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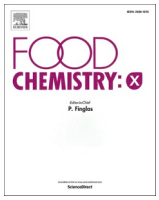

Evaluation of whey protein coating containing nanoliposome dill (*Anethum graveolens* L.) essential oil on microbial, physicochemical and sensory changes of rainbow trout fish

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ABSTRACT

The aim of this study was to investigate the effect of whey coating containing dill (*Anethum graveolens L.*) essential oil nanoliposome on the physicochemical, microbiological and sensory characteristics of rainbow trout (*Oncorhynchus mykiss*). Treatments comprise: sample without coating (control), coating containing whey, coating containing whey with essential oil (whey-EO) and coating containing whey with nano EO (whey-NEO). The particle size, zeta potential, polydispersity index and the encapsulation efficiency were ranged from 142 to 159 nm, −16.3 to −11.7 mV, 0.79 to 0.88 Mw/Mn and 45.85–70.01 %, respectively. Microbial analysis, after 21 days, the maximum and minimum of TVC (total viable counts), TPC (total psychrophilic counts) and LAB (lactic acid bacteria) counts were related to control (8.16 for TVC, 8.46 for TPC and 7.7 log CFU/g for LAB) and whey + NEO (7 for TVC, 7.3 for TPC and 6.16 log CFU/g for LAB), respectively. Also, results of pH, peroxide value (PV), thiobarbituric acid reactive substances (TBARS) and total volatile base-nitrogen (TVB-N) after 21 days were ranged from 6.3 (whey-NEO) to 7.5 (control), from 11.5(whey-NEO) to 20.9 mEq/Kg (control), from 5.23(whey-NEO) to 8.34 mg MDA/kg (control) and from 22.5 (whey-NEO) to 37 mg N/100 g (control), respectively. Finally, in all sensory evaluation items (texture, off-odor, discoloration and red color), the best result after 21 days was related to whey-NEO (score = 1). Consequently, the edible coating comprising whey and nanoliposome of EO could be effective to the maintenance of fish's microbiological, physicochemical, and sensory properties.

Introduction

Fish and products related to fisheries are known as important sources of human nutrition. Rainbow trout (*Oncorhynchus mykiss*) fish is one of the most consumed fish. Since rainbow trout contains 65–80 % moisture, high fat, amino acids (free) and 9–18 % other combinations of non-protein nitrogenous, many biochemical, physical and microbial changes occur during storage. Considering that reactions of biological like oxidation of lipids and microbial growth lead to spoilage and production of bad smell, bad taste and deterioration of quality during fish storage. Safety, delay in spoilage and maintaining good quality of fish at the storing time are the main concerns of consumers and producers. With these explanations, nowadays, the using of novel and complementary technologies for protection of foodstuff such as packaging with antimicrobial properties in the form of biodegradable films, edible coatings, as well as the use of antimicrobial composites and natural antioxidants, has attracted a lot of attention (Mehdizadeh, Shahidi, Shariatifar, Shiran, & Ghorbani-HasanSaraei, 2021; Pouryousef, Ahmady, Shariatifar, Jafarian, & Shahidi, 2022b; Yousefi et al., 2022).

The chief objective of coatings and edible films is to rise the quality, durability and safety of foodstuff by limiting the transfer of moisture and gases in them. Among hydrocolloids, proteins are a suitable option for producing coatings and biodegradable films because they have good properties of physical (flexibility and resistance), optical (transparency) and mechanical. In addition, proteins can act as a barrier for oxygen, flavors and organic compounds (Behbahani, Shahidi, Yazdi, Mortazavi, & Mohebbi, 2017; Noori, Zeynali, & Almasi, 2018).

A byproduct of the cheese making process is whey protein and it is able to create a favorable film with mechanical properties, high resistance and transparency, as well as good biodegradability (de Castro et al., 2017). Researchers have investigated different additives like flavorings, antimicrobial and antioxidants agents, spices and dyes to whey-

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based active films (Bagheri, Madadlou, Yarmand, & Mousavi, 2013; Bahram et al., 2014).

There are large amounts of essential oils in many parts of the plant (fruits, seeds and leaves) that play an antioxidant and antimicrobial role. Antioxidants and antimicrobials are the most common ingredients used to prepare an edible coating or film, which are used to delay the oxidation of fats, safety and increase the quality of foodstuff (Babri, Khokhar, Mahmood, & Mahmud, 2012; Peerakam et al., 2014; Radulescu, Popescu, & Ilies, 2010).

One of the annual plants is Dill (*Anethum graveolens L*), which contains essential oil and belongs to Umbelliferae family. Dill contains compounds such as essential oil, fatty acids, mineral elements (manganese, calcium, sodium, copper, magnesium, iron, potassium and phosphorus), vitamins, carbohydrates, proteins, fiber, flavonoid, carotenoid and phenolic compounds. Dill plant, in addition to being applied in the industry of pharmaceutical and traditional medicine (to treat digestive problems, liver, colds, spasms, anorexia, throat swelling, pain relief, anti-inflammation and blood fat reduction) as a flavoring in meat products, drinks, snacks, yogurt, cheese and condiments are used (Mannai, Elhleli, Feriani, Otsuka, Belgacem, & Moussaoui, 2023; Peerakam et al., 2014; Radulescu et al., 2010).

However, due to the hydrophobic nature of EOs and their low solubility in the polar solutions, it is crucial to coat them with a hydrophilic substances to improve their performance. The use of the encapsulation process to produce carriers or nanoparticles is being developed and expanded, which is called nanocapsules. Encapsulation of nano (nanoliposome) particles is done in order to protect antimicrobial and antioxidant agents against environmental stresses and control their release in a specific period of time and under specific conditions (Bagheri et al., 2013; Mehdizadeh et al., 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

Liposomes are vesicles that consist of one or more double-layered membranes and are necessary investigations in the industry of food regarding the encapsulation and delivery of biologically active substances like essential oils, enzymes, vitamins, and antimicrobials. Nanoliposome is one of the types of lipid-based nanocarriers that are used to surround antimicrobial and antioxidant substances (Homayounpour, Jalali, Shariatifar, Amanlou, & Khanjari, 2020; Wu et al, 2015).

