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Design of nanoemulsion systems of essential oil alongside chitosan to control gray mold spoilage in strawberries

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ABSTRACT

Strawberry is a perishable fruit and gray mold is one of the main reasons which reducing its shelf life. Due to the limitations of using chemical poisons, it seems necessary to use safe methods such as the use of essential oils (EOs) and edible coatings to control this fungus maintain the quality, and increase the storage period. However, the high volatility of EOs and organoleptic effects on agricultural products have hindered the direct application of EOs. This study is designed to enhance the shelf life of strawberries by designing nanoemulsion systems of EO with chitosan as follows: in the first experiment, the effect of nanoemulsion of the thymol, one of the main components of thyme, with a concentration of 5 g/L, alone and in combination with chitosan (CH) biopolymer was investigated on Botrytis cinerea. All treatments significantly reduced fungal growth compared to the control sample. Also, combined treatment showed the highest level of inhibition of B. cinerea. The second experiment was performed to evaluate the quality changes and post-harvest wastes of strawberries during storage. In this section, the fruits were coated with thymol 0.5%, thymol nanoemulsion 0.5%, and thymol nanoemulsion 0.5% + CH 0.5% and kept at 4 °C. Results presented that the application of the applied treatments had a positive effect on the physicochemical and biochemical indicators of strawberry fruit during the post-harvest period and caused better preservation of firmness, prevented weight loss and reduced microbial load. Also, the lowest rate of weight loss, the highest rate of firmness, and the lowest rate of growth of microorganisms were observed in covered fruits with treatment of thymol nanoemulsion 0.5% + CH 0.5%. Finally, this treatment can be suggested as a suitable cover to maintain the quality and reduce post-harvest waste of strawberries.

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1- Introduction

Strawberries are very popular worldwide due to their appealing color, aroma, and flavor. They are rich in various bioactive compounds, including flavonoids, tannins, polyphenols, anthocyanins, vitamins, and amino acids [1]. Despite their nutritional benefits, strawberries are highly perishable and vulnerable to physical damage and pathogenic attacks, particularly by fungi. This perishability limits their shelf life to about 3-4 days at room temperature [2]. One of the major threats to strawberries during storage and transportation is the fungal pathogen *Botrytis cinerea*, which causes gray mold rot. This pathogen can lead to significant losses in strawberry quality [3]. While the use of fungicides is a common method to control this fungus, there is growing consumer concern about the residues of these chemicals on fruits, leading to restrictions on their use [4].

Essential oils (EOs) naturally contain antimicrobial compounds, and their use is increasingly popular due to the growing preference for these natural substances. Thymol, a primary component of thyme (*Thymus vulgaris*) EO, is a phenolic and hydrophobic compound. It can bind to proteins in cell walls and plasma membranes, causing their degradation and increasing permeability [5]. Thymol is utilized as a natural antifungal agent in various coatings and active packaging to extend the shelf life of fruits and vegetables [5]. However, the practical use of EOs faces challenges due to their volatility, low water solubility, and physical and chemical instability. Additionally, EOs can impart sensory effects, such as odor and taste on the products [6].

Recently, encapsulating EOs using emulsion systems has been an effective method to reduce their sensory impacts. Emulsions are classified into three groups based on droplet size: microemulsions, nanoemulsions, and macroemulsions. Among these, nanoemulsions, with droplet sizes around 200 nm, are particularly notable. Their smaller droplet size offers greater stability and enhances antimicrobial properties due to increased cellular absorption [7]. Nanoemulsions offer several benefits, including the encapsulation of nutrients and reduced oxidation, lower fat content, and the use of entirely natural and biocompatible components [7]. Coating nanoparticles with various biopolymers further enhances the stability of the encapsulated compounds and facilitates the formation of dual emulsions, leading to more effective and durable applications [8]. Nanoemulsions consist of a water phase, an oil phase, and a surfactant. These nanoemulsions are used as systems to deliver water- and fat-soluble substances, flavors, and antibacterial agents in the food and pharmaceutical industries [9]. The methods for creating nanoemulsions are categorized based on energy usage into high-energy and low-energy methods. Low-energy methods rely on the spontaneous formation of droplets at the boundary between the oil and water phases, heavily depending on the nature of the surfactant. Techniques such as spontaneous emulsification, membrane emulsification, solvent displacement, and phase inversion are examples of these methods [10]. Another technique to extend the shelf life of highly perishable fruits like strawberries is the use of edible and biodegradable coatings. These coatings, made from natural sources, effectively prevent the escape of gases and water vapor from the fruit. They also help maintain the fruit's flavor, texture, color, and appearance.

