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# Experimental investigation of Zein Edible Film Effect Containing Nanoemulsion of anise (*Pimpinella anisum* L.) Essential Oil on the Shelf Life of Gouda Cheese

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ABSTRACT: In recent years, the antimicrobial properties of plant essential oils and their nanoemulsions have received significant attention. In the present study, anise essential oil and its nanoemulsion were added to Zein films at minimum inhibitory concentrations, and their mechanical properties were investigated. The prepared films were applied on the surface and sides of single-slice Gouda cheese and then placed inside polyethylene packaging. The samples were kept at 4°C in the refrigerator and subjected to testing at intervals of 0, 15, 30, 45, and 60 days. The results showed that except for day zero, the highest counts of mesophilic, psychrotrophic, enterobacteriaceae, lactic acid bacteria, molds, and yeasts were observed in the control sample, while the lowest counts were observed in the Gouda cheese covered with Zein film containing 4% nanoemulsion of anise essential oil. The highest overall taste, texture, color, and overall acceptance scores were observed in the Gouda cheese samples covered with Zein film containing 4% nanoemulsion of anise essential oil. Based on the results of this research, the use of nanoemulsion of anise essential oil in Zein films can be considered as an effective method to improve the sensory and antibacterial properties of Gouda cheese. These findings can be highly beneficial and practical for food producers and related industries in enhancing product quality, attracting customers, and improving consumer satisfaction.

**Keywords**: *Gouda Cheese, Nanoemulsion of Anise Essential Oil, Microbiological Analysis, Sensory Analysis,* Zein films

## Introduction

Nowadays, there is an increasing demand for consuming healthy food and a preference for using plant extracts (Veiga *et al*., 2020) and essential oils (Matera et al., 2023) instead of synthetic and chemical additives in food products. Due to the well-known biological properties

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and flavor enhancement of plant extracts and essential oils, this category of compounds is considered among the most widely used additives (in various forms) in different food products (Al-Maqtari *et al*., 2021; Hou *et al*., 2022). The antimicrobial properties of plant essential oils against a wide range of microorganisms, including bacteria, yeasts, and molds, have been demonstrated (Chacha *et al*., 2022; Dupuis *et al*., 2022). The search for safe and effective antimicrobial agents continues, which can be used for both therapeutic and preventive purposes against a wide range of bacterial infections (Zou *et al.,* 2023). One of these important compounds is essential oils, which exhibit numerous biological effects (Zhao *et al.,* 2022). It has been revealed that many essential oils extracted from medicinal plants possess antifungal (Afsharnia, 2023), antiparasitic (Chandrawanshi *et al.,* 2023), antibacterial (Hu *et al.,* 2023), and antiviral properties (Marquez *et al.,* 2022). These substances are rich in phenolic compounds and are widely used to increase the shelf life of food products. Their antioxidant properties prevent food items from spoilage, and their antimicrobial and inhibitory activities against bacteria contribute to extending their shelf life (Popescu *et al.,* 2023).

Anise has antimicrobial and antioxidant essential oil that can be used in the manufacture of active edible films

Edible films and coatings are thin layers of edible materials that are applied on the surface of food or between its components. They serve as one of the fundamental ways to control physiological(Yuan *et al.*, 2022), microbial(Kouser *et al.,* 2023), and physicochemical changes in food (Pouralkhas *et al.,* 2023). Hence, they are also referred to as active food packaging. Edible films and coatings should be non-toxic, neutral, transparent, clear, odorless, and tasteless, therefore they do not affect the food's sensory properties during consumption(de Paula *et al.,* 2023). Most of these films are used to prevent moisture and oxygen transfer, preserve aromas, flavor compounds, or oils, in order to enhance the quality and shelf life of food (Hakim *et al.,* 2023). Zein is a type of prolamine found in the endosperm of corn and constitutes over 50% of the total protein content. Its distinctive properties, attributed to a high concentration of non-polar amino acids relative to other plant proteins, make zein particularly suitable for creating films and edible coatings. Notable characteristics of zein biopolymer include its ability to form superior coatings and films, its solubility in organic solvents like ethanol, and its compatibility with antimicrobial and antioxidant compounds. These attributes contribute to zein's potential application in cheese packaging (Sayadi *et al.,* 2021). Embracing such innovative films in cheese packaging not only improves quality and shelf life but also proves economically advantageous by reducing packaging costs (Costa *et al.,* 2018). Among the various types of cheeses available worldwide, Gouda cheese is one of the most beloved and widely consumed cheeses. Gouda cheese is a semi-hard, yellowish cheese made from cow's milk (Düsterhöf *et al.,* 2017). Due to its popularity and high consumption, researchers have paid significant attention to improving the storage conditions of Gouda cheese using various methods, including the use of nanoemulsions and essences as films.

Nanotechnology finds applications in many areas of food science, including processing, packaging, and food enrichment with essential nutrients(Jayaweera *et al.,* 2021; Singh *et al.,* 2023). Nanoemulsions are kinetic stable systems with a unique droplet size, making them appear transparent or semi-transparent to the naked eye. They exhibit the necessary stability and resistance against sedimentation and creaming. Various methods have been proposed for the production of nanoemulsions, including high-energy methods (homogenizer, ultrasonic waves, and microfluidizer) and low-energy methods (self-emulsification and phase change) (Sugumar *et al.,* 2016; Asadinezhad *et al.,* 2019). Nanoemulsions are among the most well-known nanomaterials and serve as ideal carriers for delivering fat-soluble compounds(Duraisamy *et al.,* 2022). They are easily prepared, have small particle sizes, and enhance the accessibility, bioavailability, and stability of the encapsulated substances(Rathod *et al.,* 2024; Sharma *et al.,* 2022). Moreover, nanoemulsions inherently possess antibacterial properties because their structure allows them to sequester water particles, thereby preventing microorganisms' access to water*(Das et al., 2020; Balasubramanian et al.,* 2022).

Mohammadzadeh-Vazifeh *et al. (*2020) investigated microbial limitations in Gouda cheese by using an active nanocomposite containing chitosan and nanorose. Their study results demonstrated that the most effective antimicrobial effect was related to the nanocomposite layer with a composition of 3% (w/w) chitosan and 1% (w/w) nanorose addition, which could prevent the microbial spoilage of Gouda cheese. Therefore, the nanocomposite has the potential to be used as a surface coating not only for Gouda cheese but also for similar cheeses and other food items to enhance their microbial properties and extend their shelf life.

In a related study, Hajirostamloo *et al.* (2022) investigated soy protein isolate films that were embedded with varying ratios of microcapsules containing cardamom essential oil (CEOM). The results showed that the addition of CEOM increased the films' thickness, water vapor permeability, and flexibility, while simultaneously decreasing their tensile strength. The color parameters (L*, a*, b*, and ΔE) and optical properties of the films were also influenced by the incorporation of CEOM. Fourier-transform infrared spectroscopy results indicated the formation of hydrogen bonds within the film structure. Furthermore, the addition of CEOM significantly enhanced the antioxidant and antibacterial properties, as well as the total phenolic content of the films. Packaging Iranian white cheese with these soy protein isolate films contributed to an extended shelf life for the cheese.

In the study conducted by Kõrge *et al.* (2020) active films based on chitosan mixed with an extract rich in tannin from chestnut fiber (Castanea sativa Mill.) were used for packaging Gouda cheese contaminated with spoilage microflora including Pseudomonas fluorescens, Escherichia coli, and Penicillium commune. The microbiological analysis of the cheese revealed that the film without additives exhibited the most significant reduction in bacterial counts.

Pluta-Kubica *et al.* (2021) investigated the effect of recently developed nanocomposite films with silver nanoparticles (obtained by in-situ method) on the quality characteristics of two types of cheeses: Gouda cheese and acid-set cheese (quark). The results of their study showed that the total bacterial count during the storage of Gouda cheese remained unchanged regardless of the type of packaging used. However, the use of the nanocomposite film led to a reduction and inhibition of yeast growth in Gouda cheese, thereby improving the microbiological quality of the cheeses during storage. Gouda cheese is a soft cheese type that is store in brine. It may suffer from microbial spoilage caused by bacteria and molds during the ripening and storage period, so maintaining its desirable quality is very important. This study investigates how the use of edible films containing anise nanoemulsion in Zein can extend the shelf life and enhance the quality of Gouda cheese. The aim of the present work is investigated the effects of anise nanoemulsion on the physicochemical and antimicrobial properties of Zein film.