It is rare that encapsulated active agents are used as controlled release and systems of delivery in bioactive coatings and packaging, however studies in this field are increasing. In the past, studies have been conducted on the effect of different essential oils on Rainbow trout preservation, such as Yousefi et al.'s study on coating with thyme essential oil (Yousefi et al., 2022), Chamanara et al.'s study on coating with chitosan and thyme essential oil (Chamanara, Shabanpour, Gorgin, & Khomeiri, 2012), Mexis et al.'s study on coating with oxygen absorber and oregano essential oil (Mexis, Chouliara, & Kontominas, 2009), Ozogul et al.'s study on coating with herb essential oils (rosemary, laurel, thyme and sage) and Hosseini et al.'s study on coating with oregano essential oil (Hosseini, Rezaei, Zandi, & Ghavi, 2016), but according to our knowledge, so far there have been no results in the formulation of whey protein coating containing EO from dill as active edible coatings (nanoliposomes and free forms). Moreover, no researches have been conducted on the use of mentioned coating in fish such as rainbow trout (*Oncorhynchus mykiss*) fish. Therefore, the purpose of the present study is to investigate the effect of free forms and nanoliposome of dill essential oil with whey protein coating to increase the shelf life of rainbow trout, which was done using physicochemical tests (zeta potential, TS, EM, encapsulation efficiency, water vapor permeability, polydispersity index, z-average diameter, pH, TVB-N, TBARS and PV), microbial tests (TVC, TPC and LAB) and sensory properties (color change, texture, red color and bad smell) was evaluated.

Materials and methods

The materials used

From Merck Co. (Darmstadt, Germany) were bought sodium carbonate anhydrous, cholesterol (95 %), glycerol (>97 % purity), dichloromethane, methanol, and reagent of Folin-Ciocalteu, acetic acid. For nanoliposomes preparation, from Across Co. (USA), L-a-lecithin was obtained with 99 % pure. Other applied solvents and reagents were bought from Company of Sigma-Aldrich in grade of analytical. Whey (whey protein concentrate) with 80 % (w/w) purity was attained from Company of Fonterra, Ltd. (Auckland, Australia).

Preparation of EO

Plant of Dill was confirmed by Iranian pharmacognosy specialists (Tehran University of Medical Sciences) and acquired from Tehran city, Iran. The step of EO preparation was performed according to our prior study (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast et al., 2018).

Investigation of EO compounds with GC/MS equipment

The EO compounds were investigated by GC (gas chromatography)/MS (spectrometry of mass) equipment (Agilent 7890A, USA/5975C VL MSD with Triple-Axis detector). The GC–MS equipment conditions such as temperature, etc. were according to our previous researches (Homayonpour et al., 2021; Pabast et al., 2018).

The nanoliposomes' preparation and characterization

This step was done by the film (thin) hydration procedure and ultrasound (Homayonpour et al., 2021). This step was performed by creating four lecithin/cholesterol ratios (50:10, 30:30, 40:20 and 60:0) and dissolving them in a methanol/dichloromethane (1:1) mixture.

Whey protein film production

Eight grams of whey was dissolved in water (distilled, 100 mL) and by adding 1 % sodium hydroxide the pH of solution was set to eight. Next, for 30 min, the mixture was heated at 80 °C. Glycerol as a softener and nano-liposomed dill essential oil were added to it, and for homogenization, the solution was stirred for two minutes at a 13,000 rpm speed in a homogenizer. Then, a fixed amount of the film solution was poured into the plate and dried under ambient conditions (Asdagh et al., 2021).

Sample preparation

The fish sample of rainbow trout were achieved from markets of Tehran, Iran. Preparation of fish samples was according to prior researches (Abdollahzadeh, Rezaei, & Hosseini, 2014; Homayounpour et al., 2020). Also, our treatments were including uncoated (control); whey protein (whey); whey with EO (whey + EO); and whey with nanoliposomes of EO (whey + NEO). For each treatment, 3 samples were prepared (and stored at 4 °C) and evaluated at 7 different days (1, 4, 7, 10, 13, 16 and 21 days).

Physical properties

Evaluation of nanoliposomes encapsulation efficiency (EE %), zeta potential and particle size

According to our previous study liposomes mean diameter and particle size distribution by the particle size analyzer of Shimadzu (SALD 2101, Japan), the nanoliposomes zeta potential or surface charge by a Malvern Zeta sizer Nano ZS (Company of Malvern Panalytical, Worcestershire, UK) and the encapsulation efficacy or EE % by using a

spectrophotometer of UV (Pharmacia biotech ultraspec 2000, UK), were analyzed (Pabast et al., 2018).

SEM (scanning electron microscopy) assay

The morphology and structure of prepared film were evaluated by SEM analyzer (KYKY-EM3200; KYKY Technology Development Ltd., Beijing, China) (Behbahani, Noshad, & Falah, 2019).

FTIR (fourier transform infrared spectroscopy) analyses

Spectral measurement parameters of resolution and accumulation assessed by FTIR analysis. It can assess characteristics of chemical and provide detailed information of compositional (Cebi, Arici, & Sagdic, 2021).

Microbiological analysis (LAB (Lactic acid bacteria), total psychrophilic count (TPC) and TVC (total viable counts))

Fish fillet (25 g, aseptically) and were placed in a bag of stomacher (Seward Medical, London, the UK), comprising sterile quarter-strength Ringer's (225 mL), and by a blender (Lab Mixer 400, Seward Medical, London, the UK) were homogenized for 1 min at room temperature. A surface of dry media was smeared with 0.1 mL of serial dilution homogenates of fish samples for the purpose of enumerating microbial populations. Microbial analyzes (including TVC (total viable counts) and LAB (Lactic acid bacteria) were performed according to the study of Homayounpour et al. (Homayonpour et al., 2021). The TPC analysis was performed after incubating at 7 °C for 12 days, according to the research of Mohan et al. (Mohan, Ravishankar, Lalitha, & Gopal, 2012). Data from all mentioned analysis were converted to unite of log cfu/g (colony forming unit number).

Chemical analysis (thiobarbituric acid reactive substances (TBARS), (total volatile base (TVB-N), peroxide value (PV) and pH value)

By the procedure of micro-diffusion, TVB-N value was assessed. The fish minced samples (about 10 g) were homogenized in 100 mL of perchloric acid (6 %) for 2 min and then filtrated. Afterward, it was alkalized with NaOH solution (20 %) and finally steam distillation of the extract is done. By an acid receiver, the volatile base components were absorbed and analyzed by titration. It was expressed in mg N/100 g of fish samples for the outcomes (Tometri, Ahmady, Ariaii, & Soltani, 2020).