Various biocompatible compounds such as polysaccharides (alginate, cellulose, chitosan (Ch), proteins (gelatin, zein, isolated whey protein), and their derivatives have been used to create edible coatings [2]. In this project, simultaneously, a combination of two techniques, nanoemulsification of thymol and Ch-based edible coating (a cationic polysaccharide with a positive charge), has been employed to prevent gray mold growth and extend the shelf life of strawberries. Essentially, this combination aims to form a polymeric matrix for these purposes. The hypothesis is that the Ch coating not only controls the release rate of thymol but also provides a more effective inhibitory coating due to its antimicrobial effects.

2- Materials and methods

2.1. Effect of thymol nanoemulsion and Ch biopolymer on controlling *B. cinerea* (gray mold) on strawberry via contact method

2.1.1. Sample collection

Strawberries (*Paros* cultivar) with approximately 80% red coloration were randomly harvested in Aban 1399 (November 2020) from a farm located in Gozneh village, Sanandaj. The fruits were immediately transferred to the Faculty of

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Agriculture and stored at 4°C until treatments were applied.

2.1.2. Pathogenic fungus

Isolation of *B. cinerea* from infected parts of strawberry bushes in contaminated fields of Kurdistan province was performed. Colonies of this fungus were prepared by culturing infected samples on Sabroud Dextrose Agar (SDA) medium and incubating at 25°C for 3 to 5 days.

2.1.3. Thymol

In this experiment, to prepare the organic phase of the nanoemulsion, 500 mg of thymol and 4 g of carrier oil (MCT oil) were mixed homogeneously for 15 minutes at 800 rpm using a magnetic stirrer. Then, Tween 80 (emulsifier) was added to the oil phase at a ratio of 2:1 and mixed thoroughly for 1 hour at 800 rpm using a magnetic stirrer.

2.1.4. Thymol nanoemulsion

Nanoemulsions were prepared by combining three components: aqueous phase, carrier oil, and thymol (concentration of 500 mg mL^{-1}), along with an emulsifier in a ratio of 8:4:88 using the self-emulsification method, accompanied by titration of the organic phase into the aqueous phase [11]. The experiment was conducted inside a 100 mL beaker at room temperature. Initially, the oil phase was prepared as described in section 2-1-3 and homogenized. Then, the oil phase was slowly added dropwise to the aqueous phase. During this phase, the emulsifier along with the carrier oil and thymol (oil phase) was added drop by drop at a rate of 0.5 mL per minute. Throughout the mixing process, the two phases were homogenized at 1200 rpm using a magnetic stirrer. Finally, after completing the phase mixing, the stirring of the resulting nanoemulsion continued at the same speed for an additional 30 minutes.

2.1.5. Thymol nanoemulsion and chitosan solution (Ch) 0.5%

Similar to section 2.1.3, the organic phase for the nanoemulsion was prepared. For the Ch solution, 2 mL of acetic acid was added to 100 mL of sterile distilled water. Then, this solution was placed on a magnetic stirrer, and 0.5 g of Ch was gradually added over 45 minutes until completely dissolved. Subsequently, the organic phase of the nanoemulsion was added dropwise at a rate of 0.5 ml per minute to the Ch solution and homogenized at 1200 rpm using a magnetic stirrer until thoroughly mixed.

2.1.6. Droplet size analysis

The particle size (Z-average) and size distribution (Polydispersity Index, PDI) of the prepared nanoemulsions were determined using Dynamic Light Scattering (DLS) analysis (Zetasizer Nano ZS 3600, Malvern Instruments, Malvern, U.K.). All measurements were conducted after overnight sample storage at room temperature.

2.1.7. Preparation of pathogen inoculum

Colonies of the fungus *B. cinerea* were grown by culturing infected samples on SDA and incubating them for 10 days at 25°C. To harvest the fungal spores, 10 mL of sterile distilled water was added to the culture plates, and the colonies were gently scraped with a sterile blade. The resulting suspension was then filtered through three layers of sterile gauze to separate the fungal structures and vegetative parts. A hemocytometer was used to count and determine the concentration of the fungal conidia (spores) in the suspension.