The objectives include prolonging the cheese's storage time, comparing the antioxidant and antimicrobial properties of anise nanoemulsion with pure anise essence, examining the impact of anise essence on Zein film characteristics, and assessing the effects of Zein-based edible films containing anise nanoemulsion on the microbiological and sensory attributes of Gouda cheese under refrigeration. So far, no study has been done in the production of Zein-based films containing anise nanoemulsion. The study aims to provide insights for improving food packaging and preservation in the industry.

## Materials and Methods

- ***Materials***

This study has utilized a wide range of materials, and in this section, an overview of these materials along with their manufacturers will be provided. The materials used, which were obtained from Merck Germany, are as follows: Physiological serum, immersion oil, sulfuric acid, bromocresol green, thiobarbituric acid, chloroform, starch solution, citric acid, magnesium oxide, sodium hydroxide, boric acid, gallic acid, streptomycin antibiotic, $FeCl_3$, methanol, ethanol, acetate buffer, hexane, dimethyl sulfoxide 4% (DMSO), muller Hinton Broth (MHB), sodium chloride. Regarding gouda cheese slices, it should be mentioned that it was provided from Kalleh Company, a well-known Food processing company in Iran.

- ***Pimpinella anisum essential oil nanoemulsion preparation***

Initially, 1 mL of Anise essence (produced by Zardband Tehran Company) was well-mixed with 1 mL of Tween 80 (produced by Sigma Aldrich, Germany) in equal proportions, serving as the oil phase and surfactant, respectively, using a magnetic stirrer (Sartorius AG company, Germany). Next, the aqueous phase, consisting of distilled water (97 mL), was mildly acidified using citric acid (0.3%) (pH 6.8). This acidified aqueous phase was then slowly added to the oil phase and surfactant mixture while stirring the pre-emulsion mixture on a magnetic stirrer at 700 RPM. This process resulted in the preparation of the pre-emulsion mixture. Subsequently, the prepared pre-emulsion mixture was subjected to an ultrasonic bath (power: 100 watts, frequency: 40 kHz) for 20 mins to reduce the particle size and produce a nanoemulsion under the influence of ultrasonic waves. The nanoemulsion was stored in refrigerator at 4 °C until use.

- ***Nanoemulsion characterization***
- ***Particle size***

A Laser nanoparticle size analyzer (Mastersizer 3000, Malvern Instruments, UK) was utilized at room temperature to measure the average particle size and particle size distribution of the emulsion. The parameters were configured with a particle refractive index of 1.453, a particle absorption rate of 0.001, and a dispersant refractive index of 1.330. The droplet particle size was characterized by the mean volume diameter, with three replicates performed for accuracy.

- ***Turbidity during storage***

These samples were storage and their turbidity were measured during 60 days storage in refrigerator at 4 °C (with intervals of 0, 15, 30, 45, and 60 days).

- ***Total phenol content***

25 mg of each sample (anise essential oil and nanoemulsion loaded with anise essential oil) was immersed in 3 mL distilled water at 50 °C until completely dissolved, and the mixture was centrifuged at 6000 rpm for approximately 10 min. Then, 1 mL of the supernatant was added to 5 mL of Folin–Ciocalteu reagent and kept at ambient temperature for about 10 min. Next, 4 mL of sodium carbonate (7.5% (w/v)) was added and the absorbance number was read after 60 min at the wavelength of 765 nm. A standard curve was prepared using gallic acid solutions (10–100 µg mL−1).

- ***Zein film preparation***

As corn zein film is not water-soluble, corn zein (5.5 g) was mixed with 95% ethyl alcohol (50 mL), along with stabilizers glycerol and polyethylene glycol in a 1:1 ratio (20% by weight based on corn zein), to act as a plasticizer, preventing film fragmentation. The mixture was prepared at a temperature of 70 °C. Subsequently, the corn zein films was cooled to 60 °C and then following Table 34, the anise essence nanoemulsions (4% w/v) was added to it. Control solution (without nanoemulsion) and solution containing anise essence nanoemulsion, approximately 40 mL, was were poured into aluminum plates with dimensions of 7x5.17 cm. The films were then dried at a temperature of 25 °C and 50% humidity. The films were stored in a dryer at 25 °C and relative humidity of 50±4%.

- ***Film characterization***

The thickness of each film was measured at five 20 different points locations using a digital caliper with an accuracy of 0.001/00.01 mm.

- ***Mechanical properties***

The mechanical tests of the films were conducted based on the modified ASTM D0882-02 method. For tensile strength (TS), the films were cut into 1 × 7 centimeter cm specimens and conditioned for 24 h under 50% relative humidity and 25 °C. The testing machine (Instron) had a 50- mm distance between the two grips, with the upper grip moving at a speed of 50 per min while the lower grip remained fixed. The peak force, breaking force, elongation at break (EB), and energy to break were determined during the tensile test (ASTM, 2000b). This testing approach provides valuable information about the mechanical properties of the films and their ability to withstand stretching and breaking forces. TS and EB have estimated at least six replicates.

- ***Application of films on Gouda cheese slices***

The films were placed on the surface and the slices of Gouda cheese purchased from Kalle Company were sandwiched together, with the surface of the cheese in between. The cheese was then packaged in polyethylene packaging. It was stored in a refrigerator at a temperature of 4°C and tested at time intervals of 0, 15, 30, 45, and 60 days. Two different groups used in active packaging and their labeling are as follows:

S1. Gouda cheese samples without coating with Zein film.

S2. Gouda cheese samples coated with Zein film containing anise essential oil nanoemulsion (at a specified concentration of 4% nanoemulsion of anise essential oil).

- ***Microbiological analysis***

Twenty-five grams of the treated and control cheese samples were transferred aseptically and under a laminar hood into sterile stomacher bags. They were then homogenized in 225 mL of sterile saline solution (0.85% w/v NaCl) for 60 inside the stomacher (Pulsifier). The microbial enumeration was performed using a 10-fold serial dilution of the homogenized cheese samples. Total psychrotrophic bacteria and total mesophilic bacteria were counted using nutrient agar media by incubating the plates at 37°C for 2 days and at 10°C for 7 days, respectively. The enumeration of Staphylococcus aureus was done using Baird Parker agar medium and incubated at 37°C for 24 h. Enterobacteriaceae were enumerated using Violet Red Bile Glucose Agar after incubation at 37°C for 24 h. Lactic acid bacteria were counted using MRS agar under aerobic conditions at 28°C for 72 h. Total yeast and mold count were performed using DRBC agar at 25°C for 5 days, and the plates with 30-300 colonies were counted. The results were expressed as log10 cfu/g (colony-forming units per gram) of the samples. The microbial tests were conducted at time intervals of 0, 15, 30, 45, and 60 days.

- ***Sensory analysis***

The sensory evaluation of different treatments of cheese was conducted using the Hedonic test, which measures the degree of liking or pleasure on a five-point scale. The parameters assessed in the sensory evaluation were taste, texture, color, and odor of the cheese samples. Cheese samples (in duplicate) were allowed to equilibrate at room temperature (20 ± 2°C) for one hour prior to the sensory analysis sessions, and they were evaluated only after confirming their microbiological safety. In order to assess the cheese's appearance, the entire cheese was initially examined by the panel. It was then cut into slices approximately 2 cm thick and placed on individual Petri dishes. The samples were labeled with three-digit random codes and were provided to the evaluators, who were 30 semi-trained assessors aged between 20 and 35 years, equally distributed between both genders, familiar with Gouda cheeses. The panel had been screened previously and selected among students and staff of the university. The sensory characteristics evaluated included color change (5: no color change, 1: severe color change), odor (5: very desirable, 1: very undesirable or bad odor), texture (5: hard, 1: very soft), and taste (5: very tasty, 1: lacking desirable taste). The average scores of these characteristics were defined as overall acceptability (5: very desirable, 1: very undesirable). Sensory evaluation was performed to determine the acceptance level of the edible coating of the Gouda cheese. The sensory evaluations were performed at time intervals of 0, 15, 30, 45, and 60 days to monitor any changes in the sensory attributes of the cheese samples during storage.

- ***Statistical analysis***

The results obtained from the experimental data were expressed as mean ± standard deviation of triplicate measurements. The colony-forming unit (CFU/g) counts in all experiments were transformed into logarithmic values before statistical analysis. All microbial counts were expressed as log CFU/g. One-way analysis of variance (ANOVA) was used to compare the data from different treatments. Significant differences between the mean values (in cases where the overall treatment effect was significant) were determined using the post hoc multiple comparison test, Dunnett's test.

