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Enhancement of white cheese quality and shelf life using green synthesized zinc oxide nanoparticles (ZnO NPs) in combination with *Bunium persicum* L. essential oil

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ABSTRACT

In this research, enhancement of white cheese quality and shelf life using green synthesized zinc oxide nanoparticles (ZnONPs) in combination with *B.persicum* L. essential oil (control sample, sample containing 50 mg of *B.persicum* L. essential oil, sample containing 100 mg of *B.persicum* L. essential oil, sample containing 5 mg of zinc oxide nanoparticles with 50 mg of *B.persicum* L. essential oil, and sample containing 5 mg of zinc oxide nanoparticle with 100 mg of *B.persicum* L. essential oil). Means were compared using Duncan's multi-range test at a significant level of 5% with SPSS version 22 software. The physicochemical results showed that the addition of *B.persicum* L. essential oil and zinc oxide nanoparticles containing *B.persicum* L. essential oil to cheese have a no significant effect on acidity, pH and protein ($p>0.05$), but it showed a significant increase on the amount of dry matter ($p<0.05$). The results of the microbial test showed that, the effect of zinc oxide nano particles with *B.persicum* L. essential oil on cheese on the count of *Candida Albicans* yeast, *Staphylococcus* and *Escherichia coli* was better than *B.persicum* L. essential oil. The antioxidant activity results showed that, the lowest and highest IC_{50} values were respectively related to the cheese sample containing 5 mg of zinc oxide nanoparticles along with 100 mg of *B.persicum* L. essential oil and the control sample. Histological results showed the essential oil and zinc oxide nanoparticles containing *B.persicum* L. essential oil increased and decreased hardness and adhesion, respectively ($p>0.05$). In the colorimetric test, L* and b* index of the samples containing zinc oxide nanoparticles containing *B.persicum* L essential oil decreased significantly ($p<0.05$), but the a* index increased ($p <0.05$). The results of sensory evaluation showed that, the addition of 5 mg of zinc oxide nanoparticles along with 100 mg of *B.persicum* L. essential oil improved the overall acceptance of the cheese sample ($p<0.05$).

1- Introduction

Nowadays, due to the side effects and disadvantages of chemical preservatives, the use of medicinal plants and natural compounds as suitable alternatives for food preservation and the production of disinfectant and antimicrobial agents—free from harmful by-products—has increasingly attracted attention [1]. In this context, many nanoparticles are also produced through chemical or physical methods; however, the use of hazardous and toxic chemicals and the resulting environmental damage have raised significant concerns [2]. There are various methods for synthesizing nanoparticles, including chemical, sonochemical, electrochemical, microwave-assisted synthesis, and green synthesis methods [3]. Among these, the green synthesis of zinc oxide nanoparticles offers more advantages compared to other physical processes, as it is inspired by biological processes, relatively inexpensive, environmentally friendly, and renewable. Furthermore, it has pharmaceutical and biomedical applications and can be easily employed for large-scale synthesis. Most importantly, such processes can be carried out under normal pressure and relatively low temperatures [4].

Bunium persicum (black cumin) is a plant from the Apiaceae family and belongs to the group of flowering plants, class Dicotyledons, subclass Rosidae, and order Apiales. The Apiaceae family consists of 423 genera and about 3,000 species [5].

Cheese is among the foods with high-quality protein and is very rich in essential amino acids. Iranian white cheese is a type of brined cheese made from cow's milk curd without dry salting, with a ripening period of 40–90 days in brine [6]. Rafiei et al. (2018) reported that zinc oxide nanoparticles synthesized using eucalyptus leaf extract, as well as zinc sulfate, possess antimicrobial properties. However, the strongest antimicrobial effects were observed with zinc oxide nanoparticles [7]. Rajawat and Qureshi (2012), in a study using tea extract, synthesized silver nanoparticles. The results showed that the antimicrobial activity of silver nanoparticles combined with ampicillin was greater than when combined with gentamicin [8]. Khodadadi et al. (2015) stated that the shape and size of silver nanoparticles synthesized using aqueous extract of bilberry fruit were significantly influenced by factors such as extract concentration, silver nitrate concentration, reaction time, and light exposure. Additionally, the synthesized silver nanoparticles exhibited considerable antimicrobial effects against both Gram-negative and Gram-positive bacteria [9] Hamdi et al. (2023) showed that biosynthesized silver nanoparticles using Ephedra intermedia extract had a stronger inhibitory effect on bacterial growth compared to commercial nanoparticles. Therefore, these nanoparticles could be suitable alternatives in pharmaceutical, medical, and disinfectant applications [10]. Sousani et al. (2021) investigated the antimicrobial activity of zinc oxide nanoparticles synthesized using cell-free extract of Rhodotorula pacifica NSO2 against certain clinical isolates. The results showed that due to the small size and uniform distribution of the nanoparticles, a favorable inhibitory effect was observed against all tested bacterial isolates [11]. Heydarzadeh and Yaghoubi (2017) examined the antimicrobial effects of silver nanoparticles produced using bitter orange blossom extract. The results indicated that the extract could reduce silver ions to silver nanoparticles. Furthermore, the green-synthesized silver nanoparticles demonstrated suitable antimicrobial activity against both Gram-positive and Gram-negative bacter [12]. One of the major problems currently facing the food industry is the increasing use of chemical preservatives in food products. These substances are directly associated with the incidence of serious diseases such as cancer. Therefore, finding ways to eliminate or at least reduce the dosage of these chemical preservatives in food products, without compromising their quality, is of great importance.

The aim of this study is to use zinc oxide nanoparticles (ZnONPs) synthesized through a green method along with the essential oil of *Bunium persicum* (Iranian black cumin) and to evaluate their effects on improving the quality and shelf life of Iranian white cheese.

2- Materials and methods

2-2- Methods

2-2-2- Essential oil extraction from Iranian Black Cumin seeds

The essential oil was extracted by hydrodistillation using a Clevenger-type apparatus [13].

