

“Natural Antimicrobials and Antioxidants in Shelf-Stable Low-Moisture RTE Foods”: A Review of Synergistic Preservation Strategies

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Abstract: Shelf-stable, low-moisture foods (LMFs) and ready-to-eat (RTE) foods are increasingly in demand due to their extended shelf life, convenience, and minimal storage requirements. However, despite their low water activity, LMFs remain susceptible to quality degradation and microbial contamination over time, particularly by xerophilic and osmophilic microorganisms. This review explores the role of natural antimicrobials and antioxidants as effective preservation agents in LMFs, emphasising their synergistic interactions when used in combination. Natural compounds such as essential oils, plant extracts (e.g., rosemary, green tea), organic acids, and bacteriocins offer broad-spectrum antimicrobial activity. In contrast, phenolic compounds and tocopherols serve as potent antioxidants to retard lipid oxidation and preserve sensory qualities. The integration of these bioactive agents into edible films, coatings, or active packaging systems is also discussed, with a focus on compatibility with biodegradable matrices. Furthermore, the review assesses factors that influence the efficacy of these compounds, including concentration, food matrix interactions, and environmental conditions. By highlighting synergistic combinations and multifunctional effects, this paper provides insight into designing holistic, clean-label preservation strategies for LMFs, aligning with consumer demand for natural, sustainable, and safe food products.

Keywords: Shelf-stable, low-moisture food, ready-to-eat, antimicrobials, antioxidants, bioactive, biodegradable, clean-label

1. Introduction

LMFs are foods that are inherently low in moisture or that can be produced by drying or dehydrating foods that are higher in moisture. These types of foods have a prolonged shelf life because of their low water activity (aw). Water activity (aw) refers to the amount of water available for microbial growth; lowering the aw in food can inhibit the proliferation of various microorganisms. The incorporation of substantial quantities of salt or sugar can be considered a simulated drying process, as it decreases the accessibility of water for microbial reproduction. Microorganisms may grow at a minimum aw of 0.60, which is also the aw below which physiological processes required for cell division are hindered. While halophilic bacteria can thrive at aw as low as 0.75, most bacteria require aw of less than 0.87 for survival. For instance, whereas most bacteria cannot grow below an aw of 0.9, some yeasts and molds can grow as low as an aw of 0.6, while *Staphylococcus aureus* (*S. aureus*) can grow down to about an aw of 0.85 and may have significant requirements for particular dry items. Cereals, grains, confections (like chocolate), powdered protein products (including dairy and egg powders), dried fruits and vegetables, honey, spices, seeds, nuts, and nut-based goods (like peanut butter) are a few types of LMF items. LMF are typically regarded as safe by consumers, and many are eaten as prepared foods without the need for a consumer-performed pathogen reduction step, such as cooking [1-3].

Consumption of an emerging group of processed foods, known as ready-to-eat items, is rising in consumer markets [4]. The presence of microbes poses significant hazards to food safety, as cooked, ready-to-eat products are often consumed without additional cooking. Fresh, quick meals with fewer or no preservatives are in great demand from consumers. This demand necessitates the development of methods, such as vacuum packaging or modified atmosphere

packaging (MAP), that preserve the natural attributes of cooked, ready-to-eat foods without the use of chemical preservatives. MAP is a technique that alters the relative amounts of atmospheric gases surrounding perishable and semi-perishable food items to increase their shelf life. By inhibiting the growth of aerobic spoilage microorganisms, MAP effectively prolongs the shelf life of cooked vegetables, thereby reducing water content loss and the rates of oxidation and enzymatic degradation [5,6].