The data were collected and analyzed using Statistical Package for Social Sciences (SPSS) version 26.0 (SPSS Inc., Chicago, IL, USA). The results are presented in figures and tables as means ± standard errors. The experiments were replicated six times (n = 6 for each treatment), except for the sensory analysis, which was conducted by 30 semi-trained panelists across three trials for each treatment. The effects of packaging and storage time were evaluated using Duncan’s Multiple Range Test (DMRT) at a significance level of 0.05. One-way ANOVA and t-tests were employed to analyze the film characteristics, while the data from the sensory analysis were processed using two-way ANOVA and repeated measures ANOVA, respectively.

## Results and Discussion

- ***Pimpinella anisum essential oil nanoemulsion***

Ensuring the quality and shelf life of dairy products, particularly Gouda cheese, is essential for establishing trust between producers and consumers. This study focuses on active packaging using Zein films containing anise essential oil nanoemulsion as an innovative method to extend the shelf life and preserve the quality of Gouda cheese. In order to assess the impact of this active film on Gouda cheese shelf life, samples were prepared using two methods: one coated with Zein film (without anise essential oil nanoemulsion) and the other coated with Zein film containing anise essential oil nanoemulsion. These samples were then packaged in polyethylene wrappers and subjected to testing at 4°Cover intervals of 0, 15, 30, 45, and 60 days.

The results related to the mean volume diameter of the nanodroplets in the anise essential oil nanoemulsion are presented in this section. Dynamic Light Scattering (DLS) analysis, a physical method for particle distribution determination, was employed. DLS measures the Brownian motion of particles after interaction with laser light, evaluating scattering and intensity changes. The small size and unique properties of nanodroplets contribute to their stability, preventing creaming and sediment formation during storage. Nanodroplets also offer high permeability, making them effective as a delivery system.

The particle sizes and percentages are detailed in Table 1, with selected peaks illustrated in Figure 1. The nanoemulsions exhibited a significant difference in particle sizes in descending order of frequency: 82.43 nm (87.90%), 84.37 nm (6.9%), 75.50 nm (3.4%), 77.58 nm (1.1%), 37.32 nm (0.5%), and 06.68 nm (0.2%). These findings indicate high stability of the nanodroplets, aligning with the stability results obtained for the nanoemulsion. This outcome can be attributed to the reduction of interfacial tension between the oil and aqueous phases due to the addition of surfactants. This addition lowers the amount of free energy needed to deform and break apart the droplets, resulting in smaller droplets after the homogenization process (McClements, 2005). Similar findings were reported by (Navarro et al., 2016) who investigated the impact of different types of nanoemulsions on the physical properties of edible films made from alginate and thyme oil.

One of the other properties related to the anise essential oil nanoemulsion that was examined is their turbidity. The results of the comparison of the mean turbidity of the anise essential oil nanodroplets are presented in Figure 2. The findings of this study demonstrated a significant increase in the turbidity of the nanodroplets over time ($p \leq 0.05$). This increase in turbidity can be attributed to the enlargement of the droplet size in the dispersed phase, as larger droplets cause changes in light scattering detected by the spectrometer for nanodroplets and impact their turbidity. In a study conducted in 2021, investigated the antibacterial effects of a nanoemulsion made from extracted corn zein and garlic extract on the shelf life of Vannamei prawn (Litopenaeus vannamei) stored at refrigerated temperatures. Their findings indicated that samples prepared with Tween 20 and various concentrations of garlic essential oil exhibited significant turbidity and sedimentation during storage (Rahnama *et al.,* 2021).

**Table 1.** The results of particle size distribution using the Dynamic Light Scattering (DLS) method

| Peaks | Particle size (nm) | Content (%) |
|---|---|---|
| Peak 1 | 82.43±1.14$^{d}$ | 87.9 |
| Peak 2 | 84.37±1.02$^{e}$ | 6.9 |
| Peak 3 | 75.50±1.32$^{c}$ | 3.4 |
| Peak 4 | 77.58±1.44$^{b}$ | 1.1 |
| Peak 5 | 37.32±0.95$^{f}$ | 0.5 |
| Peak 6 | 6.68±1.56$^{a}$ | 0.2 |

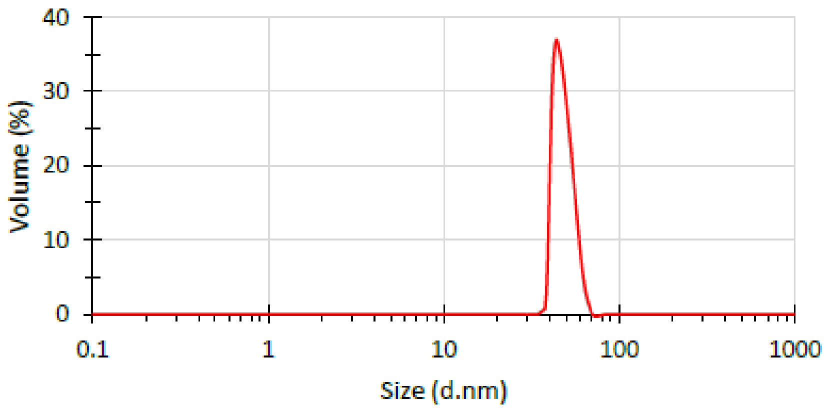


**Fig. 1.** Size distribution by volume of anise essential oil nanoemulsion.

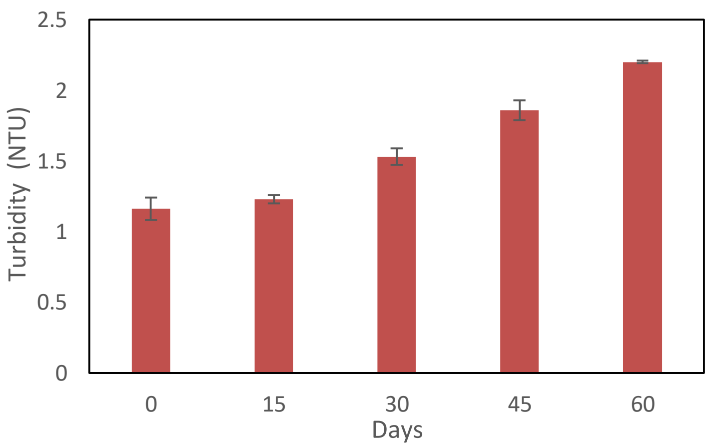


**Fig. 2.** The changes in turbidity of the anise essential oil nanoemulsion over time.

Figure 3 displays the results of the mean total phenol content comparison among the samples, with the highest content observed in the anise essential oil sample. The significant decrease ($p \leq 0.05$) in total phenol content in the nanoemulsion compared to essential oil is attributed to dilution during nanoemulsion preparation. Phenolic compounds influence the antioxidant and antimicrobial properties of plant compounds, and their quantitative analysis aids in assessing tissue quality and authenticity.

Essential oils, with high antioxidant activity, help to prevent oxidative spoilage in foods and maintain nutritional quality in oils and fats. Quantifying phenolic compounds in essential oils is crucial for understanding their potential health benefits. Spectrophotometry is preferred for assessing antioxidant properties due to its cost-effectiveness. Phenolic compound composition in plant extracts varies based on factors like harvesting season, location, and processing method. Thus, rapid and accurate analytical techniques are valuable in the food and herbal industries. These findings align with those of several studies. Rahman et al., 2021, indicated that the total phenolic content (TPC) of the extracts exhibited a significant and strong positive correlation with their free radical scavenging abilities and the inhibition of lipid peroxidation. Consequently, the polyphenolic components in the extracts are likely the primary contributors to their antioxidant activity in neutralizing free radicals and preventing lipid peroxidation.