2-2-3 Identification of compounds in the essential oil of Iranian Black Cumin

To analyze and identify the components of the essential oil, a gas chromatograph coupled with a mass spectrometer (Thermoquest-Finnigan, model Trace) was used. The device was equipped with a DB column, 60 cm in length, 0.25 mm internal diameter, and 0.25 µm film thickness. Wiley version 7 and NIST version 1.7 software were used for identifying the compounds and for searching validated libraries [14].

2-2-4- Synthesis of zinc oxide nanoparticles (ZnONPs) with Black Cumin essential oil

Zinc oxide nanoparticles were synthesized via a green synthesis method using zinc nitrate hexahydrate, 2 M sodium hydroxide, distilled water, and black cumin essential oil as precursor materials. Initially, 10 mL of black cumin essential oil was mixed with zinc nitrate hexahydrate in a flask. Then, 18 mL of 2 M sodium hydroxide was slowly added. The flask was completely sealed and stirred at 80 °C for 3 hours. After 3 hours, the solution color changed from light brown to cream. The mixture was then washed several times using a Buchner funnel with ethanol: distilled water (3:1). The resulting precipitate was placed in an oven at 80 °C for 20 hours to dry. It was then placed in a furnace at 500 °C for 3 hours to completely remove impurities and obtain the white zinc oxide nanoparticle powder [15].

2-2-5- Cheese preparation

To prepare the cheese, fresh whole cow's milk pasteurized at 65 °C for 30 minutes was used. Before beginning the cheesemaking process, the milk was brought to 35 °C. Then, the desired bacterial inoculum at a concentration of 10^3 CFU/mL was added. Next, 0.02% (w/v) of calcium chloride dissolved in 20 mL of sterile distilled water at 40 °C was evenly added to the milk. At this stage, the black cumin essential oil and the zinc oxide nanoparticles with black cumin essential oil were separately added according to Table 1. Finally, the enzyme (cheese rennet tablet obtained from IIEC company) was added at a concentration of 0.001% (w/v) after being dissolved in sterile distilled water. The cheese-containing containers were labeled according to Table 1.

Table 1. Test treatments

No	Treatments
1	Control
2	Sample containing 50 mg of *B. persicum* L. essential oil
3	Sample containing 100 mg of *B. persicum* L. essential oil
4	Sample containing 5 mg of zinc oxide nanoparticles with 50 mg of *B. persicum* L. essential oil
5	sample containing 5 mg of zinc oxide nanoparticle with 100 mg of *B. persicum* L. essential oil

2-3- Cheese Testing

2-3-1 Physicochemical tests of cheese samples

The acidity, pH, fat content, and protein of dry matter were measured based on the Iranian National Standards No. 2852, 8785, 1-9188, and 1753, respectively [16-19].

2-3-2- Evaluation of the antimicrobial effect of zinc oxide nanoparticles containing Black Cumin essential oil in cheese

The effect of the essential oil and zinc oxide nanoparticles containing black cumin essential oil on cheese shelf life was investigated. The prepared treatments, as listed in Table 1, were subjected to microbial tests at 7-day intervals, in accordance with the Iranian National Standards for cheese. Identification of Staphylococcus aureus, Escherichia coli, and Candida albicans was conducted based on standards No. 1-6806, 2946, and 1-10899, respectively [20-22].

2-3-3- Evaluation of antioxidant activity in cheese samples containing zinc oxide nanoparticles and Black Cumin essential oil

The antioxidant activity of the cheese samples containing zinc oxide nanoparticles and black cumin essential oil was determined using the DPPH free radical scavenging assay [23].

2-3-4– Measurement of textural parameters

Textural characteristics were assessed using a texture analyzer (Model QTS25 CNS Farnell, UK), equipped with dedicated computer software. The texture properties obtained from the force-deformation curve included maximum compression force (g), used as an indicator of hardness, and the negative area of the force-time curve during the probe's return, used as a measure of adhesiveness (g.s) [24].

2-3-5- Measurement of color parameters

Color was evaluated using a HunterLab colorimeter (Model ColorFlex, Germany), recording the L* (lightness), a* (red-green), and b* (yellow-blue) color indices. For each cheese formulation, three random pieces were selected, photographed, and stored in JPG format. Further image processing was performed using ImageJ software version 1.40g [25].

2-3-6- Sensory evaluation

Following preliminary training, 20 individuals were selected as evaluators. Using a 5-point hedonic scale, they assessed the cheese samples in terms of flavor, color, texture, aroma, and overall acceptability. A score of 5 represented "excellent" and 1 represented "very poor" [23].

2-3-7- Data Analysis

All analyses were conducted in triplicate using a completely randomized design. Means were compared using Duncan's multiple range test at a 5% significance level with SPSS software version 22. Graphs were created using Microsoft Excel 2013.

3- Results and discussion

3-1- Results of chemical analysis of black cumin essential oil by GC/MS

The average yield of black cumin essential oil from three replicates was approximately 2.81% ± 0.02. The essential oil had a yellow-amber color and a distinct aroma. Results showed that black cumin essential oil contained 22 compounds, accounting for 94.93% of the total oil (Table 2). Among the components, the most abundant were: Para-cymene (17.17%), Gamma-terpinene (15.14%), Cuminaldehyde (25.78%), Anethole (8.67%), Anisole Trans-anethole (5.27%), Thymol (11.23%), Carvacrol (1.70%) and Limonene (1.19%). Takayuki et al. (2007) studied the antifungal effects of volatile compounds from black cumin against Fusarium oxysporum. The results showed that the essential oil contained seven volatile compound gamma-terpinene, Limonene, P-cymene, Beta-pinene, Alpha-pinene, Cuminaldehyde, and Myrcene with Cuminaldehyde being the main antifungal component in black cumin [26].

