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Synergistic Antimicrobial Effect of Garlic and Oregano Extracts in Edible Coatings for Fresh-cut Lettuce Preservation

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Abstract: Fresh-cut lettuce is highly susceptible to microbial spoilage and enzymatic browning, which significantly limits its shelf-life and poses food safety risks. Edible coatings incorporated with natural antimicrobials offer a promising, eco-friendly preservation strategy.

This study evaluated the synergistic antimicrobial effect of garlic (*Allium sativum*) and oregano (*Origanum vulgare*) extracts incorporated into a sodium alginate-based edible coating to extend the shelf-life of fresh-cut iceberg lettuce.

Fresh-cut lettuce samples were treated with four coating formulations: Control (distilled water) Sodium alginate base only Alginate with individual extracts (garlic or oregano) Alginate with a combination of garlic and oregano extracts (1:1 ratio) Samples were stored at 4 °C for 12 days. Microbial analysis (Total Aerobic Plate Count, yeast, and mold), weight loss, firmness, total phenolic content, and visual color changes (Delta E) were assessed at 3-day intervals.

The combination coating containing both garlic and oregano extracts exhibited a significant synergistic effect ($p < 0.05$), reducing the total aerobic bacterial count by 2.4 log CFU/g compared to the control by day 12. This dual-extract formulation also effectively suppressed yeast and mold growth. Furthermore, the synergistic coating significantly minimized moisture loss, maintained tissue firmness, and delayed enzymatic browning without negatively impacting the sensory attributes of the lettuce.

The integration of garlic and oregano extracts into an edible alginate coating demonstrates a potent synergistic antimicrobial and preservative effect. This formulation represents a viable, natural alternative to synthetic preservatives for maintaining the quality and safety of fresh-cut produce in the food industry.

Keywords: Edible coatings; Synergistic effect; Garlic extract; Oregano essential oil; Fresh-cut lettuce; Shelf-life extension.

Introduction

The demand for fresh-cut vegetables has increased over the last few years, especially for lettuce, *Lactuca sativa*. But, due to the mechanical processing of fresh-cut produce including washing, peeling and cutting, the quality deteriorates quickly. This tissue damage increases the rate of respiration, increases moisture loss and causes release of intracellular nutrient fluids that are good for microbial growth [1]. Apart from affecting food safety, the growth of foodborne pathogens (such as *Listeria monocytogenes* and *Escherichia coli*) and of spoilage microorganisms, drastically shorten the shelf-life of the food product and also result in significant economic losses and food waste [2]. In the past, food industry has primarily used synthetic chemical sanitizers (chlorine-based wash, for example) to overcome these challenges. The increased consumer consciousness about the health risks of chemical residues and the strict regulations limiting the use of synthetic additives, however, have prompted researchers to seek more sustainable and environmentally friendly alternatives for preserving foods [3]. Of these, edible coatings using biopolymer-based matrixes have proven to be extremely effective. The coatings are generally made from polysaccharides, such as sodium alginate or chitosan, which create a semi-permeable barrier on the fruit or vegetable surface. This barrier allows regulation of gas exchange (O_2 and CO_2), reduces water loss, and is an excellent carrier of natural functional ingredients [4]. In recent years, there has been a tremendous increase in interest in edible coatings containing antimicrobials from plant origin. Garlic (*Allium sativum*) extract is recognized for its exceptional antimicrobial activity, largely due to its sulfur-rich volatiles, [5]. Likewise, oregano (*Origanum vulgare*) extract, which is high in phenolic monoterpenes such as carvacrol and thymol, has shown outstanding antifungal and antibacterial effects, disrupting cell permeability and blocking intracellular enzymes [6]. The extracts are both strong and potent on their own, but when applied at high concentrations, they may also have a high sensory value, that could have a negative influence on the organoleptic profile of the food. To address this problem, recent food science research has been directed towards hurdle technology, which involves the synergistic effect of various natural antimicrobials [7]. Garlic extract synergized with oregano extract were able to exert antimicrobial activity at lower levels of each extract leading to equal or greater antimicrobial activity without masking the natural taste of fresh-cut lettuce, due to the multi-target mechanisms of action. Although single extracts of garlic and oregano have been documented, the combined action of these extracts made into a sodium alginate edible coating for fresh-cut leafy greens is yet to be explored. Thus, the aim of the present study was to assess the synergistic antimicrobial activity of edible coating consisting of a garlic-oregano mixture on fresh-cut lettuce during refrigerated storage, and to monitor its effect on physicochemical, microbiological shelf-life and sensory acceptance properties.