### - *Antimicrobial activity of films*

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antimicrobial substance that results in the death of microorganisms, while the Minimum Inhibitory Concentration (MIC) is the lowest concentration that inhibits microbial growth. Bactericidal substances directly kill microorganisms, reducing their population, whereas at the MIC, microorganisms are present but unable to reproduce. Plant extracts, known for their antimicrobial properties, offer alternatives to chemical preservatives in the food industry, contributing to safer and more sustainable products. However, comparing results is challenging due to methodological variations. The minimum inhibitory concentration (MIC) and

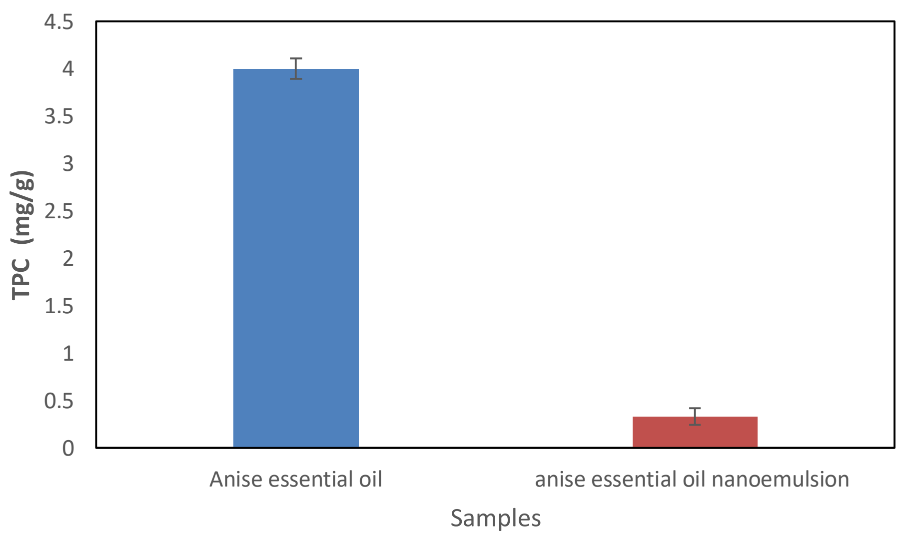


**Fig. 3.** Total phenol content of prepared nanoemulsion and anise essential oil.

minimum bactericidal concentration (MBC) were evaluated against four selected microorganisms: S. aureus, E. coli, C. albicans, and A. niger (Figures 4 and 5). S. aureus exhibited the lowest MIC value of 8.33 µg/mL when tested with anise essential oil (EO). Additionally, both S. aureus and E. coli demonstrated a lower MIC value of 6.66 µg/mL when treated with anise nanoemulsion, indicating a higher sensitivity to the nanoemulsions. The MBC for S. aureus and E. coli was found to be 13.33 µg/mL for anise EO, while it was 10 µg/mL for the anise nanoemulsion. Both anise EO and anise nanoemulsion exhibited similar MBC and MIC values against the Aspergillus strain. The growth of S. aureus was inhibited by the nano-based anise EO, which had a MIC value of 5 µg/mL (Figure 6). The nanoemulsions displayed a more pronounced bactericidal effect against this bacterium, with an MBC of 5.33 µg/mL. These findings indicate that anise EO has a stronger antimicrobial effect when formulated as a nanoemulsion. The mechanism of action of plant extracts on microorganisms is complex, involving diverse chemical groups affecting various targets within microbial cells. Investigative results, including mean MIC and MBC values, are depicted in Figures 4 and 5. Notably, the nanoemulsion of anise essence demonstrated the lowest MIC and MBC values against all tested microorganisms, suggesting its potent antimicrobial efficacy. Various mechanisms have been suggested to explain the antimicrobial activity of anise, including the inhibition of DNA, RNA, and protein synthesis (Li *et al.,* 2022).

The analysis of well diffusion in agar for the prepared nanoemulsion with anise and also the comparison of the mean diameter of the inhibition zones for the samples are shown in Figure 6. The current study's results demonstrated that the zone of inhibition diameter against all tested microorganisms was the lowest in the anise essence.

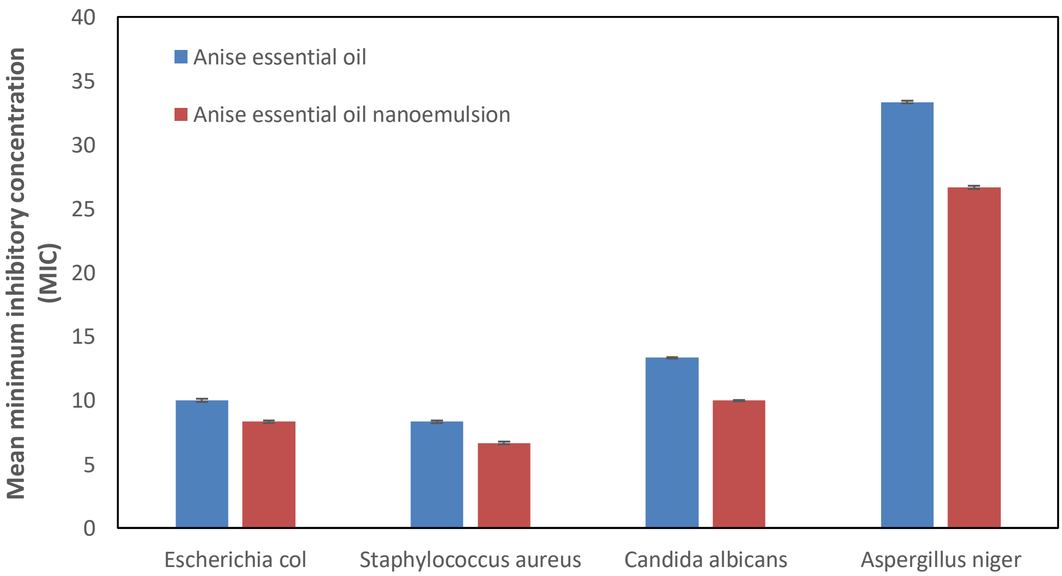


**Fig. 4.** Comparison of the minimum inhibitory concentration (MIC) of samples against Escherichia coli, Staphylococcus aureus, Candida albicans, Aspergillus niger.

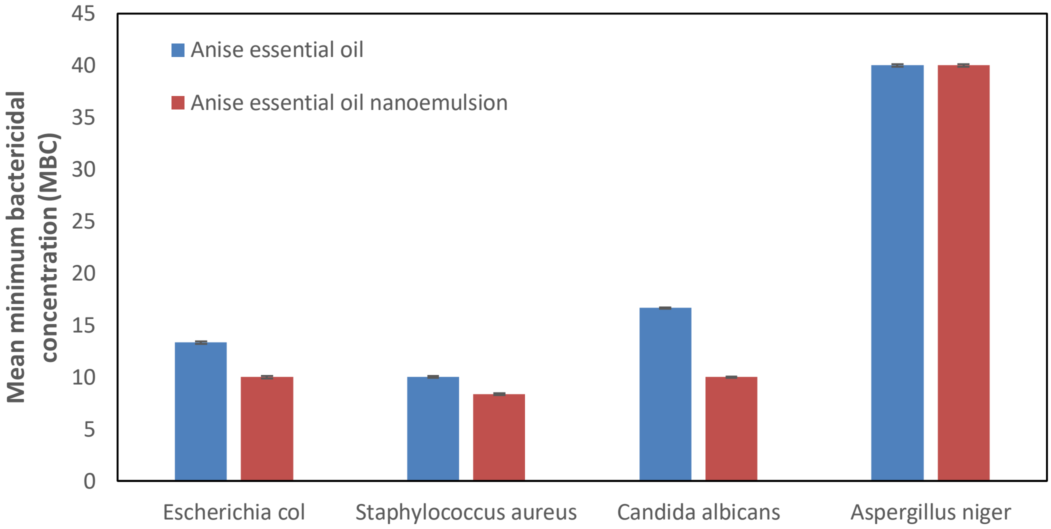


**Fig. 5.** Comparison of the minimum lethal concentration (MBC) samples against Escherichia coli, Staphylococcus aureus, Candida albicans, Aspergillus niger.

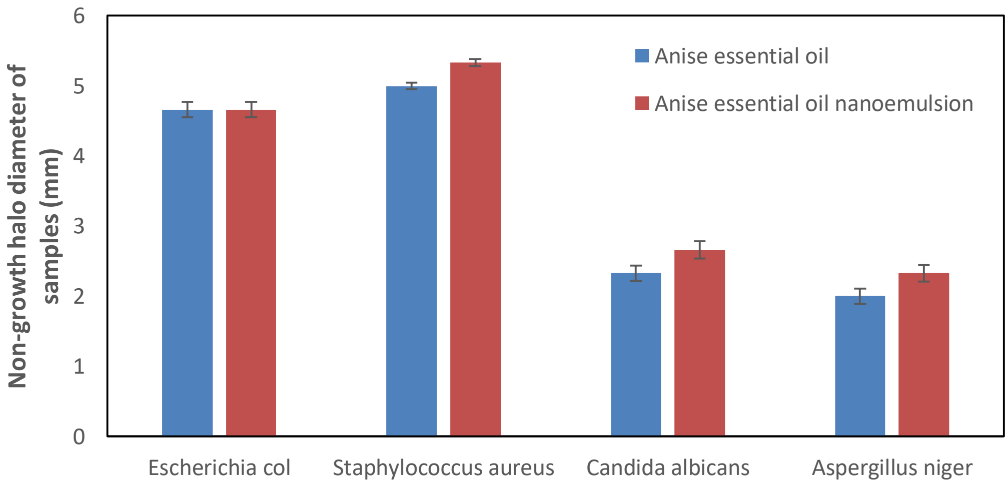


**Fig. 6.** Comparison of non-growth halo diameter of samples against Escherichia coli, Staphylococcus aureus, Candida albicans, Aspergillus niger.

