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Nanocomposite Films Based on Soy Protein Isolate-Montmorillonite Nanoclay Containing Emulsion and Nanoemulsion of Zataria Multiflora Essential Oil for Preserving Chilled Chicken Burgers

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A B S T R A C T

Introduction: This study aimed to evaluate the effect of nanocomposite film based on soy protein isolate-montmorillonite nanoclay (SPI-MMT) containing *Zataria multiflora* essential oil emulsion (ZEO) and nanoemulsion (ZNE) on the quality of chilled chicken burgers.

Method: Nanoemulsion, nanocomposite film, and chicken burgers were prepared based on instructions. The hamburgers were divided into six different groups with four replicates. The experimental groups were Control, SPI-MMT, SPI-MMT+1% ZEO, SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE, which were analyzed for microbial, physicochemical, and sensory parameters during 16 days of storage at refrigerator (days include 0, 4, 8, 12, and 16).

Result: The treated groups, including SPI-MMT+1% ZEO, SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE, showed the lower mesophilic and psychrophilic bacteria, lactic acid bacteria (LAB) and *Enterobacteriaceae* count than the control and SPI-MMT groups during storage. The treatments also reduced the increasing rate of total volatile nitrogen, lipid oxidation, pH, and cooking loss during storage. The SPI-MMT+2% ZNE treatment was the best treatment to reduce the microbial population, retard physicochemical and sensory changes, and increase the shelf-life of chicken burgers.

Conclusion: Based on the results, the nanocomposite film based on soy protein isolate-montmorillonite nanoclay containing *Z. multiflora* essential oil emulsion and nanoemulsion can improve the microbiological and physicochemical quality and is recommended for the preservation of chicken burgers during chilled storage.

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Introduction

Chicken meat is among the most popular types with desirable nutritional properties and a good source of high-value protein, minerals, and vitamins. The cholesterol content of chicken meat is lower than red meats, which increases its nutritional value and is preferred to other meat types in terms of health benefits and nutritional properties (28). The increasing world population produces chicken meat products with different ingredients and sensory characteristics. Burgers are the most common meat products consumed worldwide (25).

Microbial growth and oxidation of fats and proteins reduce the storage life of meat products during storage. There are various methods to preserve meat products. Edible films are packaging made from renewable, biocompatible, and biodegradable materials such as polysaccharides, proteins, and lipids and are one of the primary ways to control microbial and physicochemical changes in foodstuffs (10, 33).

The protein films possess higher mechanical properties than those made from carbohydrates and lipids while improving the nutritional value of foods (9). Soy protein provides a coating with a uniform and flexible texture, highly resistant to the penetration of oxygen and fat (8). Soy protein isolate (SPI) powder is prepared from defatted soybean flakes, washed in alcohol or water to remove the sugars and fiber, and then dehydrated and powdered. The protein content

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of SPI is more than 90% based on dry weight (24). Coating fried meat products with SPI reduces oil absorption and prevents moisture loss (5).

Nanocomposites are essential in improving polymer films' mechanical, physicochemical, and thermal properties compared to pure polymers or conventional composites (17). Nanoclays, such as hectorite, saponite, bentonite, and montmorillonite, have a particular surface area and unique properties in combination with biopolymers. Montmorillonite is one of the nanoclays that has recently received particular attention (3, 35).

Many studies have been done to replace chemicals with natural compounds to eliminate or reduce synthetic additives in foods, among which essential oils are a new way to preserve food (2). Shirazi thyme (*Zataria multiflora*) is a medical plant from the *Lamiaceae* family that grows in Iran (19), and its essential oil has various antiseptic, anesthetic, anti-epileptic, antibacterial, and antioxidant properties (4, 30).

Essential oils face several challenges, including increasing their stability and controlling their release during storage. In this regard, nanotechnology-based methods such as nanoemulsions are proposed. Nanoemulsions are more stable than conventional emulsions and have good physicochemical properties, which can be used to extend the storage life of commercial foods. On the other hand, the transfer of active compounds through biological membranes of nanoemulsions is higher and enhances the bioavailability of compounds (23).

This study aimed to evaluate the effect of nanocomposite film based on soy protein isolate-montmorillonite nanoclay containing emulsion and nanoemulsion of *Z. multiflora* essential oil on the quality of chilled chicken burgers.

Materials and Method

Materials

The fresh chicken burger was prepared by Dorsa slaughterhouse in Markazi province, Iran. Thyme (*Z. multiflora*) essential oil was obtained from Barij Essence Company (Kashan, Iran). Soy protein isolate was purchased from Barg-e-Sabz Company (Tehran, Iran). Monomorillonite (sodium form) was bought from Pishgaman Nanomaterials Company (Mashhad, Iran). All chemicals were obtained from Merck (Darmstadt, Germany). Microbial culture media were purchased from Condalab (Madrid, Spain).

