of carvacrol can make it difficult to bind to cellulose nanofibers, but various techniques,

such as encapsulation and emulsification, can circumvent this problem (Ben Jemaa et al., 2019).

Several essential oils from *Lamiaceae* species, especially oregano (*Origanum vulgare*), contain carvacrol, a natural monoterpene (Sharifi-Rad et al., 2018). In terms of its antibacterial activity, this compound exhibits both hydrophobic (aromatic ring) and hydrophilic (hydroxyl group) properties (Khorshidian et al., 2018). Because of its notable antibacterial efficacy, carvacrol has been subject to comprehensive research concerning its potential applications within the realms of both the food and pharmaceutical industries (Cacciatore et al., 2020; 2022; Marinelli et al., 2018; Sharifi-Rad et al., 2018). The European Commission, the World Health Organization, and the Food and Drug Administration recognize this substance as safe for use as a food additive (flavoring) and have given it the designation "Generally Recognized as Safe" (GRAS) (FDA, 2019; The European Commission, 2012).

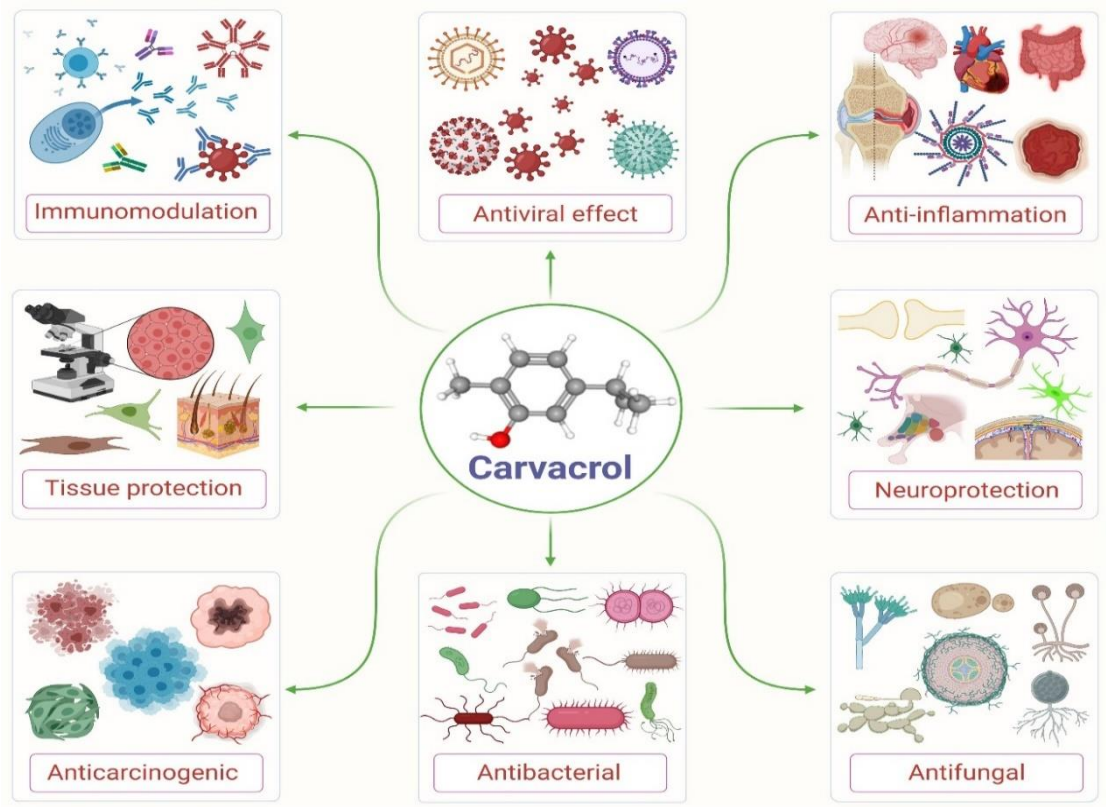

Figure 6: Antimicrobial, Anticarcinogenic and Health Beneficial Effects of Carvacrol (Javed, H.et al., 2021).

The antimicrobial agent carvacrol is able to eliminate microorganisms in food, improving food safety. However, nanotechnology can overcome carvacrol's strong odor and low water solubility, which hinder its use in food disinfection (Marinelli, L. et al., 2018).

2.3.1. Structure, Mode of Action and Applications of Carvacrol

The molecular weight of carvacrol (2-methyl-5-isopropylphenol, 2-p-cymene, 2-hydroxy-p-cymene, isopropyl-o-cresol, and isothymol) is 150.22. According to Bauer and Stoyana et al., it has the following properties: thick colorless to pale yellow liquid with a pungent, spicy odor; relative density; refractive index; melting point of 1°C; boiling point of 237–238°C; slightly soluble in water; soluble in ethanol, ethers, and alkalis; and very soluble in water (Bauer et al., 2001; Stoyanova 2022).

Carvacrol, also referred to as cymophenol ($C_6H_3(CH_3)(OH)C_3H_7$), is a phenolic substance that is a member of the monoterpenoids family. It has a characteristic, oregano-like, warm, and pungent aroma. Aromatic plants, particularly thyme and oregano, naturally produce carvacrol. It particularly exhibits strong antibacterial effects against *Salmonella* Typhimurium. Carvacrol's bactericidal properties on the foodborne pathogen Bacillus cereus have been studied in detail. Carvacrol has also been researched as a possible antibiotic feed additive replacement in female broiler chickens. Due to its low concentrations, Carvacrol has also been used as a fragrance element in cosmetic formulations and as a flavoring and preservative in food products.

This aromatic substance is mainly found in genera *Thymus, Satureja*, and *Origanum* plants, which belong to the family *Lamiaceae*. When these essential oils are stored incorrectly (high temperature, presence of water, light, etc.), precipitates and phenolates separate, causing color changes. It is well known that phenols undergo oxidation in the presence of iron, resulting in the formation of red-colored byproducts. It has been demonstrated that the production of hydroquinone is followed by oxidation to quinone and polymerization activities. The color may turn red-brown after extended exposure. A change in scent occurs along with oxidation in addition to a change in color.

Hydroperoxides are first produced. Later, they undergo a series of transformations and eventually turn into complex molecules like terpineol, carvone, and polyhydroxy.

According to studies by Zhang et al. (2018), Llana-Ruiz-Cabello et al. (2015), and Belda et al. (2005), the antibacterial effect of carvacrol is a result of the following: depletion of intracellular ATF, induction of reactive oxygen species, inhibition of efflux pump, and inhibition of bacterial biofilm (Figure 7).

Carvacrol is a bactericidal substance whose activity is influenced by exposure time and concentration (Ultee et al., 1999). Carvacrol exerts an antimicrobial impact on foodborne microorganisms by rupturing the cell membrane. It reduces intracellular ATP and membrane potential, which causes the pH gradient to dissipate and cell death. (Ultee et al., 1999). On *Streptococcus pyogenes*, carvacrol demonstrates concentration-dependent growth inhibition, with an immediate bactericidal impact seen at 2 MIC. At a concentration of 250 μg/mL (1.05 mM), carvacrol displays rapid bactericidal effects against three strains of *Streptococcus pyogenes*. Its primary mode of action involves compromising the integrity of the bacterial cell membrane, leading to the leakage of cytoplasmic contents and eventual cell demise. Carvacrol exhibits a minimum inhibitory concentration (MIC) of 125 μg/mL (0.53 mM) and a minimum bactericidal concentration (MBC) of 250 μg/mL (1.05 mM) against *S. pyogenes*. Kill curve assays affirm its immediate bactericidal potential. Transmission electron microscopy reveals dose-dependent cell rupture and demise, accompanied by the release of intracellular components like lactate dehydrogenase enzymes and nucleic acids. Notably, carvacrol's antimicrobial action showcases an additive-synergistic relationship with penicillin or clindamycin, as confirmed by checkerboard tests (Wijesundara et al., 2021).

The results show that rupture of the cytoplasmic membrane is the mechanism by which carvacrol and thymol exert their effects (Xu et al., 2008). According to Bnyan et al. (2014), the primary mechanism of carvacrol-induced growth suppression is similar in Gram-positive and Gram-negative bacteria.

Carvacrol's primary target is the cytoplasmic membrane, according to the majority of investigations on the mechanism of action of this substance (Asadi et al., 2023). Oregano, marjoram, summer savory, and thyme are all plants that contain the essential oil carvacrol (Arrebola et al., 1994; Lagouri et al., 1993). Generally recognized as a safe food additive (Leriche & Carpentier, 1995), several products, including baked goods, chewing gum, beverages, and sweets use this natural phytochemical as a flavor (Fenaroli, 1995).

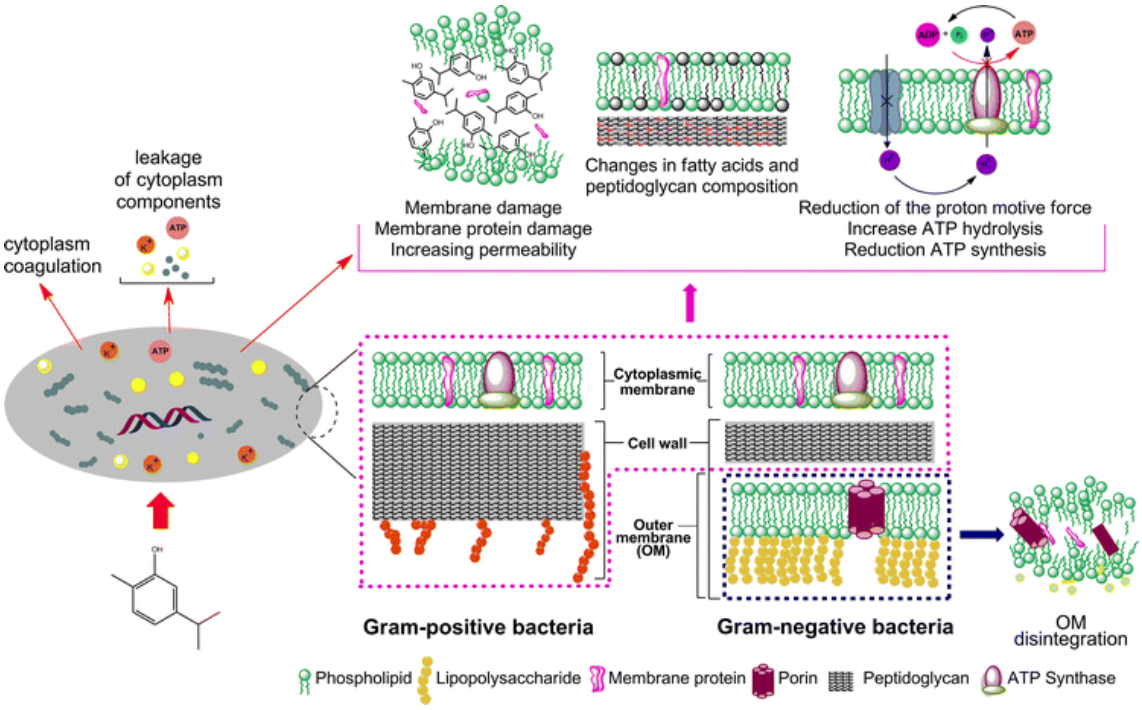

Figure 7: Schematic Representation of Carvacrol Mechanism of Action (Marinelli L., 2018).

Additionally, carvacrol is regarded as a broad-spectrum antibacterial, working well against bacteria, yeasts, and fungus (Beuchat, 1994; Davidson & Naidu, 2000; Sivropoulou et al., 1996; Thompson, 1990). Carvacrol is effective against infections, such as *S. aureus* and *S. enterica* serovar Typhimurium, to the point where it may destroy dried films of these organisms on stainless steel (Knowles & Roller. 2001; Knowles, 2002). Carvacrol is a biocidal substance that disrupts bacterial membranes, causing internal ATP and potassium ions to leak out and finally causing cell death (Ultee et al. 1999). Carvacrol and other essential oils have a wide range of antibacterial characteristics, but their intense aromatic features have restricted their use in food preservation (Rolleret al., 1995). Their potential for application in cleaning, disinfection, and biofilm reduction, however, has not received much research.

The minimum inhibitory concentration (MIC) value for carvacrol is the lowest concentration at which the growth of these organisms is inhibited. With the exception of *Pseudomonas aeroginosa*, their investigation demonstrated minimal action with a MIC value of 400 g/ml, which is the concentration that is advised for inhibiting all clinical bacterial growth. The results showed that carvacrol has the ability to significantly inhibit

both gram-positive and gram-negative microorganisms. The other proposed that the main active ingredient in thyme oils and oregano, carvacrol, appears to disintegrate the bacterial cells' outer membranes. (Lambert et al., 2001). As described by Helander et al. (1998), carvacrol possesses the capability to perturb the cell membrane of Gram-negative bacteria, leading to the release of lipopolysaccharides (LPS) and enhancing the permeability of the cytoplasmic membrane to ATP. In a similar vein, Heipieper et al. (1994) also noted that, *Pseudomonas* has adapted to ethanol and carvacrol through changes in the composition of its fatty acids. Julio et al. (2000) conducted research indicating that phenolic compounds, including carvacrol, are commonly found in essential oils with notable antibacterial properties against microorganisms. Given this, it's reasonable to expect a mechanism of action akin to that of other phenolic compounds, which typically involves perturbation of the cytoplasmic membrane, interference with the proton motive force (PMF), disruption of electron flow, hindrance of active transport, and coagulation of cellular contents (Davidson, 1997).

Infectious illnesses are treated worldwide with natural substances (Ndjonka et al., 2013). Most of these substances have high antibacterial activity against Gram-negative bacteria because they are hydrophobic. Carvacrol serves as a food additive within the food industry, and various essential oils along with their constituents demonstrate antibacterial attributes against foodborne infections (Cristani et al., 2007; Yu et al., 2012; Yang et al., 2015).

Carvacrol completely inhibited the growth of *Escherichia coli* within two hours of incubation at its minimum inhibitory concentration (MIC). Analysis using fluorescent imaging revealed an increased presence of reactive oxygen species and depolarization of the bacterial membrane, ultimately resulting in the death of *E. coli* cells when exposed to MIC value of carvacrol. Moreover, carvacrol demonstrated a pronounced disruptive effect on the bacterial membrane, leading to the release of cellular materials. Notably, even at sub-inhibitory concentrations, carvacrol significantly impacted the motility of *E. coli* cells (Khan et al., 2017).

As stated by Sarrazin et al. (2015), the effectiveness of carvacrol against Gram-positive bacteria such as *B. subtilis*, *B. cereus*, and Gram-negative bacteria like *Salmonella* Typhimurium appeared to be intricately connected with its chemical composition and structure. In a separate investigation, Du et al. (2015) established that carvacrol displayed robust antibacterial properties against pathogenic *Lactobacillus* strains, as well as Gram-positive and Gram-negative bacteria including *Clostridium*

perfringens, *Escherichia coli*, and *Salmonella* strains. Their findings suggested that carvacrol exhibited substantial inhibition against *Klebsiella pneumonia* (23 mm), *E. coli* (26 mm) and, *S. aureus* (20 mm). Variations in the chosen bacterial species and their different concentrations could contribute to the diverse outcomes observed.

The results suggest that carvacrol and essential oils containing it at higher levels could be used as an independent ingredient in food and cosmetic products, which could be the subject of further research.

2.3.2. Applications of Carvacrol on Chicken

Highly perishable foods, such as poultry meat provides a perfect environment for microorganisms such as spoilage and pathogens to grow. The GRAS (Generally Recognized as Safe) category includes essential oils that are used in food packaging. A study by Fernández-Pan et al. determined whether oregano and garlic essential oils (EOs) were effective when applied directly to chicken breast or when combined with a protein matrix. The direct application of EOs to the chicken's surface successfully reduced microbial activity after 13 days of storage, it was demonstrated. But compared to when they were applied to a structural matrix, the antimicrobials adhered to the chicken's surface less. As a result, the antimicrobials were evenly dispersed during storage but remained at ineffective quantities (Fernández-Pan, Carrión-Granda, and Maté 2014).

2.3.3 Effects of Carvacrol and Nisin Combined Applications

According to Helander et al. (1998), the protective outer membrane, which is the top layer of the cell envelope, is responsible for the nisin resistance of Gram-negative bacteria. Carvacrol and thymol are two examples of naturally occurring chemical substances that exhibit strong outer membrane dissolving properties (Helander et al. 1998). It is therefore reasonable to assume that nisin may improve the inhibitory impact against Gram-negative bacteria by passing through the outer membrane when combined

with organic chemicals that have the potential to be membrane-active (Olasupo et al. 2003). However, adding other chemical compounds to nisin did not make it more efficient at inhibiting *E. sakazakii*. Furthermore, according to other researchers, nisin and organic compounds did not work synergistically to combat *S.* Typhimurium and *E. coli* (Olasupo et al. 2003). Nevertheless, other researchers have noted that Nisin has synergistic effects with organic substances like Carvacrol or thymol against *L. monocytogenes* and *B. cereus* (Lee & Jin, 2008).

2.4. Non-thermal Control Treatments in Food Industry

Non-thermal preservation techniques inactivate microorganisms using mechanical, electromagnetic, light, or electrical energy. They typically impart more flavor and freshness to minimally processed foods and beverages while preserving health-promoting components by avoiding the changes caused by heat processing. Customer demand for high-quality, organic (preservative-free), safe (pathogen-free), and long-lasting products ensures that these technologies are becoming increasingly popular in the marketplace (Figure 8).

Non-thermal processing refers to the manipulation of food without employing heat. This approach encompasses a variety of methods such as high-pressure processing, pulsed light, pulsed electric fields, ultrasound, oscillating magnetic fields, irradiation and ultraviolet light, among others.

Non-thermal processes allow food to be processed at temperatures lower than those required for thermal pasteurization, resulting in little or no alteration of flavors, vital nutrients, and vitamins.

Non-thermal processing techniques are currently used or under development to detect potential interactions with packaging, inactivate microorganisms, and extend the microbiological shelf life of foods. Ionizing radiation, magnetic fields, dense-phase carbon dioxide, high-voltage arc discharges, ultrasound, pulsed X-rays, pulsed electric fields, ultra-high pressure, pulsed light, and hurdle technologies are among the techniques employed for non-thermal food processing (Morris et al., 2007).

Due to their minimal impact on the nutritional and sensory attributes of food products while effectively restraining or eliminating microorganisms, non-thermal techniques for food processing and preservation are generating interest among researchers, producers, and individuals engaged in the food and packaging sectors. In contrast to conventional heat-based methodologies, these non-thermal methods are regarded as more energy-efficient and better at retaining the quality features of the products. Moreover, these non-thermal procedures cater to industry requisites by presenting value-added products, novel avenues for market exploration, and heightened safety levels (Morris et al., 2007).,

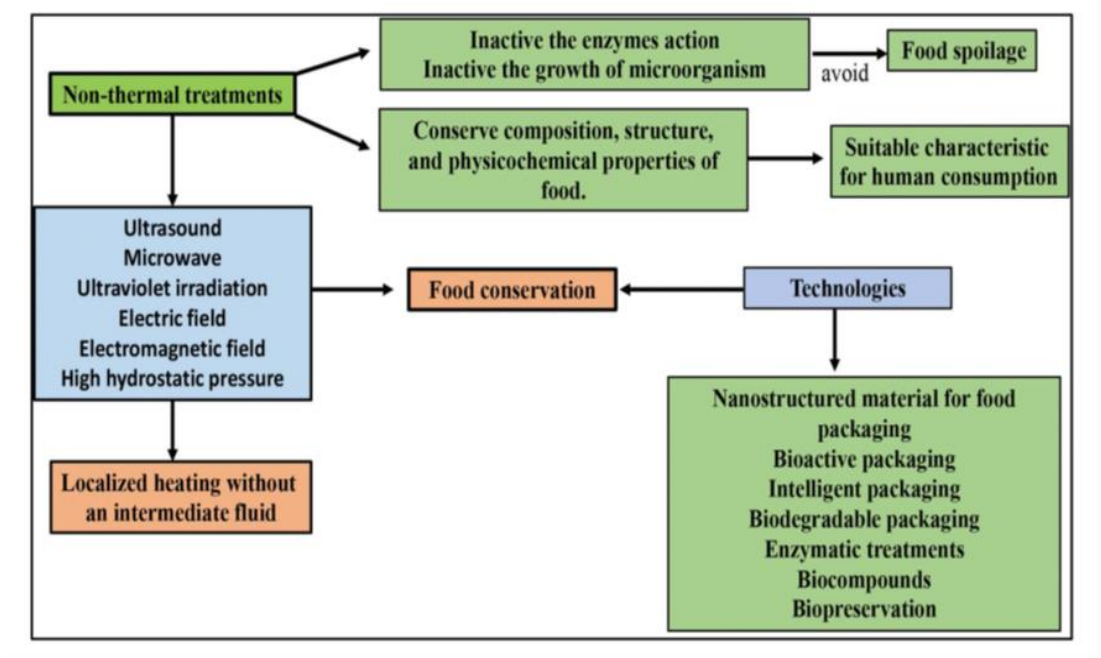

Figure 8: Non-thermal Treatments Used in the Food Industry (Buelvas-Caro et al., 2018).