2.1.8. Inoculation with fungus and treatment with thymol, thymol nanoemulsion, and thymol nanoemulsions combined with Ch biopolymer

The effect of thymol, thymol nanoemulsion, and thymol nanoemulsions combined with Ch biopolymer against *B. cinerea* on Paros variety strawberries was tested under laboratory conditions. The strawberries were disinfected using 70% ethanol for 25 seconds, then washed three times with sterile distilled water. After the strawberries dried, shallow wounds were made on the surface of each fruit, and 20 µL of the fungal spore suspension (at a concentration of 10^6 conidia/mL) was applied to the wounds. Once the inoculated wounds dried, the strawberries were dipped into the prepared treatment solutions. The treatments included thymol solution, thymol nanoemulsion, and thymol nanoemulsions combined with Ch biopolymer. For the negative control, strawberries were dipped in sterile distilled water. For the positive control, strawberries were inoculated with the fungal spore suspension without any antimicrobial treatment. All treated strawberries were placed in plastic containers with lids, sealed, and stored at 25°C for one week. The experiment was repeated three times for each treatment.

2.1.9. Infection index

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The extent of fungal infection on strawberry fruits was visually assessed using the following index [13]: **Grade 0**: 0% infection, **Grade 1**: Less than 20% infection, **Grade 2**: 20.1% to 40% infection, **Grade 3**: 40.1% to 60% infection, **Grade 4**: 60.1% to 80% infection, **Grade 5**: 80.1% to 100% infection

The infection index was calculated using the formula below:

$$\text{Infection index \%} = \frac{\sum(\text{grade} \times \text{number of infected fruits})}{4 \times 6} \times 100$$

This method provides a quantitative measure of the severity of infection across the treated fruits.

2.2.1. Application of treatments

Healthy strawberries, free from mechanical damage and uniform in color and size, were selected for treatment. After washing with sterile distilled water, the strawberries were divided into four groups for treatment: thymol 0.5% solution, thymol nanoemulsion 0.5%, thymol nanoemulsion 0.5% + Ch 0.5%, and sterile distilled water (control). The strawberries were immersed in these treatments at room temperature (25°C) for 20 seconds. After immersion, they were allowed to dry at room temperature for one hour. Each treatment group was then placed in covered plastic containers and stored in a cold room at 4°C with 75% relative humidity for 15 days. Sampling was conducted periodically on days 0, 3, 6, 9, 12, and 15 to measure the post-harvest quality and spoilage characteristics of the strawberries. Day 0 refers to the day of harvest from the farm.

2.2.2. Weight loss

To assess the weight loss of the strawberries, each batch was weighed before storage using a precision balance with an accuracy of 0.001 g. This initial weight was recorded as the primary weight. On the sampling days (days 0, 3, 6, 9, 12, and 15), the strawberries were weighed again after being removed from cold storage to record the secondary weight for that time point. The percentage of weight loss for the strawberries was calculated using the following formula as per the AOAC standard [14]:

$$\text{Weight loss \%} = \frac{\text{initial weight - secondary weight}}{\text{initial weight}} \times 100$$

This calculation helped determine how much weight the strawberries lost during the storage period.

2.2.3. Firmness

The firmness of the strawberry tissue was measured using a texture analyzer (Santam STM-1, Iran). This device was equipped with a probe that had a convex tip with a diameter of 8 mm. The probe was moved at a constant speed of 20 mm per minute. During the measurement, the probe was penetrated to a depth of 8 millimeters into the fruit. This process was repeated on the opposite side of each fruit for all samples and at all designated time points. The firmness readings for each treatment were then averaged and expressed in Newtons (N), providing a measure of how resistant the fruit was to compression over the storage period.

2.2.4. Soluble solids content

At specified sampling intervals, pieces of strawberries from each treatment were separated, and their juice was manually extracted and collected. After filtering, the juice was used to measure the total soluble solids (TSS) and titratable acidity (TA). TSS, expressed as degrees Brix was measured using a handheld refractometer (Atago ATC, Japan) at a temperature of 22°C. This measurement provided an estimate of the sugar concentration in the juice, which is a key indicator of the fruit's sweetness and overall quality during storage.

2.2.5. Titratable Acidity

To determine TA, 3 mL of the extracted juice was mixed with 27 mL of distilled water. This mixture was titrated with 0.10 N NaOH until the pH reached 8.2. The TA was then calculated as a percentage of citric acid using the following formula [15]:

$$TA = \frac{Eq_{acid} \times V_b \times 0.1}{V_j} \times 100$$

Eq_{acid}: Equivalent weight of dominant acid; V_b: Volume of NaOH used; V_j: Volume of juice used