Preparation of Nanoemulsion

The ingredients of the aqueous coarse emulsion were Tween 80 (4.5% w/w) (as surfactant or emulsifier) and *Z. multiflora* essential oil (6% w/w). High-speed homogenization (IKA, model T25D, Ultra Turrax, Staufen, Germany) at 10,000rpm for 15 minutes was used to prepare nanoemulsion of *Z. multiflora* essential oil (6).

Particle Size Measurement

The nanoemulsion's particle size and polydispersity index were measured using dynamic light scattering (HORIBA Scientific, model SZ-100, USA) (18).

Preparation of Nanocomposite Film

First, 5g of soy protein isolate (SPI) powder was added to distilled water and became uniform. The pH was adjusted to 10.5 with sodium hydroxide. Next, montmorillonite powder (1, 3, 5, 7, and 9%) was mixed with 1.25g glycerol as a plasticizer and mixed by a magnetic stirrer for 1h. The film solution (10ml) was poured into plates and was dried at 40°C for ~ 4h. Films with different montmorillonite ratios were evaluated regarding tensile strength and elongation percentage, and the most desirable film regarding these two factors was the film containing 5% montmorillonite. Finally, emulsion and nanoemulsion of essential oil (1% and 2%) were added to the SPI solution containing 5% montmorillonite. The final solutions were poured into trays and dried at 40°C for 4h, and the films were separated from the trays (15).

Chicken Burger Preparation

The sample was produced based on the formulation of the chicken burger and Iran's national standards (21). The fresh minced chicken breast meat was mixed with onion, breadcrumbs (8%), mixed spices (1%), and liquid oil (5%) for 5 minutes. The samples were placed between two films and divided into six groups after cutting the burger pieces to 100g (1 × 8cm). The first and second groups were the control and soy protein isolate-montmorillonite films (SPI-MMT), respectively. The third group was wrapped in soy protein isolate-montmorillonite film containing an emulsion of *Z. multiflora* essential oil with a concentration of 1% (SPI-MMT+1% ZEO). The fourth group was wrapped in soy protein isolate-montmorillonite film containing an emulsion of *Z. multiflora* essential oil with a concentration of 2% (SPI-MMT+2% ZEO). The fifth group was wrapped in soy protein isolate-montmorillonite film containing nanoemulsion of *Z. multiflora* essential oil with a concentration of 1% (SPI-MMT+1% ZNE). The sixth group was wrapped in soy protein isolate-montmorillonite film containing nanoemulsion of *Z. multiflora* essential oil with a concentration of 2% (SPI-MMT+2% ZNE). The samples were stored in a refrigerator (3±1°C) and tested for microbial, physicochemical, and sensory changes for 16 days.

Microbial Analysis

About 10g of the chicken burger was homogenized with 90ml of sterile normal saline. The pour-plate method was used for counting total mesophilic bacteria (TMB) and total psychrophilic bacteria (TSB) in plate count agar (PCA) after the preparation of serial dilutions. The mixture was incubated at 35°C for 72h and 7°C for ten days, respectively. The lactic acid bacteria (LAB) were enumerated in MRS agar and incubated at 35°C for 72h. Violet red bile dextrose agar (VRBA) was used after incubation at 35°C for 24h to count *Enterobacteriaceae*. The colonies were reported as log CFU/g chicken burgers (18).

Physicochemical Analysis

The pH (7), total volatile basic nitrogen (TVN), thiobarbituric acid reactive substances (TBARS) (10), and cooking loss (29) were determined based on the procedures previously characterized.

Sensory Evaluation

The sensory properties of the chicken burgers were determined by 15 trained panelists using a 7-point hedonic scale. In this method, a score of 7 shows "excellent," and a score of 1 indicates "very poor" (34).

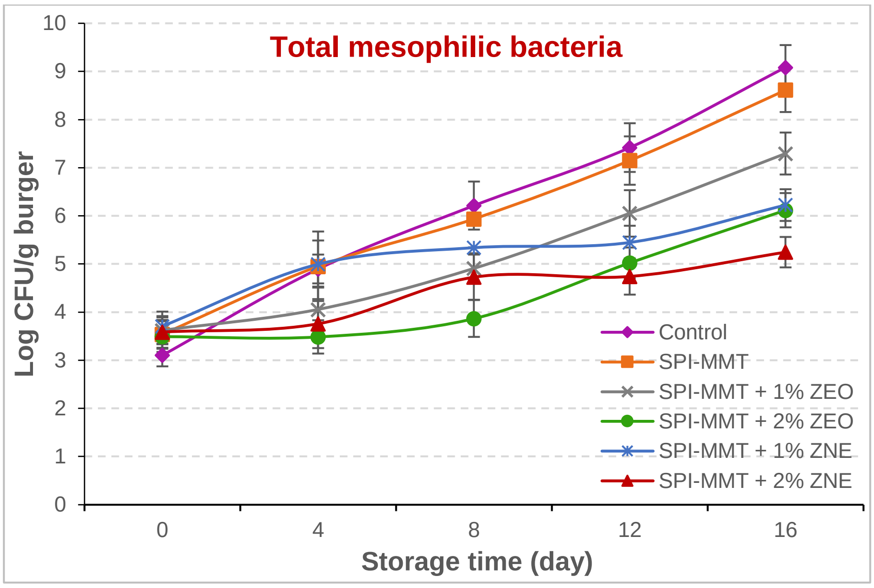
Statistical Analysis

The data of measured parameters were analyzed using analysis of variance followed by the Duncan post-test in SPSS software version 20. The statistical significance of all the variables was determined at the 5% probability level (p<0.05).