Table 2. Determination of components and elements of *Bunium persicum* (Boiss.) B. Fedtsch. essential oil by GC/MS

No	Chemical compound	Composition percentage	RI
1	Alpha.-Pinene	0.51	939
2	Beta.-Pinene	0.40	981
3	Beta.-Myrcene	0.40	991
4	Para cymene	17.17	1033
5	Limonene	1.19	1039
6	Gamma.-terpinene	15.14	1051
7	Alpha.-Thujone	0.31	1102

8	Pulegone	0.49	1237
9	Benzene	0.88	642
10	Cuminic aldehyde	25.78	1239
11	Anethol	8.67	*
12	Anisole Trans-anethole	5.27	917
13	Thymol	11.33	1290
14	Karvakrol	1.70	1298
15	Thiophene	0.37	*
16	Benzenemethanol	1.06	*
17	Beta.-F0.arnesene	0.47	1429
18	Cycloheptane	1.29	627
19	Croweacin	1.05	*
20	Caryophyllene oxide	0.54	1581
21	Dill-apiol	0.53	1622
22	Propenoic acid	0.38	*
Total		**94.93**	

3-2- Results of cheese testing

3-2-1- Evaluation of physicochemical properties of cheese samples

The results for acidity, pH, protein, and dry matter content of cheese samples containing zinc oxide nanoparticles and black cumin essential oil are presented in Table 3. As shown in Table 3, the addition of black cumin essential oil and zinc oxide nanoparticles containing black cumin essential oil had no significant effect on acidity, pH, and protein content ($p>0.05$). However, a significant increase was observed in dry matter content ($p<0.05$). The lowest and highest dry matter content belonged to the control sample and the sample containing 5 mg zinc oxide nanoparticles with 100 mg black cumin essential oil, respectively. Zinc oxide nanoparticles, due to their special surface structure, can interact with water molecules and milk proteins. The essential oil of black cumin, with its antioxidant components and antimicrobial properties, prevents tissue degradation and the growth of harmful microorganisms. This combination helps to reduce free moisture, reinforce the protein network, minimize water loss from the cheese matrix, and ultimately increase concentration and improve retention of solid materials [27]. Momeni Sarvestani and Lashkari (2018) also reported that the addition of black cumin essential oil to cheese did not have a significant effect on protein and dry matter content ($p>0.05$) [23].

Table 3- Investigation of the physicochemical properties of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

Treatments	Acidity	pH	Protein	Dry matter
Control cheese	1.02 ± 0.03^a	4.49 ± 0.05^a	13.12 ± 0.02^a	36.27 ± 0.04^d
Cheese containing 50 mg *B. persicum* L. essential oil	1.12 ± 0.04^a	4.57 ± 0.03^a	13.20 ± 0.04^a	37.17 ± 0.03^c
Cheese containing 100 mg *B. persicum* L. essential oil	1.19 ± 0.02^a	4.62 ± 0.04^a	13.24 ± 0.07^a	38.14 ± 0.05^c
Cheese containing 5 mg zinc oxide nanoparticles along with 50 mg *B. persicum* L. essential oil	1.28 ± 0.05^a	4.32 ± 0.03^a	13.39 ± 0.04^a	39.87 ± 0.06^b
Cheese containing 5 mg zinc oxide nanoparticles along with 100 mg *B. persicum* L. essential oil	1.31 ± 0.07^a	4.30 ± 0.02^a	13.43 ± 0.07^a	42.92 ± 0.04^a

*Means in a colu mn followed by the diferent superscripts are signifcantly diferent at $p\leq0.05$ by Duncan test.

3-2-2- Evaluation of the antimicrobial effect of zinc oxide nanoparticles containing Black Cumin essential oil in cheese during storage

3-2-2-1- Enumeration of *Staphylococcus aureus* in cheese samples

The statistical results of *Staphylococcus aureus* count in the cheese samples are presented in Table 4.

Table 4-Investigation of counting of *Staphylococcus aureus* bacteria (log of colony forming unit/gr) of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

Storage time	Control cheese	Cheese containing 50 mg *B. persicum* L. essential oil	Cheese containing 100 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 50 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 100 mg *B. persicum* L. essential oil
production day	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Bh}	0.00 ± 0.00^{Bh}	0.00 ± 0.00^{Ce}	0.00 ± 0.00^{Ce}
Day 1	2.98 ± 0.02^{Ag}	1.31 ± 0.04^{Bg}	1.11 ± 0.03^{Cg}	0.00 ± 0.00^{De}	0.00 ± 0.00^{De}
Day 2	3.87 ± 0.03^{Af}	2.27 ± 0.05^{Bf}	1.95 ± 0.02^{Cf}	0.00 ± 0.00^{De}	0.00 ± 0.00^{De}
Day 3	4.21 ± 0.04^{Ae}	3.11 ± 0.02^{Be}	2.93 ± 0.07^{Ce}	0.00 ± 0.00^{De}	0.00 ± 0.00^{De}
Day 4	5.16 ± 0.03^{Ad}	3.48 ± 0.03^{Bd}	3.16 ± 0.06^{Cd}	2.12 ± 0.08^{Da}	1.92 ± 0.04^{Ea}
Day 5	5.71 ± 0.09^{Ac}	4.27 ± 0.06^{Bc}	3.98 ± 0.05^{Cc}	1.39 ± 0.04^{Db}	1.57 ± 0.02^{Eb}
Day 6	6.96 ± 0.02^{Ab}	4.37 ± 0.04^{Bb}	4.11 ± 0.07^{Cb}	1.12 ± 0.03^{Dc}	1.31 ± 0.03^{Ec}
Day 7	8.18 ± 0.07^{Aa}	4.82 ± 0.03^{Ba}	4.68 ± 0.02^{Ca}	0.98 ± 0.06^{Cd}	0.86 ± 0.06^{Ed}

*Mean ± standard deviation

**Large non-common letters indicate the presence of significant differences in each row, and small non-common letters indicate the presence of differences in each column (Duncan's test, P < 0.05).