2.2.6. Microbial load analysis

In this test, a dilution (10^{-1}) was made by mixing 10 g of each treatment with 90 mL of sterile peptone water. Subsequent dilutions of 10^{-2} and 10^{-3} were prepared using peptone water. The surface plating method was used with PDA (Potato Dextrose Agar, Merck, Germany) as the culture medium for counting total molds and yeasts. The PDA medium was prepared according to the instructions (39 g of medium in 1 L of water) and sterilized at 121°C for 15 minutes. The molten solid medium was then added to the plates under sterile conditions at 40-45°C. After solidification, 100 µL of the sterile peptone water and fruit tissue mixture was

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inoculated onto the petri dishes and spread on the surface of the medium using a sterile L-shaped rod. The petri dishes were incubated upside down for 48 hours at 25°C. This test was performed in three replicates. After incubation, the colonies were counted, and the final results were reported as the logarithm of colony count per gram of fruit [16].

2.2.7. Statistical analysis

The statistical analysis of the first part of the experiments was conducted using a completely randomized design with five treatments and three replicates using MSTATC software. The least significant difference (LSD) test was used for comparing means. The second experiment was conducted with two factors, treatment and time, in a factorial design with three replicates. The first factor was treatments at four levels (thymol 0.5%, thymol nanoemulsion 0.5%, thymol nanoemulsion 0.5% + Ch 0.5%, and control) and the second factor was storage time at six levels (days 0, 3, 6, 9, 12, and 15). Statistical analysis of the data was performed using MSTATC software. After examining the ANOVA tables, means were compared at the 1% or 5% significance level, and Excel from the Office 2013 suite was used to plot the graphs.

3- Results and Discussion

3.1. Effect of thymol nanoemulsion and Ch biopolymer on controlling *B. cinerea* on strawberries using contact method

3.1.1. Particle size analysis

The particle size of the prepared nanoemulsions was determined using a DLS device. According to the results, the average particle size index for thymol nanoemulsion and thymol nanoemulsion + Ch treatments were 60.45 ± 2.1 nm and 77.61 ± 1.9 nm, respectively (Table 1). As the DLS results indicate, the production of the nanoemulsion was successfully achieved using the spontaneous method. This method has also been successfully used in other studies to produce emulsions with droplets in the nanometer range.

Table 1: Average Particle Size (Z-Average) and polydispersity index (PDI) of nanoemulsion obtained from DLS.

Treatment	Z-Average (d.nm)	PDI
NanoEm-Thy 0.5%	60.45 ± 2.1	0.5
NanoEm-Thy 0.5% + Ch 0.5%	77.61 ± 1.9	0.45

3.1.2. Evaluation of the effect of treatments on gray mold of strawberries using the contact method in laboratory conditions

According to the results, all the studied treatments significantly reduced fungal growth compared to the infected control. Among them, the combined treatment of thymol nanoemulsion 0.5% with Ch 0.5% exhibited the highest inhibition of gray mold decay on the fruit (Figure 1). Recently, extensive research has been conducted on controlling post-harvest fruit diseases using thymol combined with the Ch [19, 20]. The results of this study indicated that encapsulating thymol droplets via nano-emulsification enhances the antifungal effect of thymol against *B. cinerea*. Using nanoemulsions of EOs, due to their smaller particle sizes, plays a significant role in increasing cellular uptake, thereby enhancing the antimicrobial properties of the EOs [7]. In one study, a nanoemulsion of lemon EO, prepared by high-pressure homogenization, significantly inhibited the germination of spores and the growth of the *Phomopsis sp.* mycelium, which causes post-harvest decay of kiwi fruit [21]. Another study utilized cinnamon EO emulsion and nanoemulsion along with the fungicide thiabendazole to control the growth of two molds, *B. cinerea* and *Rhizopus stolonifer*, on strawberries. Results showed that the nanoemulsion of EO had a greater impact on reducing the fungal decay of strawberries compared to the emulsion of EO. Furthermore, at the highest concentration (0.2%), the cinnamon EO nanoemulsion showed greater antifungal activity compared to thiabendazole [22]. In this study, the greatest antimicrobial activity was observed with the thymol nanoemulsion combined with the biopolymer Ch. Additionally, the controlled release of thymol encapsulated within nano-droplets during storage could contribute to extending the shelf life of strawberries. The interaction between the positive charges of Ch and the negative charges

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of microbial cell membranes disrupts cell wall structure, ultimately leading to cell death [19].

