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The effect of free and encapsulated essential oil and extract of cinnamon with nanoliposome on *Listeria monocytogenes* and *Escherichia coli* inoculated into ground beef

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ABSTRACT

The cinnamon essential oil and extract nanoliposomes were prepared through thin layer hydration-ultrasonication technique, using lecithin and three different co-surfactants namely, glycerol, triacetin and propylene glycol, and Tween 80 as surfactant. Results showed that the propylene glycol led to production of the nanoliposomes with the smallest mean particle size (92.03 nm) with spherical-shaped and the greatest net-zeta potential value (-24.1 mV) and was selected as more suitable cosurfactant. Although antibacterial activity of cinnamon essential oil and extract were greater than those were encapsulated into nanoliposomes, both cinnamon essential oil and extract nanoliposomes exhibited high antibacterial activities against *Escherichia coli* and *Listeria monocytogenes* bacteria strains. Results indicated that based on the minimum inhibitory and bactericidal concentrations of the prepared samples, *L. monocytogenes* had higher resistance to the prepared cinnamon nanoliposomes. Then, six treatments including control, extract, nano-extract, essential oil, nano-essential oil and extract- essential oil were used for investigate the effect of cinnamon extract on shelf life of ground beef. Chemical (pH, TBA and TVN) and microbial parameters were detected periodically, as well as the effect of different treatments on ground beef inoculated with *Escherichia coli* and *Listeria monocytogenes* were examined. The results showed that the extract has an antimicrobial and antioxidant properties and the nanoencapsulation process enhances the attributes mentioned, so that bacterial spoilage and oxidation process delayed in the ground meet contains nano-extract ($p < 0.05$). The highest value of pH (6.58), TBA (0.081MDA/kg) and TVB-N (72.5mg/100g) in the control treatment on the 9th day was observed. While, the value of pH (6.09), TBA (0.002MDA/kg) and TVB-N (11.5mg/100g) was detected on the 9th day in the nanoencapsulated essence. According to the results obtained in present study nano-liposomal cinnamon extract can be used for extending shelf-life ground beef without causing undesirable effect in terms of oxidative stability and low microbial spoilage.

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1. Introduction

Meat is an important source of nutrients and is considered one of the most sensitive perishable foods because it is a very favorable environment for the activity of microbes, yeasts and molds; Therefore, meat and meat products and their optimal storage conditions are considered important. Meat products typically spoil during refrigerated storage due mainly to microbial activity [1]. Therefore, one of the most important concerns of the food industry is the control of pathogenic and spoilage microorganisms [2].

Unlike freshly slaughtered carcasses, minced meat is attacked and spoiled exclusively by bacteria, in this regard bacterial genera such as Pseudomonas, Alcaligenes, Acinetobacter, Aeromonas, Listeria and Moraxella are of great importance. It is generally believed that Pseudomonas, Acinetobacter and Moraxella are among the primary and important spoilage factors of these meats, which are the initiators of spoilage [3]. Usually, spoilage at low temperature is accompanied by the production of an unpleasant smell. In the meantime, the secondary contaminations that enter the product during the meat grinding process should not be ignored. Also, due to the more accessible surface that the minced meat provides for pathogens to attack, the percentage of contamination doubles and even multiplies [1].].

Among the rotting bacteria of minced meat, some of them cause disease in humans in addition to spoiling the meat. The high risk of contamination transmission through contaminated meat sources*Listeria monocytogenes* It has led to the general recording of slaughtered animals in most countries [4].

Pathogen*Escherichia coli* O157:H7 is of particular importance to the grounding operation, as it is considered as an adulterant in ground meat. There is recent evidence that the prevalence of*E. coli* O157:H7 in cattle is higher than previously thought [5]. General forms especially*Escherichia coli* They are considered one of the most important causes of gastroenteritis and microbial indicators of water and food contamination. In different parts of the world, the occurrence of infections and food poisoning caused by contamination*Escherichia coli* It has been studied a lot. Among these studies, Hanlon et al. reported in 2018 in England [6], Fröder et al. in 2007 in Brazil [7], Park et al. in 2015 in South Korea [8], Omar et al. in 2017 in Libya. [9], Norlis et al. in 2011 in Malaysia [10] and Gormley et al. in 2010 in England [11].

During meat storage, microbial growth can be delayed by the use of antimicrobials, increasing the storage period and maintaining its quality [1]. The use of chemical preservatives, some of which are suspected of harmful effects such as carcinogenicity or toxic residues, has caused concern to health authorities. Therefore, one of the needs of human society is to reduce or eliminate these synthesized compounds in food, and this has led to a significant approach of food manufacturers to replace chemical preservatives with natural types. Plant extracts are known as a natural antimicrobial source that have positive effects such as protection against chronic diseases, cancer, diabetes, and degenerative heart diseases. The composition, structure and functional groups of the extracts play an important role in their antimicrobial activity and usually the compounds that have phenolic groups are more effective [12].

Most herbal perfumes have an antimicrobial effect, which is mainly related to their phenolic compounds. One of the most well-known of these plants is cinnamon, which is native to Sri Lanka and has a scientific name*Cinnamomum zeylancium* Is known. Cinnamon essence has been recognized by many researchers as a good source of anti-fungal and bacterial compounds. The aroma of cinnamon is able to prevent the growth of the main microorganisms that spoil meat. In addition to its antimicrobial properties, this spice can be used as a smell and taste factor in meat products [13]. Perfume essence is an alcoholic extract with abundant oil, which mainly includes terpene compounds or terpene derivatives, while the soluble extract contains all the useful substances of the plant, including tannins, mucilages, perfumes, etc.