The two kinds of MAP are active and passive. In order to achieve the desired steady-state equilibrium, passive MAP entails putting produce in a gas-permeable packaging, sealing it, and allowing produce respiration to lower oxygen and raise carbon dioxide levels [7]. The O₂ and CO₂ concentrations in active MAP are first altered and vary dynamically according to the rate at which commodities respire and the permeability of the film enclosing the produce. Only fruits and vegetables can be treated with passive MAP. Active MAP, however, might be used in a wide variety of food items [8, 9]. With the objective to maintain an acceptable equilibrium atmosphere within the package, which is crucial for delaying ripening, maturation, and senescence and thereby extending the product shelf-life, MAP relies on striking a balance between the minimally processed product's respiration rate and film permeability [7].

Multiple research studies support the idea that diets high in functional compounds may be beneficial to human health [12]. The word functional or bioactive compound is used to indicate a class of diverse molecules that possess biological activity. These substances are found in nature as metabolites of plants and/or microorganisms. They have either essential or non-essential qualities and are a component of the food chain. The most researched functional compounds include antioxidants, primarily phenolic compounds, carotenoids, and other phytochemicals [10, 11]. These bioactive components

must be recovered from waste and by-product streams in an equally sustainable manner to be included in food and drinks. Innovative and environmentally friendly extraction techniques have been created with the goal of recovering these compounds efficiently and cost-effectively. The most researched extraction methods are natural deep eutectic solvents (NADES), pulsed electric fields (PEF), enzymatic assisted extraction (EAE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE) [13]. Phenolic compounds are the most acknowledged functional constituents in food and beverages. They consist of secondary metabolites from plants that are found in a variety of fruits, vegetables, and cereals [14]. The antioxidant properties of phenols stem from their capacity to donate hydrogen, hence preventing the generation of free radicals. Cell death, premature aging, and the onset of certain diseases, including cancer and Alzheimer's disease, are all caused by free radicals [15]. In addition, phenolic compounds exhibit antimicrobial activity against a range of pathogens, preventing their growth and extending the shelf life of food products [14].

Another class of secondary plant and microbial metabolites with a wide range of colours and potent biological activity are carotenoids. Over 1100 carotenoids have been discovered to date, and their health advantages, recovery processes, and production techniques have all been thoroughly examined. β -carotene and lycopene are the most prevalent carotenoids in nature, followed by zeaxanthin, β -cryptoxanthin, α -carotene, and lutein. Astaxanthin is the most common carotenoid present in a variety of aquatic organisms. Since many carotenoids function as precursors for the production of vitamins (such as pro-vitamin A), the inability of the human body to synthesize them makes them necessary for the human diet [16-19].

Organic acid additions to food and feed are a well-established method that is primarily used to extend the final product's shelf life. In a variety of food applications, the most commonly utilized organic acids include lactic acid, citric acid, benzoic acid, propionic acid, sorbic acid, fumaric acid, formic acid, and succinic acid. All of these organic acids have antimicrobial properties because of their capacity to penetrate the microbial cell wall. The potential of acquiring an illness from a number of bacteria, including *Salmonella*, *Clostridium spp.*, *Listeria spp.*, *E. coli*, and *Coliforms spp.*, can be decreased by adding organic acids to food [20]. Furthermore, several pathogenic bacteria in the human gut are controlled by organic acids, which function as acidifiers [21].

The primary causes of food quality degradation and shelf-life reduction are microbial growth and lipid oxidation [22]. Chemical additives are frequently used in food products to prolong their shelf life and prevent lipid oxidation and proliferation of bacteria, but consumers are becoming increasingly concerned about the potential health risks linked to the use of synthetic antimicrobial/antioxidant agents [23]. Moreover, consumers show a preference for products featuring clean labels that include natural food ingredients and additives, characterized by familiar names and perceived health benefits [24]. Plant extracts and their essential oils, enzymes, peptides, chitosan, bacteriocins, bacteriophages, fermented ingredients, and ozone present promising

alternatives to synthetic antimicrobial agents, enhancing both the shelf-life and safety of food products [25]. Herbs (rosemary, oregano, marjoram, sage, basil, etc.), spices (cinnamon, clove, nutmeg, ginger, black pepper, garlic, etc.), vitamins (ascorbic acid and α -tocopherol), and plant extracts (strawberry, blueberry, cranberry, tea, grape seed, etc.) also have antioxidant components and can be utilized as natural antioxidants to prevent lipid oxidation of food product [26].