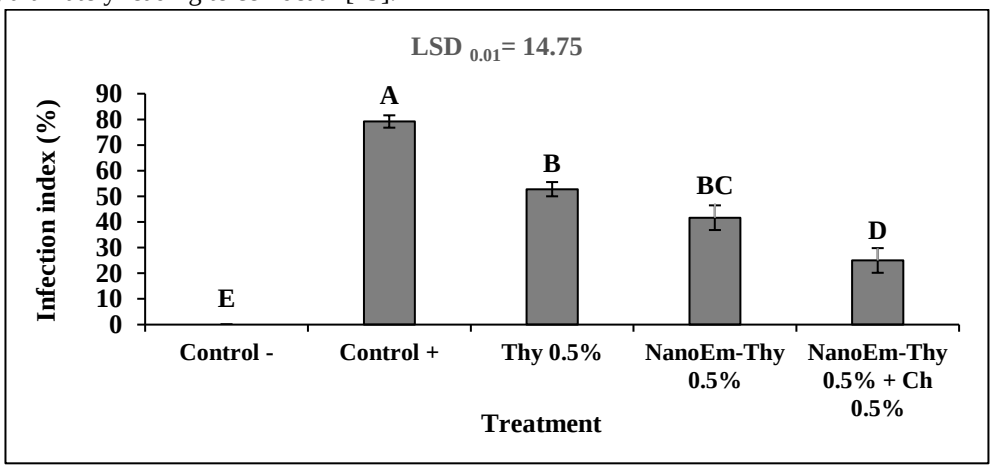

Fig 1. Influence of thymol (Thy), thymol nanoemulsion (NanoEM-Thy) and thymol nanoemulsion + chitosan (NanoEM-Thy + Ch) on strawberry fruit inoculated with *B.cinerea*. Fruit stored at 25 °C up to 7 days. The same letters are not significantly different ($p < 0.01$) according to the LSD test.

3.2. Effect of treatments on quantitative, qualitative, and biochemical indicators of strawberry fruit during storage

3.2.1. Weight loss

Throughout the storage period, the percentage of weight loss increased in all treatments. However, lower percentages of weight loss were observed in strawberries treated with coatings compared to the control. Specifically, at the end of the storage period, the highest and lowest percentages of weight loss were observed in the control fruits and the fruits treated with the thymol nanoemulsion 0.5% + Ch 0.5%, with values of 5.80% and 2.32%, respectively (Figure 2A). During the storage period, the percentage of weight loss in fruits and vegetables primarily increases due to respiration and evaporation of moisture from the surface of the fruit, which also depends on the storage temperature and humidity [23]. The percentage of weight loss can vary depending on the type of product, cultivar, and the textural properties of the product [24]. Strawberries are highly sensitive to weight loss, which results in a reduction in fruit quality [25]. Edible coatings create a physical barrier against moisture loss, protect the skin from mechanical damage, and repair minor wounds, thereby reducing weight loss [6, 23].

Results indicate positive effects of edible coatings on reducing weight loss in a wide range of fruits, such as strawberries [23], plums [26], pomegranates [27], and citrus fruits [28] during storage. Moreover, plant EOs can help control weight loss by reducing respiration rates and delaying senescence [29]. In one study, the use of eugenol and thymol delayed ripening and aging processes in grapes compared to untreated fruits [30]. Additionally, microbial contamination is also a significant factor in fruit weight loss during storage [31]. The application of Ch coatings along with cinnamon EO controlled decay and reduced weight loss during storage in jujube fruit [32].

The weight loss results demonstrated that edible coatings act as a physical barrier against moisture loss to the external environment, thereby reducing the amount of water lost from the fruit. Furthermore, the use of thymol nanoemulsion treatments combined with Ch may play a more effective role in reducing moisture loss from the surface of strawberries. It is also possible that the combined treatment reduces respiration intensity and delays aging, thereby preventing weight loss during storage [19].