Foods with little processing have become increasingly popular with consumers. Customers are looking for nutritious, natural products unspoiled by additives or excessive heating. This trend is being driven by the increasing knowledge of consumers, who are becoming more aware of how overheating foods can damage ingredients and reduce the nutritional value of the food. Because of this problem, the food industry has adapted, and

there have been numerous developments in the development of novel non-thermal preservation technologies over the past few years.

Non-thermal decontamination techniques for microbial inactivation and mycotoxin degradation have great potential to improve quality and safety. These techniques include ozone, UV and pulsed light, cold plasma, and ionizing radiation. The most critical factors affecting processing effectiveness are treatment operating parameters, food characteristics, types of bacteria, and mycotoxins. It is advisable to conduct additional studies to evaluate the toxicology of the degradation product and its interaction with food components. Particular attention should be paid to scaling up the technique for commercial use (Figure 9).

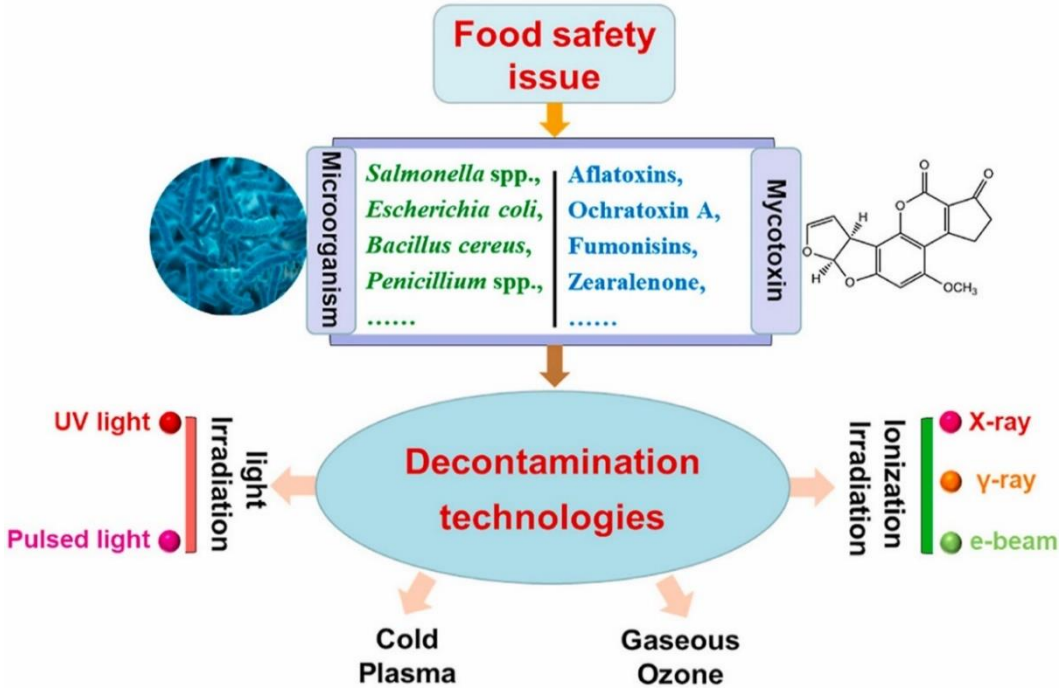

Figure 9: Non-thermal Decontamination Technologies (Deng et al., 2020).

It is possible to inactivate microorganisms using mechanical, electromagnetic, light, or electric energy in addition to thermal preservation methods. The major techniques utilized commercially include UV light (UV), high-pressure processing (HPP), pulsed electric field (PEF), membrane filtration (MF), and UV irradiation. The

implementation of these technologies has been made easier in recent years because of advancements in equipment regarding production capacity and dependability. In addition, there has been increased knowledge and awareness of the sector. However, the countries and sectors that use those technologies determine their market penetration.

In recent years, there has been a growing interest in the use of ultraviolet radiation as a method of surface decontamination (Holck et al., 2017; McLeod et al., 2018). While UV-C light emits mainly at 254 nm, pulsed UV light emits UV energy over the entire spectrum, from UV (200 nm) to infrared (1100 nm), due to bremsstrahlung. UV-C light damages nucleic acids, forming nucleotide dimers that prevent microbes from performing vital cellular functions. Pulsed UV light has been demonstrated to induce cell death through the impairment of the cell membrane (Takeshita et al., 2003) and bacterial rupture due to excessive heat generated by the absorption of UV light from the flash lamp (Wekhof et al., 2001). Additionally, pulsed UV light has been observed to create nucleotide dimers. Furthermore, according to Krishnamurthy et al. (2010), high-energy pulses can cause cell damage.

Few studies have been reported on the use of UV light and pulsed UV light in relation to salmon. In general, reductions in the range of 0-1.9 log have been achieved, depending on the type of UV treatment, fluence and product tested (Miks-Krajnik et al., 2017; Shaw, 2008; Ozer & Demirci, 2006; Cheigh et al., 2013).

The FDA gives Regulations in conjunction with conventional continuous UVC light and pulsed UV light in the USA (U.S. Food and Drug Administration, 2017).

Germany has imposed limitations on the utilization of UV-C light, reserving it for water, fruit, vegetable products, and hard cheese. The success of decontamination through UV-C and pulsed UV light hinges on multiple factors, encompassing the intensity and wavelength of illumination, the microbial resilience to UV radiation, the food surface's extent, the depth of UV light penetration, and the presence of shielding particles for microorganisms. The effectiveness of UV-C and pulsed UV light treatments against diverse strains of *L. monocytogenes* associated with fish was assessed in the context of cold-smoked salmon, raw salmon muscle, and skin, aiming to bolster food safety. The influence of UV treatments on the sensory attributes of fish products was also investigated.

UV irradiation has emerged as a non-thermal method for decontaminating and enhancing the shelf life and safety of food products (Bahrami et al., 2020; Mikš-Krajnik et al., 2017). UV radiation, a form of non-ionizing energy, possesses germicidal

properties within the wavelength range of 200-280 nm, commonly referred to as UV-C. The broader spectrum of UV light spans from 100 to 400 nm and is categorized into UV-A (315 to 400 nm), UV-B (315 to 315 nm), UV-C (200 to 280 nm), and UV-vacuum (100 to 200 nm) (Vasuja & Kumar, 2018). The mechanism underlying UV radiation involves the disruption of the genetic components of pathogens, inhibition of their replication and multiplication, and containment of their proliferation (Bahrami et al., 2020; Xuan et al., 2017). The required UV inactivation dose (measured in mJ/cm^2) to neutralize distinct types of pathogens varies based on the specific food and pathogen. For instance, bacteria, yeasts, fungi, protozoa, and algae necessitate UV inactivation doses of 1–10, 2–8, 20–200, 100–150, and 300–400 mJ/cm^2, respectively, with algae exhibiting the highest requirement (Vasuja & Kumar, 2018; Unluturk et al., 2010). The efficacy of UV radiation is influenced by diverse factors, encompassing the radiation's source and intensity, exposure duration, food type, device orientation, and bacterial characteristics (Delorme et al., 2020).

Numerous investigations have demonstrated the germicidal properties of UV light (specifically UV-C) within th*e* wavelength range of 100 nm to 280 nm. For instance, in a study involving *Salmonella*-inoculated walnuts subjected to UV light treatment at a distance of 8 cm for 45 seconds, a reduction of 3.18 log CFU/g was observed. This proved to be an essential substitute for the non-preferred chemical and thermal treatment methods, as the physicochemical properties of the walnuts were not affected (Izmirlioglu et al., 2020). Another investigation demonstrated that subjecting raw milk to UV-C treatment led to a reduction of 2 and 3 logs in the counts of total mesophilic aerobic bacteria and yeast-mold, respectively. Additionally, a significant reduction of 2–3 logs in the counts of inoculated bacteria including *Salmonella*, *S. aureus*, *E. coli*, and *L. Monocytogenes* was observed following UV treatment. Nevertheless, this study emphasized that for more effective bacterial load reduction, UV light should not be employed as a sole approach (as depicted in Figure 10), but rather integrated with other technologies (Atik & Gümüş, 2021).

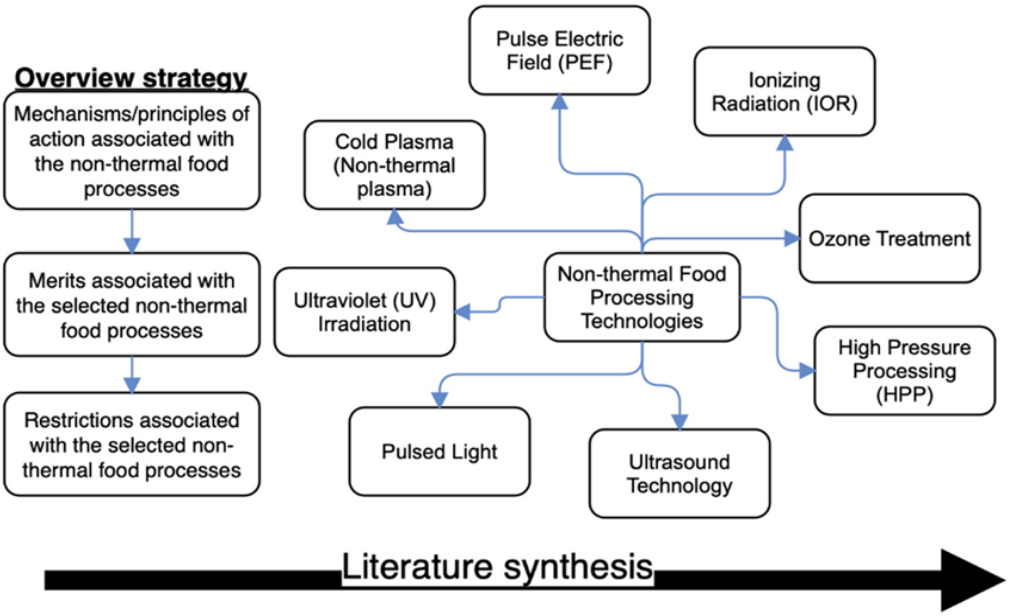

Figure 10: Schematic representation of non-thermal food processing technologies with the overview strategy (Chacha et al., 2021).

In addition, another study found that a UV-C treatment of 127.2 mJ/cm^2 for 30 second was effective in reducing the bacterial load of raw salmon (Pedrós-Garrido et al., 2018). These findings indicate that UV-C has a significant role to play in microbial stabilization. However, more research is needed to determine what other technologies could be used in addition to UV-C as a barrier to ensure that the treated food retains both its physicochemical and sensory properties during microbial inactivation.

Using UV radiation to process fruit juices (such as watermelon juice) preserves their nutrient content, according to research. As a result, it has a mild effect on lycopene, Vitamin C, phenolic compounds, and antioxidant capacity (Bhattacharjee et al., 2019).

Furthermore, UV radiation is more lethal than conventional chemicals, such as chlorine and hydrogen peroxide (Bahrami et al., 2020; Mikš-Krajnik et al., 2017), and it is characterized by its user-friendly nature and cost-effectiveness (Bhattacharjee et al., 2019; Gayán et al., 2012). It exerts minimal impact on food quality, even enhancing sensory attributes such as taste for specific foods. This technique prevents recontamination, making it applicable to pre-packaged food items. Notably, it aligns with environmental concerns and is suitable for both liquid and solid food items. UV processing is recognized for its brief processing time, and it showcases remarkable

penetration capabilities into food materials (Hernández-Hernández et al., 2019; Khan et al., 2018).

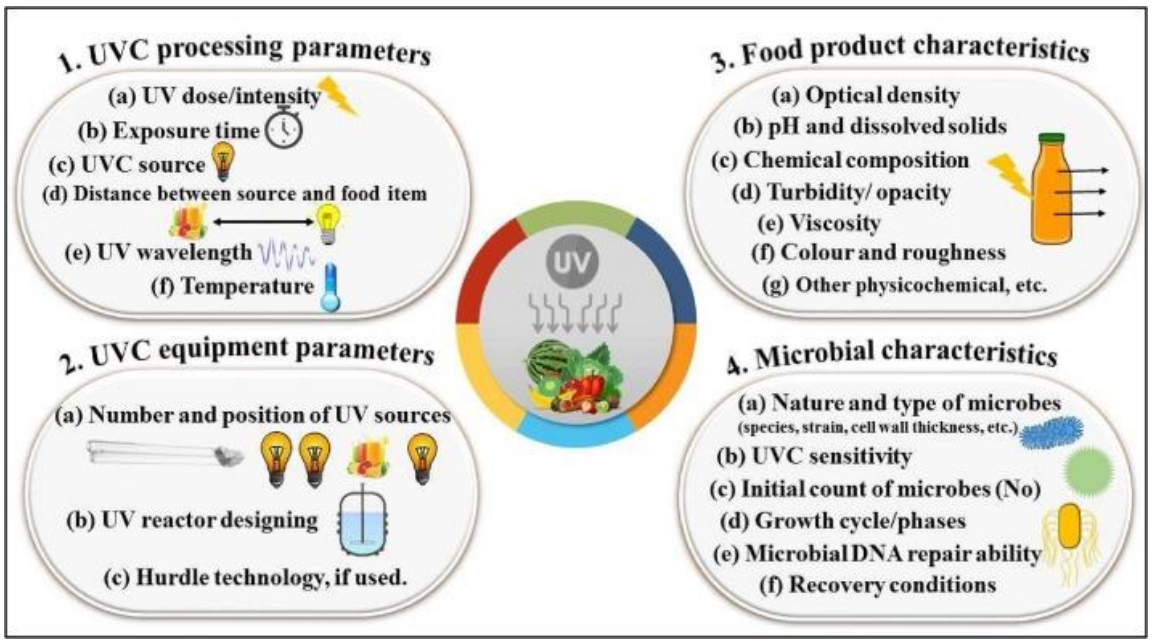

Figure 11: Important factors that affect the UV-light processing of food items (Singh et al., 2021).

One of the biggest challenges UV irradiation has faced for quite a long time is the lack of consumer acceptance and recognition. Many people are still skeptical, particularly about whether UV-treated foodstuffs are safe (Hernández-Hernández et al., 2019; Khan et al., 2018). There seems to be a concern among consumers that UV radiation might lead to radioactive materials in foods, which could pose a serious threat to their health (Bahrami et al., 2020; Xuan et al., 2017). Indeed, UV-C light could be harmful to humans if they are exposed to it.

In addition, it's important to note that UV radiation can induce isomerization and oxidation of compounds like lycopene, especially with increased radiation concentrations and longer exposure times (Bhattacharjee et al., 2019). Another limitation is that the efficacy of UV-C light application on liquid foods can be influenced by their turbidity (Pinto et al., 2016). The logistical challenges of transporting ready-made food to irradiation facilities can hinder the practicality of UV radiation for post-packaging food

treatment (Bahrami et al., 2020; Zacconi et al., 2015). When applied to food items with irregular shapes and structures, UV radiation's limited penetration capacity may lead to inefficacy; this can be improved by combining UV radiation with other non-thermal processes (Bahrami et al., 2020). Additionally, substantial investment requirements serve as a constraint in achieving widespread adoption of UV radiation processing (Hernández-Hernández et al., 2019).

Recent studies have reported that UV-C can disinfect a wide variety of fruits with slight changes in its physicochemical properties. Also, similar effects have been observed in eggs. Researchers have found that UV-C irradiation on Dragon fruit decreased the microbial growth of bacteria, coliforms, yeast, and mold. Although, the fruits exposed to values above 1.2 kJ/m^2 presented dehydration and oxidation. Likewise, the impact of this technique on the safety and quality of fresh melon during cutting and before packaging also has been evaluated. According to the results, it was produced various effects such as the reduction in the growth of *Enterobacteriaceae*, the decrease of the filtration, and no significant changes on the color and the firmness of the product during storage at 6°C for 14 days (Buelvas-Caro et al., 2018).

Studies realized fruits such as mango, pineapple and mamey minimally processed demonstrated that radiation dose UV-C (0, 7 and 14 kJ/m^2) does not alter their physico-chemical, microbiological and antioxidants characteristics. The dose of UV-C irradiation of 14 kJ/m^2 allowed to obtain higher antioxidant properties and lower microbial count of aerobic mesophilic bacteria, fungi and yeasts. Another study also has highlighted the antimicrobial effect of UV-C irradiation in pears, melons, strawberries, red raspberries melons and apples. This study the rates inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 were higher for fruits less hydrophobic with most smooth surfaces (pears and apples) compared with fruits (melon, rasberry and, strawberry) over rough surfaces. Lastly, a notable discovery has indicated that the joint application of immersion solutions incorporating malic acid along with UV-C light treatment demonstrates the ability to mitigate the growth of *L. monocytogenes* and *Salmonella* Poona in fresh-cut papaya. Moreover, Table 5 provides a comprehensive overview of several studies elucidating the utilization of ultraviolet irradiation for food preservation purposes (Buelvas-Caro et al., 2018).

2.4.1. Ultraviolet (UV-C) Light Mode of Action, Dose and Fluence

In order to eliminate bacteria linked to food contamination and deterioration, non-thermal treatments have been used on a diverse range of food items, such as ultraviolet radiation (UV), ozonation and ultrasonication (US) (Cao et al., 2010; Hernández et al., 2010; Olmez and Akbas, 2009).

Table 5: Ultraviolet light treatment for food preservation (Buelvas-Caro et al., 2018).

Food Product	Operation Conditions	Principal Results
Fresh-cut apple	UV-C light treatments at 1.2, 6.0, 12.0 and 24.0.	Germicidal effect.
Fresh-cut melon	UV-C light fluence were 0, 1200, 6000 and 12,000 J/m^2. Irradiance of 20 W/m^2 increasing time up to 10 min.	Reduction of *Enterobacteriacea* growth.
Mango, pineapple and mamey	UV-C (0, 7 and 14 kJ/m^2) stored for 15 days at 5°C and HR of 85 - 90%.	Reduction of 0.89, 1.5 y 2.26 log CFU/g in mango, pine apple and mamey, respectively.
‘‘Maradol’’ red papaya (Carica papaya L)	UV-C light (0, 0.96, 2.88, 5.76 and 8.64 kJ/m^2).	Reductions of 5.28 and 3.15 Log10 CFU/g for *Salmonella* Poona and *L. monocytogenes*, respectively.
Apples, pears, strawberries, red rasberries and cantaloupes	UV-C light does up to 11.9 kJ/m^2 at 23°C with exposure times between 0-14 min.	Reduction of *E. coli* O157 and *L. monocytogenes* in apple, pears, strawberries and rasberries.

Photoproducts are created in the DNA as part of the UV's inactivation mechanism (Figure 13). The most significant of these photoproducts is the pyrimidine dimer, which can obstruct. The process of DNA transcription and translation is impacted when adjacent pyrimidine molecules on the same DNA strand come together (Franz et al., 2009).

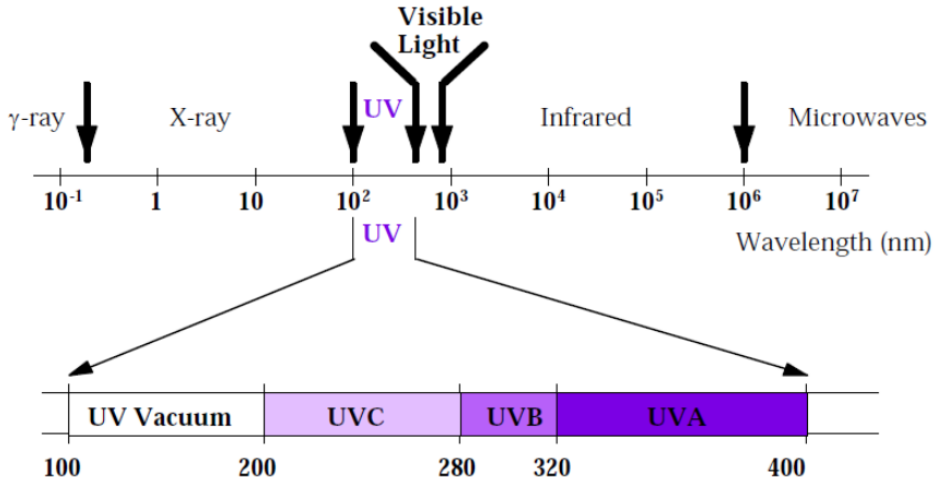

Figure 12: Schematic representation of light wavelengths and UV light ranges (Soehnge et al., 1997).

It is possible for microorganisms to migrate to these protective sites when UV radiation is applied to some surfaces (e.g. lettuce, carrots). Through dimerization between neighboring nucleoside bases in the same DNA strand, UV-C radiation inflicts DNA damage, leading to direct lethality to microbial DNA (Bintsis et al., 2000; Yaun et al., 2004).

UV light has demonstrated its efficacy in significantly reducing the microbial load present on the surfaces of various foods. For instance, in the case of walnuts, exposure to UV-C radiations at a dosage of 108 J/m^2 for 45 minutes resulted in a substantial reduction of 4.2 logs in fungal growth (Jubeen et al., 2012). The growth of *A. flavus* was similarly inhibited by a significant margin of 94.40% after 60 minutes of exposure to UV radiation (Hussein et al., 2015). Notably, a UV-C treatment at 205.6 J/cm^2 led to reductions of 1.8 and 1.3 log CFU/g in the levels of total aerobic mesophilic bacteria and total yeast/mold on thyme, respectively (Dogu-Baykut & Gunes, 2018). Importantly, these UV-C

treatments had no discernible impact on the quality attributes of thyme, including parameters such as total phenolic content, total antioxidant activity, and sensory characteristics (Dogu-Baykut & Gunes, 2018).