The manufacturing of antimicrobial packaging materials with natural antibacterial agents is gaining traction. Consumer concerns regarding issues related to health, like the usage of artificial antimicrobial agents, have sparked this interest. Using artificial antibacterial substances directly in meals can successfully prevent the growth and development of certain microbes, but consumers prefer food products that are less processed, have no preservatives, and have a longer shelf life. The last and extra barrier that can stop food-borne germs from growing is antimicrobial packaging [27, 28]. Antimicrobial agents can be directly incorporated into packaging films, coated on packaging films, or created from polymers to create an antimicrobial packaging system. Antimicrobial packaging methods are often classified as either migrating or non-migrating, with the distinction based on the antimicrobial agent utilized and how it interacts with the food matrix and packaging. They can be divided into two categories: (i) those that have an antimicrobial agent that migrates to the food's surface (migrating film) and (ii) those that effectively inhibit the growth of microorganisms on the food's surface without migrating (non-migrating film) [29- 31].

Customers prefer natural products over synthetic ones, especially in recent years. As a result, naturally derived antimicrobial agents are becoming more significant in antimicrobial packaging because they are believed to pose a lower risk to consumers [32- 34].

Plant extracts, enzymes, and bacteriocins are examples of biologically derived natural compounds that are utilized in antimicrobial packaging. Numerous kinds of bacteria synthesize peptidic antimicrobial agents called bacteriocins, which can kill other species that are typically related. Since lactic acid-producing bacteria often create the majority of bacteriocins, their use to control certain bacterial growth in food is quite alluring [35].

In the field of food packaging materials, nanostructures provide novel opportunities for the application of enzyme immobilization systems [36]. Among these enzymes is lysozyme, a single peptide protein with enzymatic activity against the beta 1-4 glycosidic bonds present in peptidoglycan between N-acetylmuramic acid and acetylglucosamine. Gram-positive and gram-negative bacteria's cell walls are composed primarily of peptidoglycan. Bacterial cells may lyse as a result of lysozyme hydrolysis of the cell wall, which may damage the wall's structural integrity. Since lysozyme is a naturally occurring enzyme that can be produced by both humans and many animals and exhibits action against bacterial cellular structure, it is of interest for application in food systems [37, 38]. Since ancient times, the antimicrobial qualities of essential oils derived from certain plant extracts, spices, and herbs have been explored and applied to the preservation of food. Due to their

high concentration of phenolic chemicals, including carvacrol, thymol, and eugenol, several plant extracts and essential oils have robust antibacterial qualities [39- 41]. Thymus essential oils, cumin, fennel, laurel, mint, marjoram, oregano, sage, savory, thyme, cinnamon, onion and garlic oils, garlic and onion extract, as well as Australian native plant oils, clove, and cinnamon oils, are all known to possess antimicrobial properties in several kinds of studies. Their constituents, which are naturally occurring antibacterial agents, are gaining popularity [42- 47].

It is possible to utilize biopolymers derived from renewable resources as active food packaging [48]. Proteins and carbohydrates are the most often utilized ingredients in edible films. Food items can also have their storage and shelf lives prolonged by edible films and coatings that prevent lipid oxidation and the growth of food-borne pathogens. Protein or carbohydrate-based edible films may include antimicrobial substances such as lysozyme, chitosan, nisin, essential oils, or their constituents [49- 52]. A renewable substance, chitosan may be found in green microalgae, fungus, yeast, protozoa, and the shells of shrimp. Because of its non-toxicity, biodegradability, and biocompatibility characteristics, chitosan also makes an excellent film. There are several types of chitosan films, including blends, edible films, and coatings that may be applied directly to food to increase food safety and shelf life [53]. Chitosan and gelatin films are notable biopolymers due to their satisfactory mechanical properties and superior gas barrier capabilities at intermediate and low relative humidity [54]. Gelatin has been documented as one of the initial materials utilized as a carrier for bioactive components [55, 56].