One of the most important issues and challenges in the field of using flavorings in food is to improve and increase their stability in the processing stages as well as controlling their release until consumption. Therefore, it is better to be microcoated before use in food and beverages in order to limit decomposition or loss of aroma during processing and storage [14]. Microencapsulation is a process in which particles and droplets of liquid, solid and gas are trapped in a membrane made of approved food and the resulting capsule releases its

contents under a controlled speed and with a specific stimulation and at a specific time. Carrier systems based on lipids include nanoemulsions, nanoliposomes, solid lipid nanoparticles and lipid nanostructure carriers. Due to the possibility of production on an industrial scale and the ability to release compounds with different hydrophilic properties, nanoliposomes can be a suitable system for the microencapsulation of fat-soluble compounds such as essential oils [15 and 16].

In this research, according to the properties of cinnamon, the effect of aroma and extract and nanoliposome, which causes the controlled release of cinnamon's antimicrobial properties and increases the solubility and more accurate delivery of these substances, has been investigated on the preservation of minced meat during its storage in the refrigerator.

2- Materials and methods

2-1- Materials

The veal meat was purchased from the slaughterhouse and transported to the laboratory under completely sterile conditions, next to the ice, and then it was cut into pieces under sterile conditions, and the pieces obtained were washed completely with 60 degrees Celsius water, dried with a sterile cloth, and ground by a meat grinder. This minced meat was stored in the refrigerator for the inoculation of bacteria, extracts, perfumes and nanoliposomes according to specific treatments.

Cinnamon sticks were purchased from the local market. Phospholipid (egg lecithin), triacetin, 1,2,3-triacetoxypropene, propylene glycol and glycerol were provided by Merck (Darmstabt, Germany). 2,2diphenyl-1-picrylhydrazyl (DPPH) (analytical base) was purchased from Sigma-Aldrich (Darmstabt, Germany). Deionized water and all solvents were purchased from Dr. Majalli Company (Tehran, Iran). Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB) were purchased from Biolife (Biolife Co., Milan, Italy).*Escherichia coli* (*E.coli*, PTCC 2015) and*Lisrteria monocytogenes (L. monocytogenes*, PTCC 1340) were provided by the Persian Microbial Type Culture Collection (PTCC, Tehran, Iran).

2-2- Preparation of cinnamon essence

The cinnamon stick was chopped into small pieces and the dried cinnamon skins were powdered by a grinder, and 70 grams were carefully weighed and transferred into a 1000 ml Erlenmeyer flask, and 600-700 ml of distilled water was added. In order to reduce the formation of bubbles resulting from boiling, some glass pearls were added inside the balloon and the cinnamon essence was distilled and extracted by steam using the Cloninger method. The results showed that the efficiency of perfume from the consumption of cinnamon was about 2.5% [17].

2-3- Preparation of cinnamon extract

The cinnamon stick was ground and turned into powder, and 60 grams of it was soaked in 300 ml of aqueous-alcohol solution (50% v/v) for 24 hours, then it was filtered and ethanol was evaporated by a rotary evaporator (Heidolph, Germany) in It was evaporated at a pressure of 0.5 atm and a water bath temperature of 50 degrees Celsius and a rotation speed of 50 rpm for 30 minutes [17].

2-4- Preparation of cinnamon extract nanoliposomes

In order to prepare cinnamon extract nanoliposomes through thin layer hydration-sonication, 2 grams of lecithin and 2 ml of Tween 80 were placed in 38 ml of cinnamon extract for 30 minutes in a bath sonicator to dissolve nano-sized particles and obtain nanoliposomes. The resulting nanoliposomes were stored at 5 degrees Celsius before diagnostic tests [17].

5-2- Preparation of nano liposomes of cinnamon essence

Nano liposomes of cinnamon essence were prepared using thin layer hydration-sonication technique according to Jabraeili et al. [18] with some modifications. 0.5 ml of cinnamon essence, 1 gram of lecithin and 1 ml of one of the selected co-surfactants named glycerol, triacetin or propylene glycol were mixed and completely dissolved in 10 ml of 50:50 V/V dichloromethane-methanol solution. (formulations A, B & C in Table 1) were sonicated (exposed to sound waves) in a bath sonicator for 5 minutes. Then the resulting mixture was transferred to a round bottom flask and the solvents were completely removed using rotary evaporation (Heidolph, Germany) at 0.5 atm, water bath temperature 45 degrees

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Celsius, rotation speed 50 rpm for 30 minutes until formation A thin layer was made on the flask wall. This thin layer was hydrated in 50 mL of distilled water magnetically stirred at 100 rpm for 60 min and then sonicated (sonicated) in a bath sonicator for 5 min in two cycles. The synthesized nanoliposomes were stored at 5 degrees Celsius.

Table 1 The formulation of prepared cinnamon essential oil and extract nanoliposomes

Sample	Amounts of essential oil (mL)	Lecithin (g)	Co-emulsifier and its amount	Solvent (1:1 v/v dichloromethanemethanol, mL)	Water (mL)
A	0.5	1	Glycerol (1 mL)	10	50
B	0.5	1	Triacetin(1 mL)	10	50
C	0.5	1	Propylene glycol (1 mL)	10	50
D	0.25	1	Propylene glycol (1 mL)	10	50
AND	0.125	1	Propylene glycol (1 mL)	10	50
F	38 mL aqueous extract of cinnamon	2	Tween 80 (2 mL)	-	-

A, B, C, D and E: Cinnamon essential oil nanoliposomes
F: Cinnamon extract nanoliposome

6-2- Tests

2-6-1- Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography (Agilent 6890N, US) with mass spectrometer (HP 5989, US) was used to describe the chemical composition of cinnamon extract and essence. The carrier gas was nitrogen, which led to the transfer of the vaporized perfume into the column (length 30 meters, inner diameter 0.025 mm and size 0.25 μm) (5MS-HP Agilent, US). The oven temperature was increased from 45°C to 250°C at a rate of 5°C per minute.