A digital pH meter was applied to analyze the pH value (HANNA, Germany). This procedure was carried out by homogenizing of sample (about 10 g) with twice the amount of water (distilled) (Homayonpour et al., 2021).

Based on the Homayonpour et al.'s research, PV value was analyzed. Fish samples (5 g) were placed in a 250 flask and then added $CHCl_3$-CH_3COOH mixture (10 mL, 2:3) and shacked until the fat enters the solution. After that, saturated potassium solution (1 mL) was added. Next, for 5 min, the lid was closed and put it in the dark place. Afterward, distilled water (20 mL) was added and then shaken. The released iodine was titrated with solution of $Na_2S_2O_3$ (0.01 mL) until it turns bright yellow. One mL of 1.5 % solution of starch was added as indicator and was titrated until colorless (Homayonpour et al., 2021).

The TBARS test was applied according to study of Pabast et al., based on mg of malondialdehyde (MDA)/kg fish samples or μg/g and shows lipid oxidation in progressive stages. Lastly, all prepared samples were read at 532 nm by spectrophotometry (Ultrospec 2000, the UK) (Pabast et al., 2018).

All mentioned analyses were carried out in triplicate.

The sensory evaluation

The sensory analyzes (by 6 panelists of semi-trained, who had a history of evaluating fish samples) was evaluated with using the procedure of Pabast et al. (Pabast et al., 2018). In this study, we analyzed 4 sensory items including texture, discoloration, off-odor and red-color. The scoring was from 1 to 5, and below 3 were accepted and above 3 were rejected. The conditions of the room used for sensory evaluation included ambient light, room temperature, dimensions of 2 x 2 m and natural ventilation.

Statistical analysis

All obtained data are demonstrated as means ± SD. By the statistical test of ANOVA (in SPSS V. 24), results were assessed. Furthermore, the significant differences by the statistical test of Duncan's multiple range, were measured (at $p < 0.05$). In all experiments, each test was repeated 3 times.

Results and discussion

Identification of essential oil composites

According to our findings (Table 1), 19 compounds account for 98.66 % of the identified compounds, which the key components were 1-phelandrene, Beta- phellandrene, limonene, Linalool, Geranyl acetate and α-Pinene. These compounds were confirmed by other researches, but with dissimilar percentages that could be due to differences in conditions of weather, geographic region, species of plant and type of soil (Babri et al., 2012; Peerakam et al., 2014; Radulescu et al., 2010).

Evaluation of nanoliposomes characteristics (polydispersity index, z-average diameter, encapsulation efficiency and zeta potential)

Table 2 shows the particle size or diameter of z-average of the prepared nanoliposomes. For the nanoliposomes to be constant and effective in releasing the composites enclosed in its core, the z-average diameter is a major issue. According to our findings, the diameter of z-average of nanoliposomes was varied from 142 to 159 nm. In prior research by Rodea-González et al. that encapsulated chia EO (*Salvia hispanica* L.) in whey protein, they stated the z-average diameter of treatments varied from 13.17 to 28.20 μm (Rodea-González et al., 2012). Furthermore, this study confirmed by research of Pabast et al. (with particle sizes 93 to 96 nm) (Pabast et al., 2018) and research of Homayonpour et al. (with particle sizes 140 to 164 nm) (Homayonpour et al., 2021).

The polydispersity index (PDI) (Table 2) was ranged from 0.79 to 0.88 Mw/Mn (Mn (number average molecular weight) and Mw (average

Table 1
Outcomes of the investigation of *Anethum graveolens L.* EO by GC/MS equipment.

Peak No.	Compound	RT (min)	A%
1	α -Thujene	4.58	0.51
2	α-Pinene	6.52	3.21
3	Camphene	7.41	0.52
4	β-Myrcene	8.03	1.28
5	limonene	9.61	21.14
6	1-phelandrene	11.57	29.27
7	Beta- phellandrene	12.96	24.35
8	p-cymene	13,27	0.87
9	Linalool	13.49	5.41
10	a-Terpinolene	13.78	0. 27
11	Citral	15.11	0.05
12	*n*-Octyl acetate	16.23	0.03
13	*trans*-Anethole	16.92	1.22
14	octyl ester	17.05	0.03
15	*N*-octyl 2-methyl butyrate	17.92	1.28
16	octyl butyrate	19.27	2.20
17	Geraniol	22.32	2.33
18	Anethole	24,37	2.58
19	Geranyl acetate	27.23	3.25
Total			98.66

Table 2
Properties of prepared nanoliposomes.

Code	Lecithin: Cholesterol	z-average diameter (nm)	Polydispersity Index (Mw/Mn)	Zeta potential (mV)	Encapsulation Efficiency%
1	60:00	159±0.18[a]	0.81±0.06[a]	−11.7 ± 0.45[a]	60.01±0.54[c]
2	50:10	142±0.41[b]	0.79±0.05[a]	−15.5 ± 0.39[a]	70.01±0.58[d]
3	40:20	146±0.39[c]	0.80±0.05[a]	−16.3 ± 0.42[b]	56.71±0.82[b]
4	30:30	149±0.84[d]	0.88±0.05[a]	−15.1 ± 0.58[c]	45.85±0.94[a]

Data are means ± SD.
Means with different letters within a column indicate significant differences ($p < 0.05$).

of weight)). This parameter is a measure of the heterogeneity of a sample based on size. This parameter can be occur owing to distribution of size in a sample or the sample agglomeration/aggregation during analysis or separation (Homayonpour et al., 2021; Pabast et al., 2018). Our results also confirmed by prior studies (Homayonpour et al., 2021; Pabast et al., 2018).

Zeta potential (ZP) is a key item in calculating the surface electrical charge of particles, characterizing colloidal systems, and evaluating the nanoliposomes' stability. The higher the absolute ZP, the greater chemical and physical stability of the colloidal suspension due to the large forces of repulsive that slow down the rate of fusion and aggregation (Homayonpour et al., 2021; Pabast et al., 2018). According to the Table 2, the ZP of nanoliposomes was varied from −16.3 mV to −11.7 mV, which was dependent on the ratio of lecithin/cholesterol. The reason for the negative ZP can be due to the existence of lipid terminals. Based on our findings, with the increase in cholesterol ratio, the negative (-) charge of nanoliposome also increased significantly. The our findings were confirmed by other researches (Homayonpour et al., 2021; Pabast et al., 2018).