## - *Mechanical properties of films*

Films play a crucial role in packaging and protecting products, and their mechanical properties can significantly impact their performance and functionality. In order to evaluate and determine the mechanical properties of films, various types of mechanical tests are employed. In general, the peak force height is related to the hardness of the films. Highly flexible films find significant applications in packaging fruits and directly wrapping food items due to their ability to conform well to the product's shape. On the other hand, rigid and tough films with high resistance to deformation are useful as packaging materials to reinforce the food structure, offering protection against potential damages and breakage during transportation and storage. The results of the comparison of the mean peak force height for the samples are presented in Figure 7. As indicated by the results presented in Figure 7, Sample 2 exhibits a lower value compared to Sample 1, which is considered as the control sample without the use of nanomulsions. In the present study, it should also be noted that the films produced were placed in direct contact with the surfaces of Gouda cheese slices. Therefore, this can be considered a positive factor. These films can serve as protective and preservative layers for Gouda cheese, potentially improving its shelf life and overall quality. Additionally, the mechanical properties of these films can play a crucial role in enhancing the structural integrity of Gouda cheese and safeguarding it against external factors such as pressure and deformation.

The results related to the tensile strength are presented in Figure 8. As shown in the figure, the value of sample number 2 is approximately 30% lower compared to sample one. The analysis of mechanical properties revealed that the addition of anise nanoemulsions to Zein film enhances its tensile strength (TS), as illustrated in Figure 8. The Zein film containing anise nanoemulsions exhibited a tensile strength of 1.33 N, which represented a significant increase ($p < 0.05$) compared to the control film without nanoemulsions, which measured 0.86 N.

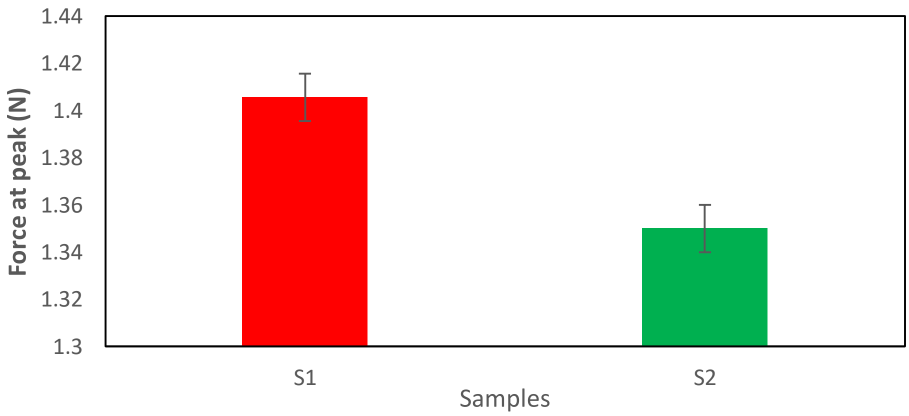


**Fig. 7.** Comparison of force at peak (N) in film samples, S1: Zein film containing anise nanoemulsions and S2: control film..

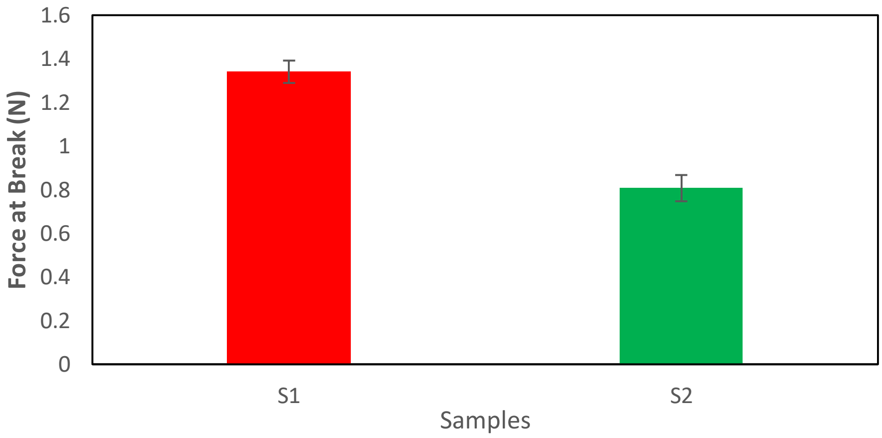


**Fig. 8.** Comparison of Force at Break (N) in film samples, S1: Zein film containing anise nanoemulsions and S2: control film.

The experimental data concerned with the elongation at break (mm) is displayed in Figure 9An opposing result was noted in the EAB of Zein films. The control film, which did not contain nanoemulsions, exhibited a significant reduction ($p < 0.05$) in EAB, decreasing from 0.46 mm (standard film) to 0.0030 mm for the Zein film with anise nanoemulsions (Figure 9). The present study's results have demonstrated that the highest tensile strength belongs to sample 2 (zein film containing 4% nanoemulsion of anise essential oil). These findings indicate that the incorporation of anise essential oil nanoemulsion in the zein film structure improves its mechanical properties and enhances its resistance to the point of rupture. This increase in mechanical strength could lead to an improvement in the shelf life and quality of packaged products, particularly in dairy products like Gouda cheese. This finding aligns with the results reported by Hajirostamloo *et al.,* 2023, who observed that the inclusion of microencapsulated cardamom essential oil in soy protein isolate-based films led to a significant decrease in tensile strength (TS) from 21.87±1.87 MPa for the standard film to 18.51±1.79 MPa. Additionally, they noted an improvement in elongation at break (EAB), which increased from 9.86±0.93% for the standard film to 31.61±3.67%.

The results shown in Figure 10 indicate that Sample 2 has a higher value compared to Sample 1. This finding demonstrates that the film containing the nanoemulsion of anise essence (Sample 2) possesses greater energy until the point of fracture compared to the film without nanoemulsion (Sample 1). The increase in energy up to the point of fracture in Sample 2 reflects better resistance and tolerance of this film against tensile forces. This property can be highly beneficial for enhancing the mechanical characteristics of essential packaging materials, especially in the dairy industry, such as Gouda cheese. By increasing the resistance to fracture, the film with anise essence nanoemulsion can protect the products inside from damages and contaminations, leading to an extended shelf life and improved product quality. These findings indicate that the incorporation of anise nanoemulsions altered the interactions with film-forming agents. This change may be attributed to a reduction in ionic and hydrogen bonds between the polymer chains, an increase in the free spaces among the macromolecule chains, and a rise in structural discontinuities within the Zein films. Enhanced hydrogen bonding leads to stronger intermolecular interactions, which, in turn, results in reduced flexibility (Nami *et al.,* 2024).

### - *Microbial analysis*

The present study results (Figure 11) demonstrated that on day zero, no statistically significant difference was observed in the total count of mesophilic viable bacteria among the samples. On the other days examined, the highest count of mesophilic viable bacteria belonged to the control sample (Sample 1), while the lowest count was observed in Sample 2 (Gouda cheese covered with Zein film containing 4% anise essence nanoemulsion). Over time, the total count of mesophilic viable bacteria in the samples significantly increased. In general, it can be concluded that the addition of anise essence nanoemulsion has led to an enhancement in the antimicrobial activity of the Zein films.

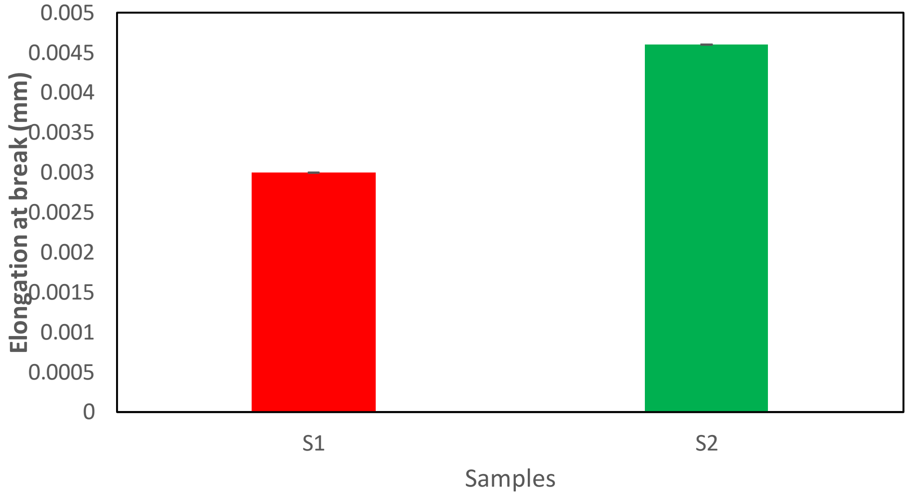


**Fig. 9.** Comparison of Elongation at break (mm) in film samples, S1: Zein film containing anise nanoemulsions and S2: control film.