Chem Station software (G1701DA MSO Chem Station, Agilent, US) was used for data analysis [18].

2-6-2- Particle size, polydispersity index (PDI) and zeta potential

The mean particle size Z, size dispersion or multiple dispersion index and zeta potential values of the produced nanoliposomes were analyzed using a particle size analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK) [19].

2-6-3- Antibacterial activity

Antibacterial activity of prepared nanoliposomes by obtaining the MIC (minimum inhibitory concentration) of the samples against the growth of bacteria*E. coli* And*L. monocytogenes*, as selected Gram-negative and Gram-positive bacteria, was evaluated using the micro-broth dilution method [18].

2-6-4- Morphology

The microstructure of selected cinnamon nanoliposomes was revealed by transmission electron microscope (TEM. Hitachi H7500, Japan). A drop of the sample was placed on the film grid and stained by 1% aqueous phosphotungstic acid solution and observed after drying [19].

2-6-5- Inoculation of microorganisms into meat

In order to stain the sample with bacteria, minced meat samples with*Escherichia coli* (About 10^6) And*Listeria monocytogenes* (About 10^5) were inoculated. The inoculated samples were completely homogenized. Different treatments were added to minced meat and after mixing and re-homogenization, it was packed in zipped nylon bags and stored at refrigerator temperature (4°C ± 1). On the test days during the storage period, three samples from each section were randomly selected and tested [20].

2- 6- 6- Total escape nitrogen (TVN)

We poured 10 grams of minced meat into the protein distillation set and added 2 grams of magnesium oxide catalyst. The tube was placed in the distillation apparatus and according to the program, at least 90 cc of distilled water was added to the sample and boiled for 4 minutes.

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Due to the heat of free nitrogen, the distilled sample was introduced into a human body containing 60 cc of 4% boric acid, and this process continued for 4 minutes. A green color was obtained due to the formation of ammonium borate. Finally, after boiling, the distillation product sample was titrated with 0.2 sulfuric acid. The green color of ammonium borate was removed and purple color was obtained. At this stage, the titration was stopped. The amount of TVN in mg per 100 g of food was calculated according to the following formula [21].

(1) = TVN (mg/100 g sample)

0.14 x 1000 x acid normality x amount of sulfuric acid consumed

2-6-7- Thiobarbituric acid index (TBA)

The amount of 5 grams of meat along with 100 ml of 10% trichloroacetic acid solution in a 250 ml beaker was completely homogenized by an electric stirrer and passed through Whatman No. 42 filter paper. The filtered solution was made up to 100 ml with the help of 10% trichloroacetic acid solution. 3 ml of filtered solution was mixed with 3 ml of 0.02 M trichloroacetic acid solution in a test tube with a lid and placed in an oven at 100 °C for 45 minutes. After cooling the samples, the amount of light absorption was measured at a wavelength of 532 nm by a spectrophotometer [21].

2-6-8- Total count of bacteria

To count gram-negative bacteria*Hey Clay* Eosin methylene blue (EMB) culture medium was selected. 37 grams of culture medium was dissolved in one liter of distilled water and boiled. After being sterilized in an autoclave, it was divided into plates at a temperature of 50 to 55 degrees Celsius. One plate was selected for each dilution [22].

7-2- Statistical analysis

In the phase of investigating the formulation characteristics of perfume and cinnamon extract nanoliposomes, the data were analyzed using one-way analysis of variance (ANOVA) and the significant difference ($P<0.05$) between the means was determined using Tukey's limited multiple comparison test. In the phase of investigating the effect of adding nanoliposomes to meat, the experiments were conducted in the form of a factorial arrangement with a completely random basic design in three replications. The experimental factors included six levels of treatment (treatments A, B, C, D, E and F) and four levels of storage time (days 1, 3, 6 and 9). This approach was used to determine the effects of different factors. Tukey's test was used to compare the averages and examine the simple and mutual effects of the factors. Minitab V.17 software was used for all statistical analyses. Graphs were drawn using Excel software.

3. Results and Discussion

3-1- The main bioactive compounds of cinnamon

The main bioactive compounds of perfume and cinnamon extract that were more than 1% are listed in Table 2. As seen in Table 2, while cinnamaldehyde is the main compound of cinnamon extract and perfume, its content in perfume is about 1.5 times higher than in the extract. This with studies

The previous one that reported cinnamaldehyde as the main constituent of cinnamon is in good agreement [1, 23]. Cinnamaldehyde is a phenylpropanoid (aromatic terpenoid aldehyde) with the formula C6H5CH = CHCHO, which is responsible for the taste and smell of cinnamon. Vanillin, ethyl vanillin, α-moverulene, δ-cadinene, 4-(4-hydroxyphenyl)-2-butanone were other compounds that were more than 1% in both cinnamon extract and cinnamon essence.