UV light has emerged as a compelling strategy for mitigating mycotoxin production by molds and for breaking down mycotoxins themselves. Notably, exposure to UV irradiation at 366 nm for a duration of 10 minutes yielded remarkable results in terms of aflatoxin total content reduction. In standard solutions, reductions of 98%, 99.5%, 99.8%, 100%, and 99.1% were achieved for various aflatoxin types (Sharareh et al., 2015). Similarly, treating almonds with UV light for a period of 30 minutes led to a substantial 49.3% decrease in AFB1 concentration (Jabłonska & Mankowska, 2014). Another study by Diao et al. (2015) demonstrated that the toxicity of AFB1 present in peanut oil was markedly diminished following a mere 10-minute exposure to UV radiation. This underscores the potential of UV light as an effective technique for the removal and detoxification of mycotoxins (Deng et al., 2020).

The effectiveness of UV light for disinfection is subject to various factors, encompassing the intensity and wavelength of the UV light, the duration of exposure, the inherent characteristics of the product, and the specific microbial strains involved. For instance, *A. flavus* growth inhibition reached 92.53% and 87.37% when subjected to UV irradiation at wavelengths of 240 nm and 365 nm for a duration of 120 minutes (Hussein et al., 2015). In another study, the presence of fungi in walnuts was curtailed by 1.2, 3.2, and 4.2 logarithmic units following exposures of 15, 30, and 45 minutes to UV-C light (Jubeen et al., 2012). Notably, the surviving microbe population displayed a diminishing trend with increasing UV intensity. The impact of UV treatment is closely tied to the type of microorganisms and food materials in question. A UV-C treatment with a dosage of 205.6 J/cm^2 resulted in a 1.77 logarithmic reduction of total aerobic mesophilic bacteria in thyme, while the count of *B. cereus* remained relatively unchanged (Dogu-Baykut & Gunes, 2018). Similarly, in the case of roasted coffee beans, UV-C irradiation for 2 hours led to a reduction of 2.16, 0.71, and 1.58 logarithmic CFU/g for *A. flavus* in round part, crack part, and whole beans respectively, and a reduction of 1.03, 0.37, and 0.72 logarithmic CFU/g for *A. parasiticus* (Deng et al., 2020).

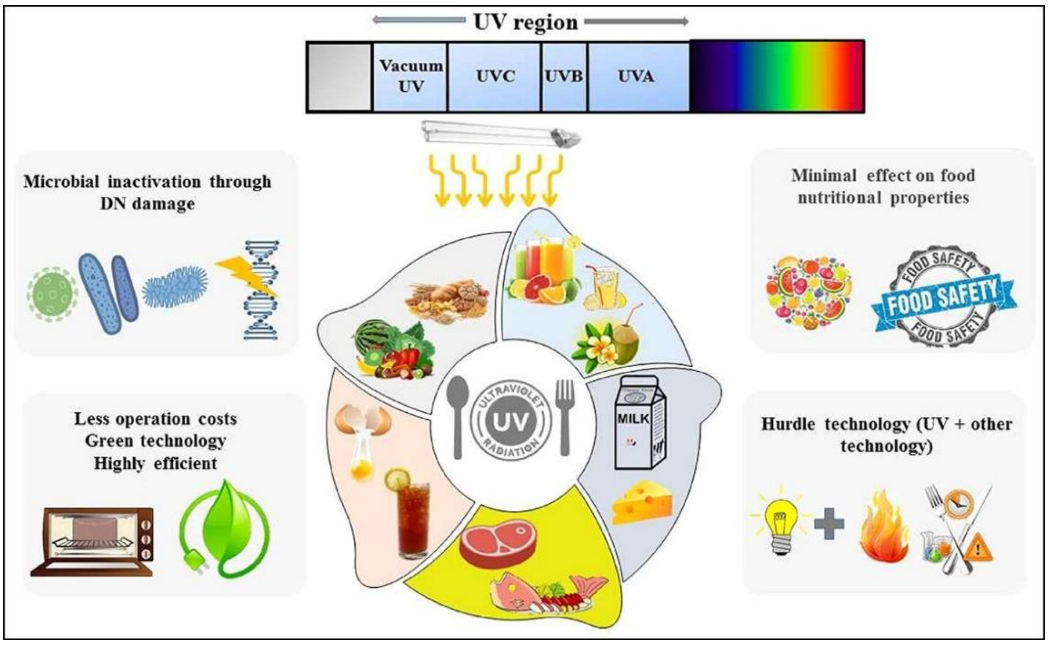

Figure 13: Inactivation Mechanism of UV (Singh et al. 2021).

2.4.2. UV-C Light and Applications on Chicken

In the realm of chicken meat processing, the establishment of critical control points is imperative in order to mitigate the potential hazards linked to spoilage and the infiltration and dissemination of pathogenic microorganisms into the end product, ultimately enhancing food safety (Barker et al., 2004). Among these control points, surface contact, encompassing the skin and carcass cavity, stands out as a predominant origin of bacterial contamination throughout the meat processing procedures (Kondjoyan and Portanguen, 2008).

Applying UV-C treatment to *Escherichia coli* UPEC on chicken suspended plastic food contact surfaces demonstrated effective inactivation, with energy doses ranging from 11.4 to 12.9 mJ/cm^2. Notably, on chicken breast meat, UV-C treatment led to a reduction of approximately 0.6 log in UPEC levels. These findings underscore the potential of existing non-thermal processing techniques like High Pressure Processing (HPP), Gamma Radiation (GR), and UV-C to substantially diminish UPEC concentrations in poultry meat or its exudate, consequently enhancing the safety of

poultry products, particularly for vulnerable consumers (Sommers, Scullen, and Sheen, 2016).

Sausages are susceptible to contamination by spoilage microorganisms during various processing stages, including post-cooking and chilling. To counteract such microbial growth, non-thermal methods like cold plasma (CP) have been explored. In a study conducted by Zeraatpisheh et al., the effects of CP treatment on sliced chicken sausages over a 60-day storage period were examined. The sausages were categorized into three groups: a negative control, ultraviolet (UV)-radiated group (positive control, subjected to 200 and 400 seconds of UV radiation), and plasma-treated group. The outcomes revealed a notable reduction in the total microorganism count, with a significant decrease of approximately 1.87 log CFU/g observed after 400 seconds of CP treatment at a power of 70 watts during the entire storage duration (Zeraatpisheh, Yazdi, and Shahidi, 2022).

Chicken carcass frames are commonly utilized to extract mechanically separated chicken (MSC), a component used in various processed food items. Instances of foodborne disease outbreaks linked to *Salmonella*-contaminated MSC have underscored the potential risk of transmitting this pathogen to consumers through such products. This study aimed to assess the effectiveness of diverse treatments applied to chicken carcass frames to mitigate microbial loads. Both non-inoculated frames and those inoculated with a mixture of *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium were examined. The treatments encompassed a rinsing with sterile water, a water rinse followed by a brief 5-second application of UV-C light, or an advanced oxidation process (AOP) involving 5% or 7% (v/v) hydrogen peroxide (H_2O_2) combined with UV-C light. The application of 7% H_2O_2 alongside UV-C light resulted in a reduction of aerobic bacteria counts by up to 1.5 log CFU per frame. No noteworthy variation was observed in *Salmonella* reductions achieved through the use of 5% H_2O_2 (1.1 log CFU per frame) compared to 7% H_2O_2 (1.0 log CFU per frame) (Jones-Ibarra et al., 2019).

CHAPTER 3

MATERIAL AND METHOD

3.1. Test Microorganisms and Preparation of Bacterial Suspensions

Test Microorganisms

The bacterial strains used in the study included both Gram-negative and Gram-positive bacteria. *Listeria innocua* was chosen as a non-pathogenic surrogate strain of *L. monocytogenes* and Gram-negative bacteria, *Cronobacter sakazakii* and *Salmonella* Typhimurium were chosen as Gram-positive bacteria, *Candida albicans* was also selected as a yeast strain. Izmir Institute of Technology, Food Engineering Department, provided *Listeria innocua*, *Cronobacter sakazakii*, *Salmonella* Typhimurium, and *Candida albicans*.

The antibacterial activity of nisin and carvacrol was tested against 3 bacterial strains, of which some were reference strains: two Gram-negative strains (*Salmonella* Typhimurium CCM5445 and *Cronobacter sakazakii Cronobacter sakazakii* (ATCC 10876), one Gram-positive strain (*Listeria innocua* NRRLB-33314), one yeast (*Candida albicans*).

L. innocua, Cronobacter sakazakii, *S.* Typhimurium, and *C. albicans* strains were propagated in Tryptic Soy Broth (TSB, Difco, BD). All microbial strains were maintained in TSB containing 20% (v/v) glycerol at −80°C.

Preparation of Bacterial Suspensions

Microbial cultures were cultivated in Tryptic Soy Broth (TSB) under controlled conditions at a temperature range of 35-37°C. Bacterial suspensions were standardized to the level of a 0.5 McFarland standard, roughly corresponding to a range of approximately

10^7 to 10^8 colony-forming units per milliliter (cfu/mL). This standardization was conducted using a Densitometer (DEN-1, HVD Life Sciences, Vienna, Austria). Furthermore, an additional dilution, reduced by a factor of ten, was performed in the TSB medium.

3.2. Nisin and Carvacrol

Nisin and Carvacrol were procured from Sigma-Aldrich (St. Louis, MO, USA). All chemicals utilized in the research were of analytical grade and were sourced from Sigma Chemical Co (St. Louis, MO, USA).

The lethal effect of Nisin on microorganisms was found by experimenting with various concentrations. Table 6 shows the concentration values in the wells of different volumes taken from the stock solution prepared by dissolving 0.1 g of nisin with 10 ml Phosphate Buffer Solution (PBS) and passing it through a 0.22 µm filter.

Table 6: Concentrations used to detect the lethal effect of Nisin on microorganisms.

Drawn Volume from (0.1 g in 10 ml PBS) Stock Solution (µl)	Final Concentration in 200µl well (mg/ml)
2	0.1
5	0.25
10	0.5
15	0.75
20	**1**
25	1.25
30	1.5
50	2.5
60	3
75	3.75
90	4.5
100	5
125	6.25
140	**7**
150	7.5

The lethal effect of carvacrol on microorganisms was found by experimenting with various concentrations. In the table below (Table 7), the different volumes taken from the stock solution are given directly.

Table 7: Concentrations used to detect a lethal effect of Carvacrol on microorganisms.

Drawn Volume from Carvacrol Stock Solution (µl)	**Final Concentration in 200µl well (mg/ml)**
2	0.01
5	0.25
10	0.05
15	0.075
20	0.1
25	0.125
30	0.15
50	0.25
60	**0.3**
75	0.375
80	**0.45**
100	0.5
125	0.625
140	0.7
150	0.75
160	0.8
175	0.875
180	0.9

Microbial strains were sourced from the Faculty of Food Engineering at IZTECH in İzmir, Turkey. For the antimicrobial assessment of various microorganisms, it is a customary practice to cultivate the cultures overnight in Tryptic Soy Broth (TSB). On the subsequent day, these cultures were transferred to test tubes and their optical density was adjusted to the 0.5 McFarland standard using peptone water with the assistance of a DEN-1 Densitometer from Riga, Latvia. Subsequently, these cultures were further diluted with TSB to attain microbial culture concentrations of 10^5 CFU mL-1, which served as the basis for the antimicrobial activity testing. Different concentrations of nisin and carvacrol were incorporated into the TSB media in which these cultures were grown. Prior to the tests, suitable concentrations for these experiments were determined, ranging between 180 ml. In addition, control samples were prepared involving the test microorganisms but without the addition of nisin and carvacrol.

The cultures were then placed in an incubator set at 37°C and 200 rpm for a duration of 24 hours. This incubation process was conducted in triplicate for each sample and bacterial culture. The initial assessment of bacterial turbidity relied on visual observation of turbidity. Following incubation, the bacterial cultures were subjected to a dilution procedure at a ratio of 1:10 with peptone water. This dilution process was performed iteratively up to the sixth dilution for each microbial culture. Afterward, aliquots of 100 μL from both the original and diluted samples were transferred onto TSA agar plates. These plates were subsequently incubated for a period of 24 hours at 37°C. The viable bacterial counts were enumerated, and the values were expressed as colony forming units (CFU mL^{-1}).

3.3. UV-C irradiation equipment and UV-C treatments

The UV-C irradiation system made use of a monochromatic lamp that emitted UV-C light with a peak radiation wavelength of 254 nm. The device was made up of a stainless steel chamber in which the samples were placed 10 cm away from the lamp. In this investigation, an indoor UV irradiation system with a fixed UV-C lamp (Germicidal et al., USA) and a cabinet measuring 95 cm by 9.75 cm was used. A tray was created to keep the samples horizontally inside the stainless steel cabinet in order to ensure constant

UV-C treatment. While variations in the distance between the UV lamps and the samples could potentially be made, it's important to note that for the specific scope of this experiment, the distance of irradiation remained constant at 10 cm. A UV-VIS radiometer was employed in the experimental setup, positioned at the identical distance from the UV lamp as the plates. This allowed for the measurement of the radiation emitted by the lamp, denoted as I0.

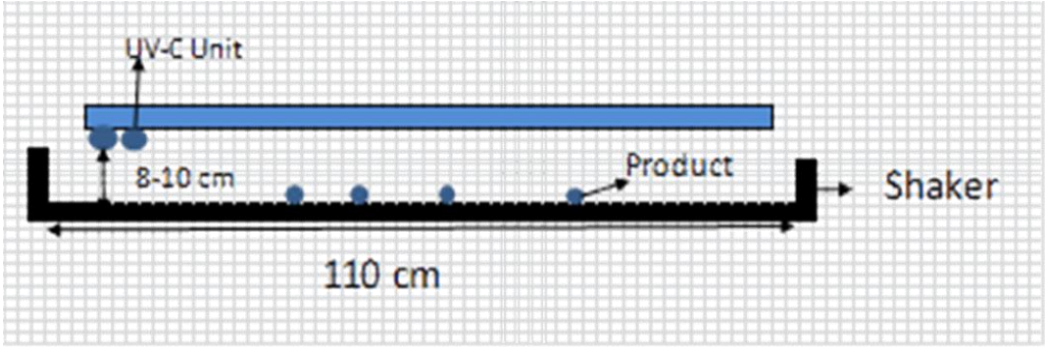

Figure 14: Vectoral image of the UV-C Irradiation Equipment.

The samples were positioned on a stainless steel tray and subjected to irradiation from a germicidal emitting lamp located 10 cm above the tray's upper surface. UV-C treatment was administered to achieve radiation doses ranging from 0 to 4 kJ/m^2. This procedure was carried out within an enclosed chamber at room temperature, utilizing distinct exposure durations and measuring UV-C light intensity using a portable digital radiometer on the surface. To maintain a consistent UV-C intensity output and reduce fluctuations, the UV-C lamp was activated for a minimum of 30 minutes before the experiment.

The device below is the industrially manufactured UV device which used in laboratory (Figure 14). Other schema is the experimental set up of UV-C Irradiation (Figure 15).

Figure 15: UV Irradiation Device.

,

Figure 16: Experimental set up for UV-C Irradiation.

3.4. Nisin and Carvacrol treatments

Determination of Antimicrobial Effect of Nisin and Carvacrol by Broth Microdilution

For the broth microdilution method, 20 μL of bacterial suspension was introduced into the wells of a sterile 96-well microtiter plate containing 180 μL of nisin or carvacrol solutions with two-fold dilutions. Control wells were also established with culture medium combined with bacterial suspension, along with nisin or carvacrol without bacterial inoculation. The plates were then placed in an incubator for 24 hours, and the turbidity was gauged using a microplate reader (Varioskan Flash, Thermo) at a wavelength of 600 nm, with measurements taken at 30-minute intervals (Klancňik et al., 2010). Subsequently, 100 μL samples were extracted from each well and evenly spread onto agar plates to monitor the growth of bacteria or yeast. The minimum inhibitory concentrations (MICs) of the antimicrobials were identified as the lowest concentrations that displayed no detectable viability in the wells of the 96-microwell plates after incubating for 24 hours.

In the case of broth macrodilution (Klancňik et al., 2010), 100 μL of bacterial suspensions were introduced into 900 μL of growth media containing the desired antimicrobial concentration, followed by shaking incubation for 24 hours at the appropriate incubation temperature. After 24 hours, 100 μL of the sample was directly spread onto agar plates, and the growth of colonies was assessed following an additional 24-hour incubation period. The absence of colonies on treatment plates was taken as the MIC values (at 37°C).

3.5. Enumeration of Test Microorganisms after treatments

To enumerate microorganisms, either control or drumstick samples were placed in a sterile stomacher bag (Gosselin SM2B-01, Villeurbanne, France), along with 90 mL of Peptone Buffer Water (PBW; Oxoid, U.K.), and homogenized for a duration of 2 minutes. Subsequently, 1 milliliter of the homogenized sample was subjected to a 10-fold

serial dilution by mixing with 9 mL of sterile BPW (0.1%). The resulting dilutions were then poured onto appropriate non-selective or selective media. All samples underwent analysis in accordance with the ISO standard methods.

Figure 17: Preparation of Chicken Drumstick samples before microbiological analyses (BioRender).

3.5.1. Total Mesophilic Aerobic Bacteria Count

In this regard, drumstick samples were subjected to homogenization using a 90 mL solution of sterile Buffered Peptone Water (Merck). Sequentially, decimal dilutions were meticulously prepared. The process of inoculation was carried out through the utilization of the spread plate technique. The enumeration of TMAB (Total Mesophilic Aerobic Bacteria) was conducted by employing Plate Count Agar (PCA, Merck). The resulting petri dishes were then subjected to an incubation period characterized by aerobic conditions at a temperature of 30°C, maintained for approximately 72±1 hours.

3.5.2. Yeast and Mould Count

To assess the yeast and mold (Y&M) count from various dilutions of homogenized drumstick samples, the spread plate technique was employed for inoculations. The enumeration of Y&M was carried out using Potato Dextrose Agar (PDA, Merck). The ensuing petri dishes were then subjected to an incubation process, maintained under aerobic conditions at a temperature of 30°C for a period of 3 to 5 days.

3.5.3. Total Coliform Count

To ascertain the counts of *Enterobacteriaceae*, 1 mL of the suitable dilutions was introduced into Violet Red Bile Agar (VRBA, Merck). Subsequently, the Petri dishes were subjected to incubation under anaerobic conditions at a temperature of 30°C for a duration of 2 days.

3.5.4. *Listeria monocytogenes* Count

Listeria inoculated plates were incubated at 37°C for *Listeria innocua* analysis. Reductions of bacteria were calculated by counting. (PALCAM & OXFORD) (Figure 18)

Figure 18: *Listeria innocua* inoculated PALCAM & OXFORD agars on Petri dishes.

3.5.5. *Salmonella* Typhimurium Count

For the *Salmonell*a enumeration, each sample was aseptically mixed with 90 mL of peptone water (0.1% peptone containing 0.02% Tween-80). Samples were homogenized by 1 min of hand massaging. From each sample, 100 µL of homogenate was spread plated on Bismuth Sulphite Agar either directly or after serial dilutions in buffered peptone water. These plates were incubated at 37°C for 48 h before CFU counts were performed. Non-inoculated and untreated samples for each chicken drumstick product type were also processed for *Salmonella* quantification as described above. Bismuth Sulphite Agar plates were incubated at 37 ± 1 °C according to ISO 6579:2002 for *Salmonella* analysis. Reductions of bacteria were calculated by counting.

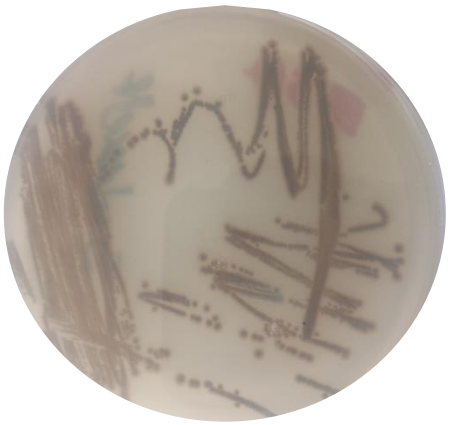

Figure 19: *Salmonella* Typimurium inoculated Bismuth Sulphite Agar on Petri dish.

The research aimed to evaluate and compare the susceptibility of specific foodborne microorganisms, namely *Listeria monocytogenes, Escherichia coli* O157:H7 *Salmonella enterica* and, *Pseudomonas aeruginosa* to ultraviolet-C (UV-C) radiation in liquid egg white. The study observed a range of decimal reduction times (D) spanning from 26.44 to 37.22 minutes, corresponding to UV-C energy doses (DUV-C) between 170.71 and 240.33 mJ/cm². Notably, Pseudomonas aeruginosa displayed the highest resistance, while *Escherichia coli* O157:H7 exhibited the least resistance. The inactivation rate of *Pseudomonas aeruginosa* was utilized to devise UV-C processes that achieve reductions of the reference organism by 99.9% (3D) and 99.999% (5D). The study recommended exploring avenues such as incorporating more UV-C lamps or combining UV-C treatment with gentle heating to achieve the desired reduction of the reference organism while maintaining acceptable product quality (Gabriel et al., 2017).