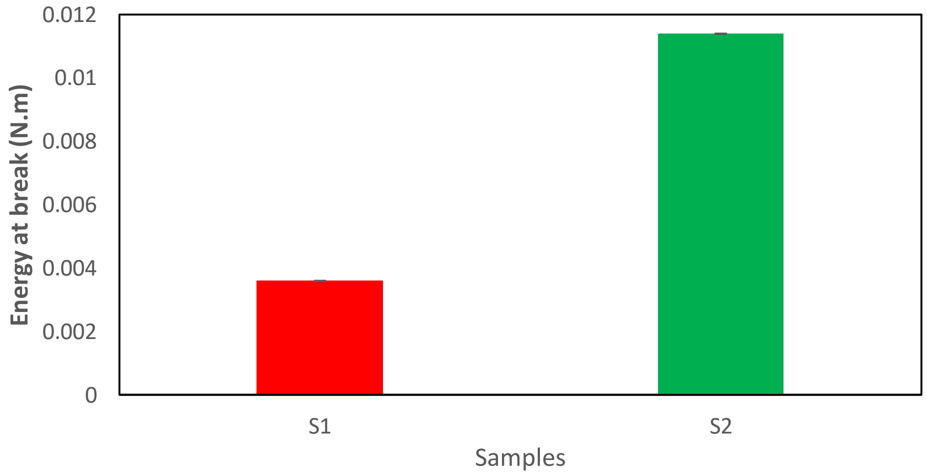


**Fig. 10.** Comparison of Energy at break (N.m) in film samples, S1: Zein film containing anise nanoemulsions and S2: control film.

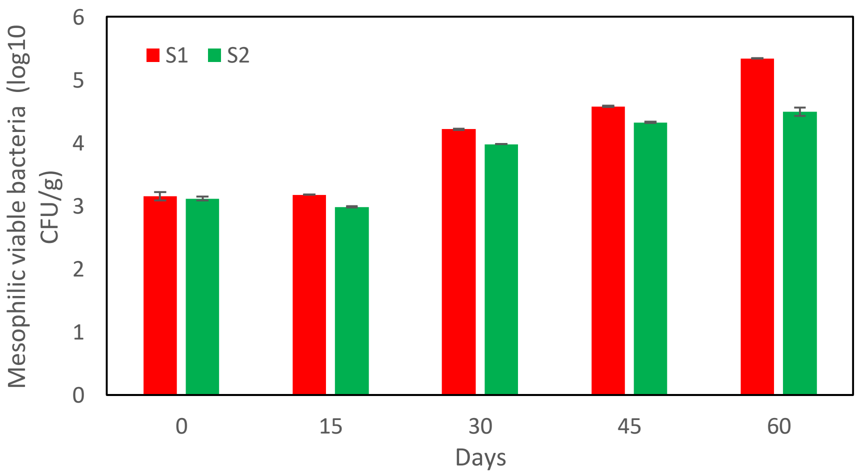


**Fig. 11.** Changes in the count of total mesophilic viable bacteria in coated cheese samples over time.

Entrobacteriaceae is considered as an indicator for judging the hygiene conditions of cheese production. The low count of these bacteria in Gouda cheese can be attributed to milk pasteurization and their sensitivity to low pH and high salt concentration. In the present study, the nanoemulsion of anise essential oil showed a positive effect in reducing Entrobacteriaceae bacteria. The average number of psychrotrophic bacteria in the samples at time intervals of 0, 15, 30, 45, and 60 days is presented in Figure 12. The present study results demonstrated that on day zero, no statistically significant difference was observed in the total count of psychrotrophic bacteria among the samples. On the other days examined, the highest count of psychrotrophic bacteria belonged to the control sample, while the lowest count was observed in Sample 2 (Gouda cheese covered with Zein film containing 4% anise essence nanoemulsion). Over time, the total count of psychrotrophic bacteria in the samples significantly increased.

The average number of lactic acid bacteria in the samples at time intervals of 0, 15, 30, 45, and 60 days is presented in Figure 13. According to the results of the average comparison, on day 0, there was no statistically significant difference in the number of lactic acid bacteria among the samples. However, on other days under investigation, the highest number of lactic acid bacteria belonged to the control sample (sample 1), and the lowest number of lactic acid bacteria was observed in sample 2 (Gouda cheese coated with zein film containing 4% anise essential oil nanoemulsion). Over time, the number of lactic acid bacteria in the samples significantly increased.

The results of the average comparison of mold and yeast populations in the samples at time intervals of 0, 15, 30, 45, and 60 days are presented in Figure 14. According to the results of the average comparison, on day 0, there was no statistically significant difference in the population of mold and yeast among the samples. However, on other days under investigation, the highest populations of mold and yeast belonged to the control sample (sample 1), and the lowest populations of mold and yeast were observed in sample 2 (Gouda cheese coated with zein film containing 4% anise essential oil nanoemulsion). Over time, the populations of mold and yeast in the samples significantly increased. An examination of the microbial properties of Gouda cheese coated with both a control film and films containing anise essential oil nanoemulsion revealed that microbial counts increased in all packages during storage. However, the rise in microbial counts was less pronounced in the films with anise essential oil nanoemulsion compared to the control film (zein film without anise essential oil nanoemulsion). This suggests that the incorporation of anise essential oil nanoemulsion positively impacts the shelf life of Gouda cheese. The results indicate that the encapsulated essential oil, which exhibits strong antimicrobial activity, is highly effective in the production of bioactive films (Molaveisi *et al.*, 2022).

## - *Sensory analysis results*

The results of comparing the average taste scores of samples over the time intervals of 0, 15, 30, 45, and 60 days are presented in Figure 15. The results indicate that on day zero, there was no statistically significant difference in the taste scores of the samples. However, on other days under investigation, the lowest taste score belonged to the control sample, while the highest taste score was observed in samples 2 (Gouda cheese covered with