Table 2 The main bioactive components of the cinnamon extract and essential oil

Chemical constituents	Retention time (min)	Cinnamon extract (%)	Cinnamon essential oil (%)
2-Ethyl-3-hydroxy-4H-pyran-4-one (Ethyl maltol)	11.21	<1	1.0
Cinnamaldehyde	13.28	33.74	50.95
2-Propenal, 3-phenyl-	15.50	<1	12.15
2H-1-Benzopyran-2-ol	15.59	1.10	<1
2H-1-Benzopyran-2-one	16.73	1.05	<1
Vanillin	16.93	2.80	1.71
Ethyl vanillin	17.08	1.64	1.61
α-Muurolene	17.9	1.33	1.93
δ-Cadiene (18.47 min, 1.69%)	18.47	1.69	2.11

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Para Methoxy Cinnamic aldahyde	19.5	<1	1.36
4-(4-Hydroxyphenyl)-2-butanone	19.82	15.16	3.91
2-Propenal, 3-phenyl-	20.1	<1	1.06
2(3H)-Furanone	22.34	1.55	<1
2H-Pyran-2-one	22.99	4.17	<1
Phenol, 2-pentyl-	25.88	1.05	<1
Benzenamine, 2,6-dimethyl-	26.61	2.04	<1
Heptanone, 6-(dimethylamino)-4,4-diphenyl-	30.76	1.36	<1
Methoxyacetic acid benzyl ester	32.28	1.08	<1
1,3-Diphenyl-1H-pyrazole-*4*-carboxaldehyde	39.63	1.14	<1
Total		70.9	77.79

Only the compounds with > 1% were reported here in this format: detected chemical constitute (retention time, percentage)

2-3- Particle size, PDI and zeta potential of prepared nanoliposome

The particle size, PDI and zeta potential values of cinnamon essence nanoliposomes prepared based on glycerol, tristane and propylene glycol cosurfactants and cinnamon extract nanoliposomes are presented in Table 3.

Table 3 Physico-chemical characteristics of the prepared cinnamon essential oil and extract nanoliposomes

Sample	Co-surfactant / surfactant	Z-average (nm)	Mean particle size (nm)	PDI	zeta-potential (mV)
A	Glycerol	523.2	104.6	1.000	-15.8
B	Triacetin	243.0	102.9	0.283	-15.8
C	Propylene glycol	243.7	92.03	0.338	-24.1
F	Tween 80	379.3	167.2	0.540	-7.73

A, B and C: Cinnamon essential oil nanoliposomes
F: Cinnamon extract nanoliposome

The obtained results show that cinnamon nanoliposome formulated with propylene glycol as a cosurfactant has the best characteristics in terms of average particle size and zeta potential values. However, cinnamon extract nanoliposome had the maximum average particle size. Lecithin with a phospholipid structure is an amphipathic compound that has two hydrophilic and hydrophobic parts. As a result, it can be used as layered structures in water systems. Accordingly, liposomes are formed when lipophilic bilayers are hydrated, liquefied, and swollen. Then, the stirring process can split these hydrated layers. The split layers assemble together to form smooth bilayer vesicles, which prevent contact with water in their hydrophobic regions [18, 24]. Hydrophilic polyol surfactants such as glycerol, triacetin and propylene glycol, as hydrotrope agents, can increase the hydration efficiency of lecithin and perfume. Consequently, these polyols can improve the stability and encapsulation efficiency of the product, especially if the liposomes are made by the thin-film hydration method. In addition, it is assumed that these surfactants can increase the stability of liposomes by improving their molecular arrangement in bilayers by creating hydrogen bonds between the polar end groups of lecithin molecules and making them cross-link. However, the formation of consecutive swelling molecules as well as increasing the swelling ability of the layers creates larger particles that in some researches, such as the present study, the particle size of the system increases with the increase in the polarity of the cosurfactant or its content [18, 25]; Therefore, the increase in the average particle size of the system with the increase in cosurfactant polarity can be related to their mentioned performance in lecithin bilayers. The smaller particle size of nanoliposomes prepared using propylene glycol as a surfactant is related to the formation of possible hydrophobic bonds between lipophilic functional groups of perfume components, fatty acyl chains of a phospholipid, and the non-polar part of propylene glycol, which can reduce the size of nanoliposomes [26]. In addition, the lipophilic portion of propylene glycol can have a

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thickening effect that reduces the surface area of each lipid more than ideal mixing. It can also increase the stability of nanoliposomes by reducing permeability and thickening their enclosing membrane [18, 27].

Glycerol and propylene glycol are relatively small molecular compounds that have 3 and 2 -OH, respectively. They can penetrate into the vesicles and affect the position or orientation of the lipid acyl chains of the bilayer. However, triestin has larger molecules with 3 –OH, which bind more on the vesicle surfaces and thus affect the interaction of nanoparticles (nanovesicles) with each other. For example, it can increase their spatial repulsion and consequently increase their physical stability; Therefore, the smaller PDI of nanoliposomes prepared using it could be related to the steric stabilization effect of estine [28]. On the other hand, Tween 80 with the tendency of both lipophilicity and hydrophilicity, relative molecular structure and three OH groups and long acyl chains can be located either inside or between the layers or on the surface of cells and interact with the components of liposomes in different ways. and produce heterogeneous liposomes; Therefore, the PDI of liposomes prepared using Tween 80 is relatively high. The high heterogeneity of samples prepared with glycerol was not anticipated. Assuming an error in this experiment, the measurement was repeated and the same result was obtained again; Therefore, it seems that an unknown interaction between glycerol and liposome components led to the generation of liposomal or micelle systems of different sizes. Since the samples with smaller PDI are more stable against the phenomenon of physical instability, it can be concluded that tristane can produce more stable nanoliposomes due to its lower PDI value [28].

The zeta potential values of nanoliposomes are also related to the electrostatic repulsion between them and as a result their physical stability. Consequently, nanoliposomes with higher zeta potential are preferred due to better physical stability [28, 29]; Therefore, as seen in Table 3, propylene glycol formulated nanoliposomes have higher zeta potential values and consequently more stable compared to other samples.

