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ORIGINAL RESEARCH PAPER

Effect of Chitosan Nano-Gel/Emulsion Containing *Bunium Persicum* Essential Oil and *Nisin* as an Edible Biodegradable Coating on *Escherichia Coli* O_{157}:H_7 in Rainbow Trout Fillet

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ABSTRACT
This research aimed to assess the effect of biodegradable coating chitosan nano-gel/emulsion loaded by *Bunium persicum* essential oil and *nisin* on *E. coli* O_{157}:H_7 in rainbow trout fillet during 12 days at refrigeration (4˚C). Trout fillet Sample was divided into 6 groups after inoculation of bacteria (*E. coli* O_{157}:H_7), including control (without any coating), coated with chitosan 2% and other groups including nano-emulsion chitosan 2%, Nano-emulsion of chitosan containing *Bunium persicum* essential oil (0.5%), Nano-gel of chitosan containing nisin (200 IU/g) and Nano-gel/emulsion of chitosan containing *Bunium persicum* essential oil (0.5%) and nisin (200 IU/g). The samples were stored at the cool condition, and the bacterial count was performed on days: 0, 1, 2, 4, 8, and 12. The mean number of the bacterial count was significantly different among treatment (p<0.001). The most significant inhibitory effect on the growth of *E.coli* O_{157}:H_7 was observed in chitosan Nano-emulsion coating containing *Bunium persicum* essential oil (0.5%) and nisin (200 IU/g). According to this study, it was concluded that the use of Nano-gel/emulsion of chitosan coating *Bunium persicum* essential oil and nisin could be effective on the decrease of *E.coli* O_{157}:H_7 growth in food.

Keywords: *Biodegradable Coating, Chitosan, Nano-Gel/Emulsion, Essential Oil, E.coli* O_{157}:H_7

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INTRODUCTION

Seafood, especially fish, one of the essential meats for human health, has high nutritional value and rich in omega-3 polyunsaturated fatty acids, protein, and minerals [1, 2]. Thus, it is sensitive to contamination by foodborne pathogens such as *E. coli* O_{157}:H_7 that could cause diverse forms of the disease that leads to severe complications, so the need for a precise control way to decrease bacterial growth is necessary [3-5]. This bacterial is liable for many outbreaks involving various types of food products due to cross-contamination particularly by post-processing contamination [6].

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The recent studies are looking for; naturally, preservatives mainly medicinal plants have increased because people are more conscious about the side effects of chemical preservatives on health and prolong the shelf life of food products; on another hand, the environment and its health are essential to humanity. In this regard, the use of edible biodegradable coatings and anti-microbial agents like chitosan and essential oil respectively is considerable [7-9]. Essential oils (EOs) that can be extracted from plants contain various compounds, such as flavonoids and phenolic acids, with antimicrobial nature against food-borne pathogens [1, 10-12].

Bunium persicum (BPEO) is a widely used spice in food preservative can be grown in different regions of Asia such as Afghanistan, Iran, Pakistan and India [13]. This essential oil has to potent anti-bacterial and oxidant effects due to the high level of oxygenated mainly ρ-cymene, monoterpenes, limonene and γ-Terpinene [14]. Other natural antimicrobial agents, for example, nisin, that known as generally recognized as safe (GRAS), recently they have been of interest [15-17].

Nisin is produced by *Lactococcus lactis spp*. lactis and is the only bacteriocin with Food and Drug Administration (FDA) approved GRAS status for use in food products as a preservative in foods [18]. Reportedly nisin is a durable substance to eliminate gram-positive bacteria and, to a lesser extent, in gram-negative [15, 19].