In a conducted study, Ultraviolet (UV-C) light-emitting diode (LED) illumination was utilized for disinfecting skinless chicken breast (CB), stainless steel (SS), and high-density polyethylene (HD) surfaces inoculated with *Salmonella enterica*. Different exposure times and irradiances of 2 mW/cm² (50%) and 4 mW/cm² (100%) were employed in treating the samples. In the case of chicken samples, the *Salmonella* reduction was the lowest, with reductions of 1.02 and 1.78 Log CFU/cm² ($p \leq 0.05$) after 60 and 900 seconds, respectively, at 50% irradiance. Enhanced reductions were achieved on chicken breast samples under 100% illumination, with over 3.0 Log CFU/cm² reduction after 900 seconds. *Salmonella* counts on stainless steel (SS) exhibited reductions of 1.97 and 3.48 Log CFU/cm² after 60 seconds of treatment under 50% and

100% irradiance, respectively. Similarly, HD showed a significant yet relatively lower reduction, with 1.25 and 1.77 Log CFU/cm² reduction for 50% and 100% irradiance after 60 seconds. Extended exposure times of HD to UV-C yielded substantial *Salmonella* reduction of up to 99.999% (5.0 Log CFU/cm²) with both irradiance levels. While the study found UV-C LED treatment effective for controlling *Salmonella* on both chicken and food contact surfaces, the researchers proposed three potential mechanisms contributing to the diminished disinfection efficacy: bacterial aggregation, presence in food and surface pores, and light absorption by fluids associated with chicken breast (Calle et al., 2021).

3.6. Statistical Analysis

All experiments were conducted in triplicate. The obtained results are presented as the mean value of individual measurements along with the corresponding standard deviation (SD).

Microbiological data were analyzed using the log(N/N0) format, where N represents the microbial load at a specific time, and N0 represents the initial microbial load of untreated samples. Statistical analysis of the data regarding the inactivation of *L. innocua* and *S.* Typhimurium through UV-C and nisin treatments was performed using SPSS 20.0 (SPSS Inc., Chicago, USA). The results were subjected to ANOVA analysis with a significance level set at ($P < 0.05$).

CHAPTER 4

RESULTS & DISCUSSION

4.1. MIC Values of Nisin and Carvacrol

The antimicrobial effects of Nisin and Carvacrol on *Listeria innocua*, *Cronobacter sakazakii*, *Salmonella* Typhimurium, and *Candida albicans* were evaluated separately under different methods and conditions by Varioskan microplate reader at 600nm at 37°C for 24h.

After 24h incubation, MIC values are confirmed by raw data visualization and TSA inoculation. The effect of antimicrobials given above as a MIC value were summarized in Table 8, respectively.

4.1.1 MIC Values of Nisin

MIC values of nisin were calculated by taking volumes of 10mg/ml stock solutions and putting them in 96-well plates with constant bacterial volume (20µl, 0.5 MF) and complete TSB.

Experimental set up for MIC assay shown in the Figure 20.

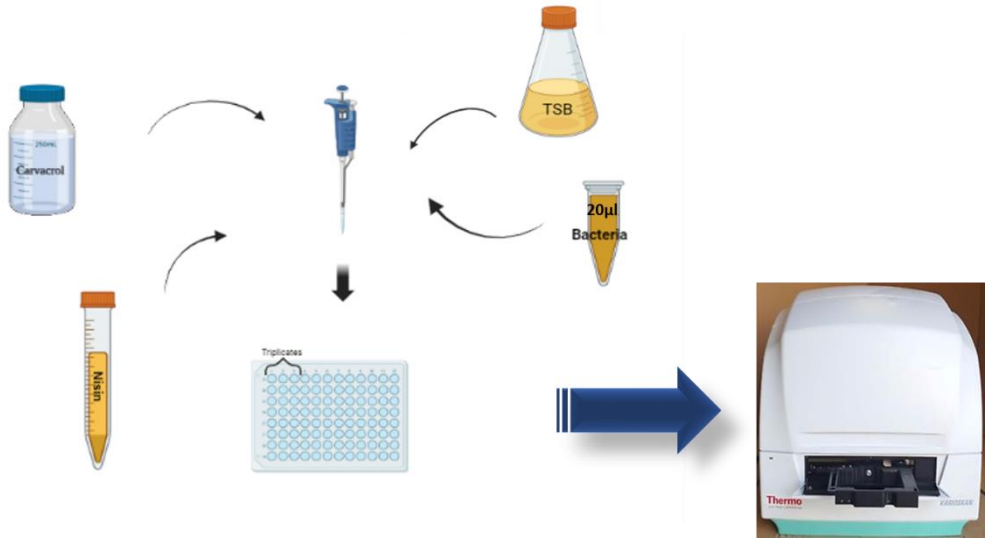

Figure 20: Experimental set up for MIC assay (BioRender).

In the growth curve, there is no growth at the values after 10 µl. Therefore, Nisin MIC value of *Listeria innocua* is 20 µl = 1 mg/ml (Figure 21).

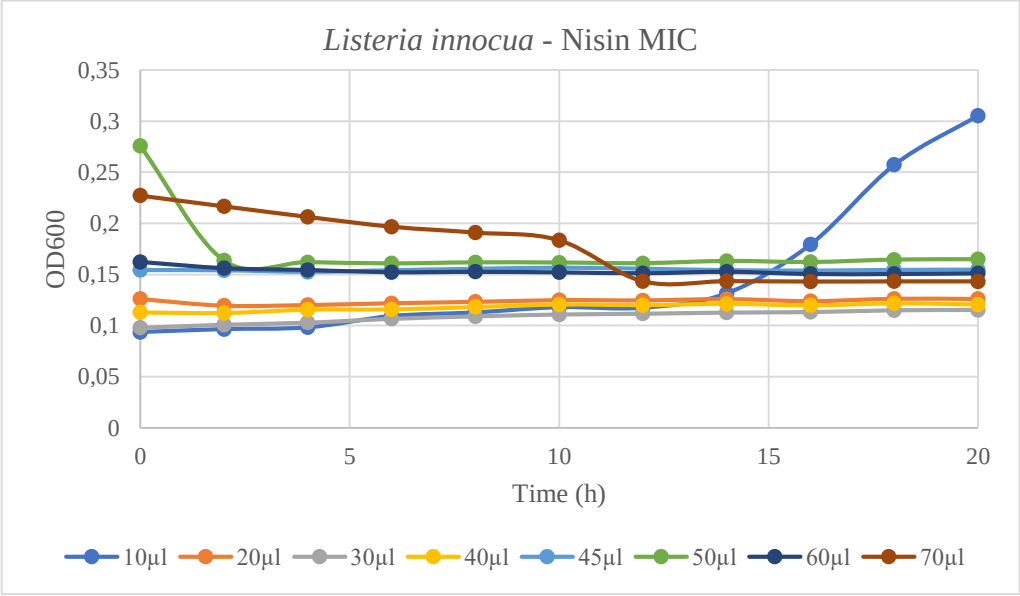

Figure 21: Minimum inhibitory concentration (MIC) value of Nisin (10-70 µl) on *Listeria innocua*

In that graph, it is seen that Control Group is working (Figure 22).

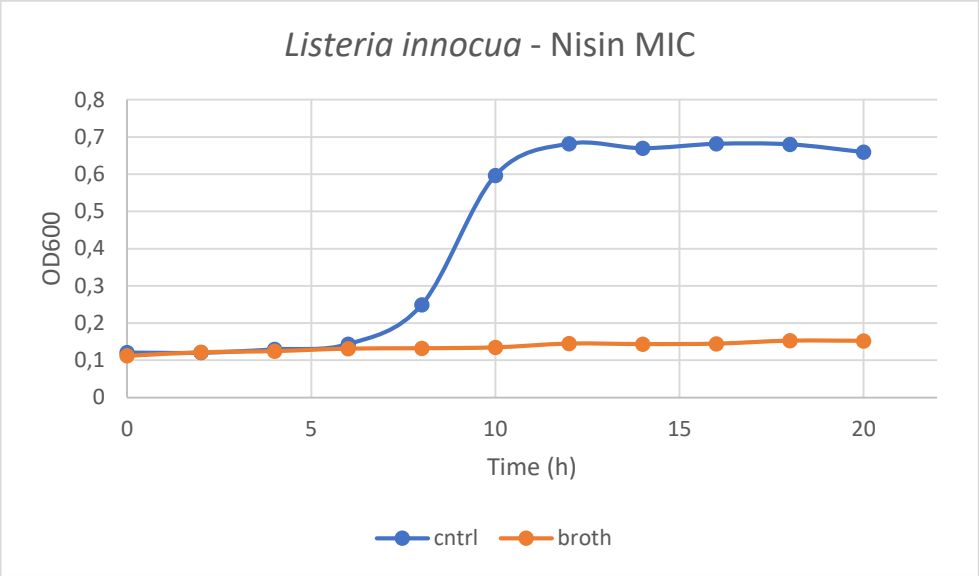

Figure 22: Minimum inhibitory concentration (MIC) value of Nisin (positive and negative controls) on *Listeria innocua*

In a thesis study, İsmail investigated the growth and antibacterial effect of lysozyme and nisin on 31 strains of *Listeria monocytogenes* isolated from various foods by using the quantitative microplate method. By testing 5 different nisin concentrations (0,465, 0, 9375, 1,875, 3,75, and 7,5 mg/ml) at 3 different temperatures (10, 25, and 37 °C), the development of *L. monocytogenes* strains was analyzed. At 3,750 mg/ml, the bacterial growth was completely inhibited at 25°C and 37°C. He Solved nisin in TSB. It is close to our results (1 mg/ml) (İsmail Kıvanç, 2020).

Hatice investigated the study on short shelf-life cream, no statistically significant bactericidal effect of nisin at a dosage of 5 mg/kg and 10 mg/kg on *L. monocytogenes* was detected in the analyses made during the 7-day storage period at +4 °C. This could be due to the high oil content of the cream (60%) or the high pH reducing the effect of 1nisin. The dosages used in their studies had a suppressive effect on the bacteria but did not allow them to grow (5mg/kg = 4500 IU = 5mg/ml) (Üstündağ and Yalçin 2022).

In this research, Haiying Cui investigated the effectiveness of nisin-loaded Poly-G-glutamic acid/chitosan (NGC) nanoparticles and NGC nanoparticles incorporated

within polyethylene oxide nanofibers against *Listeria* on cheese. Subsequently, the viable bacterial count increased gradually from 3.18 log CFU/mL to 7.89 log CFU/mL and from 3.12 log CFU/mL to 7.44 log CFU/mL at 7 days, respectively. Notably, free nisin in nisin samples lost its activity after just 1 day, suggesting a binding of nisin to cheese constituents like proteins and lipids (Haiying Cui, 2017).

In one study, a report of nisin showed enhanced antimicrobial activity against both Gram-positive and Gram-negative bacteria. Initial concentrations of wild-type nisin and mutant nisin peptides were adjusted to 7.5 mM for *L. monocytogenes.* The MIC values for both nisin types against *L. monocytogenes* LO28 was 6.28 mg/l, respectively. The MICs for *L. monocytogenes* LO28 was 3 mg/l, respectively. (7.5mM = 7,500 IU = 26,430 mg/ml) (0.00314 mg/ml and 0.00419 mg/ml) (Des Field et al, 2012).

In a research conducted by Hamdollah Moshtaghi et al., the objective was to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of combinations involving lysozyme and Nisin against bacteria such as *S.* aureus, *L. monocytogenes*, *E. coli* and *S.* Typhimurium. Additionally, they aimed to investigate the impact of various concentrations of lysozyme and Nisin on the growth rate. The study encompassed a range of pH values (5.5, 6, 6.5, 7, 7.5, and 8) for both lysozyme and Nisin, coupled with diverse concentrations (ranging from 0 mg/ml to 5 mg/ml) of these compounds. Notably, they identified a pH value of 7.5 as the most effective outcome, and considering our utilization of PBS at a pH of 7.4, the alignment of our findings is noteworthy (Moshtaghi et al., 2018).

Sahar Roshanak et al. found that *L. innocua* had the MIC for Nisin at 64 g/ml. Nisin had a bactericidal or fungicidal effect on *L. innocua* at a concentration of 256 g/ml (Roshanak et al., 2020). Negar Narimisa et al. found that the minimum inhibitory concentrations of Nisin for *L. monocytogenes* ATCC 19115 as 8 µg/ml, respectively. Their MIC value (8 µg/ml = 0.008 mg/ml) is too low from our concentration. (Narimisa et al., 2021). Laura Nyhan et al. MIC of Nisin A for *Listeria innocua* is 3.75µM. (0.013215 mg/ml) (lower than 75.67 fold than us) (Nyhan et al., 2021).

The study conducted by Martinez et al. revealed that the residual concentrations of nisin A present in processed cheese, when nisin is added, can serve as a predictive indicator for the growth of *Listeria monocytogenes*. They determined 13 minimum nisin inhibitory concentrations (MIC) between pH 5.5 and 6.5 for 11 *L. monocytogenes* isolates. The cheese exhibited nisin A concentrations ranging from 0.56 to 5.28 ppm.

Nisin caused an extension of lag periods at temperatures below 15°C. This approach supports the development of processed cheese recipes that contain nisin A and inhibit *L. monocytogenes* growth (Martinez-Rios et al., 2021).

In the trials for *Cronobacter sakazakii*, lethal dose, MIC value was also found 1 mg/ml (Figure 23).

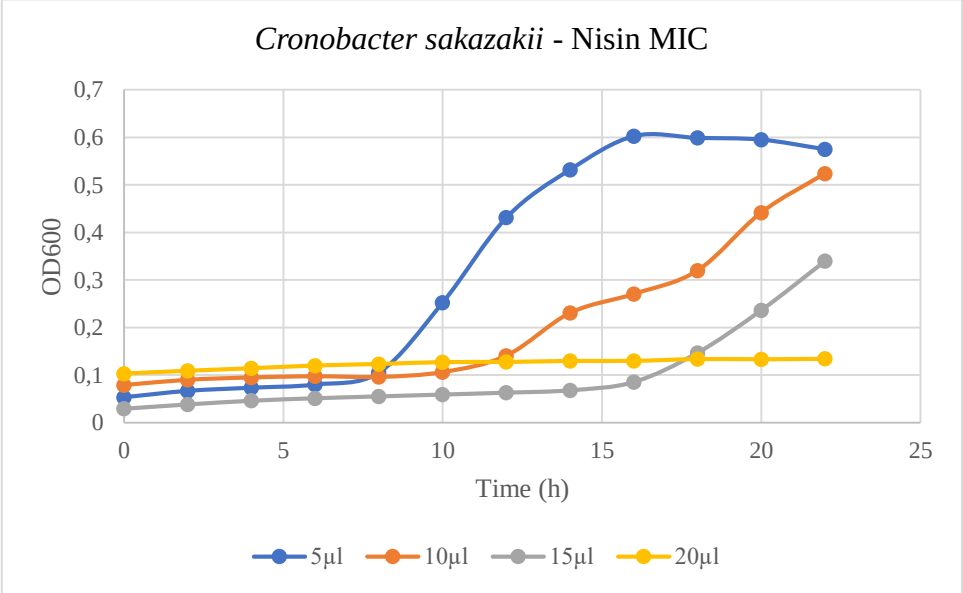

Figure 23: Minimum inhibitory concentration (MIC) value of Nisin (5-20 µl) on *Cronobacter sakazakii*.

In the growth curve, there is no sign of life at values after 10 µl. Therefore, MIC value of Nisin is 20 µl, 1 mg/ml again for *Cronobacter sakazakii*. In the Figure 24, it can be seen that Control Group is working.

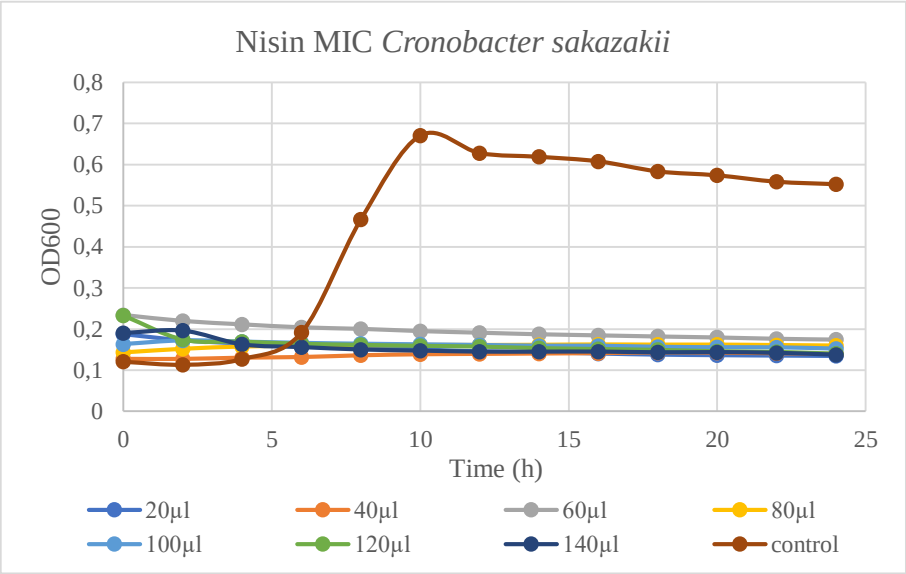

Figure 24: Minimum inhibitory concentration (MIC) value of Nisin (20-140 µl and control) on *Cronobacter sakazakii*.

According to Al-Nabusi et al., the antimicrobial activity of Nisin against *Cronobacter* spp. cells in 0.2% peptone water is influenced by concentration and temperature. A concentration of 1500 IU/ml resulted in a reduction of 4 log10 CFU/ml at both 21°C and 37°C. Interestingly, drieed *Cronobacter* spp. cells in the same peptone water exhibited higher sensitivity to lactoferrin but greater resistance to Nisin compared to undried cells (Al-Nabulsi et al., 2009).

Lee et al. found that a 25 mg/ml concentration of nisin was effective in *Cronobacter sakazakii* in their studies. It is 25 fold much than our concentration (Lee & Jin, 2008).

Nisin has been found to be effective against *Candida albicans* at a lethal dose and minimum inhibitory concentration (MIC) of 7 mg/ml in trials. (Figure 25)

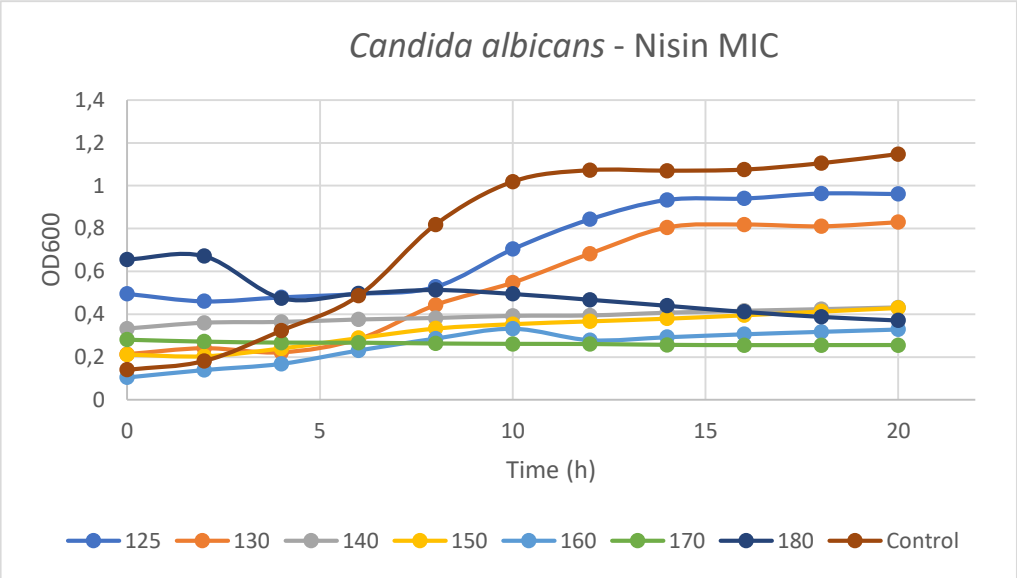

Figure 25: Minimum inhibitory concentration (MIC) value of Nisin (125-200 µl) on *Candida albicans.*

Katharina Enigk et.al found that the *Candida* activity of nisin at a concentration of 1 µg/ml and 4 µg/ml was effective. Konstantina et.al found the *Candida* activity of nisin as >125mg/L. Letícia Coli Louvisse de Abreu et.al found the *Candida albicans* MIC value of Nisin as 5 µg/ml (Enigk et al. 2020; Kourmentza et al. 2021; De Abreu et al. 2016).

4.1.2 MIC Values of Carvacrol

MIC values of Carvacrol, calculated by taken volumes from 99% purity stock soluiton and applied in 96 well plate with constant bacteria volume (20µl 0.5 MF) and completive TSB.

In the trials for *Listeria innocua*, our lethal dose, minimum inhibitory concentration (MIC) value of carvacrol is 60 µl (Figure 26). a growth is seen at the values before 60µl.