3-3- Morphology of cinnamon aroma nanoliposomes

The microstructure of selected cinnamon nanoliposomes was revealed by transmission electron microscopy. The morphology of cinnamon essence nanoliposomes prepared using 0.5% of cinnamon essence and propylene glycol as surfactant is shown in Figure 1. As can be clearly seen in this figure, the formed nanoliposomes were less than 100 nm in size and spherical in shape. The obtained results showed that the average particle sizes are consistent with the results of particle size analysis based on DLS.

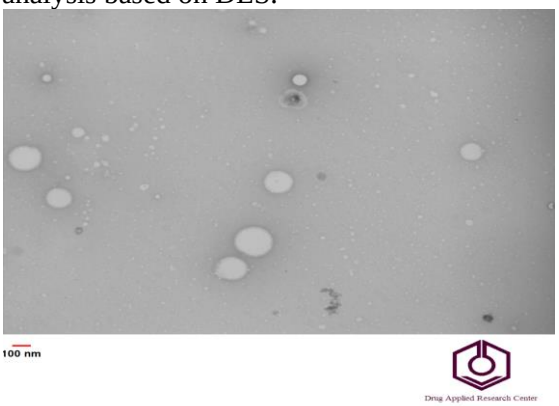

Fig 1 TEM image of the prepared cinnamon essential oil nanoliposomes using propylene glycol as co-surfactant

3- 4- Antibacterial activity of selected cinnamon nanoliposomes

The antibacterial activity of perfumes is related to their active phenolic, alcoholic, terpenoid and aldehydic compounds. Since the main compound of cinnamon is cinnamaldehyde with an aldehyde, phenyl and terpenoid structure, a very strong antioxidant activity is predicted from its extract or perfume, especially most previous researches have reported a high effect between the antibacterial activity of plant derivatives and their aldehydes and terpenes compounds. The research results showed a positive correlation between the phenolic compounds of plant extracts and their bactericidal abilities [30].

According to the measurement of the antibacterial activity of the samples (Table 4), it can be concluded that the bacteria*E. coli* Than*L. monocytogenes* It is much more sensitive to cinnamon nanoliposomes. According to the research of Guerra-Rojas et al. [31], perfumes can strongly bind to the cytoplasmic membrane and cytoplasm of cells.*E. coli* damage and cause cell death. However, cinnamon extract nanoliposomes can be grown*L. monocytogenes* compared to*E. coli* restrain more effectively. These observations

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can be explained by the possible interaction of the lipophilic active compounds of cinnamon, such as terpenoids and aldehydes, with the lipid layer of the cell membrane of gram-negative strains and disrupting its regular function. However, the antibacterial effect of cinnamon extract against*Escherichia coli* It could be related to its hydrophilic bioactive compounds that have a lower affinity to bind to the lipid layer in order to interfere with its normal function [32]. Surprisingly, cinnamon nanoliposomes with lower aroma contents were more effective against bacteria than those with higher aroma contents.*E. coli*, had lower MIC; In other words, cinnamon essence with lower concentration has more growth inhibitory activity against bacteria*E. coli* shows. These observations can be related to different mechanisms of action of cinnamaldehyde against bacteria where it inhibits cytokinesis at low concentration but suppresses bacterial cell division at high concentrations [30].

Table 4 The MIC and MBC of the prepared cinnamon essential oil (based on co-surfactant of propylene glycol) and extract nanoliposomes against *E. coli* and *L. monocytogenes*

Sample	Formulation	MIC (ppm)		MBC (ppm)	
		E. coli	*L. monocytogenes*	*E. coli*	*L. monocytogenes*
Cinnamon essential oil nanoliposomes	C	0.00625	0.0125	0.0125	0.025
	D	0.00312	0.0125	0.00635	0.025
Cinnamon extract nanoliposomes	AND	0.00312	0.0125	0.00625	0.025
	F	0.001	0.0125	0.01	0.025
Controls	cinnamon essential oil	0.000195	0.00039	0.000195	0.00039
	cinnamon extract	0.00039	0.00625	0.00039	0.00625

The possible loss of some terpene and aldehyde compounds and other active compounds during their nanoencapsulation in nanoliposomes can explain the decrease in antibacterial activity of fragrances during nanoencapsulation [33]. The lower antibacterial activity of nanoliposomal perfumes compared to non-encapsulated perfumes was also reported by Jabraeili et al. [18]. However, some previous studies reported long-term antibacterial activity for nanoencapsulated perfumes compared to macro-sized perfumes [30]. According to their observations, at the beginning of the storage period, the antibacterial activity of micro-sized cinnamaldehyde was higher than that of cinnamaldehyde nanoparticles. However, while the antibacterial activity of both decreased significantly during storage, at 10 days of storage, the antibacterial activity of nano-sized cinnamaldehyde was stronger than that of micro-sized cinnamaldehyde. It seems that during storage, the volatile components of the samples decrease, but the rate of reduction of volatile compounds in nanoencapsulated perfumes was significantly lower than the samples with free perfumes. In confirmation of the results of this research, Guerra-Rojas et al. [31] showed that the overall antimicrobial effect of perfumes depends more on the type of volatile compounds. In addition, they reported a positive correlation between the retention of volatile compounds and the bactericidal activity of nano-sized perfumes during 56-day storage. On the other hand, some other researchers such as Jabraeili et al. [18] reported an increase in antibacterial activity for nano-sized perfumes compared to small perfumes. They believe that the penetration of active compounds into the cell is improved after encapsulation in liposomes; But this result was not obtained in the present study.