Three important factors in the EE percentage value include lipid ratio, inner volume of vesicles vesicle and type (Homayonpour et al., 2021; Pabast et al., 2018). Table 2 shows the EE % of nanoliposomes that ranged from 45.85 to 70.01 % In prior research by Rodea-González et al. that encapsulated chia EO (*Salvia hispanica* L.) in whey protein, they stated the EE of treatments ranged from 70.70 to 80.70 % (Rodea-González et al., 2012).

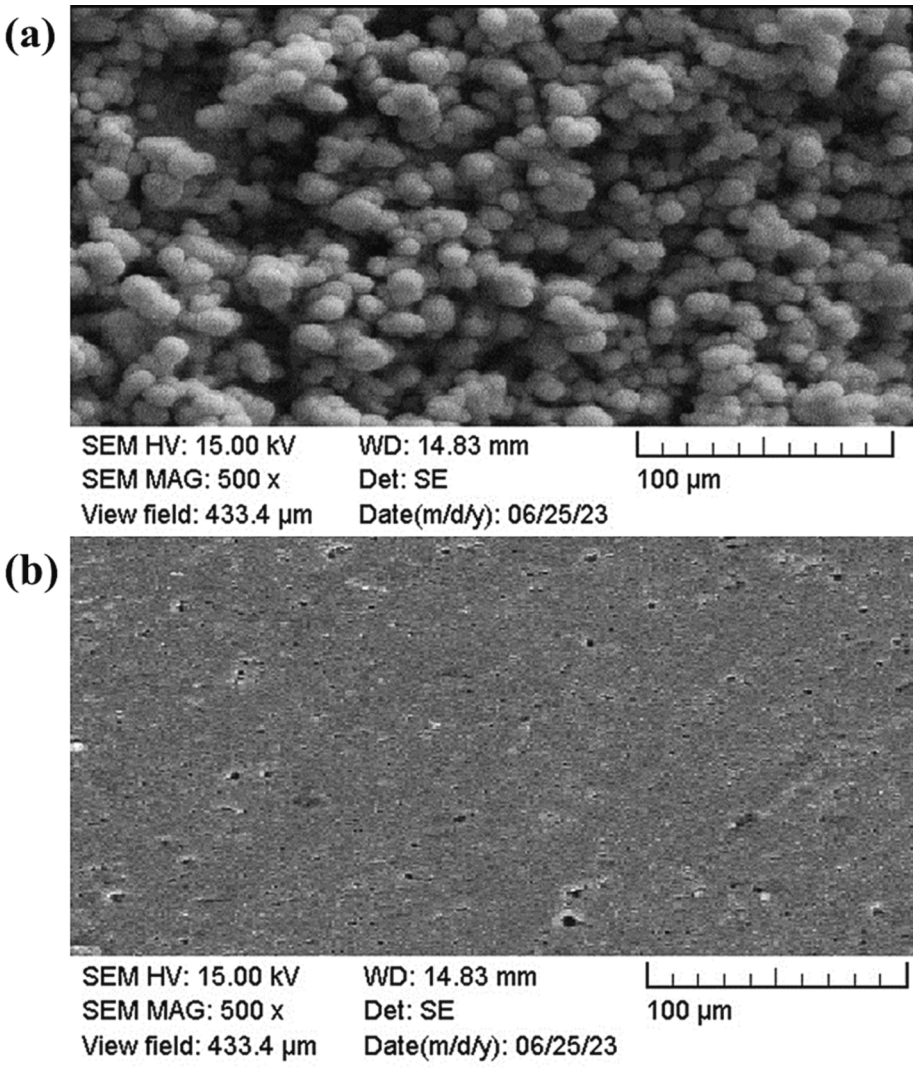

Fig 1. SEM images of whey-NEO film (a), and whey-EO film (b).

SEM assay

Fig. 1a and b shows SEM assay of whey-NEO and whey-EO, respectively. To assess the morphology of whey-NEO (Fig. 1a) and whey-EO (Fig. 1b), the SEM image was applied (with the minimum droplet size and the maximum EE% (60:00 cholesterol/lecithin)). As shown in Fig. 1a, the particles of nanoliposome were formed in the semispherical form. Our findings were similar to the prior findings (Pabast et al., 2018; Pouryousef et al., 2022a; Pouryousef et al., 2022b).

FTIR assay

To understand the composition of the whey protein film containing EO and NEO, FTIR (Fourier Transform Infrared Spectroscopy) analysis can be utilized (Fig. 2). This analytical technique allows to recognize the functional groups existing in the film and provides insights into its chemical structure. By analyzing the infrared absorption spectrum, the presence of specific bonds and compounds can be determined, shedding light on the film's properties and behavior. In this regard, whey protein film containing NEO (Fig. 2a) and EO (Fig. 2b) showed a fairly similar IR pattern. Also, FT-IR is a simple method for determining the formation of nanoliposome complexes. Mohammadi, Hamishehkar, and Piruzifard (2021) demonstrated that lecithin exhibits several peaks in its FT-IR spectrum. These include: a) A broad peak at ~3300 cm-1, which corresponds to the stretching of the free O—H group in alcohol esters; b) Peaks at ~2925 – 2856 cm-1, which are related to the stretching vibration of C—H groups; c) A peak at ~1740 cm-1, which is associated with the vibration of carbonyl groups (C═O); d) A peak at ~600 cm-1, which is attributed to PO stretching (Mohammadi et al., 2021). In the FT-IR spectra of whey-EO and whey-NEO, distinct peaks can be observed. Specifically, there is a peak at ~3270 cm-1, which corresponds to the stretching vibrations of O—H groups. Additionally, an absorption band at 1363 cm-1 is observed, which is attributed to the bending vibrations of O—H planes (Ghadetaj, Almasi, & Mehryar, 2018). The characteristic peaks between 1400 cm and 1 and 1600 cm-1 are attributed to the C═C stretching vibration in the aromatic ring. The absorption band between 1200 cm and 1 and 1300 cm-1 is caused by the C—O/C—C stretching vibration (Mohammadi, Mirabzadeh, Shahvalizadeh, & Hamishehkar, 2020). Moreover, the appearance of Amide I peaks at ~1700 – 1600 cm-1, and amide II at ~1550 – 1530 cm-1 are prominent distinctive bands of proteins (Mohammadi et al., 2020), which were observed in the FTIR spectrum of whey-based films loaded with EO and NEO. IR spectroscopy analysis revealed that the incorporation of EO and NEO into the whey protein film led to the formation of peaks corresponding to hydroxyl groups, confirming interaction between film matrix with EO and NEO. The findings of this study align with previous research on composite film matrices based on whey protein biopolymer/TiO_2 nanoparticles/cellulose nanofibers/rosemary EO (Alizadeh-Sani, Khezerlou, & Ehsani, 2018), whey protein isolate/Grammosciadium ptrocarpum Bioss. nanoemulsion essential oil (Ghadetaj et al., 2018) and whey protein isolate/chitosan nanofiber/nanoformulated cinnamon EO (Mohammadi et al., 2020).