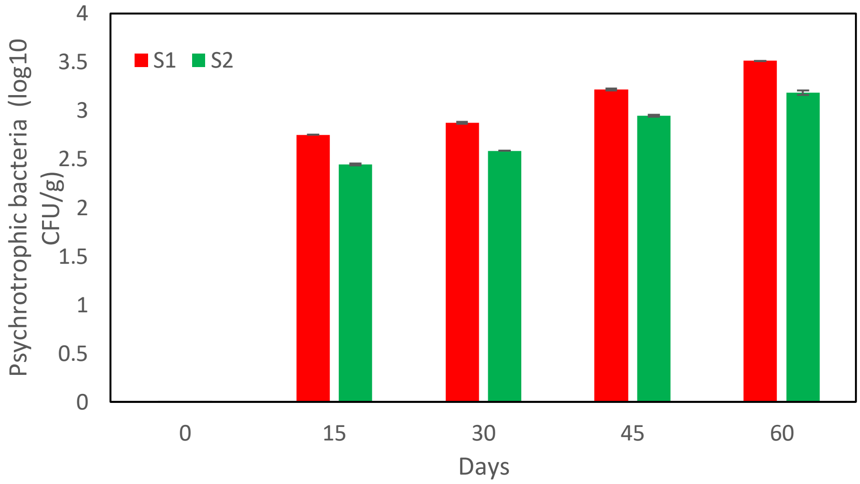


**Fig. 12.** Changes in the count of total psychrotrophic bacteria in coated Gouda cheese samples over time.

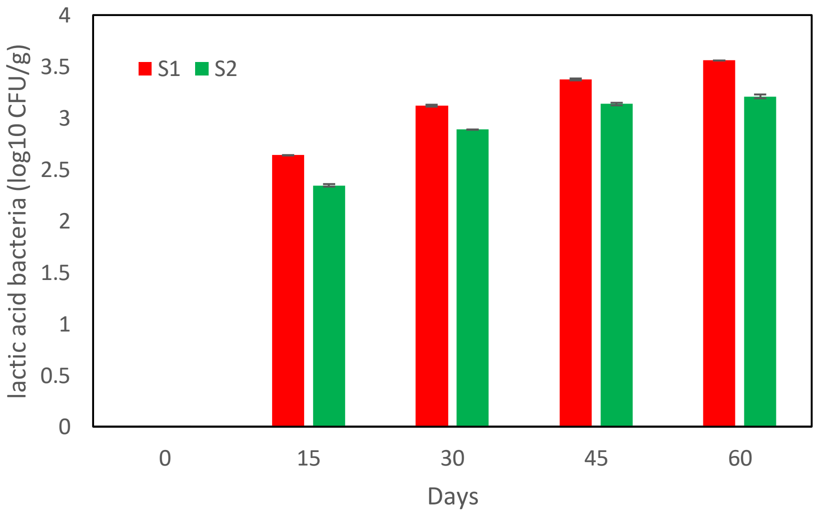


**Fig. 13.** Changes in the count of total lactic acid bacteria in coated Gouda cheese samples over time.

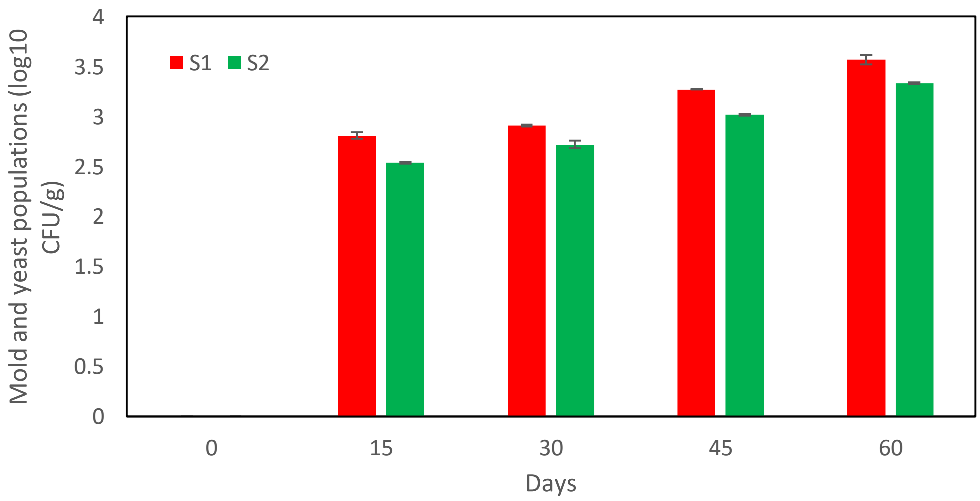


**Fig. 14.** Changes in the count of total mold and yeast populations in coated Gouda cheese samples over time.

Zein film containing 4% nano emulsion of anise essence) ($p \leq 0.05$). As time passed, the taste scores of the samples significantly decreased. These findings demonstrate that the use of nano emulsion of anise essence in Zein film can have a positive effect on the taste of the product and improve its sensory properties. Adding anise essence to the Zein film can enhance the taste of the product and make it more appealing and desirable from a sensory perspective. This can play a crucial role in improving the product's quality and attracting consumer satisfaction. Furthermore, the decrease in taste scores over time indicates changes in the sensory characteristics or chemical transformations in the product's taste, which can be tailored to meet specific product needs and preferences.

The results of the present study (Figure 16) showed that on day zero and day 15, there was no statistically significant difference in the odor scores of the samples. However, on the other days under investigation, the lowest odor score belonged to the control sample, and the highest odor score was observed in samples 2 (cheddar cheese covered with zein film containing 4% nano emulsion of anise essential oil). Over time, the odor scores of the samples significantly decreased. These findings indicate that adding nano emulsion of anise essential oil to the zein film can have a positive impact on the odor of the product and improve its sensory attributes. The addition of anise essential oil to the zein film can enhance the product's odor and make it more appealing and desirable to consumers. This can play a significant role in attracting customers and creating a pleasant buying experience for them. Additionally, the decrease in odor scores over time suggests changes in the sensory characteristics of the product's odor or chemical transformations in its odor, which can lead to improvements or alterations in the sensory experience of consumers. These results can serve as an essential guideline for producers and industry professionals in improving the product's quality and competitiveness in the market.

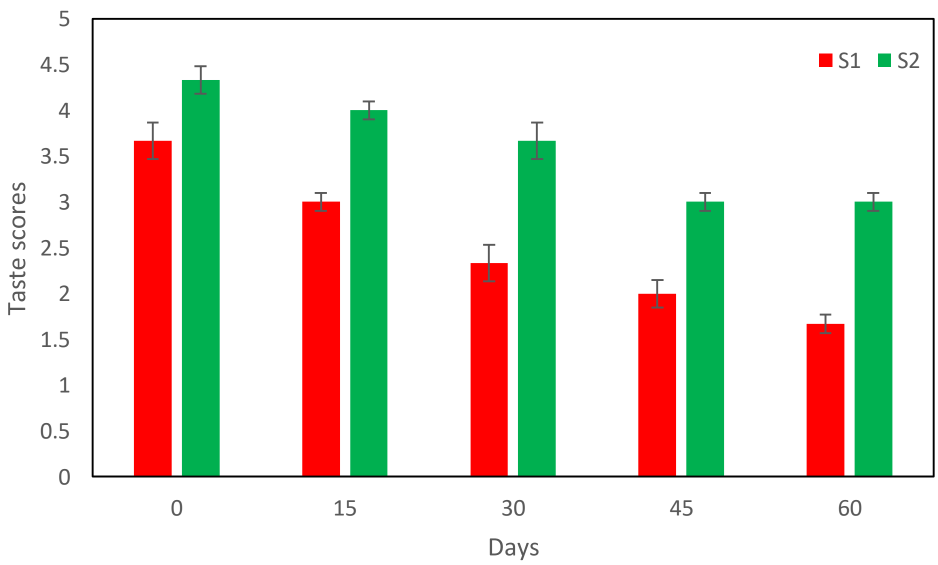


**Fig. 15.** Changes in average taste scores in coated Gouda cheese samples over time.

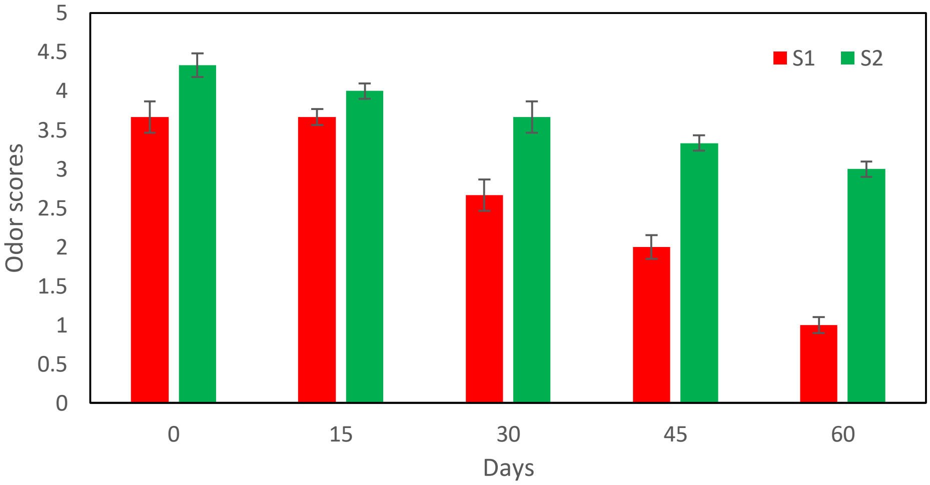


**Fig. 16.** Changes in average odor scores in coated Gouda cheese samples over time.

Figure 17 showed that on day zero and 15th days, no statistically significant difference was observed in the color scores of the samples. However, on other days under investigation, the highest color score belonged to the control sample, while the lowest color score was observed in sample 2 (Gouda cheese covered with zein film containing 4% nanoemulsion of anise essence). With the passage of time, the color scores of the samples increased significantly. These findings indicate that the use of nanoemulsion of anise essence in the zein film can have a positive effect on the color of the product and improve its color properties. Adding anise essence to the zein film can enhance the color of the product, making it more visually appealing and attractive to consumers. This aspect can play a crucial role in product marketing and consumer acceptance. Moreover, the increase in color scores over time suggests improved color stability and resistance to various factors, which can positively impact consumer satisfaction and the product's shelf life. These results can contribute significantly to enhancing the quality and appearance of packaged products and gaining customer satisfaction.