3- 5- Complete escape of nitrogen

Interaction effect of treatment and storage time on total volatile nitrogen of minced meat samples inoculated with*Escherichia coli* It was significant ($P<0.05$). By increasing the storage time in each treatment, the amount of total volatile nitrogen increased over time (Figure 2). As it is clear from the figure, the highest total volatile nitrogen corresponds to the control sample on the 9th day and the lowest total volatile nitrogen corresponds to the first day samples.

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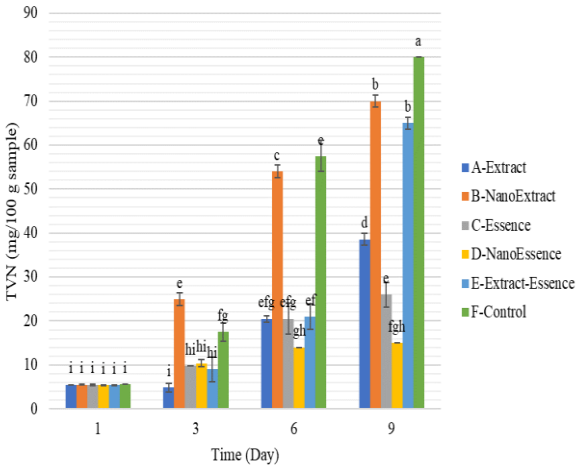

Fig 2 Changes in total volatile base nitrogen of different treatment during storage (*E. coli* inoculated into ground beef)

Interaction effect of treatment and storage time on total volatile nitrogen of minced meat samples inoculated with*Listeria monocytogenes* It was significant (P<0.05). With increasing storage time in each treatment, the amount of total volatile nitrogen increased over time (Figure 3). As it is clear from the figure, the highest total volatile nitrogen corresponds to the control sample on the 9th day and the lowest total volatile nitrogen corresponds to the sample treated with the extract on the third day.

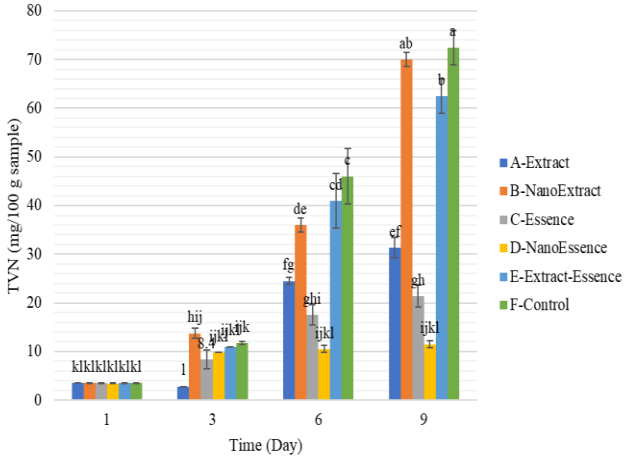

Fig 3 Changes in total volatile base nitrogen of different treatment during storage (*L. monocytogenes* inoculated into ground beef)

TVN is an important criterion in meat quality evaluation. The estimation of volatile nitrogen helps to evaluate the process of spoilage and the increase of TVN during storage is related to the activity of spoilage bacteria. From day 0 to day 9, there was a significant increase in the amount of TVN in all treatments, and statistically, a significant difference (P < 0.05) was observed between the different days of the experiment, and the increase of this index during storage in the refrigerator temperature could probably be the result of deamylation of amino acids. .

Volatile nitrogen bases are produced from the decomposition of protein and non-protein nitrogen compounds, which are mainly the result of microbial activity. These compounds are widely used to evaluate meat quality. The amount of volatile nitrogen bases is 25-35 mg/100 grams as the critical acceptance range [34]. A large percentage of volatile nitrogenous bases are products of microbial catabolism [35]. The lower amount of volatile nitrogen bases in the treated sample compared to the control sample is due to the presence of phenolic compounds that have an inhibitory role against microorganisms, especially spoilage microorganisms. Basiri et al. [36] obtained similar results regarding the effect of pomegranate peel extract on volatile nitrogenous bases in shrimp during storage in the refrigerator.

Total Volatile Nitrogen Bases (TVB-N) is one of the main indicators of the quality of meat foods and one of the main indicators of meat degradation and decomposition, which consists of trimethylamine, dimethylamine, ammonia and other volatile nitrogenous compounds related to the spoilage of meat foods. It is produced by spoilage bacteria, autolytic enzymes, deamination of amino acids and nucleotides, which is often done by the activity of microorganisms and to a lesser extent by autolytic enzymes [37 and 38].

In total, all treatments with increasing time, amount Volatile nitrogen bases increased. Increase the rate Volatile nitrogenous bases in meat may be due to various enzymatic processes such as deamination of free amino acids, breakdown of nucleotides and oxidation of amines [39].

6-3- thiobarbituric acid index

Interaction effect of treatment and storage time on thiobarbituric acid index of minced meat samples inoculated with*Escherichia coli* It was significant (P<0.05). With increasing storage time in each treatment except the control, the amount of thiobarbituric acid index decreased slightly until the third day and then increased (Figure 4). As it is clear from the figure, the highest index of thiobarbituric acid corresponds to the control sample on day 9 and the lowest index of thiobarbituric acid corresponds to the

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sample treated with nano extract on the third day.