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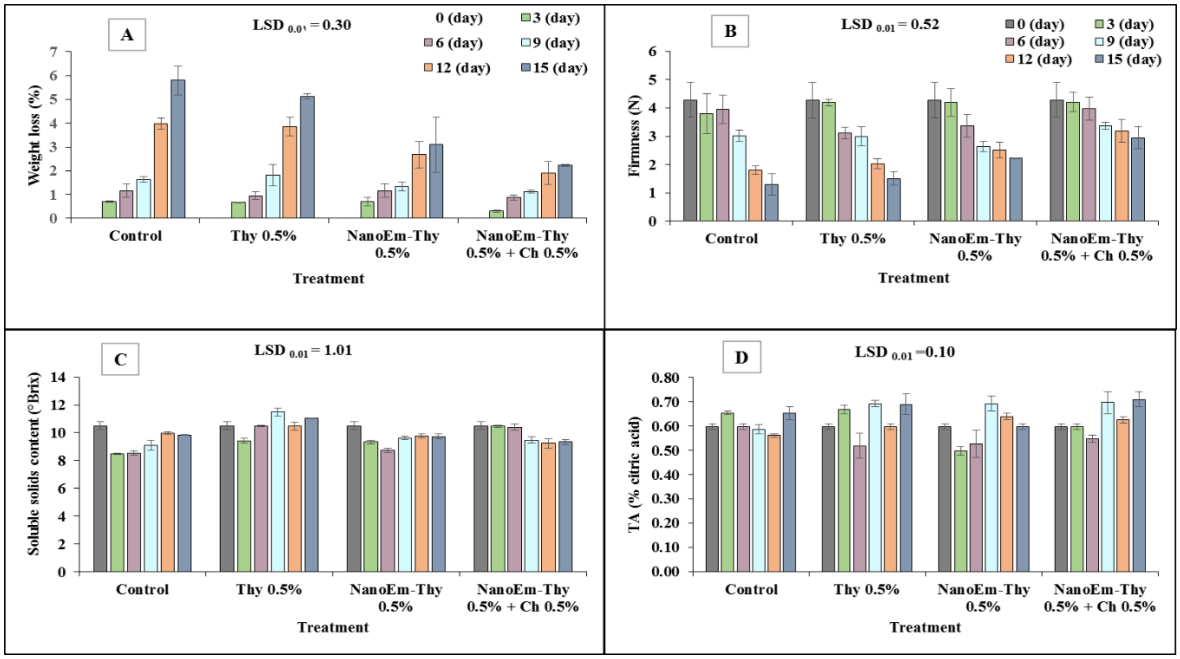

Fig 2. Weight loss (A), firmness (B), Soluble solids content (C) and titratable acidity (D) in strawberry fruit untreated or treated with thymol (Thy), thymol nanoemulsion (NanoEM-Thy) and thymol nanoemulsion + chitosan (NanoEM-Thy + Ch). Fruit stored at 4 ± 1 °C up to 15 days. Data represent the means ± SE, n = 3.

3.2.2. Firmness

The firmness of the fruit tissue in both coated and uncoated fruits decreased during the storage period. However, the rate of firmness reduction was slower in fruits treated with the combined nanoemulsion of thymol and Ch. The highest tissue firmness was observed in fruits treated with thymol nanoemulsion 0.5% + Ch 0.5% (2.95 N) after 15 days of storage at 4 ºC, while the lowest firmness was found in the control fruits (1.30 N) and fruits treated with thymol 0.5% (1.5 N) (Figure 2B). Firmness is one of the most important quality attributes of strawberries. During storage, the activity of pectin-degrading enzymes such as polygalacturonase and pectin methyl esterase typically leads to the breakdown of the cell wall and a reduction in tissue firmness [33]. Additionally, a decrease in respiration rate results in reduced activity of cell wall-degrading enzymes [34].

In general, edible coatings slow down the softening process of the fruit, likely due to the barrier properties of the coating against gas exchange between the fruit and the environment, which leads to reduced metabolic activities, including respiration, and consequently increases the shelf life of the product [35]. In fact, the firmness of strawberry tissue is directly related to weight loss and decay [36]. EOs play an effective role in delaying fruit softening by maintaining turgor pressure and consequently controlling weight loss [37]. It has been reported that the application of Ch with rue EO reduces transpiration, maintains turgor pressure, and ultimately preserves the firmness of guava fruit during storage [38]. Additionally, it has been reported that coating avocado fruit with CMC prevents tissue softening by reducing fungal activity and the growth of fungal mycelium on the fruit surface [39].

The results of this study showed that the use of thymol nanoemulsion, both alone and in combination with Ch, can help maintain the firmness of strawberries during the storage period. This reduction in firmness changes may be due to the formation of a barrier against the transfer of moisture, oxygen, and carbon dioxide, as well as the reduction of the activity of cell wall-degrading enzymes.

3.2.3. TSS

TSS remained relatively constant across all treatments during the storage period, with no significant difference observed between the day of harvest and the end of the storage period (Figure 2C). Soluble solids contain important components, especially sugars and organic acids, which are responsible for the flavor and thus the acceptance of the product by consumers [40]. In strawberries, a

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non-climacteric fruit, soluble solids are consumed in the respiration process after harvest, which consequently decreases their levels [41]. Additionally, water loss and the resulting increase in concentration can lead to an increase in Brix [42]. On the other hand, Brix levels can increase with the hydrolysis of starch into glucose, which intensifies with fruit ripening and cell wall degradation [43]. The use of Ch and CMC coatings in citrus fruits helped maintain acidity and soluble solids content throughout the storage period by affecting respiration [44].