Results

Particle Size of ZNE

The mean particle size and polydispersity index of ZNE were 82.7 and 0.385nm, respectively.



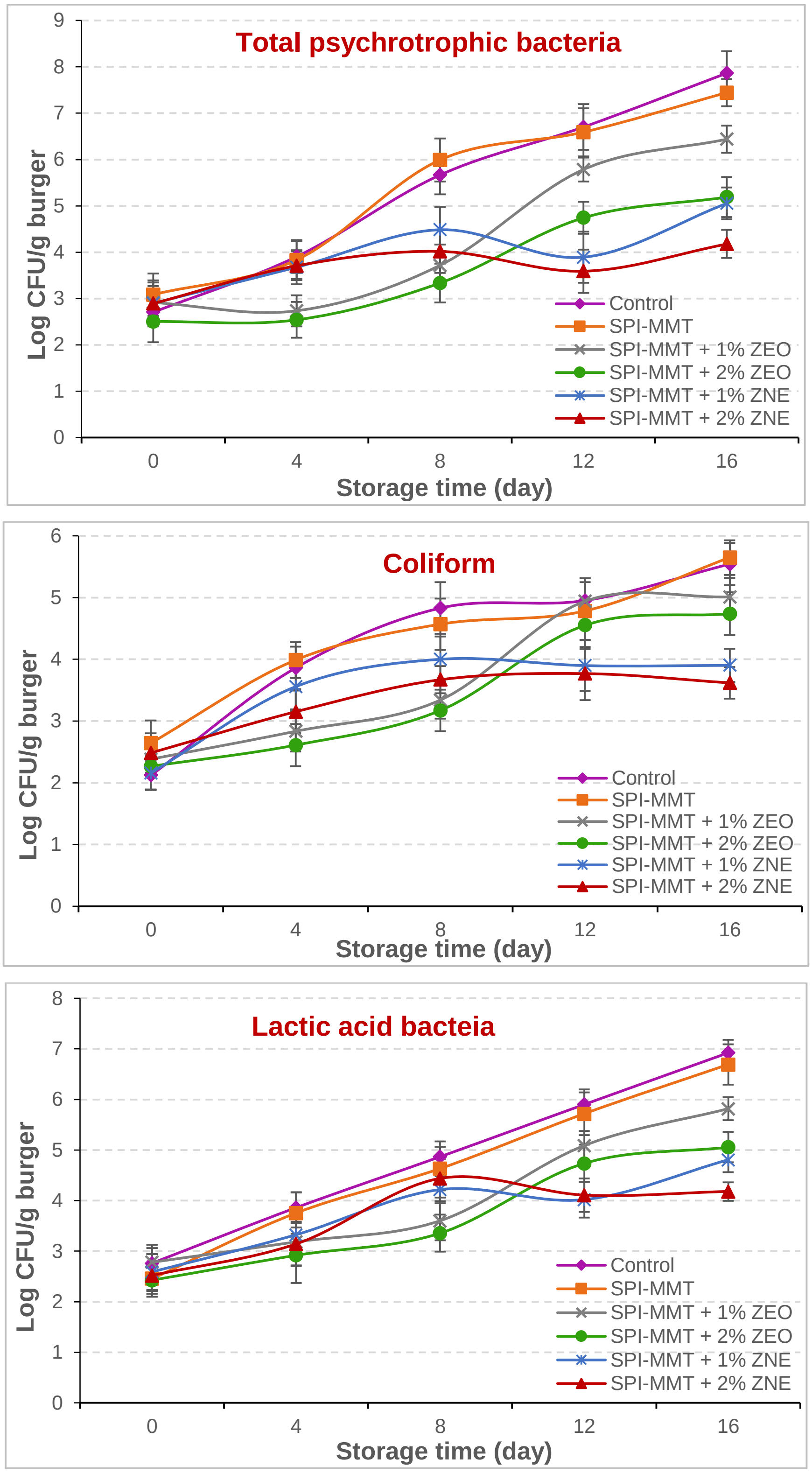
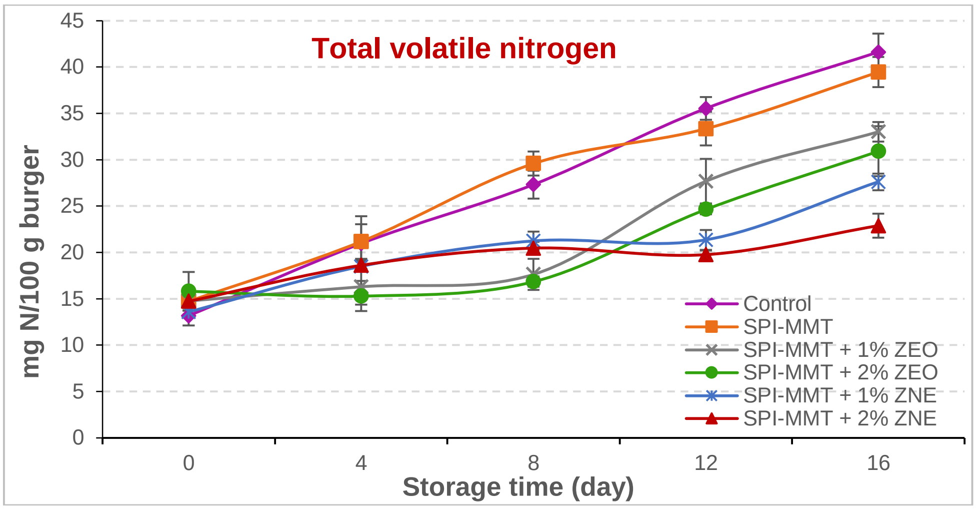
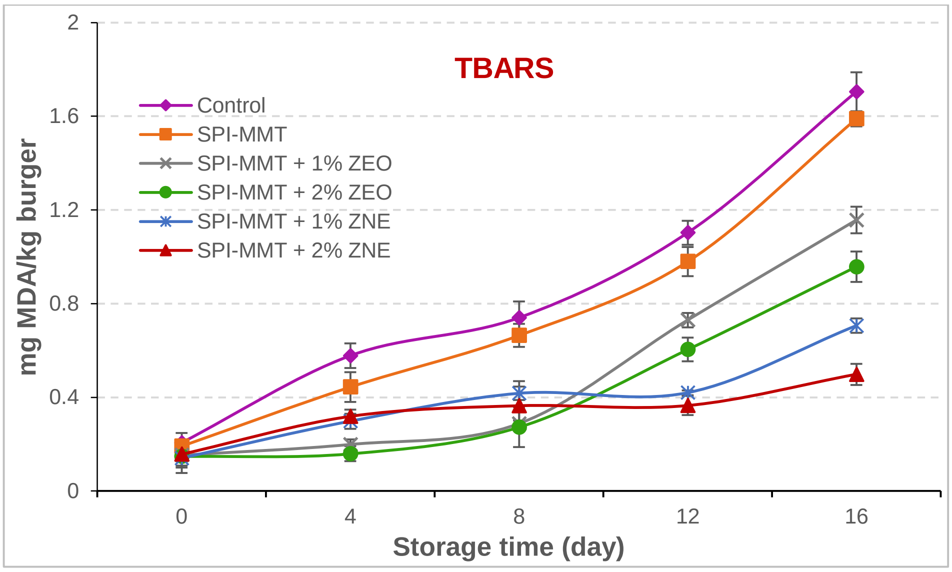