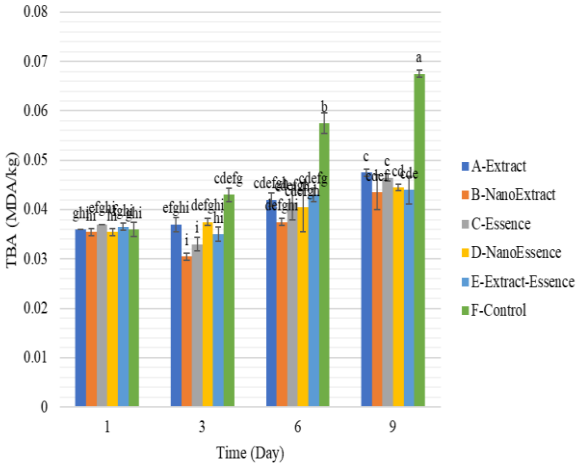

Fig 4 Changes in TBA of different treatment during storage (*E. coli* inoculated into ground beef)

Interaction effect of treatment and storage time on thiobarbituric acid index of minced meat samples inoculated with*Listeria monocytogenes* It was significant (P<0.05). With increasing storage time in each treatment, the amount of thiobarbituric index increased (Figure 5). As it is clear from the figure, the highest index of thiobarbituric acid corresponds to the control sample on day 9 and the lowest index of thiobarbituric acid corresponds to the samples of the first day.

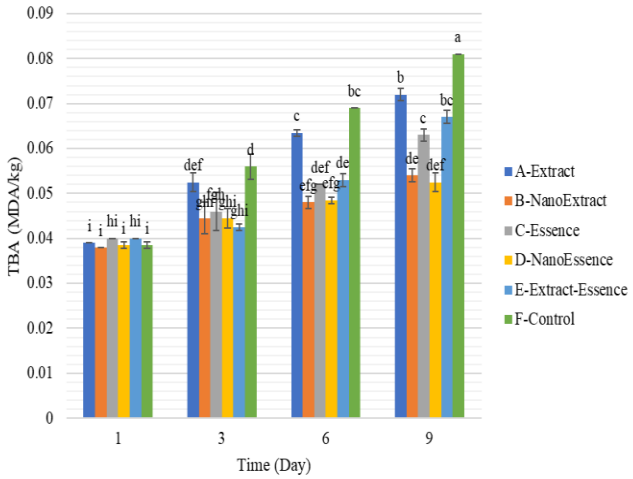

Fig 5 Changes in TBA of different treatment during storage (*L. monocytogenes* inoculated into ground beef)

Oxidative pungency is one of the important organoleptic features to confirm or reject the quality of meat storage for long periods of time. TBA is an index for the degree of secondary oxidation of fat, which is used obsessively and is due to the production of thiobarbituric acid reactive substances (TBARs) during autoxidation in the second phase, where peroxides are oxidized to aldehydes, ketones, and lactones. The presence of these compounds in minced meat causes changes in sensory characteristics such as smell and taste. The increase in TBA during storage resulting from the conversion of peroxides to substances such as aldehyde and the increase in TBA during refrigeration may also be due to the dehydrogenation of minced meat and the increase in the oxidation of fatty acids. From day 0 to day 9, there was a significant increase in the amount of TBA in all treatments, and a statistically significant difference ($p<0.05$) was observed between different days of the experiment.

The lower thiobarbituric acid index of the treated sample compared to the control sample can be due to the large amount of phenolic compounds present, which creates a strong antioxidant ability. The accepted sensory threshold for thiobarbituric acid is 1 mg/kg [40]; Therefore, the amount of thiobarbituric acid after 9 days of storage was less than acceptable even for the control sample at the end of the storage period. The results obtained in this experiment were consistent with the commercial results of Gilani et al [41]. They investigated the effect of pomegranate juice on keeping chicken in the refrigerator and stated that large amounts of phenolic compounds in pomegranate juice cause high antioxidant power. Kim et al. [42] also obtained similar results regarding the effect of garlic on pork. They observed that samples containing garlic showed lower thiobarbituric index than the control sample during storage.

In the second stage of autoxidation, when hydroperoxides are oxidized to aldehydes and ketones, malondialdehyde is formed. Secondary oxidation products cause unpleasant taste and smell in the product. Thiobarbiotic acid number is one of the oldest methods for measuring secondary oxidation products in meat and seafood products [43].

With increasing time, the amounts of thiobarbiotic acid increased in all treatments. The increasing trend of this index is due to the increase of free iron and other peroxides in meat, as well as the production of aldehydes from secondary products resulting from the breakdown of hydroperoxides [44].

In general, the amount of thiobarbiotic acid of 2 mg of malondialdehyde/g of meat is considered as the consumption limit, and that is when the smell of spoilage in meat will be

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detectable [45]. The results of the present study are consistent with the results of Rashidai et al. [44] regarding the addition of rosemary extract on the thiobarbiotic values of beef. They also stated that increasing the concentration of the extract slows down the changes in the thiobarbiotic acid number during the storage period.

7-3- Total count of bacteria

Interaction effect of treatment and storage time on total bacteria count of minced meat samples inoculated with*Escherichia coli* It was significant (P<0.05). With the increase of storage time in each treatment, the total number of bacteria increased (Figure 6). As it is clear from the figure, the highest total bacteria count is related to the control sample on the 9th day and the lowest total bacteria count is related to the samples on the third day.