Nano-emulsion is one of the most exciting applications for food products because they can act as delivery systems for lipophilic compounds, such as flavors, drugs, nutraceuticals, antioxidants, and antimicrobial agents[9, 20]. Also, Nano-emulsion of coating solutions containing antimicrobial agents indicates higher anti-bacterial activity compared with course emulsions [21, 22]. Various techniques can provide nano-emulsions, for example, low and high-energy. The high-energy methods in this regard are ultrasonic emulsification which could be effectively applied to prepare Nano-emulsions with small droplet diameters and also low size distributions [23-26]. Nano-emulsions are very stable, and some reports have used an electrical field method to separate them from each other [27, 28].

Several studies were concentrating on the shelf life and safety of food with the application of natural anti-microbial such as edible coating [1, 29-31]. The use of chitosan-based edible coatings reported in previous studies in many kinds of food; although, there have been few studies in the use of nano-chitosan solution with antimicrobial agents in kinds of seafood [32-36]. This research aimed to assess the effect of biodegradable coating nano-gel/emulsion solutions with *nisin* and *Bunium persicum* essential oil in rainbow trout fillet.

MATERIALS AND METHODS

Experimental Materials

The essential oil was purchased from medicinal plants, Karaj, Iran. Chitosan with Low molecular weight (LMW; 1.03×10^5) was also optioned from Sigma-Aldrich Company (St. Louis, MO, USA). Nisin and other Experimental Materials with analytical grade were prepared from the Sigma. The pathogen bacteria (NCTC 12900) was obtained from the department of food hygiene, faculty of veterinary medicine, Amol University of Special Modern Technologies, Amol, Iran.

Preparation of bacteria and chitosan coatings

E. coli O_{157}:H_7 was cultured in 9 mL of brain heart infusion (BHI) broth, then incubated at 37°C for 24 and 18 hours at 37°C [37]. To prepare 0.5 McFarland turbidity standard (containing 1.5×10^8 CFU/mL) and diluted (1:10) to density of 1.5×10^7 CFU/mL [37]. To prepare chitosan solution 2% (w/v), 2g of chitosan in 1% (v/v) acetic acid thus blended with 90ml of distilled water and mixed by magnetic stirrer at 40C. Then glycerol (0.75ml/gr) was added to the chitosan solution as a plasticizer and stirred for 10 min [38].

Preparation of edible biodegradable coating

At this stage, the chitosan (2% w/v) was prepared in sterile distilled water with acetic acid (1%, w/v), and glycerol, then the solutions stirred for 15 min in order to obtain explicit solutions. Following that, nisin (50 mg) was dissolved in hydrochloric acid (0.02 mol/L) to prepare a stock solution of nisin. The BPEO (0.5% V/W) and nisin (200 IU/g) were mixed in the chitosan solutions. At this stage, tween 80 (0.2% w BPEO), as an emulsifier, added to chitosan solutions to become uniform, stable, and transparent emulsion. Then coating solutions were subjected to ultraturrax for 3-5 min at 3000 rpm, then ultrasonic sonicator (60 ˚C, pulse; 45s and rest; 15s) for six min [39]. Here the transparent Nano-emulsion was prepared (Fig.1) and finally, Particle size was measured by DLS device (Nanophox Sympatec GmbH, Clausthal, Germany) as well.

Preparation of trout fillets and inoculation of the bacterial

Fresh rainbow trout fish were purchased from a local fish farm at Amol (Mazandaran, Iran), and transferred to the laboratory. Then the fillets were washed and slimed and dried. The fillets were cut to pieces with 10g weight then burnt to exterminate the surface microorganisms. *E. coli* O_{157}:H_7 were inoculated (using adjustable volume micropipettes) on each side of separate