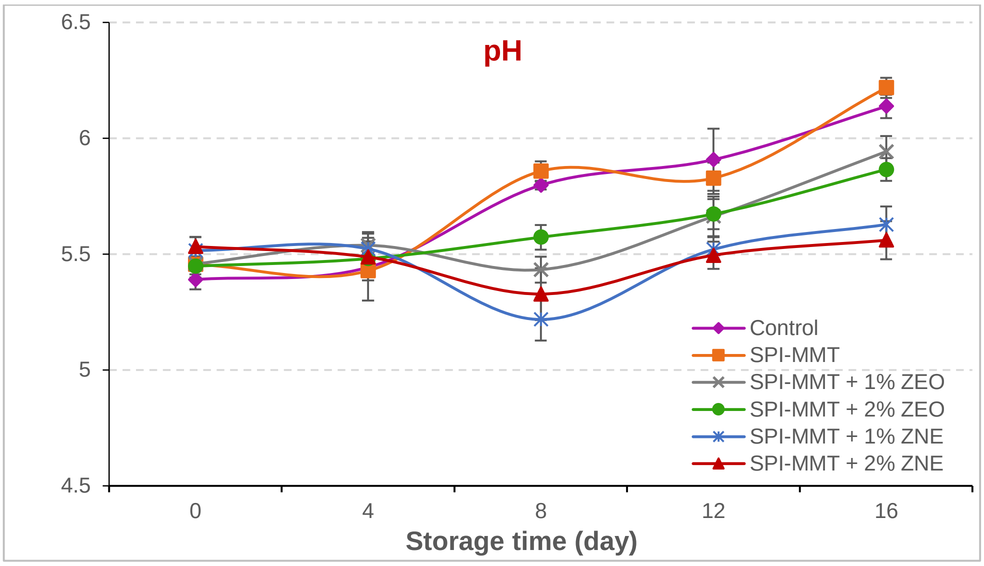
Figure 1. Microbial parameters of chicken burgers treated with SPI-MMT, ZEO, and ZNE