This potential strategy to enhance the safety, quality, and shelf stability of low-moisture RTE foods is presented by the synergism, which may be a result of the combined antimicrobial and antioxidant activity of natural compounds. Additionally, these blends provide a clean label substitute, fulfilling the growing demand from consumers for products free of artificial additives.

The function of natural antimicrobials and antioxidants in the preservation of shelf-stable low-moisture RTE foods is examined in this review, with an emphasis on synergistic approaches that improve oxidative stability and microbial safety. By compiling the most recent studies in this field, I aim to shed light on the possible advantages and limitations of using these natural preservation techniques in the food sector.

2. Review of Literature

Any scientific research must include an in-depth review of the literature. Using citations from earlier studies, the researcher draws conclusions from the literature review. The study on the value addition of natural antioxidants and antimicrobials in shelf-stable low-moisture RTE foods is briefly summarized under the following headings:

2.1 Shelf-Stable Low-Moisture RTE Foods

Beuchat et al. (2013) reported that low-moisture foods (LMFs), with water activity below 0.6, are generally resistant

to microbial growth but remain vulnerable to contamination by xerophilic molds and osmophilic pathogens like *Salmonella* and *Aspergillus* species. Their study emphasized that low water activity does not ensure absolute microbial safety, especially for Ready-to-Eat (RTE) formats. Numerous bacteria, particularly foodborne pathogens, exhibit resistance to desiccation processes. Rehydrating low-aw foods prior to consumption results in a chance that bacterial pathogens could proliferate if products are eventually stored improperly, possibly reaching populations large enough to induce sickness or intoxication.

Research by Fellows (2009) explained that LMFs such as jerky, biscuits, snack bars, and dried fruits are popular for their convenience and long shelf life. However, oxidative degradation, nutrient losses, and microbial spore activation during post-processing are critical concerns. These risks have spurred interest in natural preservation strategies, particularly the synergistic use of antimicrobials and antioxidants derived from plant and microbial sources.

2.2 Natural Antimicrobials Used in LMFs

2.2.1 Plant-Based Antimicrobials

Burt (2004) studied the antimicrobial activity of essential oils (EOs) and found that oils from oregano, thyme, and clove exhibit broad-spectrum antimicrobial effects due to the presence of bioactive compounds like thymol, carvacrol, and eugenol. These substances cause microbial cell membranes to rupture, allowing cellular contents to leak out. A variety of essential oil components have been recognized as effective antibacterials, including carvacrol, thymol, eugenol, perillaldehyde, cinnamaldehyde, and cinnamic acid, with minimum inhibitory concentrations (MICs) ranging from 0.05 to 5 $\mu\text{l ml}^{-1}$ in vitro. In foods, a greater concentration is required to attain the same result. According to studies conducted on fresh meat, meat products, fish, milk, dairy products, vegetables, fruit, and cooked rice, the concentration required for achieving a noticeable antibacterial impact in foods is between 0.5 and 20 $\mu\text{l g}^{-1}$ and between 0.1 and 10 $\mu\text{l ml}^{-1}$ in fruit and vegetable washing solutions. The hydrophobic nature of EOs allows them to separate in the mitochondrial and cell membrane lipids, making them permeable and allowing cell contents to leak out. Low pH, low temperature, and low oxygen levels are physical circumstances that enhance the effect of essential oils.