3.2.4. TA

The TA increased from 0.06% on the day of harvest to 0.69% and 0.71% in fruits treated with thymol 0.5% and 0.5%, respectively. However, in other treatments, no significant differences in TA were observed between the day of harvest and the end of the storage period (Figure 2D).

Previous studies have reported a decrease in TA during the storage period of strawberries due to metabolic changes and the consumption of organic acids during cellular respiration. However, in this study, due to controlled respiration through appropriate coating and prevention of fungal activity, such a trend was not observed [45, 46]. It has also been reported that strawberries coated with Ch showed a reduced decline in acidity, attributed to decreased respiration rates [2]. Additionally, the maintenance of acidity and prevention of changes in grapes and bananas coated with thyme EO has been reported [47]. In contrast, coating strawberries with cumin EO had a significant effect on increasing acidity during the storage period [48].

3.2.5. Microbial analysis

The total mold and yeast population in strawberries was 3.76 Log CFU/g on the day of harvest. By the end of the storage period, the total mold and yeast population increased in the control, thymol 0.5%, and thymol nanoemulsion 0.5% treatments, all of which were statistically similar. However, in the treatment with thymol nanoemulsion 0.5% + Ch 0.5%, there was no significant difference in the total mold and yeast population between day 0 and day 15 of the storage period. The lowest total mold and yeast population at the end of the storage period was observed in fruits treated with thymol nanoemulsion 0.5% + Ch 0.5% (4 Log CFU/g) (Figure 3).

This suggests that the combination of thymol nanoemulsion and Ch was effective in inhibiting the growth of molds and yeasts, thereby maintaining the microbial quality of the strawberries over the storage period. Molds and yeasts are among the most significant causes of quality reduction and increased post-harvest losses in strawberries. Therefore, reducing their levels on the fruit surface can help maintain quality and extend the shelf life of the products [49]. The growth of microorganisms on the fruit surface produces abundant extracellular enzymes such as pectinase and cellulase, leading to cell wall degradation, increased spoilage, and reduced product quality [50]. Storing strawberries at low temperatures delays the growth of microorganisms [51]. Active edible coatings are widely used to extend the shelf life of horticultural products. The antifungal and antibacterial properties of plant EOs against various microorganisms have been reported [52]. EOs can also delay tissue softening and aging, reducing tissue susceptibility to microorganisms [53]. In one study, the use of an edible coating containing thymol and calcium chloride significantly increased the shelf life of strawberries [54]. Another study showed that an edible coating containing Ch on strawberries could prevent rapid microbial growth during storage [2]. Additionally, using a CMC coating on pears during storage significantly reduced fungal growth [55]. Therefore, edible coatings can prevent the growth and activity of microorganisms, reduce respiration rates, delay spoilage, and ultimately increase the storage life of strawberries.

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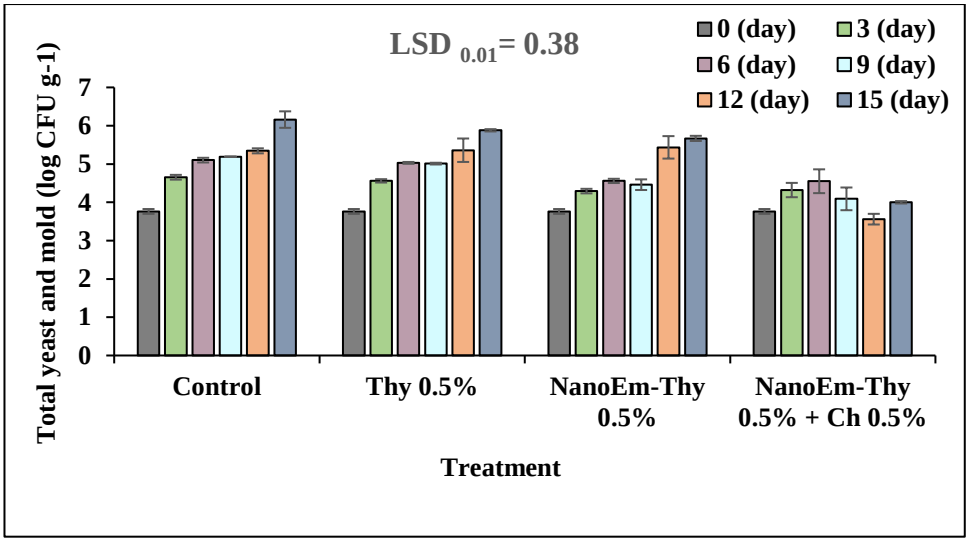

Fig 3. Total yeast and mold in strawberry fruit untreated or treated with thymol (Thy), thymol nanoemulsion (NanoEM-Thy) and thymol nanoemulsion + chitosan (NanoEM-Thy + Ch). Fruit stored at 4 ± 1 °C up to 15 days. Data represent the means ± SE, n = 3.