3-2-2-1- Enumeration of *Staphylococcus aureus* in cheese samples

As shown in Table 4, the *Staphylococcus aureus* counts in both the control sample and the sample containing 100 mg of black cumin essential oil exhibited a significant upward trend at all time points ($p<0.05$). In contrast, a decrease in bacterial growth was observed throughout the storage period in samples containing 5 mg zinc oxide nanoparticles combined with 50 mg and 100 mg of black cumin essential oil, compared to the control. The strongest antimicrobial effect of the zinc oxide nanoparticles was observed at 72 hours, indicating their ability to effectively inhibit bacterial growth in the short term. However, over time, the antimicrobial effect gradually diminished, leading to a significant increase in bacterial count ($p<0.05$). Interestingly, for the samples containing zinc oxide nanoparticles alone, *S. aureus* counts increased during the first 72 hours but then significantly decreased from day 5 to day 7 ($p<0.05$). This behavior could be attributed to the gradual release of antimicrobial compounds from the ZnO nanoparticles in combination with black cumin essential oil over the storage period. Furthermore, the cheese sample containing 5 mg ZnO nanoparticles with 100 mg black cumin essential oil showed a significantly greater inhibition of *S. aureus* growth compared to the sample with 5 mg ZnO with 50 mg essential oil ($p<0.05$). These findings align with those of Moghaddar et al. (2009), who reported that black cumin essential oil possesses notable antibacterial activity, primarily due to its high content of terpinene and cuminaldehyde compounds [28].

3-2-2-2 Enumeration of *Escherichia coli* in cheese samples

The statistical results for the enumeration of *Escherichia coli* in the cheese samples are presented in Table 5.

Table 5-Investigation of counting of *Escherichia coli* bacteria (log of colony forming unit/gr) of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

Storage time	Control cheese	Cheese containing 50 mg *B. persicum* L. essential oil	Cheese containing 100 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 50 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 100 mg *B. persicum* L. essential oil
production day	0.00 ± 0.00^{Ah}	0.00 ± 0.00^{Ah}	0.00 ± 0.00^{Ah}	0.00 ± 0.00^{Ag}	0.00 ± 0.00^{Ag}
Day 1	3.11 ± 0.04^{Ag}	1.48 ± 0.02^{Bg}	1.37 ± 0.04^{Cg}	0.00 ± 0.00^{Dg}	0.00 ± 0.00^{Dg}
Day 2	3.98 ± 0.07^{Af}	3.58 ± 0.03^{Bf}	3.31 ± 0.07^{Cf}	2.97 ± 0.09^{Da}	2.47 ± 0.09^{Ea}
Day 3	4.67 ± 0.02^{Ae}	3.29 ± 0.07^{Be}	2.99 ± 0.06^{Ce}	2.54 ± 0.04^{Db}	2.17 ± 0.04^{Eb}
Day 4	5.58 ± 0.07^{Ad}	3.97 ± 0.04^{Bd}	3.67 ± 0.03^{Cd}	2.34 ± 0.08^{De}	2.04 ± 0.08^{Ee}

Day 5	6.97 ± 0.03^{Ac}	4.57 ± 0.03^{Bc}	4.25 ± 0.06^{Cc}	1.72 ± 0.06^{Dd}	1.97 ± 0.06^{Ed}
Day 6	7.53 ± 0.06^{Ab}	5.19 ± 0.07^{Bb}	4.94 ± 0.04^{Cb}	1.57 ± 0.08^{De}	1.37 ± 0.08^{Ee}
Day 7	8.84 ± 0.02^{Aa}	5.57 ± 0.03^{Ba}	5.27 ± 0.07^{Ca}	1.25 ± 0.03^{Df}	1.04 ± 0.03^{Ef}

*Mean ± standard deviation

**Large non-common letters indicate the presence of significant differences in each row, and small non-common letters indicate the presence of differences in each column (Duncan's test, P < 0.05).

As shown in Table 5, the number of *Escherichia coli* bacteria in the control sample and the sample containing 100 mg of black cumin essential oil exhibited a significant upward trend at all time intervals (p<0.05). In contrast, the samples containing 5 mg zinc oxide nanoparticles combined with 50 mg and 100 mg of black cumin essential oil showed a significant reduction in bacterial growth throughout the entire observation period compared to the control. The strongest antimicrobial effect of the ZnO nanoparticles against *E. coli* was observed at 24 hours. However, over time, this antimicrobial property gradually diminished, and the bacterial count showed a significant increase (p<0.05). Specifically, *E. coli* levels initially increased during the first 24 hours in ZnO-treated samples, but then experienced a significant decrease from day 2 to day 7 (p<0.05). This pattern suggests that the antimicrobial compounds were gradually released from the ZnO nanoparticles combined with black cumin essential oil over the storage period. Additionally, as shown in Table 5, the sample containing 5 mg ZnO nanoparticles with 100 mg essential oil exhibited a significantly greater inhibition of E. coli growth than the sample with 5 mg ZnO with 50 mg essential oil (p<0.05). *E. coli* is known to cause early bloating in Iranian white cheese, which often indicates unsanitary production conditions. Furthermore, E. coli is considered a hygienic indicator in many foods, including dairy products [29].

3-2-2-3 -Enumeration of *Candida albicans* yeast in cheese samples

The statistical results for *Candida albicans* yeast count in the cheese samples are presented in Table 6. As shown in Table 6, both the control sample and the sample containing 100 mg of black cumin essential oil exhibited a significant increase in *C. albicans* counts across all time intervals (p<0.05). In contrast, the samples containing 5 mg of ZnO nanoparticles combined with 50 mg and 100 mg of black cumin essential oil showed a significant decrease (p<0.05) in yeast growth throughout the entire storage period compared to the control. The strongest antifungal effect was observed at 96 hours. However, similar to the antibacterial results, the antifungal activity of ZnO nanoparticles gradually declined over time, leading to a significant increase in yeast count (p<0.05).