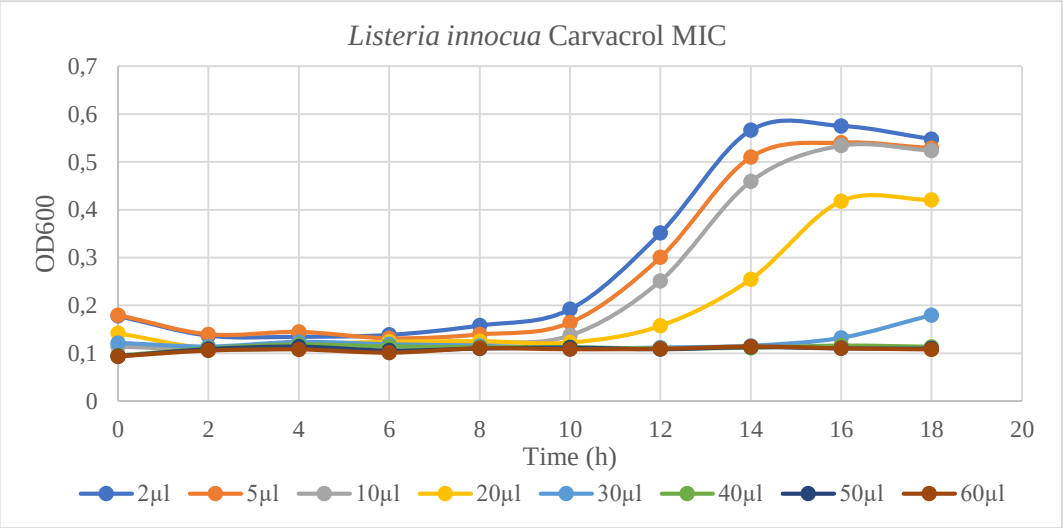

Figure 26: Minimum inhibitory concentration (MIC) value of Carvacrol (2-60 µl) on *Listeria innocua.*

Therefore, MIC values of the carvacrol is 60 µl for *Listeria innocua*. In the second one (Figure 27) it is seen that Control Group is working.

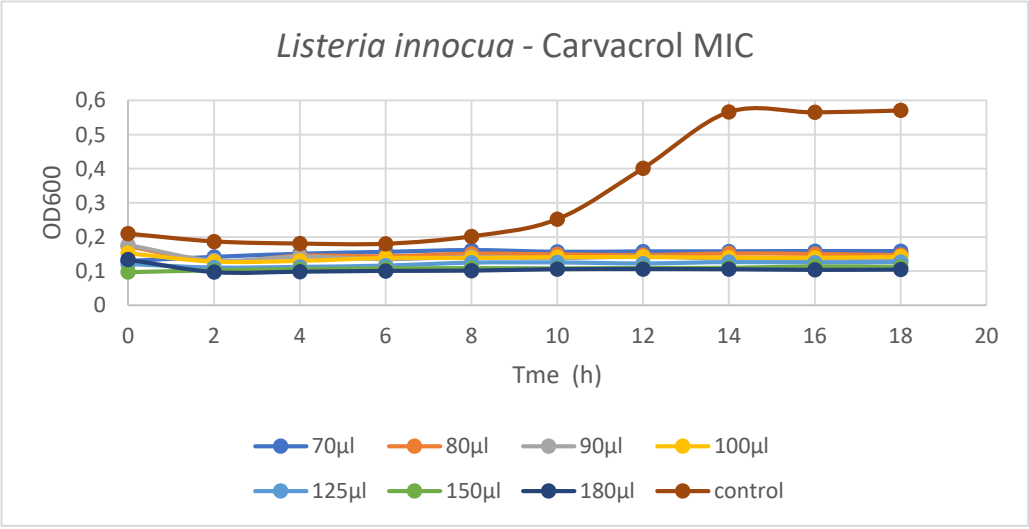

Figure 27: Minimum inhibitory concentration (MIC) value of Carvacrol (70-200 µl) on *Listeria innocua*.

Doohyun Chung et al., in their study with citrus fruit extracts and carvacrol, found that CFEs + 2mM carvacrol reduced *L. monocytogenes* by 7 log CFU/ml. Sergio et al., in their study with different essential oils, found that approximately 200 µg/ml MIC carvacrol was effective on *L. monocytogenes*. Zanini et al., in their study with carvacrol and citral, found that carvacrol was effective on *L. monocytogenes* at doses of 0.100 and 0.175 µl/ml. In their research focused on the impact of carvacrol on *L. monocytogenes* and *L. innocua*, Silva Angulo et al. observed a reduction of 3 log CFU/ml at a minimum inhibitory concentration (MIC) value of 0.175 µl/ml. This finding aligns with similar studies by Chung, Cho, and Rhee (2018), Andrade-Ochoa et al. (2021), Zanini et al. (2014), and Silva-Angulo et al. (2014).

In the trials conducted for *Cronobacter sakazakii*, lethal dose, minimum inhibitory concentration (MIC) value is 60µl like *L. innocua* (Figure 28). Also there are no life sign after 60µl (Figure 29).

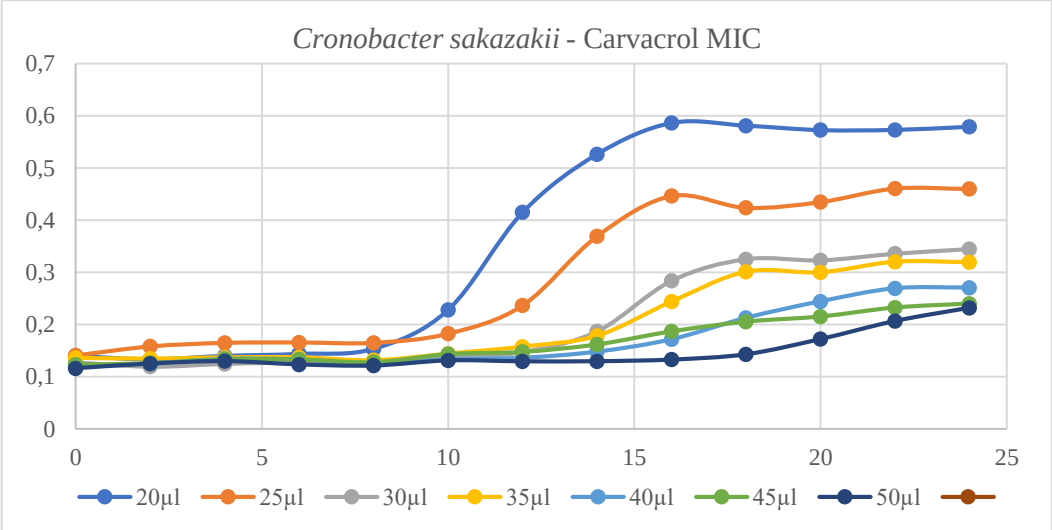

Figure 28: Minimum inhibitory concentration (MIC) value of Carvacrol (20-50 µl) on *Cronobacter sakazakii*.

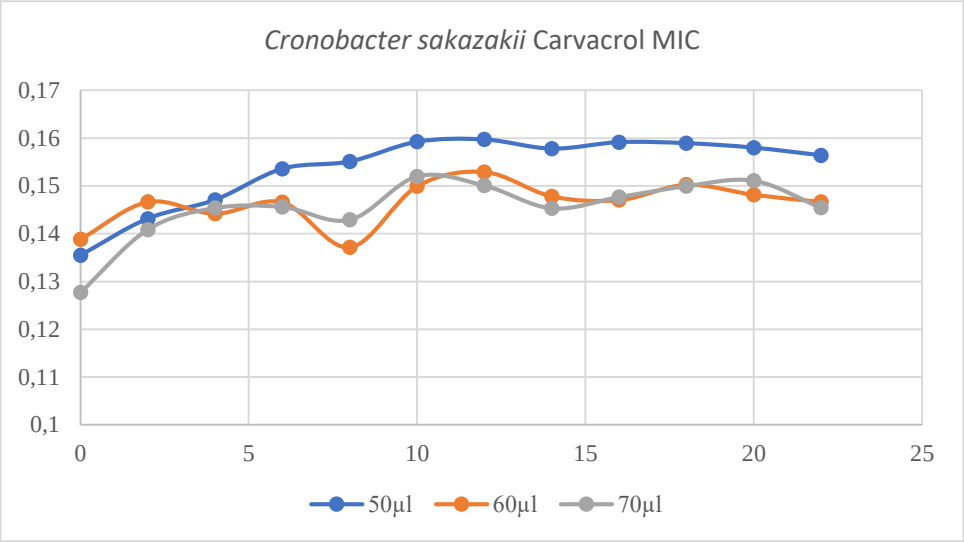

Figure 29: Minimum inhibitory concentration (MIC) value of Carvacrol (50, 60,70 µl) on *Cronobacter sakazakii.*

One study examined the antibacterial properties of plant-derived and essential oil compounds against *C. sakazakii* and *C. malonaticus*. It was found that both strains were susceptible to carvacrol. Their minimum inhibitory concentrations were 0.2 and 0.1 mg/mL (Fraňková et al., 2014).

There is less information on *Cronobacter* and carvacrol in the literature. This study may be promising for future research.

In the Carvacrol trials for *Candida albicans*, Carvacrol MIC value is 80µl (Figure 30).

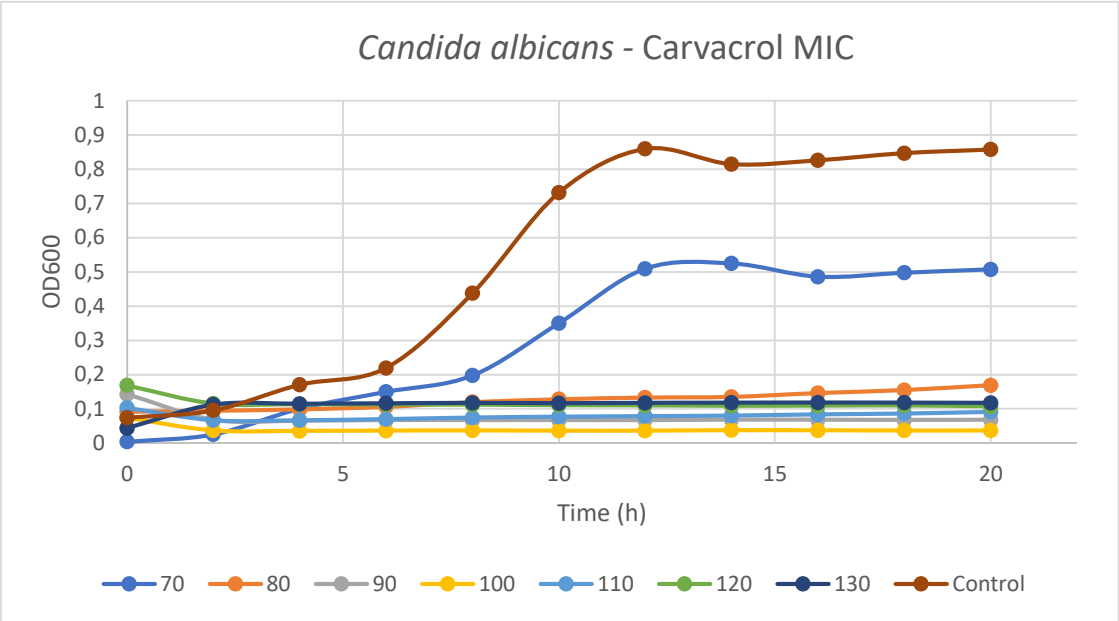

Figure 30: Minimum inhibitory concentration (MIC) value of Carvacrol (70 -130 µl) on *Candida albicans***.**

Manohar et.al, in their study examining the antifungal effect of carvacrol on *Candida albicans*, found a reduction in 0.5 mg/ml MIC value (Manohar et al., 2001). Suvidha et.al, in their study on terpenoids, found that 1.2 and 2.4 mg/ml Carvacrol reduced *C. albicans* (Menon et al. 2021). Alberto Vitali et.al, in their study of carvacrol loaded chitosan nanoparticles, found that 1200 µg/ml Carvacrol was effective on *C. albicans* (Vitali et al. 2021). Arantxa Aznar et.al found that 1 mmol/L Carvacrol was effective on *Candida lusitaniae* in their study (Aznar et al. 2013). Allali Aimad et.al found that the EO concentration of 3.125 µg/ml was effective in the Anti-*Candida* activity studies of essential oils (Aimad et al. 2022).

MIC values of antimicrobial substances may vary according to parameters such as the brand of the substance used, the difference in density and content, solubilization with different solvents, methods such as direct or disk diffusion, nanoparticle or application by methods such as spraying or dipping. Since this work was a food application, Nisin is dissolved it in PBS buffer instead of the commonly used HCl acid. But normally Nisin is a substance resistant to acidity and high temperatures or freezing due to its compositional structure and dissolves better at low pH.

In table 8, the effect / MIC values of two antimicrobial substances (Lantibiotic Nisin & Essential Oil Carvacrol) that we used on test microorganisms, *Listeria innocua*, *Cronobacter sakazakii* and *Candida albicans* are given.

Table 8: MIC Values of Nisin and Carvacrol.

MIC VALUES	Nisin	Carvacrol
Listeria innocua	1 mg/ml	60µl
Cronobacter sakazakii	1 mg/ml	60µl
Candida albicans	7 mg/ml	80µl

Nisin and carvacrol are generally more effective against gram-positive bacteria than gram-positive bacteria, while MIC values for *Listeria* and *Cronobacte*r were found to be the same for both antimicrobials. As seen in the literature, *Candida* requires higher doses for inhibition due to it's cellular morphology.

4.2. Effects of UV-C, Nisin and Carvacrol Treatment on Chicken Drumstick

Chicken Drumstick trails are made due to antimicrobial activity test. MIC values are used as a reference. Because of these low doses in vitro, the doses for use in food applications must be increased for the next experiments.

4.2.1. First Trials of Nisin, Carvacrol and UV-C Treatment on Chicken Drumstick

Nisin MIC + UV-C 128 second

Nisin was dripped onto chicken legs at MIC value, waited for 1 minute, then UV-C irradiation was done. Experimental sample groups were coded as Control, Nisin MIC, and Nisin MIC + UV_{128}. The microbial population was determined on the PCA, PDA, and VRBA media. Results were obtained as CFU. All of them were found as >300. This experiment determined that the combined application of Nisin at MIC level and 128 seconds UV-C dose had no effect by spraying method.

Carvacrol MIC + UV-C 128 second

Chicken drumsticks were dipped in carvacrol prepared at MIC concentration, then exposure to UV-C irradiation for 128 seconds. Experimental groups were coded as Control, Carvacrol MIC, UV_{128}, and Carvacrol MIC + UV_{128}. The microbial population was determined on the PCA, PDA, and VRBA media. Results were obtained as CFU. This experiment determined that the combined application of Carvacrol at MIC level and 128 seconds UV-C dose had no effect by dipping method.

Carvacrol 2 MIC + UV-C 256 second

This time, firstly chickens dipping in 2 MIC Carvacrol (20-30 mins), then UV-C irradiation was applied to both sides of skin for 256 seconds. Experimental groups; (Control, Carvacrol 2 MIC, UV_{256}, Carvacrol 2 MIC + UV_{256}).

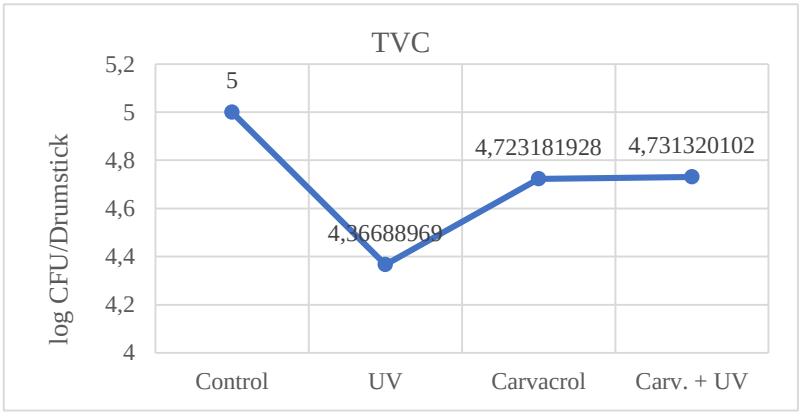

Figure 31: Total Viable Count of Control, Carvacrol MIC, UV_{256}, and Carvacrol MIC + UV_{256}.

Total Live Count results are shown in Figure 31, 256-second UV-C irradiation caused 0.633 log CFU/Drumstick reduction, 2 MIC Carvacrol caused 0.277 log CFU/Drumstick reduction, when two applications, which is a synergistic trial, UV + Carvacrol, there is 0.268 log CFU/Drumstick reduction.

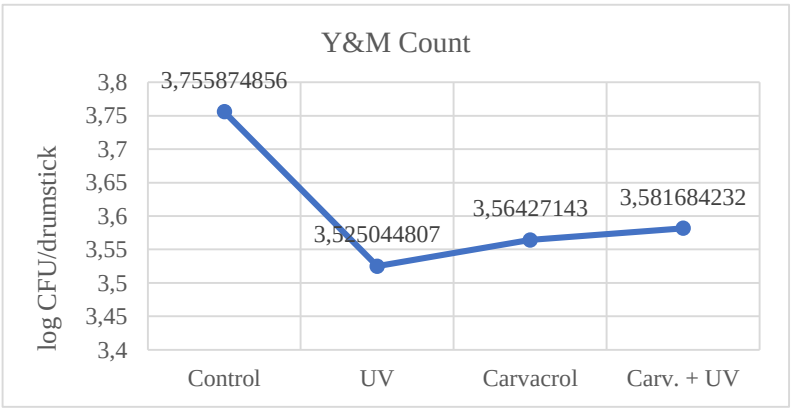

Figure 32: Total Yeast and Mold Count of Control, Carvacrol MIC, UV_{256}, and Carvacrol MIC + UV_{256}.

Total Yeast and Mold Count results shown in Figure 32, it is observed that UV-C irradiation has 0.235 log CFU/Drumstick reduction, 2 MIC Carvacrol has 0.191 log CFU/Drumstick reduction, and in the combined application cause 0.174 log CFU/Drumstick reduction.

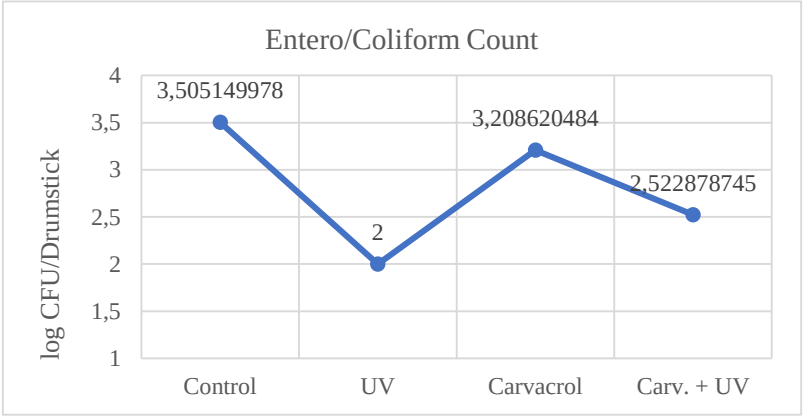

Figure 33: Total Coliform Count of Control, Carvacrol MIC, UV_{256}, and Carvacrol MIC + UV_{256}.

Coliform Count results (Figure 33) show that 256-second UV-C irradiation provided 1.5 log CFU/Drumstick, 2 MIC Carvacrol 0.3 log CFU/Drumstick, UV-C and 2 MIC Carvacrol 1 log CFU/Drumstick reduction.

Table 9: Log numbers of microorganisms from Carvacrol 2 MIC & UV 256 second experiment.

PCA	Control	UV	Carvacrol	Carv. + UV
CFU	100000	23275	52866,6667	53866,667
log	5	4,36688969	4,72318193	4,7313201

PDA	Control	UV	Carvacrol	Carv. + UV
CFU	5700	3350	3666,667	3816,6667
log	3,755875	3,525045	3,564271	3,5816842

VRBA	Control	UV	Carvacrol	Carv. + UV
CFU	3200	100	1616,667	333,3333333
log	3,50515	2	3,20862	2,522878745

Since the MIC results, found on the microorganisms did not give the effect like expectation on the meat surface, Also UV time increased from 128 to 256 seconds, the amount of Nisin to 2 MIC (2mg/ml), and the amount of Carvacrol to 2 MIC (120µl).

4.2.1. Storage Trials of Nisin, Carvacrol, and UV-C Treatment on Chicken Drumstick on 9 days at 4°C

Storage Carvacrol 2 MIC + UV-C 256 second

Experimental set: Control - UV-C_{256} Irradiation, - UV-C_{256} & Carvacrol 2 MIC / (0, 3, 6 and 9. days)

Next 3 graphs show the results of Carvacrol 2 MIC + UV-C 256 second experiment's results (Figure 34, 35, 36, and 37).