4- Conclusion

This study demonstrated that the use of thymol nanoemulsion alone or in combination with the biopolymer Ch can play a significant role in maintaining quality and reducing post-harvest losses of strawberries. The laboratory tests on the efficacy of the treatments against *B. cinerea*, the causal agent of gray mold in strawberries, showed that all treatments effectively reduced fungal growth. However, the combined treatment of thymol nanoemulsion with Ch exhibited the highest inhibition percentage against *B. cinerea* growth.

The study's findings on the quantitative, qualitative, and biochemical indices of strawberries during the storage period indicated that the highest weight loss percentage after 15 days of storage at 4°C was observed in the control group, while the lowest was in the strawberries treated with thymol nanoemulsion 0.5% + Ch 0.5%. Additionally, the thymol nanoemulsion 0.5% + Ch 0.5% treatment helped maintain fruit firmness and more effectively reduced microbial growth during the storage period. Therefore, the thymol nanoemulsion 0.5% + Ch 0.5% coating can be recommended as the most suitable coating for maintaining quality and reducing post-harvest losses of strawberries.

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مقاله علمی–پژوهشی

طراحی سامانه‌های نانوامولسیونی اسانس روغنی همراه با کیتوزان جهت کنترل فساد کپک خاکستری در توت‌فرنگی

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چکیده

توت‌فرنگی میوه‌ای فسادپذیر بوده و بیماری کپک خاکستری یکی از عوامل کاهش طول دوره نگهداری این محصول است. بدلیل محدودیت‌های استفاده از سموم شیمیایی، جهت کنترل این قارچ و حفظ کیفیت و افزایش طول دوره نگهداری، بکارگیری روش‌های ایمن مانند استفاده از اسانس‌ها و پوشش‌های خوراکی ضروری به نظر می‌رسد. همچنین فراریت بالای اسانس و ایجاد تغییرات حسی در محصولات کشاورزی استفاده مستقیم آن‌ها را برای این منظور دچار چالش کرده است. این پژوهش به منظور افزایش ماندگاری میوه توت‌فرنگی با طراحی سامانه‌های نانوامولسیونی تیمول همراه با کیتوزان بدین شکل طراحی شده است. در اولین آزمایش اثر نانوامولسیون تیمول، از اجزاء اصلی گیاه آویشن (*Thymus vulgaris*)، با غلظت ۵ گرم در لیتر، به تنهایی و نیز در ترکیب با زیست پلیمر کیتوزان روی قارچ *Botrytis cinerea* بررسی شد که تمامی تیمارها در مقایسه با شاهد آلوده، رشد قارچ را به‌طور مؤثری کاهش دادند. در این میان تیمار ترکیبی بیشترین میزان بازدارندگی را نشان داد. دومین آزمایش جهت بررسی تغییرات کیفی و تلفات پس از برداشت میوه‌های توت‌فرنگی پوشش داده شده با تیمول ۰/۵ درصد، نانوامولسیون تیمول ۰/۵ درصد و نانوامولسیون تیمول ۰/۵ درصد در ترکیب با کیتوزان ۰/۵ درصد در دمای ٤ درجه سلسیوس انجام شد. بررسی نتایج حاصل از این آزمایش نشان داد بکارگیری تیمارهای مورد مطالعه بر شاخص‌های فیزیکوشیمیایی و بیوشیمیایی میوه توت‌فرنگی طی دوره پس از برداشت، اثر مثبتی داشتند و سبب حفظ بهتر سفتی، جلوگیری از کاهش وزن و کاهش بار میکروبی شدند. به‌طوری که کمترین میزان کاهش وزن، بیشترین میزان سفتی بافت میوه و کمترین میزان رشد ریزاندامگان در میوه‌های پوشش داده شده با تیمار نانوامولسیون تیمول ۰/۵ درصد+کیتوزان ۰/۵ درصد مشاهده شد. بنابراین این تیمار می‌تواند به عنوان پوشش مناسبی جهت حفظ کیفیت و کاهش ضایعات پس از برداشت میوه توت‌فرنگی پیشنهاد شود.

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