Preparation of treatments

The samples that inoculated with *E. coli* O_{157}:H_7

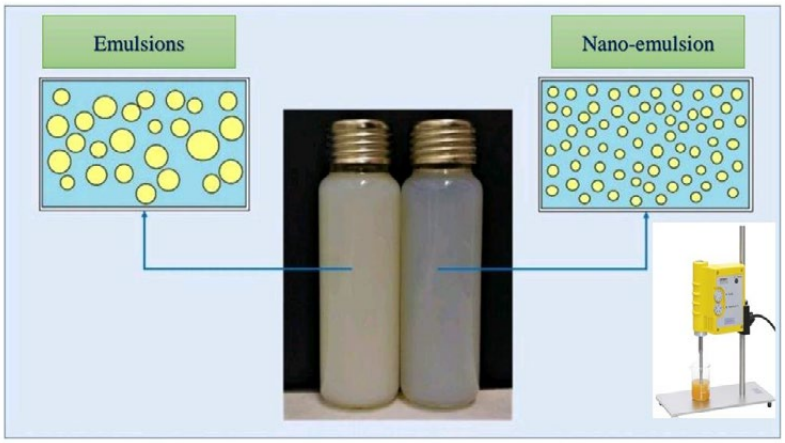

Fig. 1: Image of emulsion and nano-emulsion

Table 1: List of treatments in the present study

No	Treatment	Description
1	CON	Control
2	Ch 2%	Coated with chitosan
3	Nano Ch 2%	Coated with sonicated chitosan
4	Nano Ch+ BPEO	Coated with Nano-emulsion of chitosan containing 0.5% (w/v) BPEO
5	Nano Ch+ Nisin	Coated with Nano-gel of chitosan containing nisin 200IU/g
6	Nano Ch+Nisin+ BPEO	Samples coated with Nano-gel/emulsion of chitosan containing 0.5% (w/v) BPEO and nisin 200IU/g

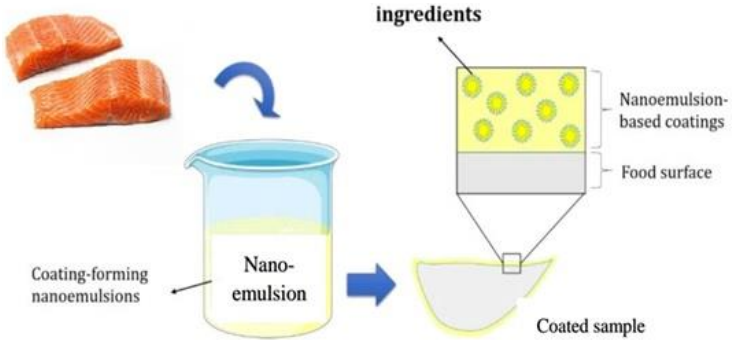

Fig. 2: Schematic of nano-emulsion coating on fresh rainbow trout fish

were divided into six groups (Table 1) and then were treated by immersing in Nano-gel/emulsion (Fig. 2) for 1 minute, drained for 15 minutes and stored at 4 ± 1°C for 12 days to be analyzed at 7-day intervals: 0, 1, 2, 4, 6, 8 and 12 [1].

Enumeration of bacteria

Firstly, the fillets (10 g) were brought to a final volume of 90 mL with 0.1% sterile peptone water and then homogenized by a stomacher (Seward Medical, London, UK) for 3 minutes. Then, serial dilutions of homogenates were plated on Sorbitol MacConkey)SMAC) Agar for enumeration of bacteria after incubation at (35-37°C for 24 h) [40].

Statistical analysis

The procedure of changes in the logarithmic bacterial count was analyzed through Repeated measure ANOVA in 12 days periods of time, and the double-two comparison of groups was done by Bonferroni post hoc test. The SPSS ver. 21 software was employed for Statistical analysis, and a P-value of less than 0.05 is considered as significant.