Tiwari et al. (2009) demonstrated the efficacy of cinnamon and clove EOs against *Listeria monocytogenes* and *E. coli O157:H7* in low-moisture meat snacks. Their study highlighted the potential of EOs in RTE foods but also noted that high concentrations could adversely affect sensory attributes. Natural antimicrobials can help replace conventional methods by being employed either alone or in conjunction with other advanced preservation technologies.

Gyawali and Ibrahim (2014) reported that phenolic acids, such as ferulic acid and caffeic acid, act as natural antimicrobials by damaging the bacterial cell membrane and interrupting energy production. Their findings suggest that such compounds are more effective when incorporated into food-grade coatings. Despite the fact that a lot of natural

products are presently being utilized to preserve food and increase its shelf life, many more sources remain undiscovered. Using natural substances from algae, mushrooms, and plant waste could lead to their potential use as new antimicrobials. The use of natural antimicrobials in food systems is still restricted, despite their potential, primarily because of their unfavourable flavor or aroma.

2.2.2 Microbial-Derived Antimicrobials

Gálvez et al. (2007) examined bacteriocins such as nisin and pediocin, produced by *Lactococcus lactis* and *Pediococcus acidilactici*, respectively. The authors reported that these peptides are particularly effective against Gram-positive bacteria and are suitable for use in low-moisture dairy and meat snacks. Various bacterial species create bacteriocins, which are ribosomally-synthesised peptides or proteins with antibacterial properties. Numerous lactic acid bacteria (LAB) produce bacteriocins with a wide range of inhibitory effects. Bacteriocins can be made in situ by bacteriocinogenic starters, adjuncts, or protective cultures, or they can be incorporated into food as concentrated preparations, food preservatives, shelf-life extenders, additives, or ingredients.

Delves-Broughton et al. (1996) confirmed the safety and broad-spectrum efficacy of nisin, which is currently one of the few bacteriocins approved by regulatory authorities for use in foods. Their work showed that nisin can be used in combination with other preservation methods to reduce required dosages. Nisin has been used more recently as a preservative in pasteurized liquid eggs, hot baked flour goods (crumpets), and high moisture products. The antilisterial qualities of nisin in food have been extensively studied, and several uses have been suggested. Nisin has been found to be used in the manufacturing of wine, beer, and alcohol as well as low pH foods like salad dressings, to reduce lactic acid bacteria that cause spoilage. Future developments for nisin are probably going to involve its usage as an adjunct in novel food processing technologies like electroporation and high-pressure sterilization, as well as its synergistic activity with chelators and other bacteriocins.

2.3 Natural Antioxidants for Lipid Stability in LMFs

Shahidi and Ambigaipalan (2015) studied polyphenols and flavonoids such as catechins, quercetin, and rosmarinic acid. Their research indicated that these compounds scavenge free radicals and inhibit lipid peroxidation, thereby extending the oxidative shelf life of LMFs. There is growing evidence that the antioxidant activity of some phenolic chemicals found in natural foods may reduce the risk of major health diseases, among other causes. Natural antioxidants derived from edible sources, by-products, and co-products are alternate sources of interest because of safety concerns and other limitations associated with the use of synthetic antioxidants.

Frankel et al. (1996) compared rosemary extract with synthetic antioxidants like BHA and BHT and found that rosemary—especially its carnosic acid and carnosol components—was more effective in stabilizing fats in dehydrated meat and bakery products. The hydrophilic rosemary antioxidants may be more oxidation-resistant in bulk oil systems where oil is the primary phase because they are oriented in the air-oil interface. These hydrophilic

rosemary antioxidants, on the other hand, stay in the water in oil-in-water emulsion systems where water is the primary phase and are less potent antioxidants at the oil-water interface where oxidation occurs. This mechanism states that the effective antioxidant concentrations in the water and oil phases as well as the interface determine the formation and decomposition of hydroperoxide in emulsion systems. Their higher affinity for the more polar oil-water interface of fish oil systems may account for the increased antioxidant activity of rosemary antioxidants found in fish oil emulsions as opposed to vegetable oil emulsions.