Microbial analysis

The fish shelf-life and quality can be evaluated using microbial and

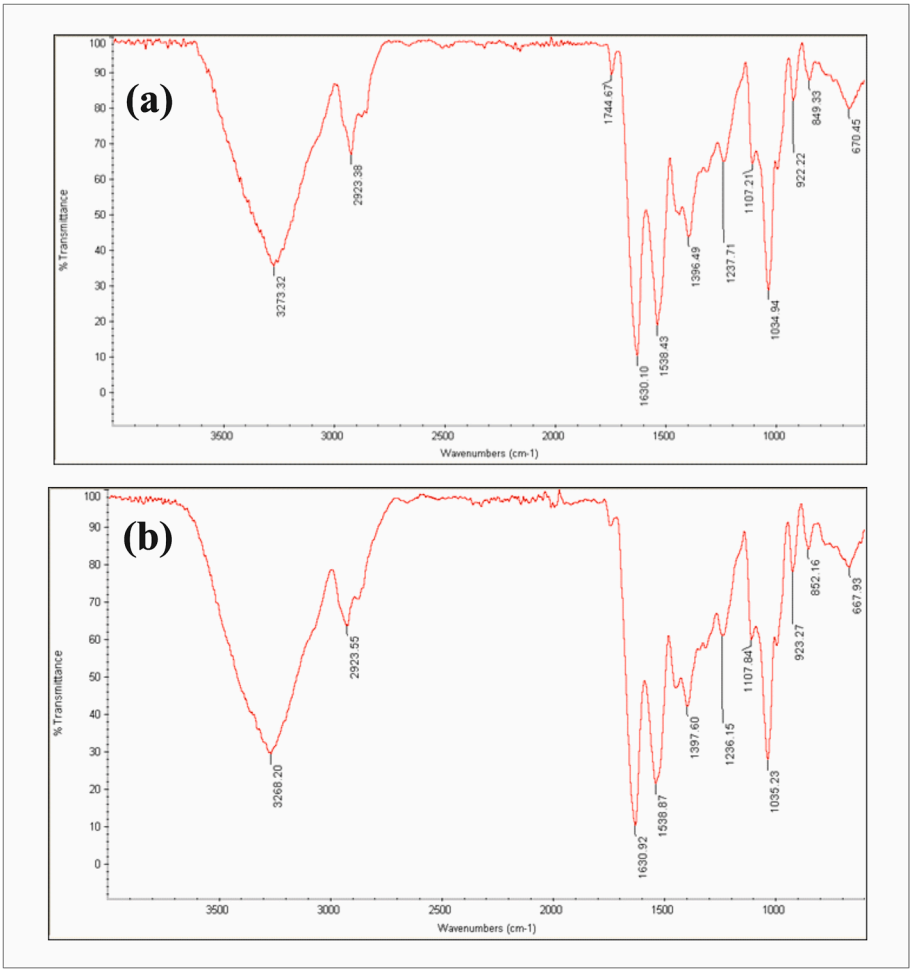

Fig 2. FTIR spectra of whey-NEO film (a), and whey-EO film (b).

chemical indexes. TPC, LAB and TVC analysis were calculated during 21 days at 4 °C, and the mean count is stated as log CFU/g.

TVC

Fig. 3a shows TVC value of different treatments (whey, whey-EO and whey-NEO) and control samples during 21 days storing at 4 °C. For fresh fish meat the acceptable TVC limit is 7 log CFU/g, which the amount of TVC on the first day for all samples of this study was 2.4 log CFU/g, it means that they had very good conditions. By the 16th day, only the control samples had exceeded 7. But on the 21st day, all of them went above 7, except whey-NEO. The lowest amount of TVC was observed after 21 days in whey-NEO treatment, which may be due to the antibacterial property of the EO of *Anethum graveolens* L. (due to the existence of polyphenols composites in it), which is effective against mesophilic lactobacilli, yeasts, lactococcus, enterobacteriaceae, enterococci and aerobic mesophilic bacteria (Radjabian, Salimi, Rahmani, Shockravi, & Mozaffarian, 2013). Antimicrobial property of whey protein was mentioned by previous studies (Kanatt, Rao, Chawla, & Sharma, 2013). Fig. 3 also showed that the treatment of nanoliposome (whey-NEO) increased the properties of antimicrobial of the films