Table 6- Investigation of counting of *Candida albicans yeast* bacteria (log of colony forming unit/gr) of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

Storage time	Control cheese	Cheese containing 50 mg *B. persicum* L. essential oil	Cheese containing 100 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 50 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 100 mg *B. persicum* L. essential oil
production day	0.00 ± 0.00^{Ah}	0.00 ± 0.00^{Ah}	0.00 ± 0.00^{Ah}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ad}
Day 1	1.24 ± 0.07^{Ag}	1.05 ± 0.04^{Bg}	0.95 ± 0.02^{Cg}	0.00 ± 0.00^{Dd}	0.00 ± 0.00^{Dd}
Day 2	2.37 ± 0.06^{Af}	1.68 ± 0.02^{Bf}	1.15 ± 0.07^{Cf}	0.00 ± 0.00^{Dd}	0.00 ± 0.00^{Dd}
Day 3	3.57 ± 0.03^{Ae}	1.97 ± 0.04^{Be}	1.87 ± 0.07^{Ce}	0.00 ± 0.00^{Dd}	0.00 ± 0.00^{Dd}
Day 4	4.36 ± 0.06^{Ad}	2.48 ± 0.06^{Bd}	2.24 ± 0.08^{Cd}	0.00 ± 0.00^{Dd}	0.00 ± 0.00^{Dd}
Day 5	4.95 ± 0.07^{Ac}	2.91 ± 0.03^{Bc}	2.75 ± 0.05^{Cc}	1.22 ± 0.02^{Da}	1.02 ± 0.04^{Ea}
Day 6	5.37 ± 0.06^{Ab}	3.48 ± 0.02^{Bb}	3.24 ± 0.03^{Cb}	1.01 ± 0.06^{Db}	0.84 ± 0.02^{Eb}

Day 7	6.21 ± 0.07Aa	3.95 ± 0.05Ba	3.78 ± 0.04Ca	0.88 ± 0.02 Dc	0.67 ± 0.03Ec

*Mean ± standard deviation

**Large non-common letters indicate the presence of significant differences in each row, and small non-common letters indicate the presence of differences in each column (Duncan's test, $P < 0.05$).

Regarding the effect of zinc oxide nanoparticles, the number of *C. albicans* yeasts increased during the first 96 hours, but then a significant reduction in yeast count was observed from day 5 to day 7 ($p<0.05$). This result can be attributed to the gradual release of antimicrobial compounds from the zinc oxide nanoparticles combined with black cumin essential oil over the storage period. As seen in table 6, the cheese sample containing 5 mg ZnO nanoparticles and 100 mg black cumin essential oil showed a significantly greater inhibition of *Candida albicans* growth compared to the sample with 5 mg ZnO and 50 mg essential oil ($p<0.05$). The greater sensitivity of Gram-positive bacteria to antimicrobial compounds may be due to structural differences in their cell walls. Gram-positive bacteria possess a single-layered cell wall, whereas Gram-negative bacteria have a multi-layered wall that offers more resistance to external substances [30]. Yousef et al. (2016) reported that biocomposites containing zinc oxide nanoparticles exhibited strong antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli*), and fungi (*Candida albicans*). They also found that packaging films made with these composites extended the shelf life of soft white cheese, suggesting their potential in food packaging applications. They demonstrated that the zones of growth inhibition for pathogens followed this order: *C. albicans* < *E. coli* < *B. subtilis* < *B. cereus* < *S. aureus* < *P. aeruginosa* < *A. niger*; furthermore, the higher the ZnO nanoparticle concentration, the greater the inhibition zone [31]. The results of the present study are in agreement with these findings. Here, we also observed microbial colony reduction in the following order: *E. coli* < *S. aureus* < *C. albicans*. Another study investigating the effect of black cumin essential oil on pathogens in Gouda cheese including yeasts reported that at a 0.4% concentration, the number of yeasts on day 90 was 3.4 log units, indicating a 2-log reduction compared to the control sample [32].

3-2-3 Evaluation of antioxidant properties in cheese samples containing zinc oxide nanoparticles and Black Cumin essential oil

The free radical scavenging activity (DPPH assay) of the cheese samples was measured and presented as IC_{50} values (Figure 1). Lower IC_{50} values indicate higher antioxidant activity. The results showed that adding zinc oxide nanoparticles containing black cumin essential oil to the cheese significantly enhanced its free radical scavenging activity ($p<0.05$). As seen in Figure 1, the lowest IC_{50} was observed in the sample with 5 mg ZnO nanoparticles and 100 mg black cumin essential oil, while the highest IC_{50} belonged to the control.

he IC_{50} values of the control sample, cheese containing 50 mg black cumin essential oil, cheese containing 100 mg black cumin essential oil, cheese containing 5 mg zinc oxide nanoparticles with 50 mg black cumin essential oil, and cheese containing 5 mg zinc oxide nanoparticles with 100 mg black cumin essential oil were 24.28 ± 1.23, 19.34 ± 1.08, 15.32 ± 1.57, 10.65 ± 1.41, and 6.32 ± 1.19 µg/mL, respectively.

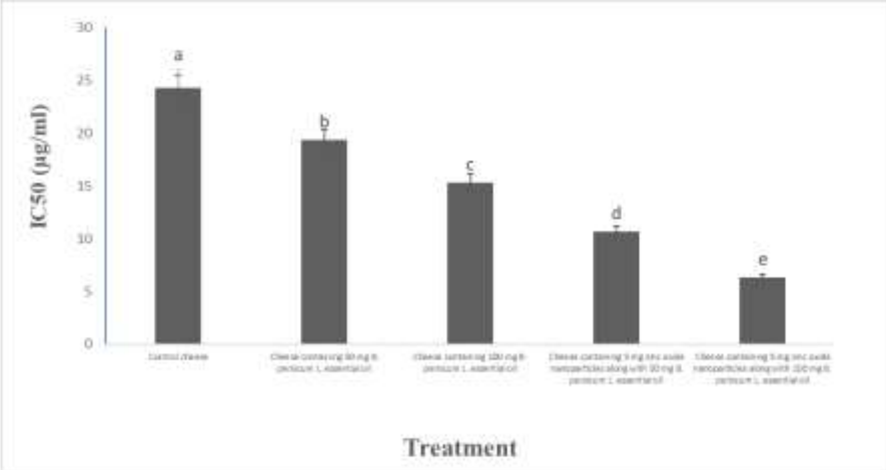

Figure 1- Investigating the antioxidant property of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

*Means in a column followed by the diferent superscripts are signifcantly diferent at p≤0.05 by Duncan test.