Oussalah et al. (2006) tested milk protein-based edible films containing oregano and rosemary oils on beef products and concluded that such films provided both antioxidant and antimicrobial protection during storage. Beef muscle slices were coated with milk protein-based edible films containing 1.0% (w/v) oregano, 1.0% (w/v) pimento, or 1.0% oreganopimento (1:1) essential oils blend. This was done to prevent the formation of harmful microbes and extend the shelf life while being stored at 4 °C. For microbiological and biochemical examination, meat and film were tested at regular intervals over the course of seven days. Thiobarbituric reactive substances (TBARS) were measured in order to assess the meat's capacity for lipid oxidation. Pimento-based films had the strongest antioxidant activity, while oregano-based films stabilized lipid oxidation in beef muscle samples. Bioactive films containing oregano were found to be the most effective against *Escherichia coli* O157:H7 or *Pseudomonas* spp. when applied to meat surfaces that contained 103 colony-forming units/cm². In contrast, films containing pimento oils appeared to be the least effective against these two bacteria.

Rojas-Graü et al. (2007) evaluated the use of apple purée films containing essential oils and found them effective in reducing oxidative rancidity and microbial growth in low-moisture snacks. Their results supported the use of bio-based films as active packaging. The study evaluated the effects of oregano, cinnamon, and lemongrass oils on *Escherichia coli* O157:H7, focusing on both the mechanical and physical properties of the films. This assessment was conducted using an edible film derived from an apple puree solution (APEF) as well as the apple puree film-forming solution (APFFS). The apple puree and the film both included oregano oil, which proved very efficient against *E. coli* O157:H7. According to the data, (a) oregano oil had the highest antimicrobial activity followed by lemongrass oil and cinnamon oil, and (b) adding the essential oils to the film-forming solution increased oxygen permeability and decreased water vapor permeability without appreciably changing the films' tensile characteristics.

2.4 Synergistic Strategies in Preservation

Research by Leistner and Gorris (1995) introduced the concept of “hurdle technology,” which combines multiple preservation methods to enhance food safety without compromising quality. Their theory supports combining natural antimicrobials and antioxidants to create multiple obstacles for microbial growth. Hurdle technology was created a few years ago as a novel idea for producing food that are stable, safe, wholesome, delicious, and cost-effective. It promotes the wise application of several preservation factor

or technique combinations (also known as "hurdles") to provide multi-target, moderate but dependable preservation effects.

Tiwari et al. (2009) further demonstrated that combining green tea catechins with thyme oil in dried meat snacks resulted in a synergistic effect, reducing microbial load while delaying lipid oxidation. The efficacy of chemical and biochemical antimicrobial compounds obtained from these natural sources against a variety of food-related pathogenic and spoilage microorganisms, as well as their effects on the organoleptic qualities of food, are described.

Bonilla et al. (2013) studied starch and chitosan-based films loaded with natural antioxidants like rosemary extract. They reported that the films exhibited dual functionality—antioxidant and antimicrobial—and could be applied to dried fruits, cereals, and protein bars. Limiting oxygen interactions is necessary to keep food products from losing quality. This could be accomplished by the use of edible coatings and films, which work through two mechanisms: the specific activity of the incorporated antioxidant agents and the oxygen barrier effect.

Ramos et al. (2012) evaluated the Antimicrobial active films based on polypropylene (PP). Thymol and carvacrol were added in three different weight percentages to create these, as well as an equimolar blend of the two chemicals. Additives reduced the PP samples' oxygen barrier and crystallinity but had no effect on their thermal stability. With higher oxidation induction parameters, the inclusion of thymol and carvacrol also improved stability against thermo-oxidative degradation. Furthermore, compared to carvacrol, thymol demonstrated more inhibition against food-borne bacterial strains, resulting in increased antibacterial activity.