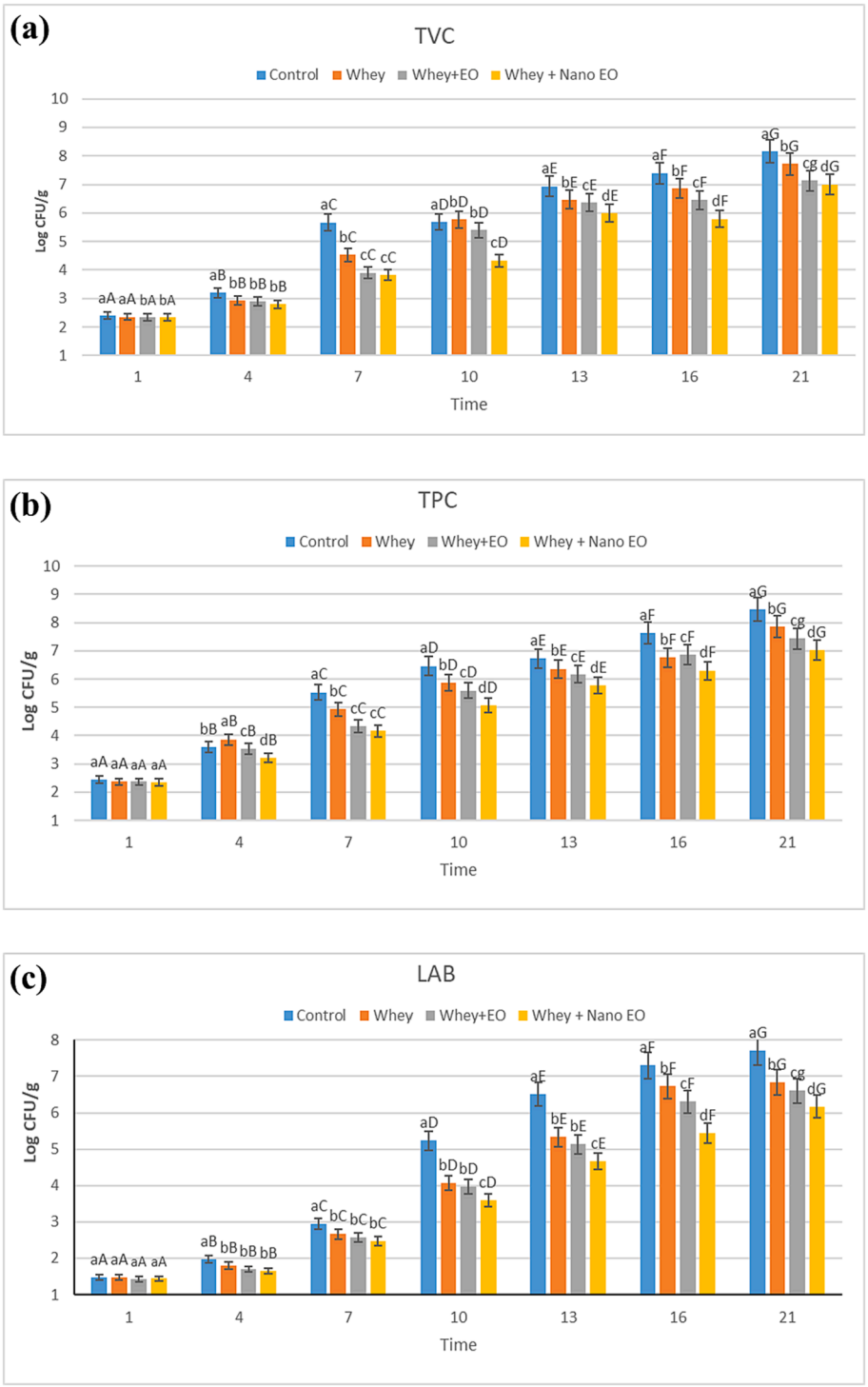

Fig 3. The TVC (a), TPC (b), and LAB (c) count of control and treatments storing for 21 days at 4 °C.

compared to the free treatment (whey-EO), which is probably due to the reduction of EO evaporation, the encapsulation of the compounds in the nanoliposome and the greater protection of the compounds and their easier transfer to the cell wall of bacteria (Homayonpour et al., 2021). Previous studies confirmed this increase in antimicrobial properties of nanoliposome EO compared to the free form (Ghaderi-Ghahfarokhi, Barzegar, Sahari, Gavlighi, & Gardini, 2017). In previous study by Socaciu et al. that analyzed effects of whey-protein film combined with EO of tarragon, they stated the TVC value of control and treatments after 15 days was ranged 6.68 to 8.65 log CFU/g (in samples of control) (Socaciu et al., 2021). Also, analogous outcomes were expressed by Wu et al. (evaluated effects of gelatin films combined with EO of cinnamon nanoliposomes) (Wu et al., 2015) and by Homayonpour et al. (assessed effects of chitosan film combined with nanoliposomes *cuminum cyminum* L. EO) (Homayonpour et al., 2021).

TPC

Fig. 3b shows TPC value of different treatments (whey, whey-EO and whey-NEO) and control samples during 21 days storing at 4 °C. The initial quality of the fish samples was good and the microbial load was low (2.34–2.45 log CFU/g). Counting the number of bacteria during the experiment indicated a rising trend, in all treatments. In this study, the highest bacteria growth (8.46 log CFU/g) was related to the control sample, and whey + NEO had minimum growth of bacteria (7.03 log CFU/g), which was owing to the several causes like its lipopolysaccharide layer disruption of the outer membrane of bacteria, whey and EO antibacterial effect (owing to the phenolic and other compounds of antibacterial), the interaction with groups of anionic on the bacterial cell surface and function as a barrier against transmission of oxygen (Mohan et al., 2012). In previous study by Socaciu et al. that analyzed effects of whey film combined with EO of tarragon, they stated the psychrotrophic count of control and treatments after 15 days was ranged 7.49 to 8.57 log CFU/g (control) (Socaciu et al., 2021).

LAB

Fig. 3c shows LAB value of different treatments (whey, whey-EO and whey-NEO) and control samples during 21 days storing at 4 °C. The initial microbial load was low (about 1.4 log CFU/g). Counting the number of bacteria during the experiment indicated a rising trend in all treatments. Based on our findings, during 21 days storge, the lowest and highest LAB value were shown in whey-NEO treatments (6.16 log CFU/g) and control samples (7.7 log CFU/g). Our mentioned treatments by disrupting the bacterial cell membrane and the polycationic nature of the treatments, inhibited growth of bacterial, which confirmed by other studies (Homayonpour et al., 2021; Pabast et al., 2018). In prior research by Socaciu et al. that analyzed effects of whey film combined with EO of tarragon, they stated the LAB value of control and treatments after 15 days was ranged from 6.29 to 6.54 log CFU/g (in sample of control) (Socaciu et al., 2021). Also, our findings were consistent with other researches in the field of other essential oils (Ghaderi-Ghahfarokhi et al., 2017; Homayonpour et al., 2021).