The use of essential oil led to a significant increase in the DPPH inhibition percentage in the cheese, which is attributed to the high levels of phenolic compounds such as thymol and carvacrol, known for their strong antioxidant activity. El-Kholy et al. (2017) reported that the addition of thyme essential oil to UF cheese resulted in enhanced antioxidant activity [33].

3-2-4-Textural properties of cheese samples containing zinc oxide nanoparticles and black cumin essential oil

The results of the texture hardness evaluation of the cheese samples containing zinc oxide nanoparticles and black cumin essential oil are presented in Figure 2. The findings showed that increasing the percentage of black cumin essential oil along with zinc oxide nanoparticles led to an increase in texture hardness. However, no significant differences were observed among the different treatments (p>0.05).

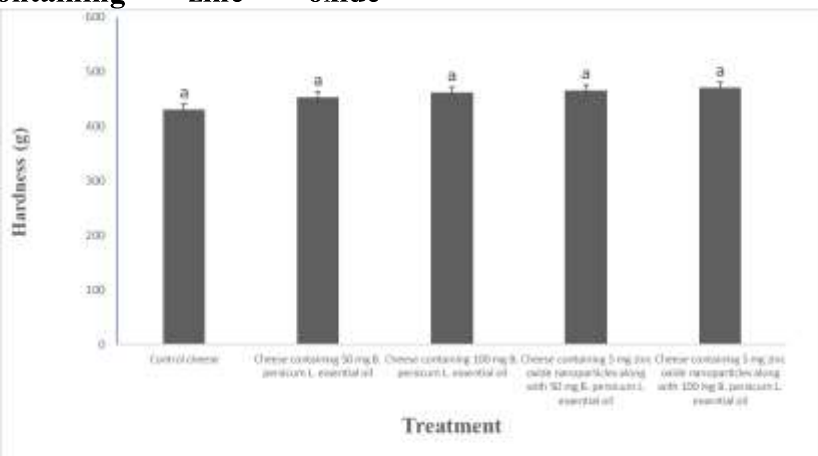

Figure 2- The hardness changes of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

*Means in a column followed by the diferent superscripts are signifcantly diferent at p≤0.05 by Duncan test.

El-Kholy et al. (2017) reported that the addition of thyme essential oil to UF cheese resulted in a reduction in texture hardness [33]. The results of the adhesiveness evaluation of the cheese texture containing zinc oxide nanoparticles with black cumin essential oil are presented in Figure 3. The findings showed that increasing the percentage of black cumin essential oil along with zinc oxide nanoparticles led to a decrease in texture adhesiveness. However, no significant differences were observed among the different treatments (p>0.05).

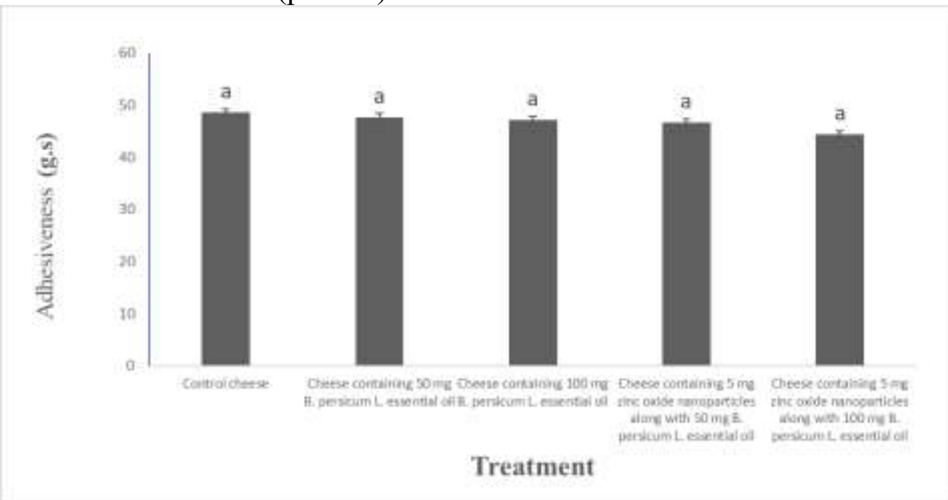

Figure 3- The Adhesiveness changes of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

*Means in a column followed by the diferent superscripts are signifcantly diferent at p≤0.05 by Duncan test.

Adhesiveness indicates the flowability or liquidity of materials. In other words, it reflects the influence of microstructure on macroscopic properties and is defined as the work required to overcome the attractive forces between the surfaces of the food product and the surfaces it comes into contact with. This parameter is considered one of the most important characteristics of cheese [34].

3-2-5- Colorimetric results of cheese samples containing zinc oxide nanoparticles and Black Cumin essential oil

The effect of zinc oxide nanoparticles containing black cumin essential oil on the color characteristics of cheese samples is shown in Figures 4, 5, and 6. As seen in these figures, the L^* and b^* values of the samples containing zinc oxide nanoparticles with black cumin essential oil significantly decreased compared to the control sample (p<0.05), while the a^* value significantly increased (p<0.05). The lowest L^* and b^* values were observed in the cheese sample containing 5 mg of zinc oxide nanoparticles along with 100 mg of black cumin essential oil, while the highest values were recorded in the control sample (Figures 4 and 5). Conversely, the lowest a^* value was found in the control sample, and the highest was observed in the cheese sample containing 5 mg of zinc oxide nanoparticles and 100 mg of black cumin essential oil (Figure 6).