Total Viable Counts of Storage Experiment

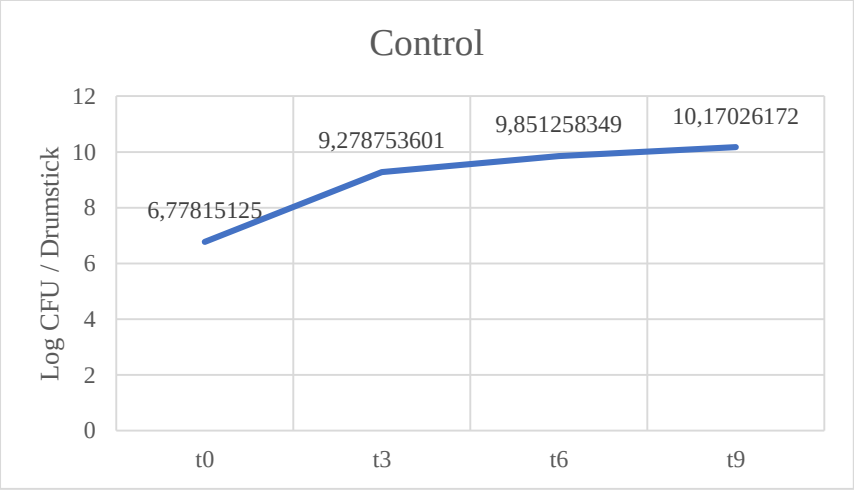

Figure 34: Total Viable Count of Control Samples during Storage.

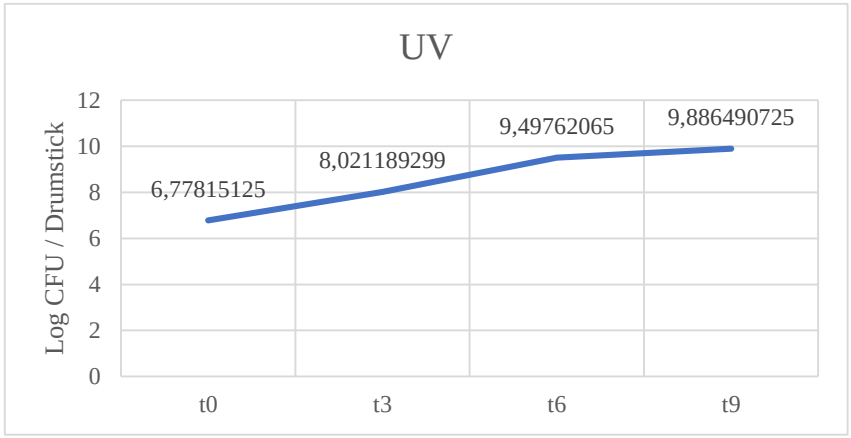

Figure 35: Total Viable Count of UV_{256} Samples during Storage**.**

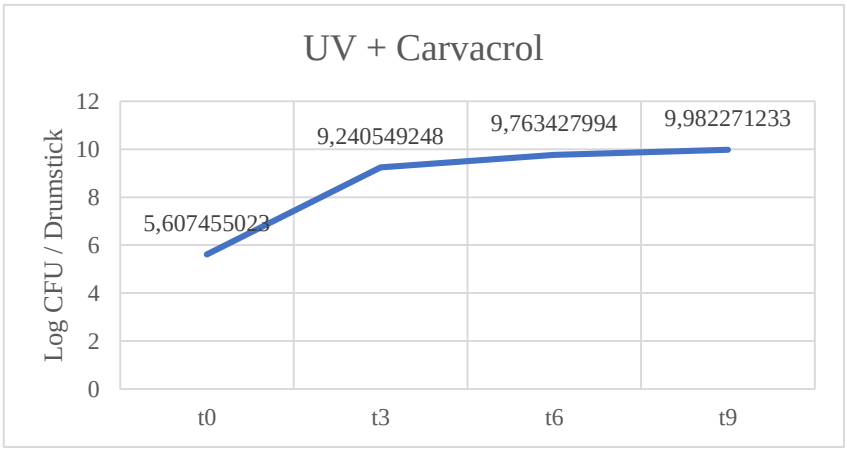

Figure 36: Total Viable Count of UV_{256} + Carvacrol MIC Samples during Storage.

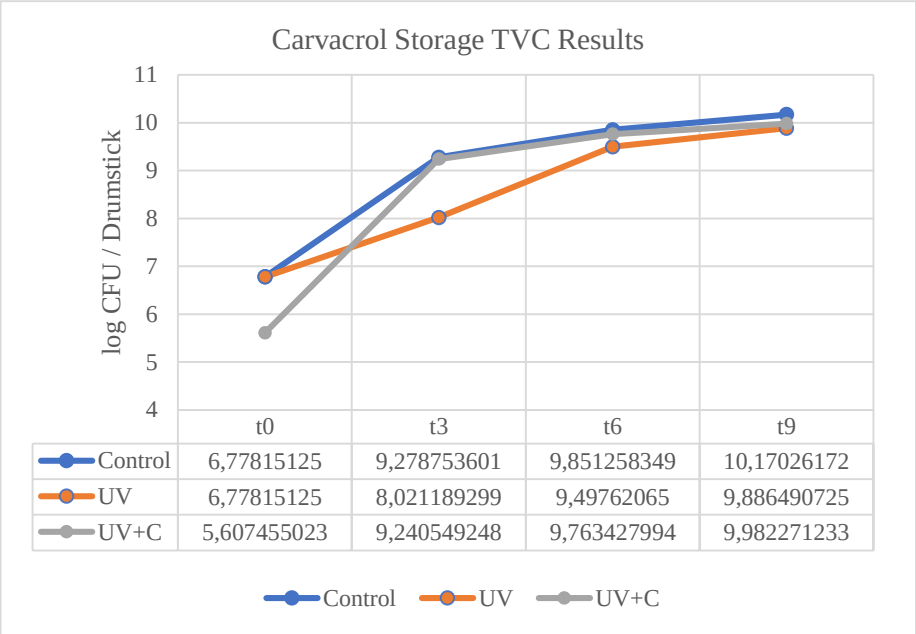

Figure 37: Total Viable Count of Control, UV_{256}, and UV_{256}+Carvacrol MIC Samples during Storage.

The logarithmic change of the total microorganisms on drumsticks' during 9 day storage period were given in the Table 10 and Figure 38.

Table 10: Logarithmic Change of the Total Microorganisms on Drumsticks' during 9 day storage period.

PCA / Log CFU/Drumstick	Control	UV	UV + C
t_0	6,77815125	6,77815125	5,607455023
t_3	9,278753601	8,021189299	9,240549248
t_6	9,851258349	9,49762065	9,763427994
t_9	10,17026172	9,886490725	9,982271233

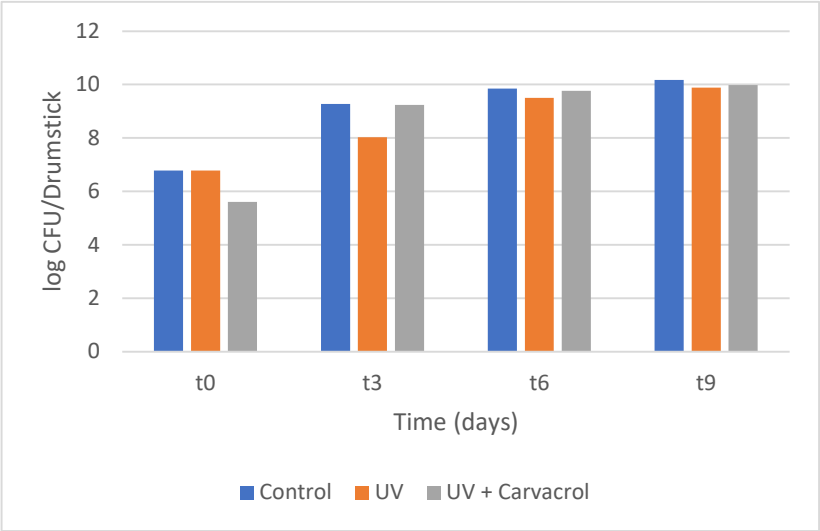

Figure 38: Logarithmic Change of the Total Microorganisms on Drumsticks' during 9 day storage period.

Total Live Count results, the control group and UV were the same on the first day, and UV-C + Carvacrol application caused a 1.17 log CFU/Drumstick reduction on total microbial load. On the third day, UV-C caused 1,257 log CFU/Drumstick and UV-C + Carvacrol caused a 0.038 log CFU/Drumstick reduction. On the sixth day, UV-C caused a 0.354 log CFU/Drumstick and UV-C + Carvacrol caused a 0.088 log CFU/Drumstick reduction. On the ninth day, UV-C decreased by 0.284 log CFU/Drumstick and UV-C + Carvacrol caused a 0.188 log CFU/Drumstick reduction.

The immediate effect values of the applied antimicrobials are given in the table above. Looking at the results, when applied together with carvacrol, it reduced 0.17 log CFU/Drumstick value.

These 4 graphs show the Yeast Counting results of Carvacrol 2 MIC + UV-C 256 experiment (Figure 39, 40, 41, 42).

Yeast & Mold Counts of Storage Experiment

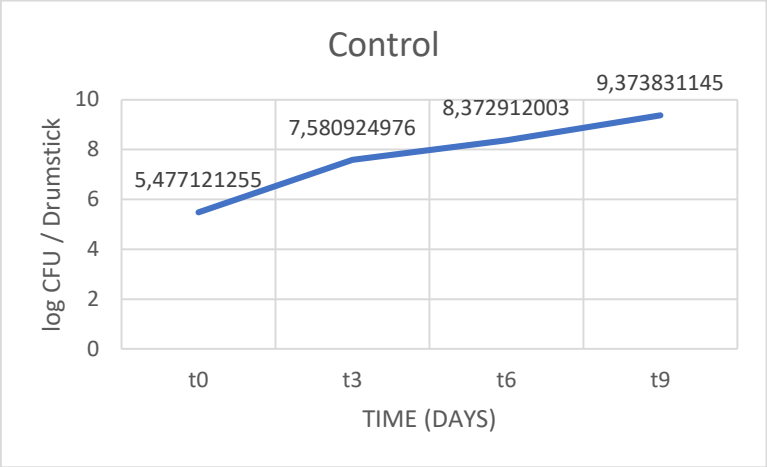

Figure 39: Total Yeast and Mold Count of Control.

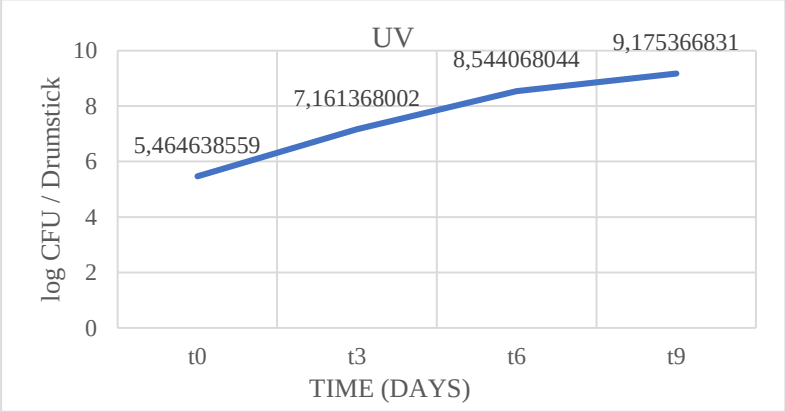

Figure 40: Total Yeast and Mold Count of UV_{256}.

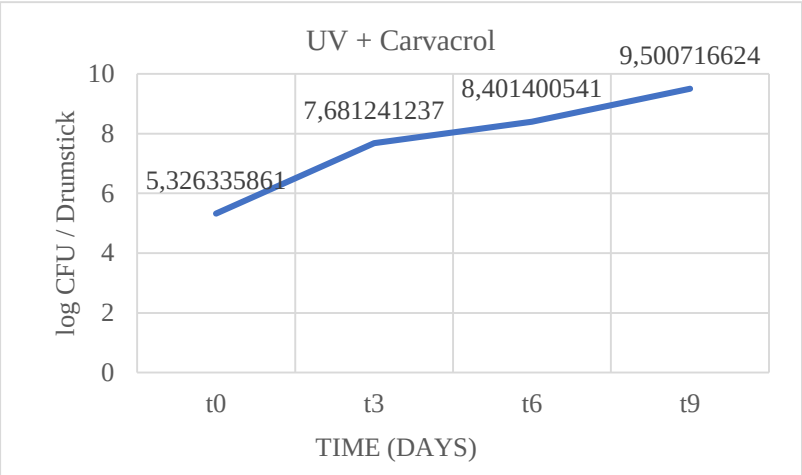

Figure 41: Total Yeast and Mold Count of Control, Carvacrol MIC + UV_{256}.

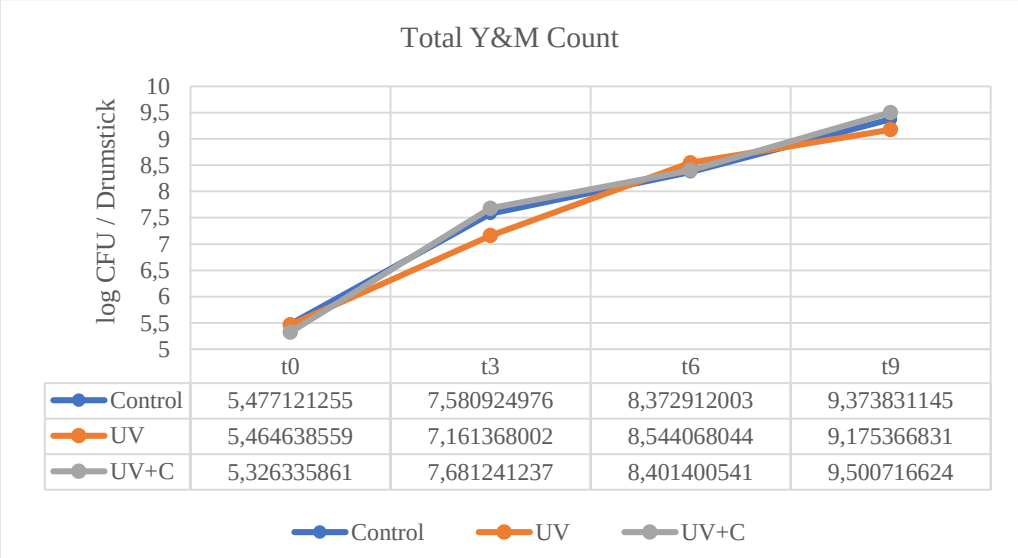

Figure 42: Total Yeast and Mold Count of Control, UV_{256}, and Carvacrol MIC+UV_{256}.

The results of the 9-day Total Yeast & Mold Counts are given above. On the first day, UV-C caused a 0.013 log CFU/Drumstick reduction and UV + Carvacrol caused a 0.151 log CFU/ Drumstick reduction. On the third day, UV irradiation caused a 0.418 log CFU/ Drumstick and UV + Carvacrol increased a 0.1 log CFU/ Drumstick. On the sixth day, it was observed that UV increased a 0.171 log CFU/ Drumstick and UV + Carvacrol

increased a 0.28 log CFU/ Drumstick. And on the last day, it was observed that UV increased a 0.198 log CFU/ Drumstick and UV + Carvacrol increased 0.126 log CFU/ Drumstick.

The logarithmic change of the total yeast amount on drumsticks' during 9 day storage period were given in the Table 11 and Figure 43.

Table 11: Logarithmic Change of the Yeasts' on Drumsticks' during 9 *day* storage period.

PDA/ Log CFU/Drumstick	Control	UV	UV+Carvacrol
t_0	5,477121255	5,464638559	5,326335861
t_3	7,580924976	7,161368002	7,681241237
t_6	8,372912003	8,544068044	8,401400541
t_9	9,373831145	9,175366831	9,500716624

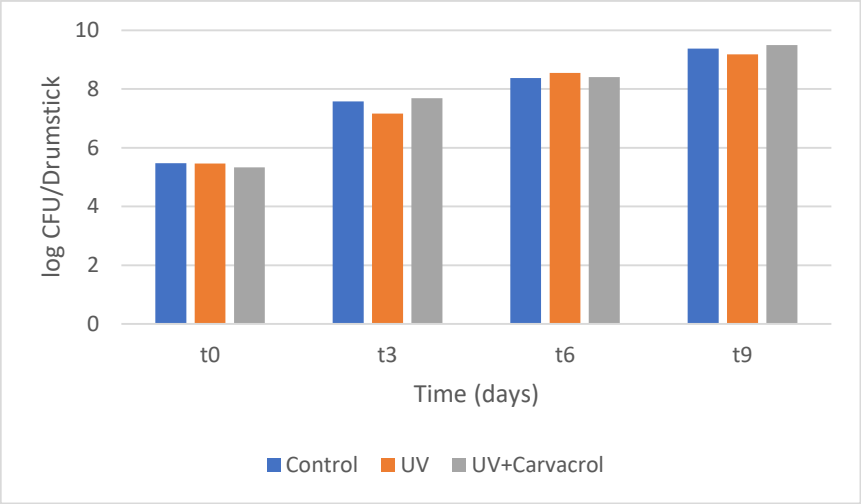

Figure 43: Logarithmic Change of the Yeasts' on Drumsticks' during 9 day storage period.

During the storage period, UV-C had a 0.185 log reduction effect on the Total Yeast amount, while the effect of UV+Carvacrol did not work from day t3.

The immediate effect of UV is 0,012 log CFU/Drumstick and UV+Carvacrol is 0.15 log CFU/Drumstick respectively. Their combination has more effective, it is ~12 fold higher.

These 4 graphs show the Coliform Counting results of Carvacrol 2 MIC + UV-C 256 second experiment (Figure 44, 45, 46, 47).

Total *Enterobactericae* / Coliform Count of Storage Experiment

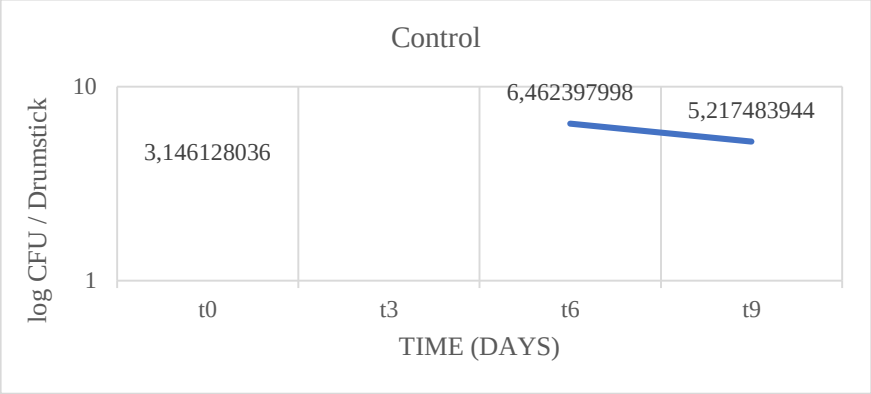

Figure 44: Total Coliform Count of Control.

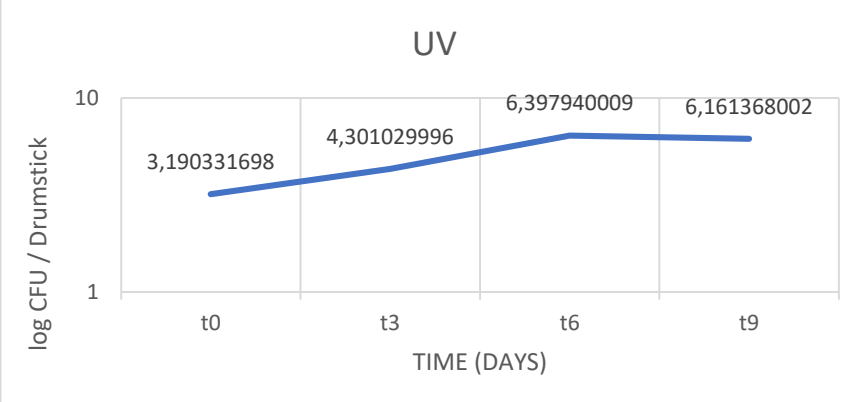

Figure 45: Total Coliform Count of UV_{256}.

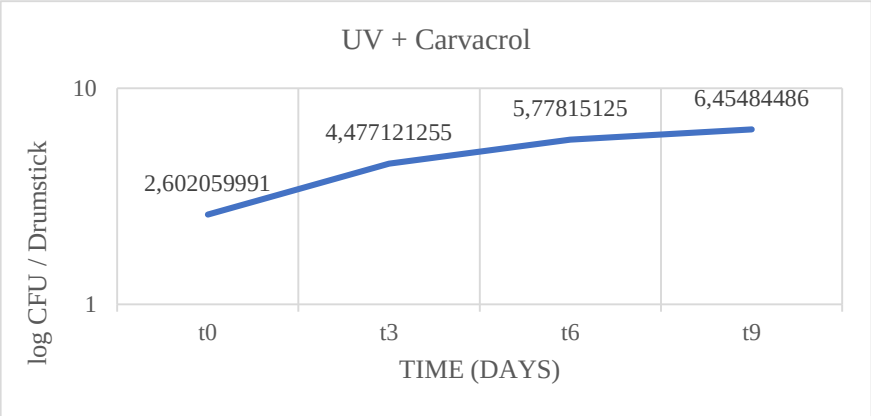

Figure 46: Total Coliform Count of UV_{256} + Carvacrol.