RESULTS AND DISCUSSION

Nano-gel/emulsion characterization

As can be seen in Table 2 and Fig. 3, the mean droplet size and PDI also decreased after the preparation of different Nano-gel/emulsion solutions. The mean droplet size decreased in the

Table 2: Particle size and distribution of different Nano-emulsion coatings

Group	z-average (d.nm)	PDI
Ch	3159	0.544
Nano Ch	531.1	0.311
Nano Ch+ BPEO	490.2	0.292
Nano Ch+ Nisin	503.5	0302
Nano Ch+Nisin+ BPEO	242.4	0.433

Nano-chitosan solution in comparison with that of the chitosan solution. The largest particle size was observed in the chitosan solution (3159 nm), and the lowest was observed in Nano-gel/emulsion of chitosan solution with nisin and BPEO (242.4 nm). Other researchers fabricated Nano-emulsion by the incorporation of EOs and antibacterial agents in the biopolymer, such as alginate solutions [41] and chitosan [42] before the sonication process. All of these researchers reported droplet sizes smaller than 1000 nm than that obtained in this research (the lowest was 242.4 nm). As can be seen, the PDI also decreased after Nano-gel/emulsion fabrication. The PDI obtained in this research was less than 0.550 in different groups. Noori et al. (2018), observed that the PDI was recorded as 0.584 for GEO emulsion and decreased to 0.222 when conventional Nano-emulsion was prepared. Also, some other researchers reported that the PDI decreased after sonication (in all of them, PDI less than 0.600 reported) [43, 44]. The lower PDI of Nano-emulsion approved the efficiency of the ultra-sonication method information of a uniform size distributed Nano-emulsion [43].

Enumeration

Fig. 4 and Table 3 represent the effect of the treatments on the growth of *E. coli* O_{157}:H_7 during the 12 days of storage. The initial count of *E. coli* O_{157}:H_7 was 6.55 ± 0.11 log CFU/g, which decreased during the storage in all samples especially for Nano-emulsion of chitosan containing 0.5% (w/v) BPEO samples (4.19 ± 0.18) and Nano-emulsion of chitosan containing 0.5% (w/v) BPEO and nisin 200IU/g (3.20 ± 0.12) that similar result was obtained in previous studies [37]. The reduction of the bacterial count was considerable in the Nano-gel/emulsion chitosan+ Nisin+ BPEO due to the significant impact of the Nano-chitosan solution and essential oil and Nisin [45]. Also, in the control

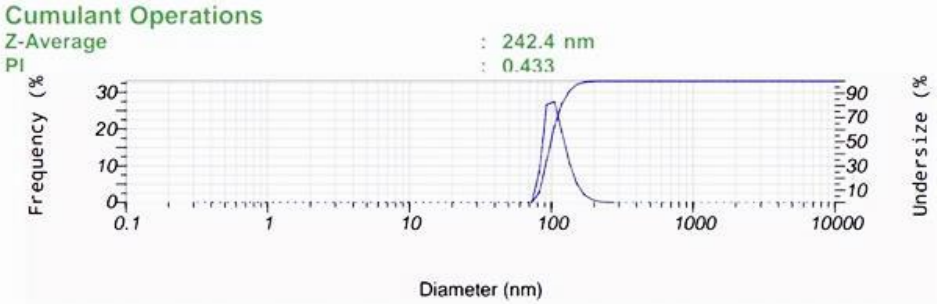

Fig. 3: The best particle size distribution of the nano-gel/emulsion coating

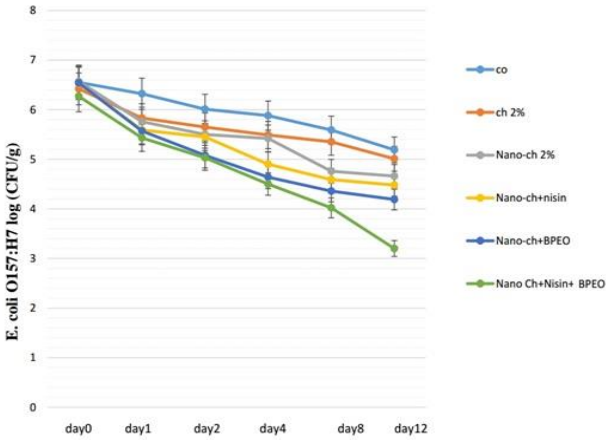

Fig. 4: Effect of the treatments on the growth of *E. coli* O_{157}:H_7 during storage