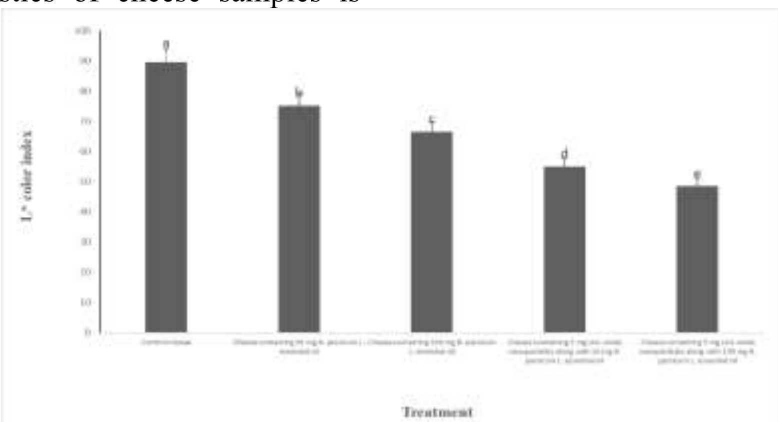

Figure 4- Evaluation of the L^* color index of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

*Means in a column followed by the diferent superscripts are signifcantly diferent at p≤0.05 by Duncan test.

L*, a*, and b* are qualitative color parameters, typically used to describe color dimensions such as reddish, greenish, and so on [35]. The a* parameter indicates a color shift from red (positive values) to green (negative values), b* reflects a shift from yellow (positive values) to blue (negative values), and L* represents brightness, ranging from black to white [36]. Color parameters significantly affect the marketability of products and consumer acceptance. Even though functional foods are recognized as health-promoting, without visual appeal, they may not achieve sufficient market acceptance. Therefore, the color of enriched products should remain stable throughout production and storage [37].

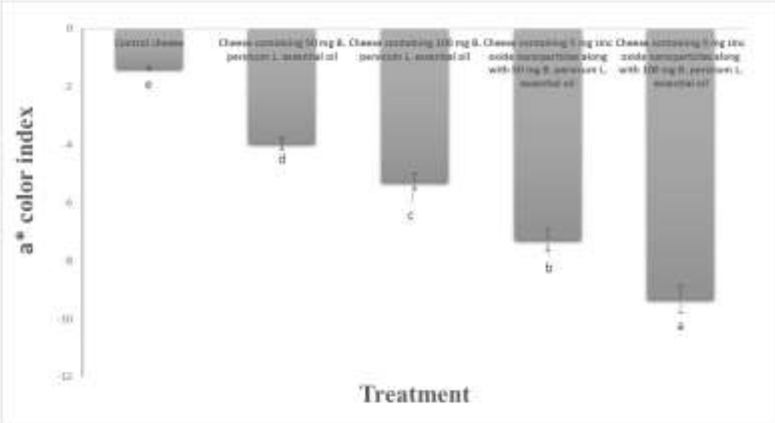

Figure 5- Evaluation of the a* color index of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

*Means in a column followed by the diferent superscripts are signifcantly diferent at p≤0.05 by Duncan test.

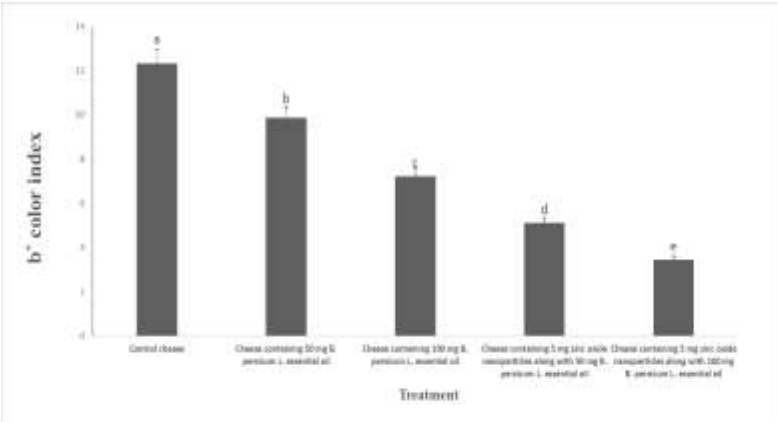

Figure 6- Evaluation of the b* color index of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

*Means in a column followed by the diferent superscripts are signifcantly diferent at p≤0.05 by Duncan test.

3-7-6- Sensory Evaluation

The effect of zinc oxide nanoparticles containing black cumin essential oil on the sensory evaluation of the cheese samples is presented in Table 7. As shown in Table 7, the lowest and highest color scores were related to the cheese sample containing 5 mg zinc oxide nanoparticles with 100 mg black cumin essential oil and the control sample, respectively. A significant difference in color scores was observed among all samples ($p<0.05$). Additionally, the cheese sample with 5 mg zinc oxide nanoparticles and 100 mg black cumin essential oil showed a significantly higher aroma and flavor score compared to the other samples ($p<0.05$). The addition of black cumin essential oil and zinc oxide nanoparticles containing the essential oil at any concentration had no significant effect on the texture score ($p>0.05$). Overall, the addition of 5 mg zinc oxide nanoparticles with 100 mg black cumin essential oil improved the overall acceptability of the cheese sample ($p<0.05$). The lowest and highest overall acceptability scores belonged to the control sample and the sample containing 5 mg zinc oxide nanoparticles with 100 mg black cumin essential oil, respectively.