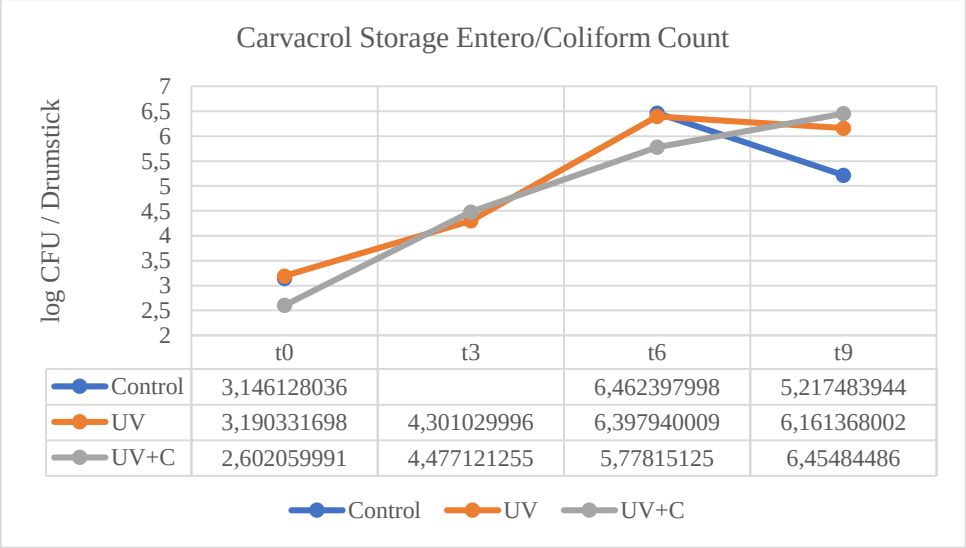

Figure 47: Total Coliform Count of Control, UV_{256} and UV+Carvacrol.

The logarithmic change of the Coliform amount on drumsticks' during 9 day storage period were given in the Table 12 and Figure 48.

Table 12: Logarithmic Change of the Coliforms on Drumsticks' during 9 day storage period.

VRBA Log CFU/DRUMSTICK	Control	UV	UV+Carvacrol
t_0	3,14613	3,19033	2,60206
t_3		4,30103	4,47712
t_6	6,4624	6,39794	5,77815
t_9	5,21748	6,16137	6,45484

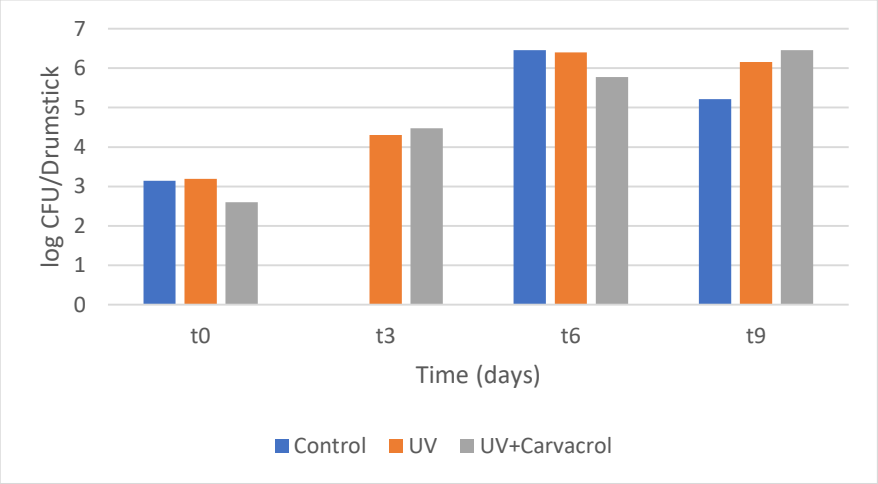

Figure 48: Logarithmic Change of the Coliforms on Drumsticks' during 9 day storage period.:

Results shows that UV-C and Carvacrol application caused 0.544 log CFU/Drumstick reduction on the first day.

Storage Nisin 2 MIC + UV-C 256 second

- Control, UV-C_{256} & Nisin 2 X MIC, Nisin 2 X MIC & Carvacrol 2 X MIC / (0, 3, 6 and 9 days)

These 4 graphs show the Total Microorganism Counting results of Carvacrol 2 MIC + UV-C 256 second experiment (Figure 49, 50, 51).

Total Viable Count of Storage Experiment

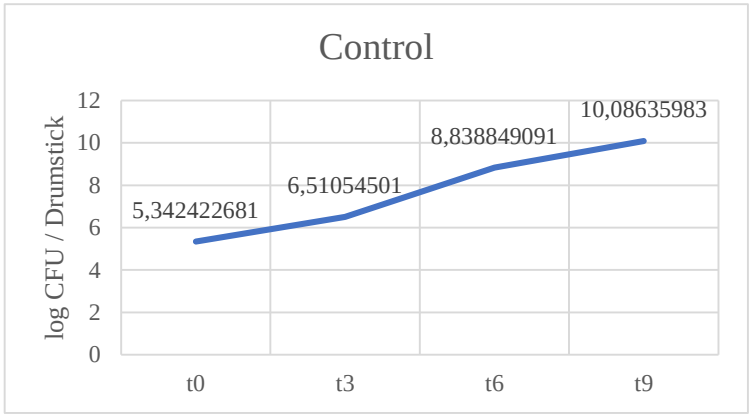

Figure 49: Total Viable Count of Control.

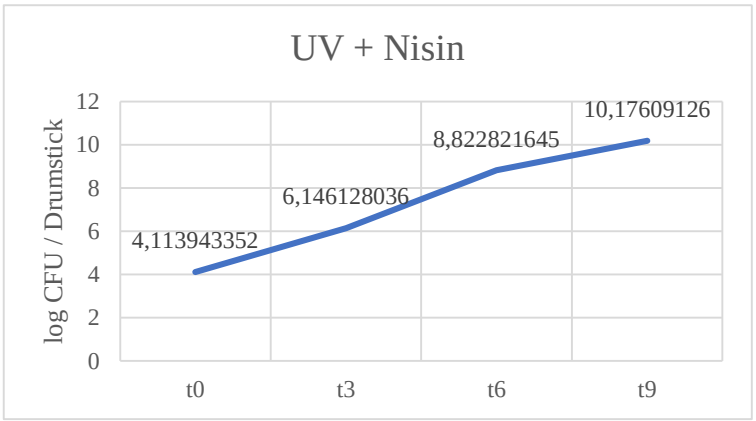

Figure 50: Total Viable Count of UV_{256} + Nisin.

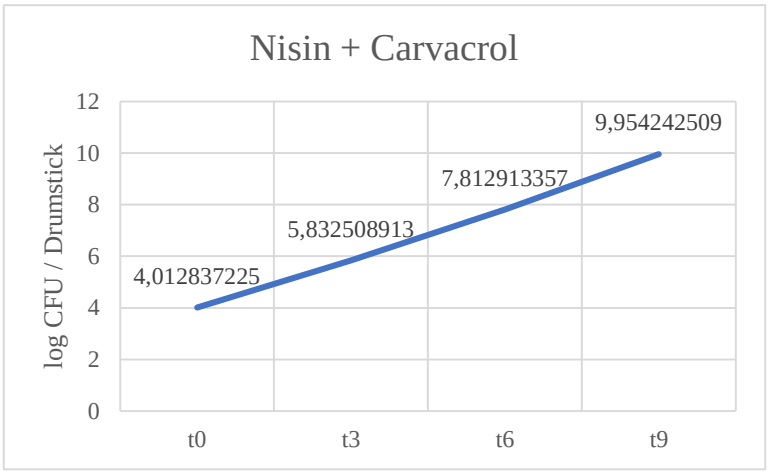

Figure 51: Total Viable Count of Nisin 2 MIC + Carvacrol 2 X MIC.

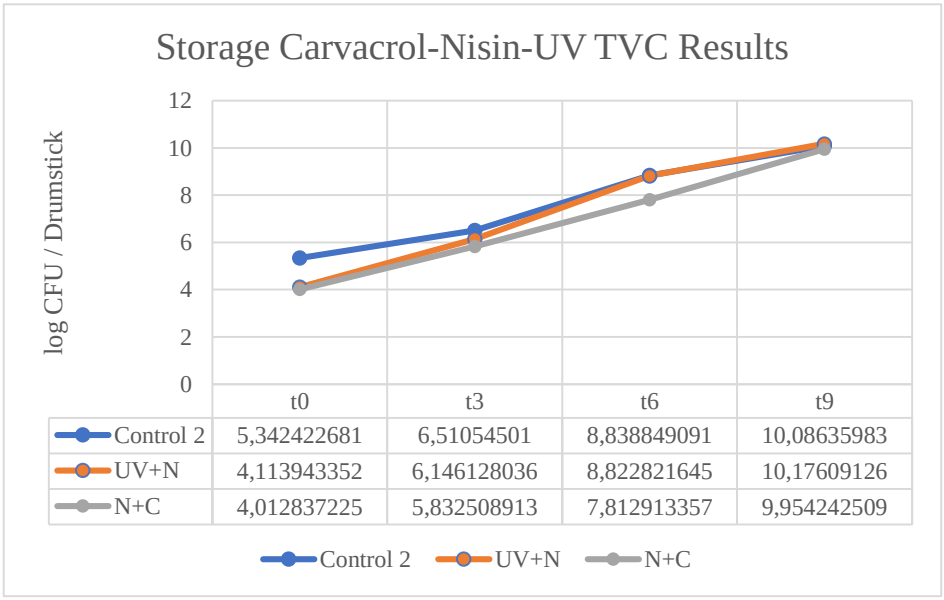

Figure 52: Total Viable Count of Control, Nisin 2 MIC + UV-C_{256}.

The logarithmic change of the total microorganism amount on drumsticks' during 9 day storage period were given in the Table 13 and Figure 53.

Table 13: Logarithmic Change of the Total Microorganisms on Drumsticks' during 9 day storage period.

PCA	Control	UV+N	N+C
t_0	5,342423	4,113943	4,012837
t_3	6,510545	6,146128	5,832509
t_6	8,838849	8,822822	7,812913
t_9	10,08636	10,17609	9,954243

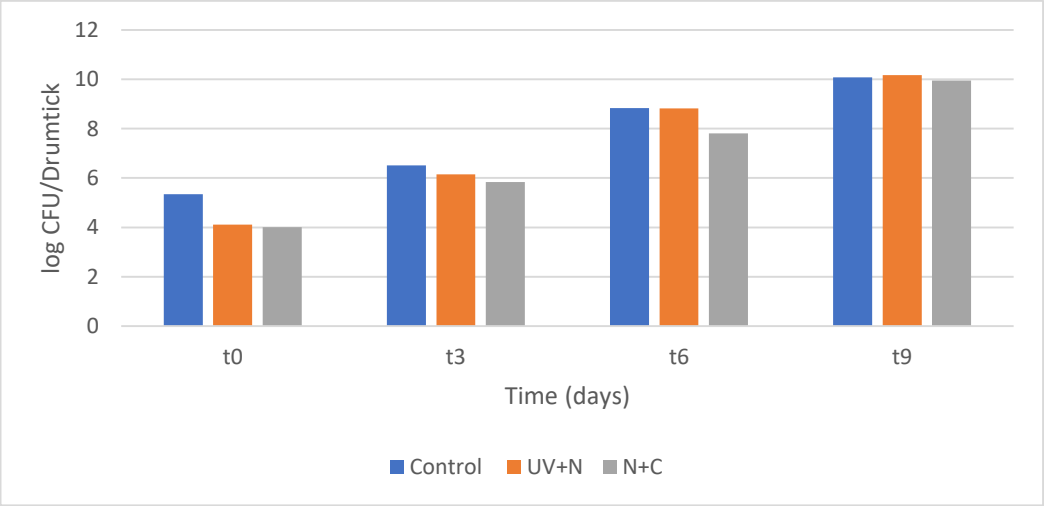

Figure 53: Logarithmic Change of the Total Microorganisms on Drumsticks' during 9 day storage period.

The results of the 9-day Total Live Count experiments are given in the table.

On the first day, UV-C + Nisin caused a 1,128 log CFU/Drumstick reduction, and Nisin + Carvacrol caused a 1,329 log CFU/Drumstick reduction. On the third day, UV-C + Nisin caused 0.364 log and Nisin + Carvacrol 0.678 log CFU/Drumstick reduction. On the sixth day, UV-C + Nisin caused a 0.015 log CFU/Drumstick and Nisin + Carvacrol caused a 1.025 log CFU/Drumstick reduction. On the last day, it was observed that UV-

C + Nisin caused a decrease of -0.089 log CFU/Drumstick, and Nisin + Carvacrol caused a 0.132 log CFU/Drumstick reduction.

From the first day to last day, logarithmic CFU increase of the Control, UV+Nisin, Nisin+Carvacrol applications is 4.74, 6.06, and 5.94 log CFU/Drumstick, respectively.

The immediate effect of UV+Nisin and Nisin+Carvacrol is 1.22 and 1.32 log CFU/Drumstick, respectively.

The next 5 graphs show the Yeast Counting results of the Carvacrol 2 MIC + UV-C_{256} second experiment (Figure 54, 55, 56).

Yeast&Mold Count of Storage Experiment

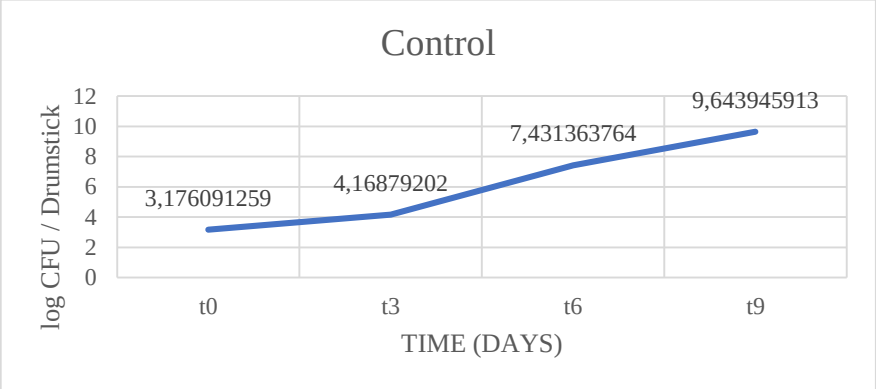

Figure 54: Yeast and Mold Count of Control.

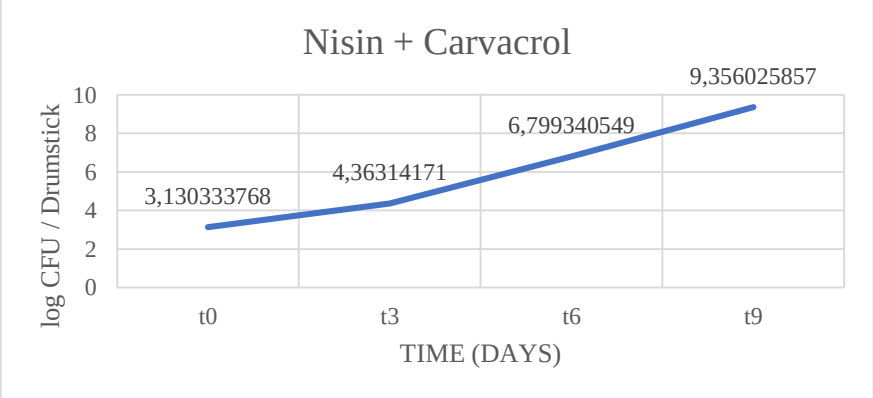

Figure 55: Yeast and Mold Count of Nisin 2 MIC + Carvacrol 2 MIC.

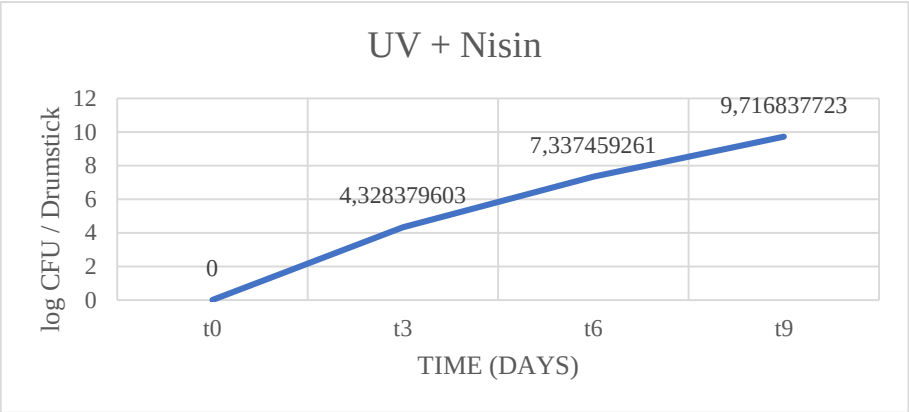

Figure 56: Yeast and Mold Count of UV_{256} + Nisin.

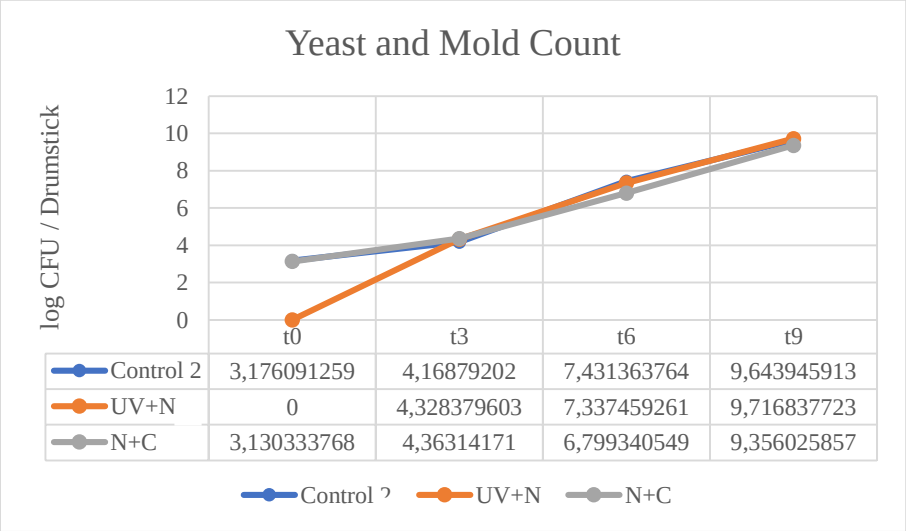

Figure 57: Yeast and Mold Count of Control, UV_{256} + Nisin, Nisin + Carvacrol 2 MIC.

The logarithmic change of the total microorganism amount on drumsticks' during 9 day storage period were given in the Table 13 and Figure 58.

Table 14: Logarithmic Change of the Yeasts' on Drumsticks' during 9 day storage period.

PDA	Control	UV+Nisin	Nis+Carv
t_0	3,176091	0	3,130334
t_3	4,168792	4,32838	4,363142
t_6	7,431364	7,337459	6,799341
t_9	9,643946	9,716838	9,356026

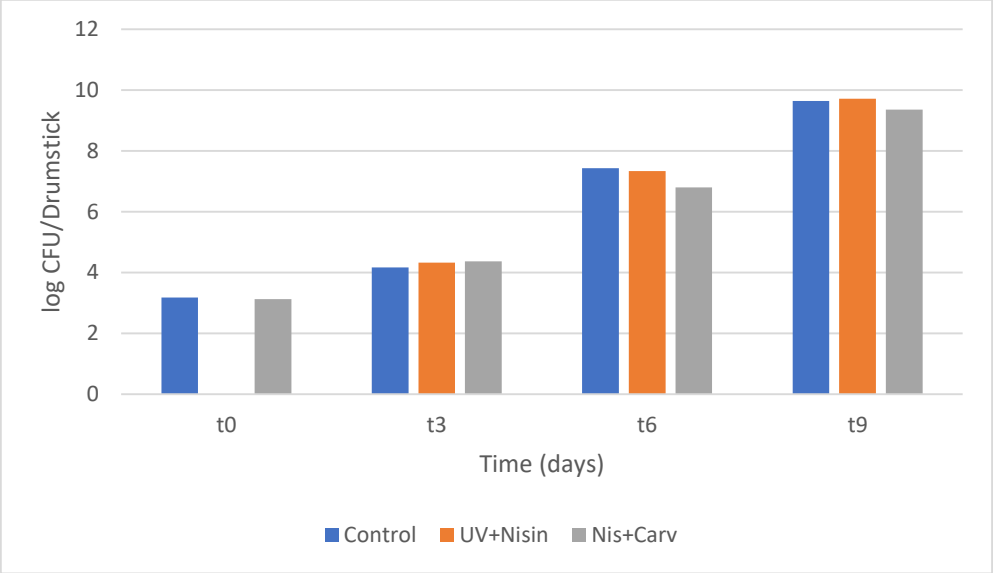

Figure 58: Logarithmic Change of the Yeast and Mold numbers on Drumsticks' during 9 day storage period.

Total *Enterobactericae* / Coliform Count of Storage Experiment

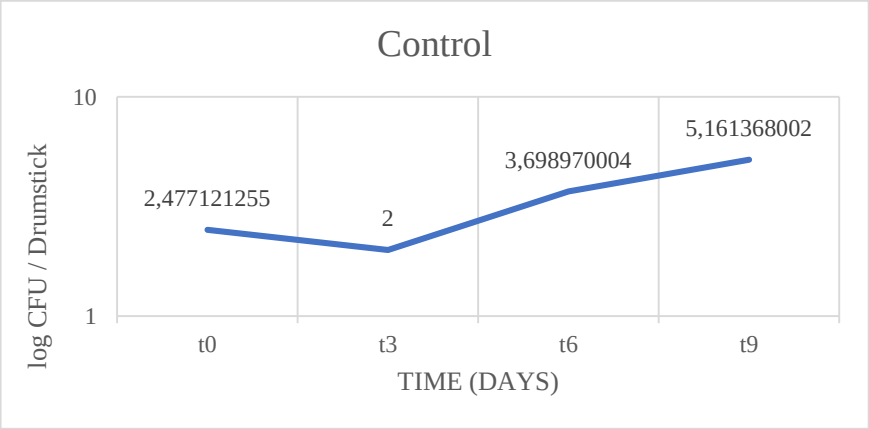

Figure 59: Total Coliform Count of Control.