Microbiological Analyses

The results of microbial analysis for chicken burger samples are shown in Figure 1. At the beginning of the test (day 0), no significant difference (p>0.05) was observed between the TMB, TSB, LAB, and Enterobacteriaceae counts among the different groups. During storage, TMB and TSB increased in all groups (Figure 1a, b). On day 16, TMB and TSB were significantly higher in the control and SPI-MMT groups than in the treated groups (p<0.05). Samples containing free essential oil (SPI-MMT+ZEO) reduced the microbial population until day 8. Then, the function of samples containing nanoemulsion essential oil (SPI-MMT+ZNE) was better due to the gradual and controlled release of essential oil during storage in these groups (10). SPI-MMT+2% ZNE was the best group for controlling TMB and TSB in burgers. According to Iran's national standard, the limit of TMB in burgers is 6 log CFU/g burgers, which was higher than the standard limit on day 8in the control samples. In SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE groups, TMB did not exceed the standard limit until the end of the experiments (16 days).







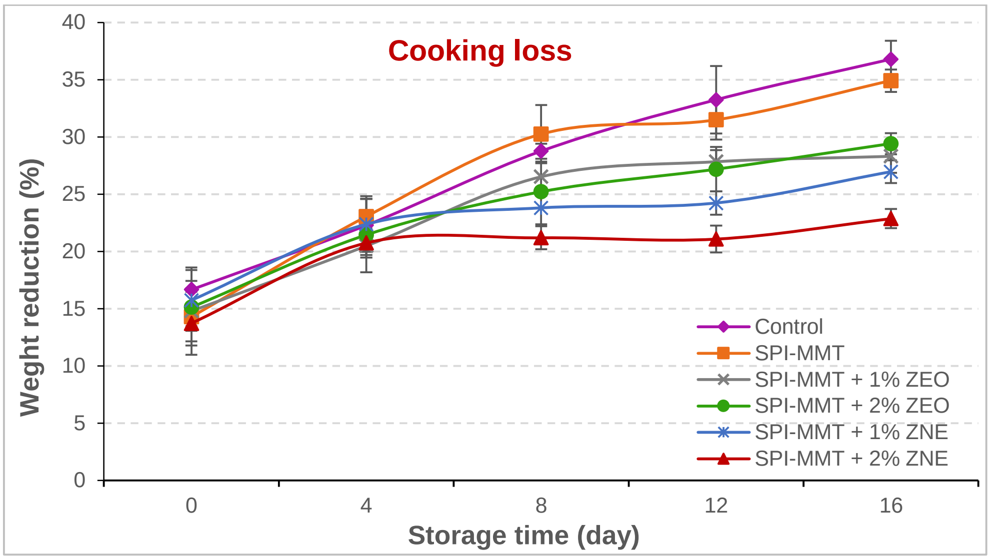


Figure 2. Physicochemical parameters of chicken burgers treated with treated with SPI-MMT, ZEO, and ZNE.

Physicochemical Analyses

A similar pattern was shown for the results of chemical parameters. TVN is a spoilage indicator that contains primary, second, and third amines (31). At day 0, TVN content and TBARS were not significantly different ($p>0.05$) in different groups (Figure 2a, b). The initial content of TVN in the studied groups ranged from 13.16 to 14.73mg N/100g burger, indicating the high quality of the burger samples. TVN content increased significantly during the storage period ($p<0.05$), but this increase was slower in the treated samples than in the control sample due to inhibition of microbial activity (28, 10, 18). SPI-MMT+2% ZNE showed the lowest TVN during storage. On day 8, the TVN content exceeded the standard limit in the control and SPI-MMT groups (25mg N/100g burger). In SPI-MMT+2% ZEO, SPI-MMT+1% ZNE, and SPI-MMT+2% ZNE groups, TVN content did not exceed the standard limit during 16 days.

The TBARS showed the lipid oxidation by-products, especially aldehydes (18). Initial TBARS levels were 0.20-0.15mg MDA/kg in all groups and gradually increased during storage. At day 16, TBARS in SPI-MMT+2% ZNE was significantly lower than the other groups ($p<0.05$).

There was a more significant reduction in TVB-N and TBARS levels in samples containing free essential oil (SPI-MMT+ZEO) than in samples containing essential oil nanoemulsion. From the next day, the TVB-N and TBARS content was lower in samples containing essential oil nanoemulsion (SPI-MMT+ZNE) due to the controlled release of essential oil (10).