The results of texture scores, shown in Figure 18, indicated that there was no statistically significant difference in the texture scores of the samples on day zero. However, on subsequent examination days, the control sample obtained the highest texture score, while sample 2 (Gouda cheese coated with a Zein film containing 4% nanoemulsion of anise essential oil) had the lowest texture score. As time passed, the texture scores of the samples significantly increased. These findings suggest that incorporating anise essential oil nanoemulsion into the Zein film can positively impact the product's texture, resulting in improved textural properties. The addition of anise essential oil to the Zein film can enhance the texture of the product, making it more appealing and firmer. This improvement can play a pivotal role in elevating the product's quality, extending its shelf life, and motivating consumers to make purchases. Additionally, the progressive increase in texture scores over time reflects the admirable textural attributes of the product, contributing to consumer satisfaction. These results can contribute to the admiration and popularity of packaged goods, thereby enhancing the overall consumer experience.

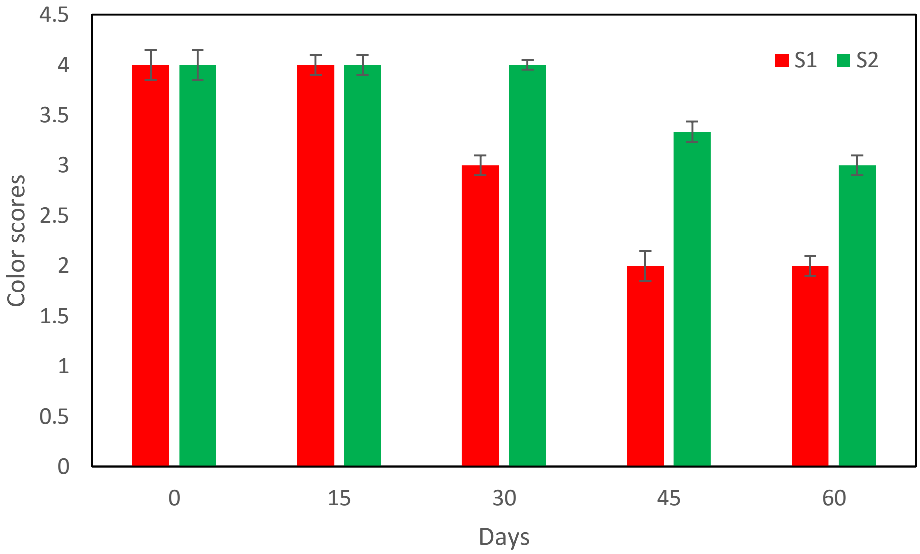


**Fig. 17.** Changes in average color scores in coated Gouda cheese samples over time.

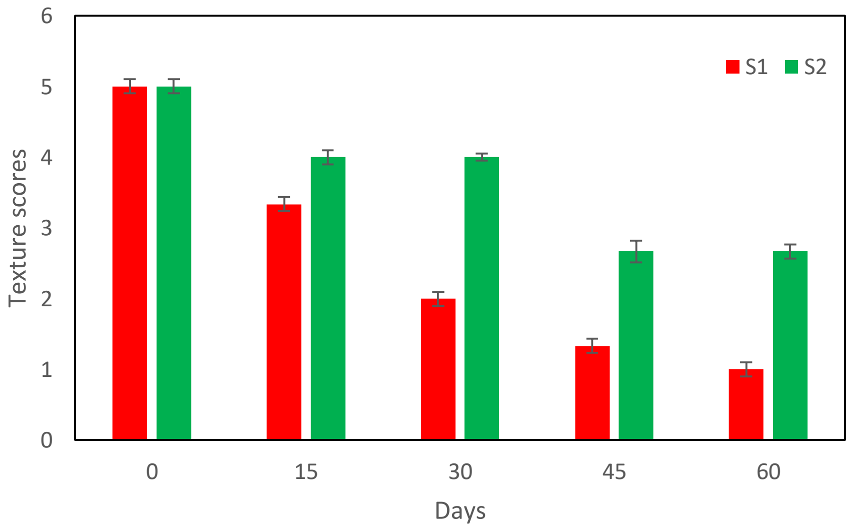


**Fig. 18.** Changes in average texture scores in coated Gouda cheese samples over time.

According to Figure 19, there was no statistically significant difference in the overall acceptance scores of the samples on day zero. However, on the other days of evaluation, the control sample had the lowest overall acceptance score, while sample 2 (Gouda cheese coated with a Zein film containing 4% nanoemulsion of anise essential oil) had the highest overall acceptance score. As time passed, the overall acceptance scores of the samples significantly increased. These findings suggest that incorporating anise essential oil nanoemulsion into the Zein film positively influenced the overall acceptance of the product, leading to a relative improvement in consumer liking and preference. The addition of anise essential oil to the Zein film enhanced the overall acceptance of the product and motivated customers to make purchases. This, in turn, can contribute to increased customer satisfaction, loyalty, and repeat purchases. Furthermore, the increasing overall acceptance scores over time demonstrate the product's growing value and appeal, which can positively impact the business's success, boost demand, and increase product sales. These results can be invaluable in making marketing and advertising decisions, helping to choose the most effective strategies to promote and market the products. The results obtained can be attributed to the bactericidal properties of anise and the fungicidal effects of anise essential oil nanoemulsion. These activities led to a reduction in the growth of bacteria, yeast, and molds in the coated cheese samples, which, in turn, diminished the production of off-flavor compounds resulting from the hydrolysis of proteins and lipids. This study's findings align with those reported by (Hajirostamloo *et al.,* 2023; Del Nottagh *et al.,* 2020).

## Conclusion

In this study, researchers examined the impact of anise essential oil nanoemulsion incorporated into Zein film on the antibacterial and sensory qualities of Gouda cheese. Mechanical tests revealed increased strength and hardness in the Zein film with anise essential oil, suggesting enhanced film stability. These improvements can potentially extend product shelf life and enhance packaging quality. Initially, there were no significant differences in antibacterial and sensory properties of Gouda cheese samples. However, over time, variations emerged, with the anise essential oil nanoemulsion-containing sample exhibiting superior taste, aroma, color, and texture scores. This suggests that the use of anise essential oil nanoemulsion in the Zein film positively influenced the sensory experience of consumers, improving product quality. The study suggests that this technique could be valuable for food factories, leading to higher-quality products and increased customer satisfaction. The authors propose future research avenues, including comparing postbiotic derivatives from various probiotic bacteria for antimicrobial and antioxidant effects. They also suggest evaluating the application of postbiotic derivatives in films and coatings, studying their effects on different food products. Additionally, combining plant essences and extracts with postbiotic compounds or

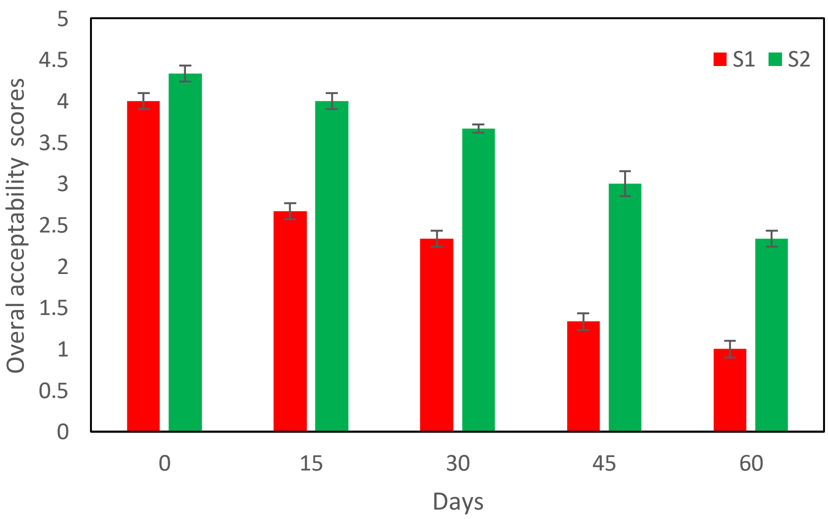


**Fig. 19.** Changes in average overal acceptability scores in coated Gouda cheese samples over time.

natural antimicrobials in nanoemulsions could be explored for synergistic effects. These suggestions aim to advance research on antimicrobial and antioxidant properties of food substances, potentially leading to improved methods and products in the food industry. Investigating synergistic effects may enhance efficiency and contribute to safer and higher-quality food products, offering innovative solutions for the industry and benefitting consumers. In conclusion, the use of their free-form anise essential oil nanoemulsion in food products is subject to several limitations. These include low solubility in aqueous systems, uncontrolled release and dosage, the potential for undesirable taste profiles, low stability, high volatility, limited effectiveness, and heightened sensitivity to adverse environmental conditions.

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