2.5 Active Packaging as a Carrier of Natural Preservatives

Han (2000) investigated how antimicrobial packaging technologies can help increase food safety and prolong the shelf life of packaged foods by preventing the growth of harmful and spoiling bacteria. Although there are many variables to consider when creating an antimicrobial packaging system, the majority of them have a relation to the properties of the target microorganisms, packed foods, and antimicrobial chemicals.

Bonilla et al. (2013) also emphasized that chitosan films, when combined with rosemary or oregano extract, significantly reduce microbial growth on dried apple slices and low-moisture nuts. The authors concluded that such films are suitable for shelf-stable, high-fat food applications.

Kumar et al. (2017) investigated the impact of incorporating essential oils into biodegradable films on the quality of low-moisture food products. The research found that adding essential oils to these films can affect their properties, potentially enhancing or diminishing their effectiveness as food packaging materials.

2.6 Challenges and Considerations

Ture et al. (2011) investigated the possibilities of employing zein films, a protein derived from corn, as edible packaging materials by adding functional extracts and essential oils (EOs) to improve their functionality. In vitro and on minced beef, zein films containing ZEO (*Zataria multiflora* essential oil) and ML (monolaurin) successfully suppressed the growth of foodborne pathogens such as *Listeria monocytogenes* and *E. coli* O157:H7. Higher ZEO and ML concentrations enhanced the films' antibacterial activity. The antioxidant activity of the zein films was considerably increased by the addition of ZEO. According to the research, by preventing microbial growth and slowing down oxidation processes, these functionalized zein films could potentially be able to increase the shelf life of food products, especially meat.

Davidson and Taylor (2007) highlighted the importance of regulatory frameworks governing the use of natural preservatives. While many plant extracts are GRAS, their permitted usage levels vary across countries, affecting formulation options. Traditional food antimicrobials are a crucial tool for preserving foods from microbes' detrimental and hazardous impacts.

López-Malo et al. (2005) stressed that water activity alone does not guarantee microbial safety, and thus combining it with natural hurdles is essential, especially in minimally processed LMFs intended for long-term storage. In potato dextrose agar (PDA), the combined effects of water activity ($[a_w]$ 0.99 or 0.95), pH (4.5 or 3.5), and antimicrobial agent concentration (0, 100, 200 up to 1800 ppm) (potassium sorbate, sodium benzoate, sodium bisulfite, carvacrol, citral, eugenol, thymol, or vanillin) on *Aspergillus flavus* growth were assessed. As the concentration of the antimicrobial agent increased and when the pH and a_w dropped, the germination period increased. At pH 3.5, *A. flavus* showed greater sensitivity to potassium sorbate, sodium bisulfite, thymol, eugenol, carvacrol, and sodium benzoate than to vanillin or citral.

2.7 Research Gaps and Future Prospects

Recent research by Sharma et al. (2022) proposed the use of agricultural waste materials such as banana fiber, rice husk cellulose, and corn husk starch for developing biodegradable films infused with natural antimicrobials. Their work aligns with the global shift toward sustainable packaging.

Despite the growing body of work, there is limited data on the long-term performance of these combined systems under variable humidity and temperature conditions typical of retail supply chains. More studies are required on compound stability, release kinetics, and consumer acceptability in real food matrices.