Table 3: Changes in the bacterial count (Log CFU/g) of rainbow trout fillet samples inoculated with *E. coli* $O_{157}H_7$ during storage (M ± SD)

Day	CON	Ch 2%	Nano Ch 2%	Nano Ch+ Nisin	Nano Ch+ BPEO	Nano Ch+Nisin+ BPEO
0	6.55 ± 0.11	6.42 ± 0.18	6.57 ± 0.17	6.53 ± 0.25	6.54 ± 0.28	6.27 ± 0.24
1	6.32 ± 0.14	5.83 ± 0.07	5.76 ± 0.07	5.59 ± 0.19	5.57 ± 0.14	5.43 ± 0.16
2	6.01 ± 0.15	5.65 ± 0.22	5.50 ± 0.09	5.45 ± 0.07	5.08 ± 0.25	5.03 ± 0.18
4	5.88 ± 0.15	5.49 ± 0.13	5.42 ± 0.12	4.90 ± 0.50	4.64 ± 0.05	4.50 ± 0.15
8	5.59 ± 0.09	5.35 ± 0.18	4.76 ± 0.44	4.59 ± 0.10	4.36 ± 0.15	4.02 ± 0.18
12	5.19 ± 0.18	5.01 ± 0.16	4.66 ± 0.08	4.48 ± 0.11	4.19 ± 0.18	3.20 ± 0.12

Table 4: The average reduction rate of the *E. coli* $O_{157}H_7$ counts (Log CFU/g) among treatments when compared with each other during the study period

Mean Difference I-J Group (I)	Group (J)	CON	Ch 2%	Nano Ch 2%	Nano Ch+ Nisin	Nano Ch+ BPEO	Nano Ch+Nisin+ BPEO
CON			0.29*	0.64*	0.66*	0.86*	1.18*
Ch 2%				0.34*	0.36*	0.56*	0.88*
Nano Ch 2%					0.02	0.21*	0.53*
Nano Ch+ Nisin						0.19*	0.51*
Nano Ch+ BPEO							0.32*

group during 12 days, the bacterial count was decreased from 6.55 ± 0.11 to 5.19 ± 0.18 because this bacterial is mesophilic [46]. A comparison of the chitosan and Nano-chitosan treatments showed a higher decrease in bacterial count in Nano-chitosan treatment than chitosan treated samples. Therefore, the use of combinational antimicrobial agents is more effective against microbial growth than their individual use [15, 37].

The mean reduction rate of *E. coli* O_{157}:H_7 count in different treatments is shown in Table 4 when treatment was compared to each other. The maximum reduction rate was related to Nano-gel/emulsion chitosan+ Nisin+ BPEO (1.18 log CFU/g) and Nano-chitosan+ BPEO (0.86 log CFU/g), when they were compared to Samples without any coating (CON). Also, in Table 4 showed that the use of combinational antimicrobial agents is more effective against microbial growth than their individual use [15, 37]. Several previous studies (Raeisi et al., 2016; Shahbazi et al., 2015) confirmed the above finding; nevertheless, they may have synergistic, antagonistic, or additive effects according to the type of antimicrobial agent and microorganism.

CONCLUSION

According to the results from the present research, the use of Nano-gel/emulsion of chitosan solution with nisin and BPEO (Nano ch+nisin + BPEO) has a potential anti-microbial effect against foodborne pathogens like as *E. coli* O_{157}:H_7 in rainbow trout fillet at 4°C. Also, the treatments had an acceptable effect on high doses of this foodborne pathogen and could effectively accelerate its reduction rate in contaminated rainbow trout fillets stored in at 4°C. According to these results, given the preference of producer and consumer for using natural food additives, we recommend the administration of Nano-gel/emulsion of chitosan enriched with BPEO and nisin in rainbow trout fillet to increase its safety against the pathogenic bacteria.

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CONFLICT OF INTERESTS

There is no conflict of interest in this study.

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