Table 7- Investigation of sensory evaluation of cheese samples containing zinc oxide nanoparticles and *B. persicum* L. essential oil

Sensory properties	Control cheese	Cheese containing 50 mg *B. persicum* L. essential oil	Cheese containing 100 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 50 mg *B. persicum* L. essential oil	Cheese containing 5 mg zinc oxide nanoparticles along with 100 mg *B. persicum* L. essential oil
Color	4.91 ± 0.03^a	4.62 ± 0.06^b	4.35 ± 0.04^c	4.13 ± 0.02^d	4.03 ± 0.07^e
Flavor	4.03 ± 0.02^e	4.88 ± 0.03^d	4.78 ± 0.05^c	4.81 ± 0.04^b	4.95 ± 0.02^a
Texture	4.62 ± 0.07^a	4.61 ± 0.04^a	4.60 ± 0.06^a	4.63 ± 0.03^a	4.68 ± 0.07^a
General acceptance	4.11 ± 0.03^e	4.32 ± 0.06^d	4.52 ± 0.05^c	4.87 ± 0.03^b	4.96 ± 0.02^a

Means in a column followed by the diferent superscripts are signifcantly diferent at $p \leq 0.05$ by Duncan test.

As shown in Table 7, the cheese containing 5 mg of zinc oxide nanoparticles with 100 mg of black cumin essential oil had a significantly higher overall acceptability score compared to the control sample ($p<0.05$). Hosseinin et al. (2014) evaluated the effects of using black cumin essential oil on the sensory properties of soft cheeses during a 30-day storage period. The results indicated that over time, the sensory changes across treatments followed a consistent pattern, and the continued production of lactic acid and other organic acids caused brittleness in the cheese texture. This negatively affected the scores for consistency, texture, and appearance [38].

4- Conclusion

In this study, the antimicrobial properties of zinc oxide nanoparticles combined with black cumin essential oil on the shelf life of white cheese were investigated. The physicochemical analysis of the cheese showed that the addition of black cumin essential oil and zinc oxide nanoparticles containing black cumin essential oil had no significant effect on acidity, pH, or protein content ($p>0.05$), but significantly increased the dry matter content ($p<0.05$). Microbiological tests demonstrated that the combination of zinc oxide nanoparticles and black cumin essential oil was more effective in reducing the populations of *Candida albicans, Staphylococcus aureus,* and *Escherichia coli* compared to black cumin essential oil alone. The antioxidant activity results indicated that the addition of zinc oxide nanoparticles containing black cumin essential oil significantly enhanced the DPPH radical scavenging activity of the cheese samples ($p<0.05$). Texture analysis showed that increasing the concentration of black cumin essential oil along with zinc oxide nanoparticles increased hardness and decreased adhesiveness, though these changes were not statistically significant across treatments ($p>0.05$). In the colorimetry test, the L^* and b^* indices of the cheese samples with zinc oxide nanoparticles and black cumin essential oil significantly decreased compared to the control ($p<0.05$), while the a^* index increased ($p<0.05$). Sensory evaluation results showed that the addition of 5 mg of zinc oxide nanoparticles with 100 mg of black cumin essential oil improved the overall acceptability of the cheese sample ($p<0.05$). In conclusion, zinc oxide nanoparticles combined with black cumin essential oil can be used in cheese production to enhance its shelf life.

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افزایش کیفیت و ماندگاری پنیر سفید با استفاده از نانوذرات اکسید روی (ZnONPs) سنتز شده به روش سبز به همراه اسانس گیاه زیره سیاه ایرانی (*Bunium persicum* L.)

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چکیده

در این پژوهش، خاصیت ضد میکروبی نانو ذره اکسید روی به همراه اسانس زیره سیاه (نمونه شاهد، نمونه حاوی اسانس زیره سیاه ۵۰ میلی‌گرم، نمونه حاوی اسانس زیره سیاه ۱۰۰ میلی‌گرم، نمونه حاوی نانو اکسید روی ۵ میلی‌گرم با اسانس زیره سیاه ۵۰ میلی‌گرم و نمونه حاوی نانو اکسید روی ۵ میلی‌گرم با اسانس زیره سیاه ۱۰۰ میلی‌گرم) بر ماندگاری پنیر سفید مورد بررسی قرار گرفت. مقایسه میانگین‌ها به روش آزمون چند دامنه‌ای دانکن در سطح معنی‌دار ۵ درصد با نرم‌افزار SPSS نسخه ۲۲ انجام شد. نتایج بررسی ویژگی‌های فیزیکوشیمیایی پنیر نشان داد، افزودن اسانس زیره سیاه و نانو ذرات اکسید روی حاوی اسانس زیره سیاه تاثیر معنی‌داری بر میزان اسیدیته، pH و پروتئین نداشت ($p>0.05$)، اما بر میزان ماده خشک افزایش معنی‌داری نشان داد. نتایج آزمون میکروبی نمونه‌های پنیر سفید نشان داد که اثر نانو ذره اکسید روی به همراه اسانس زیره به پنیر بر کاهش جمعیت شمارش مخمرکاندیدا آلبیکنس، استافیلوکوکوس و اشرشیاکلی بیشتر از اسانس زیره به تنهایی بود. نتایج فعالیت آنتی‌اکسیدانی نشان داد، کمترین و بیشترین میزان IC50 به ترتیب مربوط به نمونه پنیر حاوی نانو ذرات اکسید روی ۵ میلی‌گرم به همراه اسانس زیره سیاه ۱۰۰ میلی‌گرم و نمونه شاهد بود. نتایج بافت‌سنجی نشان داد، که با افزایش درصد اسانس زیره سیاه و به همراه نانو ذرات اکسید روی سختی و چسبندگی بافت به ترتیب افزایش و کاهش یافت اما تاثیر معنی‌داری بین تیمارهای مختلف مشاهده نشد ($p>0.05$). در آزمون رنگ‌سنجی، شاخصL^* و b^* نمونه‌های حاوی نانو ذرات اکسید روی حاوی اسانس زیره سیاه نسبت به نمونه شاهد به صورت معنی‌داری کاهش یافت ($p<0.05$) اما میزان شاخص a^* افزایش پیدا کرد ($p<0.05$). نتایج ارزیابی حسی نشان داد، افزودن نانو ذرات اکسید روی ۵ میلی‌گرم به همراه اسانس زیره سیاه ۱۰۰ میلی‌گرم باعث بهبود پذیرش‌کلی نمونه پنیر شد ($p<0.05$).