Chemical analysis

pH value

According to the Fig. 4a, the pH of control and all treatments was 6.2 on day zero, which the pH control gradually increased in the following days, but the other treatments remained somewhat constant or even decreased. On the 21st day, the maximum pH value was measured in the control sample (7.5) and the lowest pH was measured in the whey-NEO (6.3). Using whey and EO to coat the fish surface reduced the pH value in all treatments that was probably due to their effect of antimicrobial. The similar reductions have stated in pH value in other foods with different coating such as for tilapia (Chen et al., 2011), sardine fillet (Homayonpour et al., 2021). As a consequence of the protein degradation and growth of microorganisms (M.O), pH value rises in the samples of fish as a result of metabolites of M.O and autolysis of alkaline (like composites of nitrogenous) (Kılıç, Şimşek, Claus, & Atılgan, 2014). According to prier researches, edible coatings may have a protective effect against substrate decomposition and growth of M.O, causing a nearly constant pH behavior during storage (Kılıç et al., 2014). Fig. 4a also showed that the nanoliposome (whey-NEO) treatment decreased the pH value compared to the free (whey-EO) treatment, which was probably due to the reduction of EO evaporation, the encapsulation of the compounds in the nanoliposome, providing greater protection of the compounds during the study and their easier transfer to the cell walls of M.O (Homayonpour et al., 2021). In previous study by Socaciu et al. that analyzed effects of whey film combined with EO of tarragon, they stated the pH value of control and treatments after 15 days was ranged 6.5 to 6.7 (control) (Socaciu et al., 2021). Behbahani et al. assessed the effect of coting comprising *Anethum graveolens* L. EO (D) and PMSM (Plantago major seed mucilage) on beef shelf-life and expressed treatment of PMSM +1.5 % D had lowest value of pH compared to control samples, after storing for 18 days (Behbahani et al., 2017).

Peroxide value

Fig. 4b shows PV of different treatments (whey, whey-EO and whey-NEO) and control samples during 21 days storing at 4 °C. The value of PV changes indicates the amount of products of the initial stage of lipid oxidation during the fish storing period. Based on our findings, no

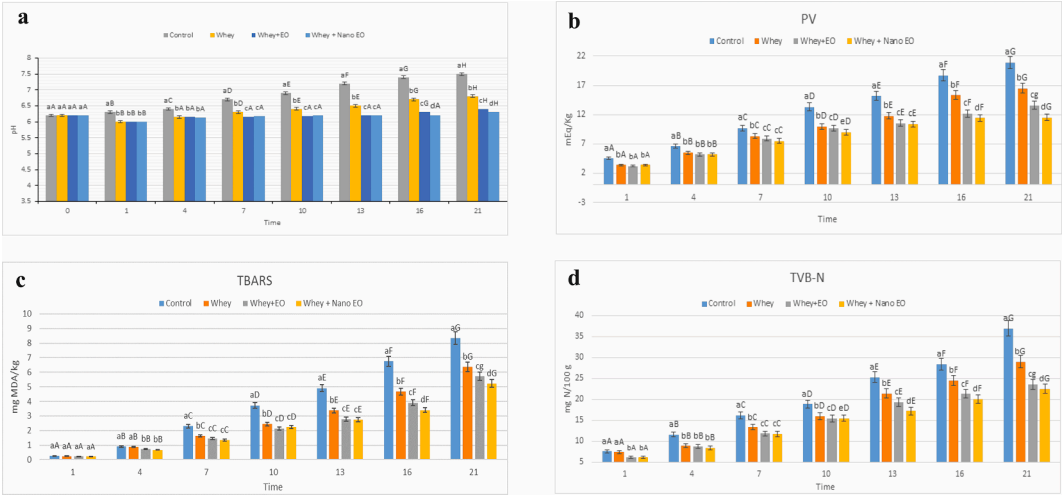

Fig 4. The pH (a), PV (b), TBARS (c), and TVBN (d) changes of control and treatments storing for 21 days at 4 °C.

difference was found on first day (3.21–4.5 mEq/Kg) about PV. The PV value during the experiment indicated a rising trend in all treatments (especially control samples). During the experiment, a lot of difference was detected among the PV value in the control and other treatment (whey, whey-EO and whey-NEO). On day 21, the maximum and minimum value of PV was identified in the control sample (20.9 mEq/Kg) and whey-NEO sample (11.5 mEq/Kg), respectively. Our outcome has also been confirmed by other researchers, which expressed nanoliposomes compared to the free form had better result on PV (Homayonpour et al., 2021; Pabast et al., 2018). During the storage period, in fish muscles, fatty acids (FAs) are oxidized to form peroxides/hydroperoxides, which are thought to contribute to increased PV values. Furthermore, as hydrogen is eliminated from the FAs double bond, free radicals are formed, which can react with oxygen and rise the value of PV (Homayonpour et al., 2021; Pabast et al., 2018). Boghori et al. (in 2020) analyzed effect of coating containing whey and *Zataria multiflora* (Shiraz thyme) EO and stated the PV of control and treatments after 12 days was ranged 1.31 to 4.22 mEq/Kg (control) (Boghori, Latifi, Ebrahimi, Mohamadi Kartalaei, & Dehghan, 2020).

TBARS value

Fig. 4c shows TBARS of different treatments (whey, whey-EO and whey-NEO) and control samples during 21 days storing at 4 °C. In the shelf-life of fish and other seafood, the TBARs value shows the products of secondary oxidation (that are the chief limiting items for rancidity) (Mehdizadeh et al., 2021; Noori et al., 2018). Based on our findings, no difference was found on first day (about 0.2 mg MDA/kg) about TBARS value. Identifying off-odors and off-taste from foodstuffs is a sign of TBARs equal or greater to two mg MDA/kg (Homayonpour et al., 2021; Pabast et al., 2018). The value of TBARS during the experiment showed an increasing trend, in all treatments (especially control samples). On day 21, the maximum and minimum amount of TBARS value was identified in the control sample (8.34 mg MDA/kg) and whey-NEO sample (5.23 mg MDA/kg), respectively. Fish samples, by combining EO and whey in film coating, were maintained from the procedure of oxidation. This might be owing to components of polyphenol in essential oil can give hydrogen electrons or atoms, consequently inhibiting oxidation of lipid thereby inhibiting production of free radical (Taherkhani, Noori, Akhondzadeh Basti, Gandomi, & Alimohammadi, 2015). According to our findings, whey + NEO treatment had better effect on TBARS. Consequently, the outcome can be concluded the encapsulation is capable of maintaining the samples of fish (during storage) from degradation and evaporation. Nanoliposomes lead to a rise specific surface area for EO, causing in an effective and rapid effect of antioxidant (Noori et al., 2018). In previous study by Socaciu et al. that analyzed effects of whey film combined with EO of tarragon, they stated the TBARS value of control and treatments after 15 days was ranged 0.75 to 0.83 mg MDA/kg (control) (Socaciu et al., 2021).