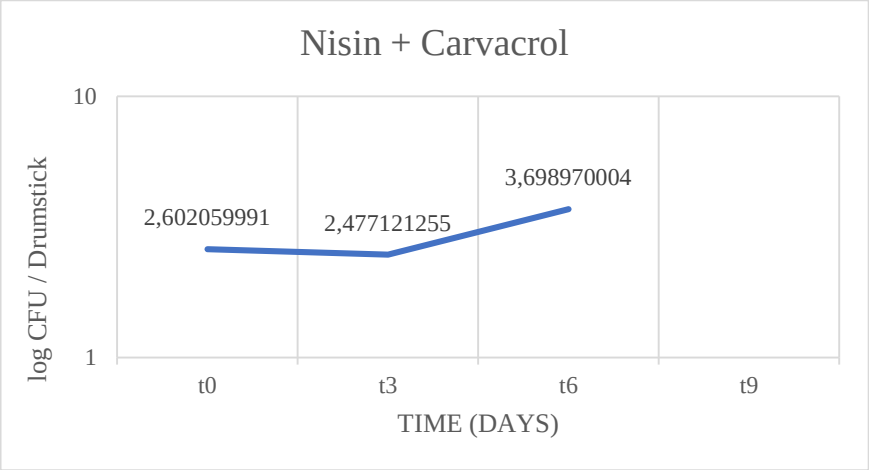

Figure 60: Total Coliform Count of Nisin + Carvacrol.

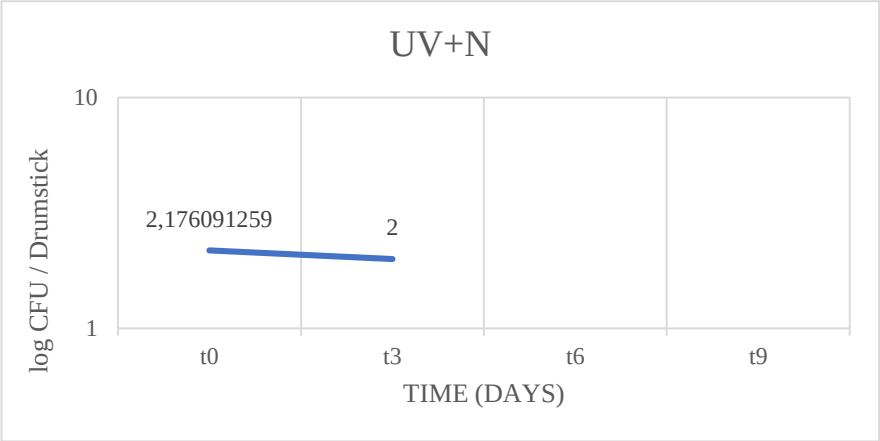

Figure 61: Total Coliform Count of UV + Nisin.

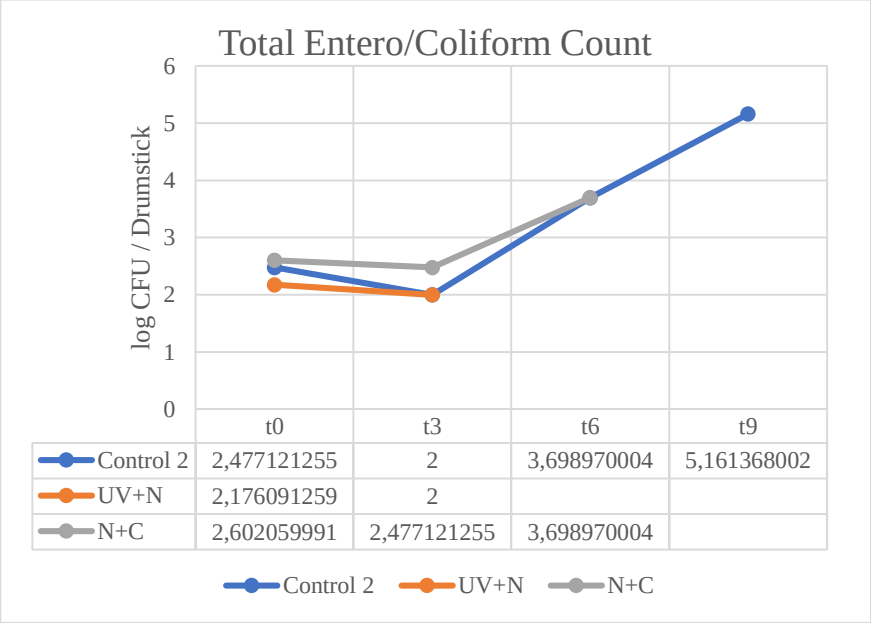

Figure 62: Total Coliform Count of Control, UV_{256} + Nisin, Nisin + Carvacrol.

The logarithmic change of the the coliforms on Drumsticks' during 9 day storage period were given in the Table 15 and Figure 62 and 63.

Table 15: Logarithmic Change of the Coliforms on Drumsticks' during 9 day storage period.

VRBA	Control	UV+N	N+C
t_0	2,477121	2,176091	2,60206
t_3	2	2	2,477121
t_6	3,69897		3,69897
t_9	5,161368		

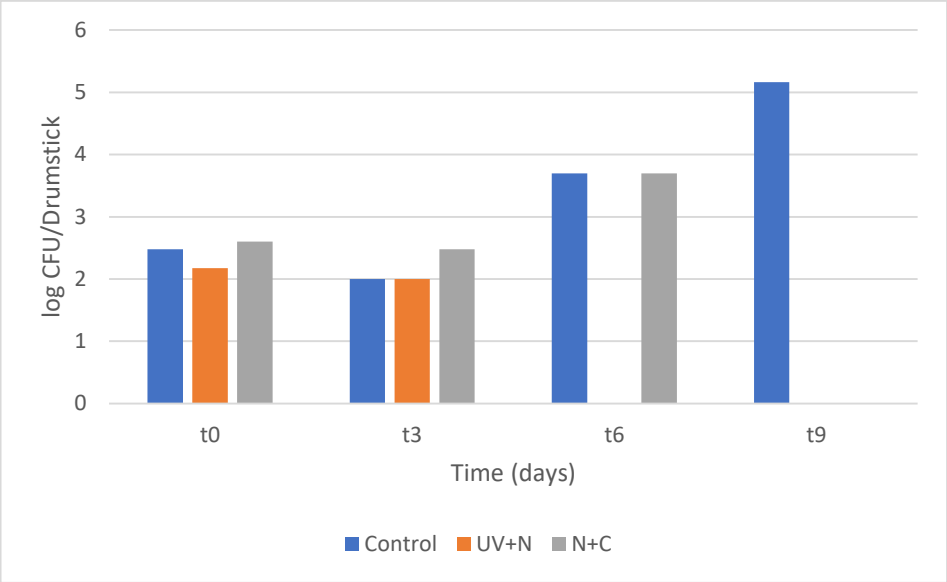

Figure 63: Logarithmic Change of the Coliform numbers on Drumsticks' during 9 day storage period.

The immediate effect of th UV radiation 0.3 log CFU / Drumstick.

Since Nisin application was made with the spraying method, its effect is not so much, there will be more effective results if it will be applied like the dipping method like Carvacrol, there will be more reduction in the future.

While counting the total bacteria and yeast in the storage experiments, it was observed that the yeast count was superior to the bacteria after the 3rd day.

4.2.2. Efficacy of Nisin and UV-C Against *Listeria* in Chicken Drumstick

Listeria innocua Nisin 2 MIC + UV-C 256 second

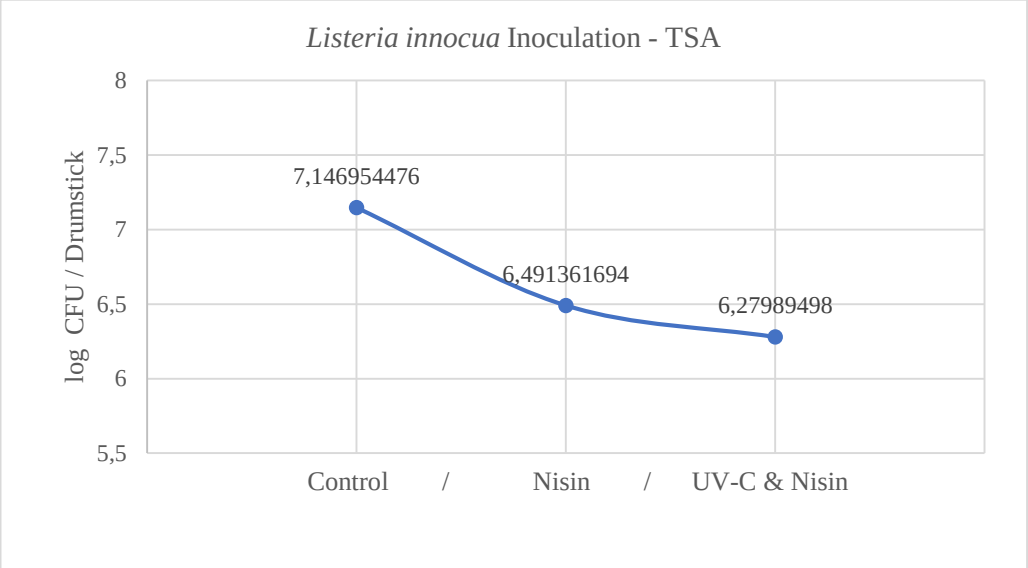

Figure 64: The results of a Nisin and UV_{256} Treatments on *Listeria innocua* Inoculated Drumstick.

Table 16: Logarithmic Numbers of the Total Microorganisms on *Listeria innocua* Inoculated Drumsticks' on TSA.

	Bacteria Load (log CFU/Drumstick)
Control	7,146954476
Nisin	6,491361694
UV/Nisin	6,27989498

In these results, Nisin application caused a 0,655 log CFU/Drumstick, UV-C + Nisin caused a 0,867 log CFU/ Drumstick and, UV-C caused a 0,211 log CFU/Drumstick reduction. UV and Nisin has a synergitic effect and more effective against microorganisms than nisin alone.

Selective *Listeria innocua* counts failed due to poor dilution inoculation on PALCAM and OXFORD agars.

The impact of different circumstances on the inactivation of foodborne pathogens and the quality of freshly cut lettuce under ultraviolet (254 nm, UV-C) radiation was discovered by Yoon-Hee Kim et al. After being injected with a mixture of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, lettuce was exposed to various UV intensities (1.36 to 6.80 mW/cm^2), exposure durations (0.5 to 10 min), and sample-to-lamp distances (10 and 50 cm), all in turn. *L. monocytogenes* surface-inoculated on lettuce experienced a 2.12-log reduction after receiving UV irradiation at 25°C for 1 minute (Kim et al., 2013).

Sungho Shin et al. conducted an assessment of disinfection effectiveness on three different surface types. They utilized UV-C light at a wavelength of 275 nm, with energy densities ranging from 4.5 to 22.5 mJ/cm^2 and exposure times spanning 1 to 5 seconds to achieve microorganism disinfection (Shin et al., 2023).

Alonzo Gabriel et al. conducted a study to determine and compare the resistance of such as *E. coli* O157:H7, *S. enterica*, *P. aeruginosa,* and *L. monocytogenes* to UV-C treatment in a turbulent flowing liquid egg white. The UV-C energy doses in the range of 170.71 to 240.33 mJ/cm^2 (Gabriel et al., 2017).

4.2.3. Efficacy of Nisin and UV-C Against *Salmonella* in Chicken Drumstick

Salmonella Typhimurium Nisin 2 MIC + UV-C 256 second

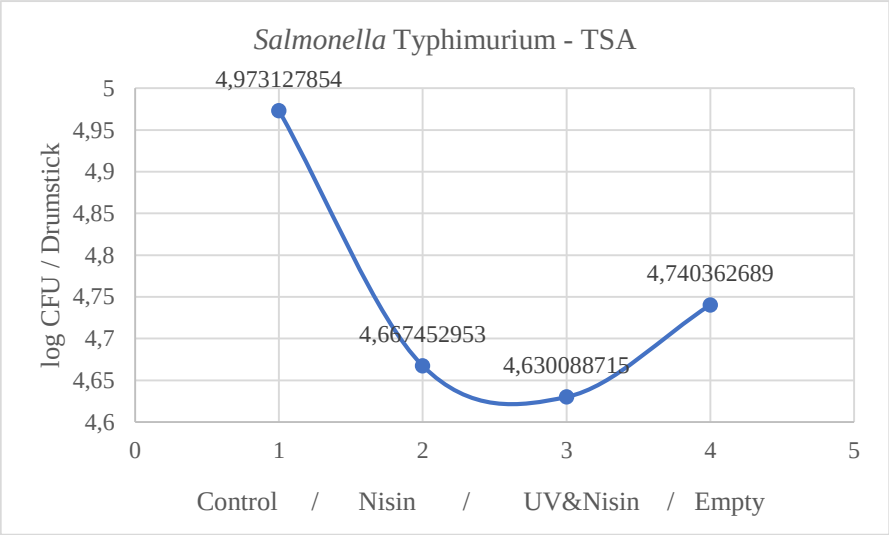

Figure 65: The results of a Nisin and UV_{256} Treatments on *Salmonella* Typhimurium Inoculated Drumsticks' on TSA.

Table 17: Logarithmic Numbers of the Total Microorganisms on *Salmonella* Typhimurium Inoculated Drumsticks' on TSA.

	Bacterial Load (log CFU/Drumstick)
Control	4,973127854
Nisin	4,667452953
UV/Nisin	4,630088715
Empty	4,740362689

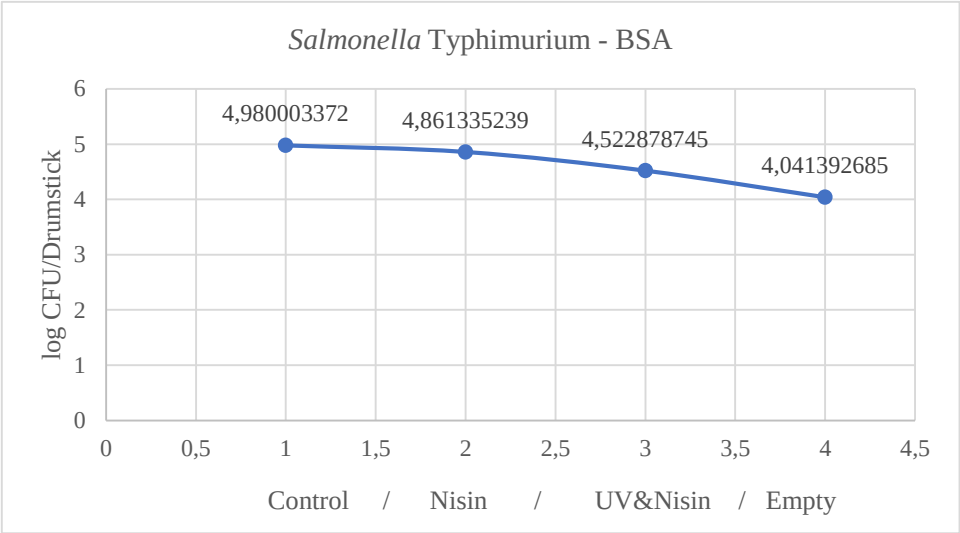

Figure 66: The results of a Nisin and UV_{256} Treatments on *Salmonella* Typhimurium Inoculated Drumsticks' on BSA.

Table 17: Logarithmic Numbers of the Total Microorganisms on *Salmonella* Typhimurium Inoculated Drumsticks' on BSA.

	Bacterial Load (log CFU/Drumstick)
Control	4,980003372
Nisin	4,861335239
UV&Nisin	4,522878745
Empty	4,041392685

In Pathogen Inoculation experiments, when compared with the control group, Nisin 2 MIC 0.655 log CFU/Drumstick and UV-Nisin 2 MIC application provided 0.867 log CFU/Drumstick reduction for *Listeria innocua*. For *Salmonella* Typhimurium, Nisin 0.3 log CFU/Drumstick, Nisin and UV irradiation provided 0.34 log CFU/Drumstick reduction.

The skinless chicken breast (CB) inoculated with *Salmonella enterica* was disinfected by ultraviolet (UV-C) LED light at a wavelength of 250–280 nm, according to Alexandra Calle et al. A different exposure time was used for samples exposed to irradiances of 2 mW/cm^2 (50%) and 4 mW/cm^2 (100%). It was observed that after 60 and 900 seconds of treatment with 50% irradiance, chicken samples exhibited the lowest reduction in *Salmonella*, with reductions of 1.02 and 1.78 log CFU/cm^2, respectively. The use of UV-C LED treatment effectively controlled *Salmonella* on both chicken surfaces and food contact surfaces in this study (Calle et al. 2021).

In a study conducted by Kye-Hwan Byun et al. (2022), it was demonstrated that combining peroxyacetic acid (PAA) or lactic acid (LA) with UV-C treatment was effective against *S. enteritidis* biofilms formed on food contact surfaces and chicken skin. The combination treatment of PAA (at concentrations ranging from 50 to 500 μg/ml) with UV-C (at exposure times of 5 and 10 minutes) resulted in reductions of 3.10–6.41 log CFU/cm^2, while LA (at concentrations of 0.5–2.0%) combined with UV-C (at exposure times of 5 and 10 minutes) led to reductions of 3.35–6.41 log CFU/cm^2 of *S. enteritidis* biofilms on the food contact surface (Byun et al., 2022).

Lázaro C. A. conducted a study to determine the optimal exposure time for reducing bacteria on chicken breasts. They used 39 chicken breasts inoculated with a mixture of *Salmonella* spp. and subjected them to three different levels of UV-C intensities (0.62, 1.13, and 1.95 mW/cm^2) for up to 120 seconds. The optimal exposure time of 90 seconds was identified. Subsequently, the effects of UV-C treatment on various parameters such as biogenic amines, total aerobic mesophilic bacteria, *Enterobacteriaceae* in 84 chicken breasts that were exposed to UV-C at different intensities (0.62, 1.13, and 1.95 mW/cm^2) and then stored at 4°C for 9 days. Notably, the highest UV-C intensity (1.95 mW/cm^2) resulted in a reduction of the initial bacterial load, as well as an extension of the lag phase and the shelf life of the chicken breasts during storage (De La Torre et al., 2014).

In artificial inoculation experiments on TSA, control groups have 7.14 and 4.97 log CFU/ Drumstick microorganism. By the results of Nisin treatment, there are 0.65 and 0.3 log CFU/ Drumstick reduction, and UV – Nisin combined treatments are showed 0.86 and 0.34 log CFU/ Drumstick reduction for *L. innocua* and *S.* Typhimurium, respectively. It shows Nisin and UV combination works better than Nisin alone, also applications are more effevtgive against *Listeria* than *Salmonella,* although the initial bacterial loads were different.

In the *Salmonella* experiment, there was not much difference between the total live count counted in TSA and *Salmonella* count counted in BSA. While the TSA count was 4.74 log in the uninoculated blank chicken, the BSA count was 4.04 log CFU/ Drumstick, and in the untreated control chicken with bacteria inoculation, the numbers were 4.97 log CFU/ Drumstick and 4.98 log CFU/ Drumstick. Thus, it can be said that chicken has a large amount of *Salmonella*. While nisin was more effective on the total number of organisms, it showed a more lethal effect on *Salmonella* when combined with UV.

In applications on meat, the microorganisms initially found on chickens affected some of our results. Since *Enterobacter* and Coliform group microorganisms should not be abundant in chicken meat sold, no significant growth was observed in the results. Generally, in Yeast and Mold count results, there is any mold, mainly yeast overgrowth occurred.

In the experiments, 3 drumsticks were used for each group except for storage, as a parallel, but since the size and microorganism load of each chicken is different from each other, sometimes seen that there were more bacteria in the results of the applied substances than control group.

Since the drumsticks we are working with are chicken meat with skin, the substances we applied may not have fully penetrated the meat although they were thoroughly homogenized in the stomacher bag. More effective results will be obtained if future studies are performed.

CHAPTER 5

CONCLUSION

In summary, this study represents the pioneering investigation into the inactivation of *L. innocua*, *Cronobacter sakazakii*, and *Candida albicans* in vitro by using Nisin and Carvacrol .

This study demonstrated that combined treatments effectively ensured product safety and quality against the tested bacteria and did not adversely affect food structure and color.

These treatments present themselves as promising alternatives to the existing preservative techniques employed in the poultry industry. Not only are they cost-effective, but they also require minimal equipment and consume low amounts of energy.

The effectiveness of these methods was influenced by the dose, exposure time and the surface of the food product.

However, a large scale of experiment will be needed to determine the process conditions for industrial application.

Nisin, as a lantibiotic, should not be solely relied upon as the main method for food preservation. Instead, it can be integrated into the broader "hurdle concept" strategy for food preservation and safety. This approach involves combining various intrinsic and extrinsic treatments to create multiple barriers that collectively enhance the preservation of food by impacting microbial growth.

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