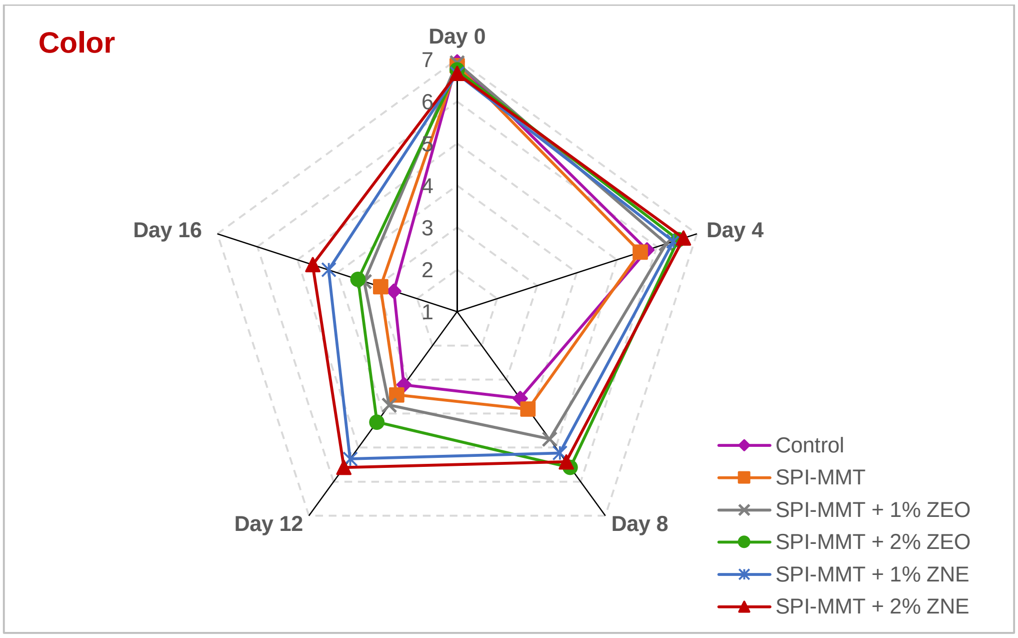
PH

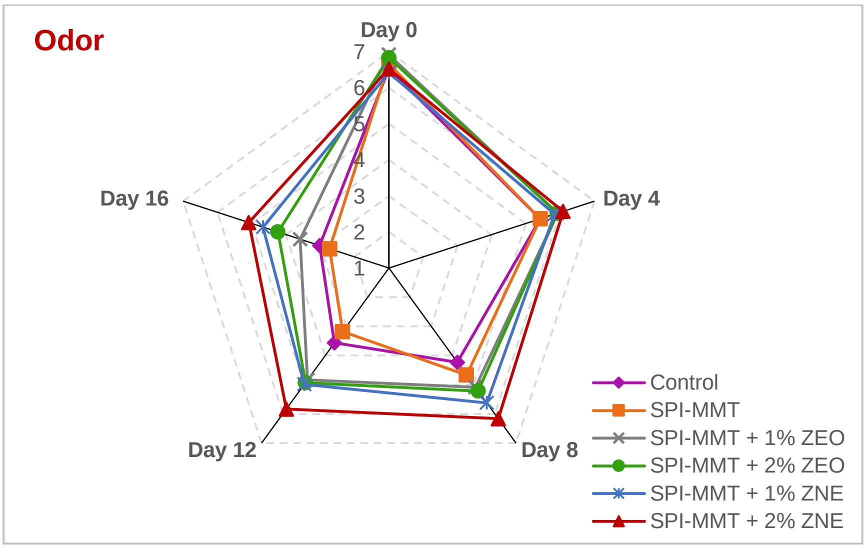
No significant difference was found in pH value among the experimental groups at days 0 and 4 ($p>0.05$) (Figure 2c). Then, the pH value was significantly higher in the control and SPI-MMT groups compared to the other groups ($p<0.05$). In all nanoemulsion groups (SPI-MMT+1% ZNE and SPI-MMT+2% ZNE), changes in pH were not significantly different at the end of the experiments. In contrast, in emulsion groups, the pH changes were significant ($p<0.05$). MMT+1% ZNE and SPI-MMT+2% ZNE were the best treatments to control pH changes in burger samples.