TVB-N value

Fig. 4d shows TVB-N value of different treatments (whey, whey-EO and whey-NEO) and control samples during 21 days storing at 4 °C. The TBARS during the experiment showed an increasing trend, in all treatments (especially control samples). This increase can be owing to deamination of free amino acid (FAA), some enzymatic procedures like oxidation of amines, degradation of nucleotides and activity of M.Os (Tometri et al., 2020). On day 21, the maximum and minimum amount of TVB-N value was identified in the control sample (37 mg N/100 g) and whey-NEO sample (22.5 mg N/100 g), respectively, which can be owing to the reduction of the bacterial counts or their ability of oxidative to remove amines from compounds of volatile nitrogen. In the case of encapsulation, this process may have helped retain the antibacterial characteristics of the EO for a longer period. In previous study by Socaciu et al. that analyzed effects of whey film combined with EO of tarragon, they stated the TVB-N value of control and treatments after 15 days was ranged from 3.64 to 4.55 mg N/100 g (control) (Socaciu et al., 2021). Also, Sayyari et al. by investigating the *Foeniculum vulgare* EO (forms of nanoliposomes and free) on fillets of fish, confirmed these outcomes about TVB-N analysis (Sayyari, Rabani, Farahmandfar, Esmaeilzadeh Kenari, & Mousavi Nadoshan, 2021).

Sensory evaluation

Table 3 displays the outcomes obtained from sensory evaluation. In this examination, the score was varied from one to five, where a score of five means the worst condition (lowest score) and a score of one means the best condition (highest score). In this study, the texture, odor, color (in the sample of control), became unacceptable (score higher than 3) after 16, 13 and 16 days, respectively. For all treatments, texture was acceptable after 21 days. After 21 days, 3 items of sensory (including off-odor, discoloration and red color) were acceptable for whey-EO and whey-NEO. A difference (significant) among the experiments could be elucidated by a protective coating on fish by whey and EO (free and nano form) that efficiently prohibited growth of microbial in fish samples and decreased the rate of protein degradation as well as the accumulation of volatile combinations in fish (Mohan et al., 2012). Finally, in all sensory evaluation items, the best result after 21 days was related to whey-NEO (score = 1). In previous study by Socaciu et al. that analyzed effects of whey film combined with EO of tarragon, they stated the sensory of treatments after 15 days was better than control samples (Socaciu et al., 2021). Also, our findings were confirmed by similar studies on edible coatings containing EO (Homayonpour et al., 2021; Pabast et al., 2018).

Conclusion

This study is the first investigation of the effects of whey protein coating containing dill (*Anethum graveolens L.*) EO nanoliposomes on rainbow trout fish. All treatments including whey, whey-EO and whey-NEO were all effective in protecting fish during the storage period. The results of SEM image and FTIR spectroscopy showed the proper structure of the produced film coatings. Based on our results, all tests (microbial, physicochemical and sensory evaluations), coatings containing whey and NEO (whey-NEO) had the best effect compared to the control sample and other treatments (whey and whey-EO). Therefore, according to our findings, the EO of plants such as dill especially in the form of nanoliposomes combined with whey protein, can be used as a good edible coating to protect fish (at refrigerated temperature or in temperature of 4 degrees Celsius). Among the limitation of our study, can be mentioned the lack of investigation of the extract of dill (forms of aqueous and ethanolic). Finally, it can be suggested, the effects of the extract of mentioned plant (in the form of ethanolic and aqueous) should also be evaluated in future researches.

Ethics and consent of sensory evaluation: In sensory evaluation, appropriate protocols have been used to protect the rights and privacy of all participants during the implementation of the research. In this research, the sensory panel participants have given permission to participate and use their data/answers. Authors will adhere to the Ethical Responsibilities of Authors and the COPE Rules. All authors consent to publication. This study does not involve any human or animal testing.

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CRediT authorship contribution statement

Mozhgan Azizi: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft. **Kambiz Jahanbin:** Conceptualization, Project administration, Resources, Writing – review & editing. **Nabi Shariatifar:** Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing.

Table 3
The sensory changes of control and treatments storing for 21 days at 4 °C.

Sensory items	Treatments	Days						
		1	4	7	10	13	16	21
Texture	Control	1±0.01	1±0.02	1±0.02	2±0.01	3±0.02	4±0.03	5±0.02
	Whey	1±0.01	1±0.01	1±0.01	1±0.01	1±0.01	2±0.03	2±0.01
	Whey + EO	1±0.02	1±0.01	1±0.02	1±0.01	1±0.02	1±0.02	2±0.02
	Whey + Nano EO	1±0.01	1±0.02	1±0.02	1±0.02	1±0.01	1±0.01	1±0.01
Off-odor	Control	1±0.01	1±0.02	2±0.01	3±0.02	4±0.03	5±0.04	5±0.03
	Whey	1±0.02	1±0.02	1±0.02	2±0.01	2±0.02	3±0.03	4±0.02
	Whey + EO	1±0.01	1±0.01	1±0.01	1±0.01	1±0.01	1±0.02	2±0.02
	Whey + Nano EO	1±0.02	1±0.01	1±0.01	1±0.02	1±0.01	1±0.01	1±0.02
Discoloration	Control	1±0.02	1±0.01	1±0.01	2±0.02	3±0.03	4±0.03	5±0.02
	Whey	1±0.01	1±0.01	1±0.02	1±0.02	2±0.03	4±0.04	5±0.03
	Whey + EO	1±0.01	1±0.02	1±0.01	1±0.01	1±0.02	1±0.01	2±0.02
	Whey + Nano EO	1±0.02	1±0.02	1±0.02	1±0.02	1±0.01	1±0.02	1±0.02
red color	Control	1±0.01	1±0.02	1±0.01	2±0.02	3±0.03	4±0.02	5±0.03
	Whey	1±0.01	1±0.01	1±0.01	1±0.02	2±0.02	3±0.02	4±0.03
	Whey + EO	1±0.01	1±0.02	1±0.01	1±0.01	1±0.02	1±0.01	2±0.03
	Whey + Nano EO	1±0.01	1±0.01	1±0.01	1±0.02	1±0.01	1±0.02	1±0.01

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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