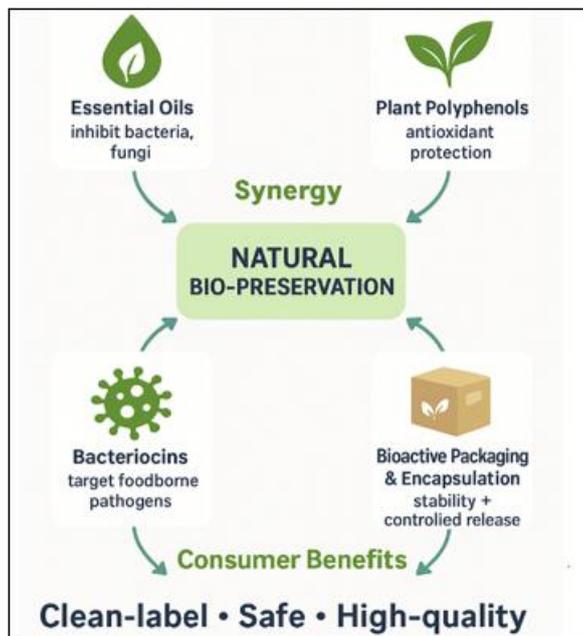


Figure 1: Synergistic role of Natural Antimicrobials and Antioxidants in Shelf-stable Low-Moisture RTE Foods

Table 1: Summary of Key Studies on Natural Antimicrobials and Antioxidants in Shelf-Stable Low-Moisture RTE Foods

Author (Year)	Bioactive Used	Food Matrix	Effect Observed
Burt (2004)	Oregano essential oil	Dried meat	~2-log CFU/g reduction in <i>E. coli</i>
Tiwari et al. (2009)	Cinnamon + Clove EO	RTE meat snack	3-log reduction in <i>Listeria</i> , flavor retention
Gálvez et al. (2007)	Nisin (bacteriocin)	Low-moisture dairy/meat	Inhibition of <i>Clostridium botulinum</i>
Frankel et al. (1996)	Rosemary extract	Dehydrated fish powder	Better oxidative stability than BHA
Rojas-Graü et al. (2007)	Oregano EO in apple-based film	Dried apple rings	TBARS < 1.0 mg/kg after 28 days
Oussalah et al. (2006)	Oregano + Rosemary EO	Beef muscle	~2-log microbial reduction; 70% DPPH scavenging
Bonilla et al. (2013)	Rosemary extract in starch-chitosan film	Dried mango strips	Antioxidant + antimicrobial protection for 60 days
Sharma et al. (2022)	Banana peel-based EO film	Snack food model	>80% DPPH; 4-log <i>E. coli</i> reduction
Shahidi & Ambigaipalan (2015)	Green tea catechins, rosemary, sage	Dehydrated snacks and powders	40% delay in lipid oxidation vs. BHT
Delves-Broughton et al. (1996)	Nisin	Powdered dairy, protein mixes	Stable bacteriocin activity in shelf-stable matrices
Leistner & Gorris (1995)	Nisin + rosemary (hurdle approach)	Intermediate-moisture sausages	Improved oxidative and microbial shelf life
Ramos et al. (2012)	Cinnamon oil + grape seed extract	Baked biscuits	30% fungal inhibition and better oxidative stability
Han (2003)	Oregano EO in biopolymer film	RTE dried products	EO extended the activity for 3 months
Kumar et al. (2021)	Thyme EO in zein-based film	Low-moisture bakery items	6-week microbial inhibition and aroma retention

3. Conclusion

Low-moisture, ready-to-eat (RTE) foods are valued for their convenience and long shelf life, but they remain susceptible to oxidative deterioration, quality loss, and microbial survival. The use of natural antimicrobials and antioxidants offers a sustainable alternative to synthetic preservatives, enhancing both safety and stability while aligning with clean-label demands. Essential oils, plant-derived polyphenols, and microbial peptides have shown considerable potential in extending shelf life by inhibiting microbial growth and delaying lipid oxidation. Importantly, synergistic combinations of these bioactive compounds with conventional preservation hurdles can provide broader protection and reduce the need for high dosages, minimizing sensory alterations.

Looking ahead, the development of advanced delivery systems, such as nanoencapsulation and bioactive packaging, is crucial to improve the stability, controlled release, and functionality of natural preservatives in complex food matrices. By integrating natural antimicrobials and antioxidants with modern processing and packaging strategies, it is possible to design safe, high-quality, and sustainable shelf-stable RTE foods that meet both industry needs and consumer expectations.

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