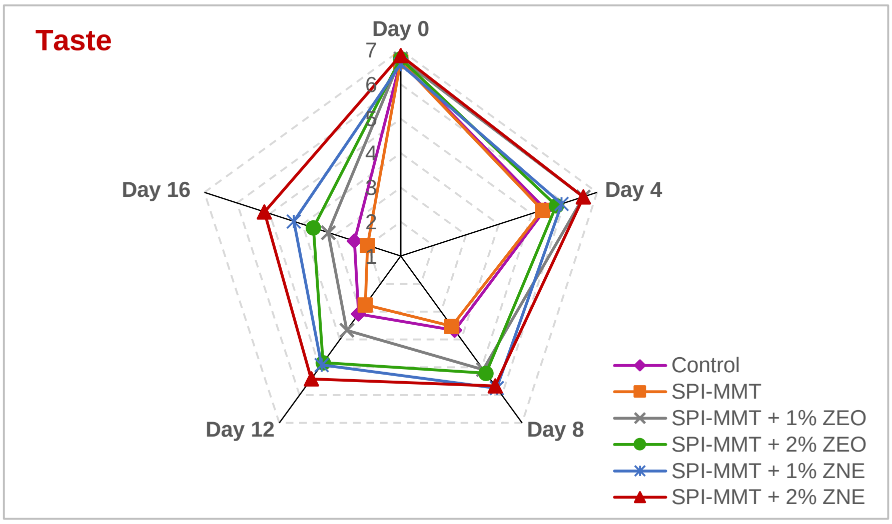
Cooking Loss

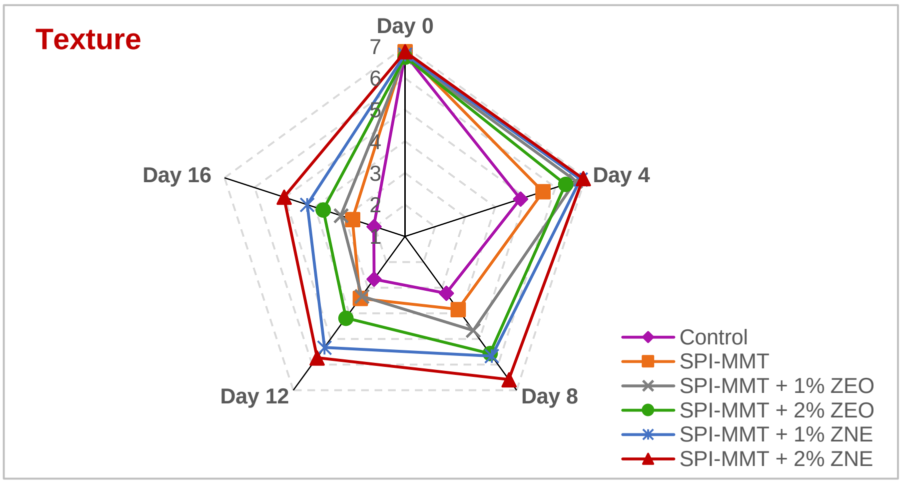
Juiciness and cooking loss are negatively correlated, implying that a high cooking loss results in low juiciness. Juiciness variation is partly explained by cooking loss, but it also influences the appearance of meat. A high cooking loss gives an expectation of a less optimal eating quality. There is also significant economic importance to cooking losses in the catering industry (11). The cooking loss is a combination of liquid and soluble matters lost from the meat during cooking and is calculated as the difference in weight between the uncooked and cooked burger divided by the weight of the uncooked burger (29).

Until the fourth day of storage, the cooking loss in different groups was not remarkably different ($p>0.05$), but there was a considerable increase during the storage ($p<0.05$), and this increase was slower in the treatment groups (Figure 2d). SPI-MMT+2% ZNE was the most effective treatment to reduce the cooking loss in burgers over 16 days of storage.




Odor
Day 0
Day 4
Day 8
Day 12
Day 16
7
6
5
4
3
2
1
Control
SPI-MMT
SPI-MMT + 1% ZEO
SPI-MMT + 2% ZEO
SPI-MMT + 1% ZNE
SPI-MMT + 2% ZNE


Taste
Day 0
Day 4
Day 8
Day 12
Day 16
7
6
5
4
3
2
1
Control
SPI-MMT
SPI-MMT + 1% ZEO
SPI-MMT + 2% ZEO
SPI-MMT + 1% ZNE
SPI-MMT + 2% ZNE


Texture
Day 0
Day 4
Day 8
Day 12
Day 16
7
6
5
4
3
2
1
Control
SPI-MMT
SPI-MMT + 1% ZEO
SPI-MMT + 2% ZEO
SPI-MMT + 1% ZNE
SPI-MMT + 2% ZNE

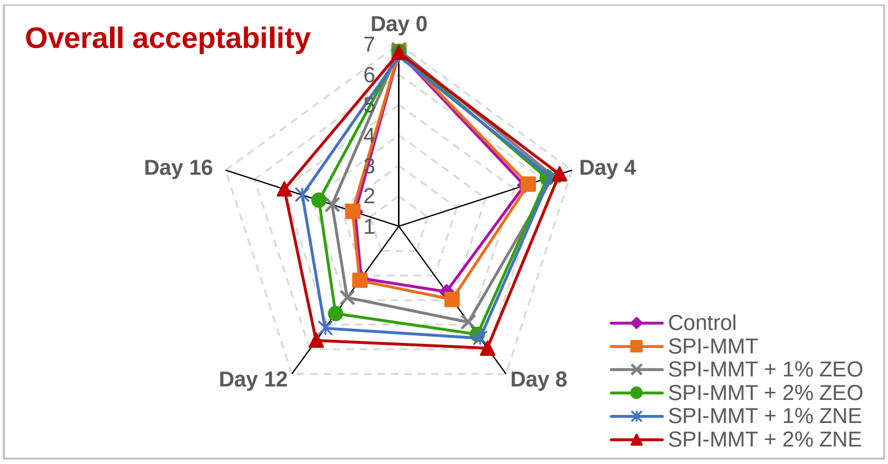


Figure 3. Sensory properties of chicken burgers treated with treated with SPI-MMT, ZEO, and ZNE.