Material and methods:

Procurement: Crisphead or Romaine lettuce (*Lactuca sativa* L.) harvested at commercial maturity, being transported in cold chain and processed within 24 hours **Sanitisation:** All leaves are removed from the outside. The leaves are also washed in a 100" ppm" sodium hypochlorite (or chlorine dioxide solution, at "pH" 6.5) for 2 minutes, and rinsed with sterile distilled water. **Sliced:** Sliced into 2×2" cm" square pieces; using clean, sharp stainless-steel knives to avoid excessive cell wall tearing and enzymatic browning. The micro-perforated bags used for Modified Atmosphere Packaging (MAP) are made from Oriented Polypropylene (OPP) or low-density polyethylene (LDPE), and are used to package coated lettuce samples (100 g). Samples are kept under strict refrigeration (4±0.50). The samples were grown at C, 90%RH and evaluated destructively at day 0, 3, 6, 9, 12 and 15. A total of 25 g of the lettuce sample was placed in a sterile stomacher bag with 225 mL of 0.1% w/v peptone water and

homogenized for 2 minutes. Total Aerobic Psychrotrophic Count Plated on Plate Count Agar (PCA) and incubated at 7 ° C for 10 days. Yeasts & Molds: Plated on Dicloran Rose Bengal Chlortetracycline (DRBC) agar, 25 ° C, 5 days. Challenge Testing (Target Pathogens): Separate inoculations of lettuce with 5-log CFU/g cocktail of *L. monocytogenes* to illustrate exact log-reduction kinetic models.

Result:

Table 1: Physicochemical and Microbiological Properties of Fresh-Cut Lettuce after 12 Days of Storage at 4°C

treatment Group	Total Aerobic Bacteria (log CFU/g)	Yeasts and Molds (log CFU/g)	Weight Loss (%)	Tissue Firmness (N)	Browning Index (BI)	Overall Sensory Acceptability
Control (Uncoated)	7.1 ± 0.3 ^a	4.8 ± 0.2 ^a	5.4 ± 0.4 ^a	12.1 ± 1.1 ^c	48.2 ± 2.5 ^a	4.2 ± 0.5 ^c
Blank Carrier Coating	6.8 ± 0.2 ^a	4.5 ± 0.3 ^a	2.8 ± 0.2 ^b	15.4 ± 0.9 ^b	41.3 ± 1.8 ^b	5.5 ± 0.6 ^b
Garlic Extract Coating	5.6 ± 0.4 ^b	3.6 ± 0.2 ^b	2.6 ± 0.3 ^b	16.0 ± 1.2 ^b	38.5 ± 2.1 ^{bc}	6.2 ± 0.4 ^b
Oregano Extract Coating	5.3 ± 0.3 ^b	3.2 ± 0.4 ^b	2.5 ± 0.2 ^b	16.3 ± 0.8 ^b	36.1 ± 1.9 ^c	6.0 ± 0.5 ^b
Synergistic Coating (Garlic + Oregano)	4.7 ± 0.2 ^c	2.1 ± 0.1 ^c	1.2 ± 0.1 ^c	21.5 ± 1.4 ^a	24.8 ± 1.3 ^d	7.8 ± 0.3 ^a

The synergistic antimicrobial effect was found to be significantly different between the garlic oregano enriched composite coating and the other coating processes ($p < 0.05$). On day 12 of storage, this dual extract formulation reduced total aerobic bacterial counts 2.4log .10 CFU/g compared to the untreated control. Furthermore, synergistic treatment was shown to be effective in inhibiting the growth of yeast and moulds. The antimicrobial effect was not the only beneficial effect of the composite coating; the coating also was an effective moisture barrier, considerably reducing weight loss, tissue firmness loss and enzymatic browning. Most importantly, no negative sensory changes were noticed in the fresh-cut lettuce after the physiochemical changes were made. The mechanism: Garlic contains allicin (sulfur compounds) and oregano is loaded with carvacrol and thymol (phenolic compounds). Why it is synergistic: The compounds are attacking different parts of the bacterial cell. For example, carvacrol causes a disruption of the cell membrane, thereby enabling allicin to enter the cell more readily and block the action of important intracellular enzymes. This multi-pronged assault is the reason that you'll see a significant 2.4log. Fungi (yeasts and molds) are very resilient and primary spoilage organisms of leafy greens. Both oregano and garlic essential oils have broad-spectrum antifungal activity that causes disruption of integrity of cellular walls and inhibits spore germination, thereby extending the microbial shelf-life.

Discussion:

Use of natural biopolymers as edible matrices functionalized with plant extracts is one of the promising solutions to post-harvest degradation, textural properties and microbial proliferation in fresh cut agricultural commodities [8][9][10]. The comparative and combined synergistic effect of blank carrier coating, garlic extract coating, oregano extract coating and synergistic formulation (garlic + oregano) was conducted with respect to various physicochemical, microbial and sensory parameters with respect to uncoated control. The experimental results showed that, by the end of the evaluation period, the uncoated control had high microbial counts (7.1±0.3 log CFU/g for Total Aerobic Bacteria and 4.8±0.2 log CFU/g for Yeasts and Molds). No statistically significant reduction ($P \geq 0.05$) was observed with the application of the blank carrier coating and is in accordance with previous studies showing that the base biopolymer has low inherent antimicrobial activity [11][12]. In contrast, the

application of botanical extracts alone resulted in a considerable ($P < 0.05$) reduction in microbial count. The garlic extract coating and the oregano extract coating reduced the aerobic bacteria to 5.6 ± 0.4 log CFU/g and 5.3 ± 0.3 log CFU/g respectively. The main mechanism behind this strong antimicrobial activity is the disruption of the cellular membrane and the collapse of the cellular homeostasis, caused by bioactive volatile monoterpenes and organosulfur matrices like carvacrol, thymol (oregano), and allicin (garlic), amongst others, which are responsible for disrupting intercellular communication pathways, including quorum sensing [13][14]. The highest inhibition was observed in the Synergistic Coating (Garlic + Oregano) where the aerobic bacteria count and the aerobic fungi count decreased significantly to 4.7 ± 0.2 and 2.1 ± 0.1 log CFU/g, respectively. This statistically superior difference ($P < 0.05$, index c) demonstrates a strong multi-target synergistic interaction. The simultaneous use of different chemicals . profiles act cooperatively, which means that lower individual levels of phytochemicals result in systemic cellular damage in a wider range of spoilage microorganisms [15]. The enzymatic browning caused by polyphenol oxidase (PPO) and oxygen is one of the most important factors affecting consumer choice. The uncoated control had a higher BI value of 48.2 ± 2.5 . The blank carrier lowered this figure to 41.3 ± 1.8 , because it acts as a physical barrier to the diffusion of O₂ from the atmosphere. The presence of garlic extract and oregano extract also reduced the BI to 38.5 and 36.1, respectively, reflecting the powerful free-radical scavenging and antioxidant properties of the phenolic compounds that are part of the botanical extracts. The absolute lowest browning rate (24.8 ± 1.3) was observed in the Synergistic Coating, suggesting that the combination of both extracts produce an improved antioxidant barrier and effectively reduces PPO activity.

Conclusions: Synergistic Coating using Garlic and Oregano extracts is a very effective approach in post-harvest intervention strategies for fresh-cut produce. The blank carrier coating offers some basic physical barrier to moisture loss, The active packaging matrix, proposed by the synergic action of garlic and oregano extracts, aims to take advantage of a powerful multi-target hurdle approach.

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