Sensory Evaluation

The sensory analysis for chicken burgers is shown in Figure 3. the sensory parameters were highly desirable in all samples at the beginning of storage (scores≥6.5). The control sample was acceptable in terms of color, texture, taste, and overall acceptability until day 4, but it was acceptable from the point of view of odor until day 8. SPI-MMT+2% ZNE and SPI-MMT+1% ZNE groups were acceptable until day 16, but SPI-MMT+2% ZEO and SPI-MMT+1% ZEO were acceptable until days 12 and 8, respectively.

Discussion

This study showed that TMB and TSB increased in all experimental groups. On day 16, TMB and TSB were significantly higher in the control and SPI-MMT groups than in the other treated groups ($p<0.05$). Samples containing free essential oil (SPI-MMT+ZEO) reduced the microbial population until day 8. However, the function of samples containing nanoemulsion essential oil (SPI-MMT+ZNE) was better due to the gradual and controlled release of essential oil during storage in these groups. Dini et al. (10) showed that composite chitosan films containing nanoemulsion are more effective in reducing mesophilic and psychrophilic bacteria in beef loins. Hasani-Javanmardi et al. (18) showed that nanoemulsions reduced TMB, TSB, LAB, and Enterobacteriaceae in the lamb loins. Hassanzadeh et al. (20) reported similar results concerning chitosan-coated chicken breast containing grape seed extract. The results agree with other researchers who reported decreased mesophilic bacterial counts in coated beef with caseinate-whey protein (22).

During 16 days of storage, LAB counts increased in all groups, while the rate of increase was higher in the control group than in the treatment group (Figure 1c). SPI-MMT+2% ZNE group was the best sample for LAB control in burger samples. These results are consistent with those of Sarmast et al. (31), in which coated trout fillets had lower LAB counts than uncoated samples.

Enterobacteriaceae counts in the treated groups were significantly ($p<0.05$) lower than the control (Figure 1d). Abdeldaiem et al. (1) did not identify Enterobacteriaceae in coated carp fish fillets with calcium caseinate film containing essential rosemary oil during 12 days of refrigerated storage. In previous studies, nanoemulsions of various plant oils and essential oils reduced TMB and TSB, Enterobacteriaceae, and LAB counts in fish (12, 26), chicken (28), beef (18), and pork (11).

The different nanoemulsions were shown to have antibacterial effects against various microorganisms (18, 13). Nanoemulsions integrate with lipid membranes, destabilizing cytoplasmic membranes, releasing essential oils, and causing cell death in microorganisms (18, 12).

Carvacrol and thymol are responsible for the antibacterial properties of ZEO (4), which are the main constituents of the essential oil. The lipophilic property of the essential oil, especially monoterpene compounds, enables the oil to penetrate the cytoplasmic membrane of the microorganisms and disrupts its function, thereby causing cell death (10).

Microorganisms' proteolytic enzymes have produced volatile nitrogenous compounds (10, 31, 14). These results align with those of Abdeldaiem et al. (1), who reported that coated carp fillets with calcium caseinate film had low TVN levels during storage.

The phenolic compounds, including carvacrol and thymol, neutralized free radicals and thus reduced lipid oxidation (10, 16). Nanoemulsion formation reduced the droplet size and increased the specific surface area, so radical scavenging occurred faster and more effectively. SPI-MMT+2% ZNE was more effective than other groups in controlling the oxidation of burger samples due to the essential oil's gradual release (10). Hassanzadeh et al. (18) reported decreased TBARS levels in chitosan-coated chicken breasts containing grape seed extract and showed a delay in increasing total volatile nitrogen and lipid oxidation in coated lamb (18).

The activity of microorganisms and the increasing volatile bases can increase the pH values. Similar findings have been reported in the literature (31, 32).

The scores of all sensory properties in all experimental groups decreased significantly during 16 days ($p<0.05$), but it was slower than the control group in the treated groups. These results confirmed those of microbial and chemical analysis. Previous studies have shown that treating different types of meat with different nanoemulsions based on essential oils increased their shelf life during refrigeration (28, 10, 18, 12, 13).

Conclusion

The results showed that soy protein isolate-montmorillonite nanoclays containing ZEO nanoemulsion effectively controlled the population of microbial flora and delayed physicochemical changes in chicken burgers. In this regard, SPI-MMT+2% ZNE was the most influential group for increasing the shelf life of chicken burgers. As a result, the nanocomposite film containing nanoemulsions of ZEO may be suggested to preserve chicken burgers during chilled storage.

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Conflict of Interest

The authors declare the existence of any conflict of interest in this study.

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