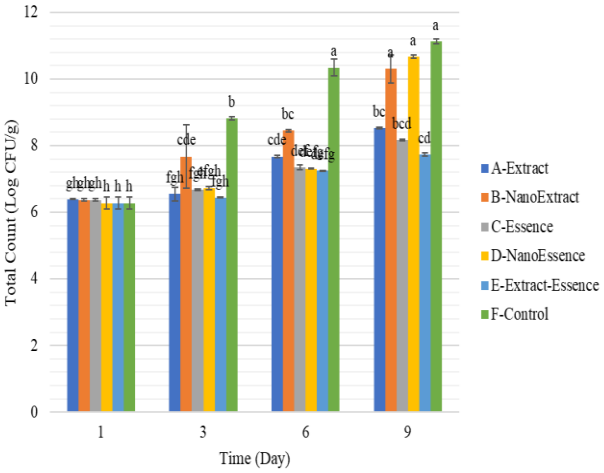

Fig 6 Changes in total count of different treatment during storage (*E. coli* inoculated into ground beef)

Interaction effect of treatment and storage time on total bacteria count of minced meat samples inoculated with*Listeria monocytogenes* It was significant (P<0.05). With increasing storage time in each treatment, the total number of bacteria increased (Figure 7). As it is clear from the figure, the highest total bacteria count is related to the control sample on the 9th day and the lowest total bacteria count is related to the samples on the third day.

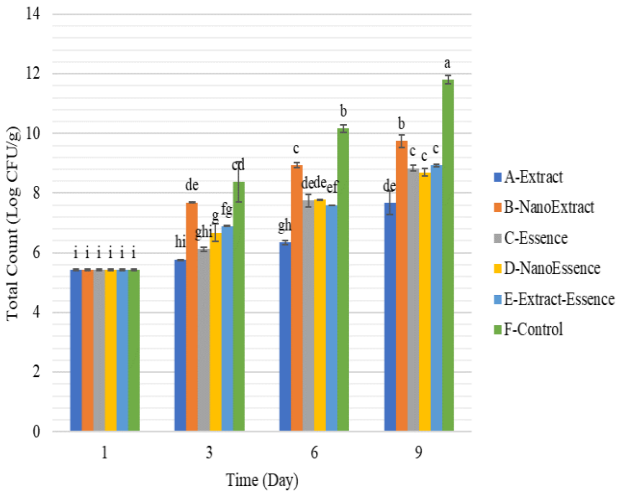

Fig 7 Changes in total count of different treatment during storage (*L. monocytogenes* inoculated into ground beef)

From the results of the microbial analysis of the sample, it can be concluded that the antimicrobial activity observed in the treated samples is related to the phenolic compounds and flavonoids present. The antimicrobial activity of phenolic compounds depends on several factors. The type of microorganism and its cell wall structure play an important role. Phenolic compounds can denature enzymes and can also connect with substrates such as minerals, vitamins and carbohydrates and remove them from the reach of microorganisms [46-48]. Gilani Trading and colleagues [41] observed similar results regarding the effect of phenolic compounds of pomegranate juice on inhibiting the growth of psychrotrophic bacteria. Daham et al [49] investigated the antibacterial and antifungal effect of pomegranate. They reported that the antimicrobial effect of pomegranate is due to the presence of polyphenols (containing several hydroxyl groups), polytannins and flavonoids (flavonols, flavanols and anthocyanins). In another study, the growth of several species of gram-positive pathogenic microorganisms such as*Staphylococcus aureus* And*Streptococcus mutans* and gram-negative bacteria such as*Escherichia coli* And*Pseudomonas aeruginosa* It was inhibited by walnut varieties [50]. Also, studies have shown that many Gram-positive and Gram-negative bacterial species can be significantly inhibited by garlic and some strains by allicin extract [51].

5- General conclusion

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Nanoliposomes of perfume essence and cinnamon extract were prepared by thin layer hydration-ultrasound method using lecithin and three different cosurfactants namely glycerol, triacetin and propylene glycol and Tween 80 as surfactant. The results showed that propylene glycol led to the production of nanoliposomes with the smallest average size of spherical particles (92.03 nm) and the highest value of net zeta potential (-24.1 mV) and was chosen as the most suitable cosurfactant. The antibacterial activity of cinnamon essence and extract was more than those encapsulated in nanoliposomes, both cinnamon essence and extract nanoliposomes had high antibacterial activity against bacteria.*Escherichia coli* And*Listeria monocytogenes* Showed. In order to study the effect of cinnamon in increasing the shelf life of minced meat, the effect of different treatments of extract, nano extract, aroma essence, nano aroma essence and extract with aroma essence on various characteristics of minced meat (pH, values of thiobarbituric acid and volatile nitrogenous bases) and also the effect of this Treatments in microbial population control*Escherichia coli* And*Listeria monocytogenes* inoculated in minced meat were investigated. The results showed that cinnamon extract has antimicrobial and antioxidant properties and nanoencapsulation of the extract increases its antimicrobial and antioxidant properties. According to the results obtained in the present study, nanoliposomal cinnamon extract can be used to increase the shelf life of minced meat without causing adverse effects and in terms of oxidative stability and microbial spoilage.

6-Resources

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مقاله علمی_پژوهشی

تأثیر عطرمایه و عصاره آزاد و ریز پوشانی شده با نانولیپوزوم دارچین بر *لیستریا مونوسایتوژنز* و *اشرشیاکولی* تلقیح شده به گوشت چرخ شده گاو

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چکیده

نانولیپوزوم‌های عطرمایه و عصاره دارچین با استفاده از روش هیدراتاسیون لایه نازک- فراصوت با استفاده از لسیتین و سه کوسورفکتانت مختلف به نام‌های گلیسرول، تری استین و پروپیلن گلیکول و Tween 80 به‌عنوان سورفکتانت تهیه شدند. نتایج نشان داد که پروپیلن گلیکول منجر به تولید نانولیپوزوم‌هایی با کوچک‌ترین میانگین اندازه ذرات کروی شکل (۹۲/۰۳ نانومتر) و بیشترین مقدار پتانسیل خالص زتا (۲٤/۱- میلی‌ولت) شد و به‌عنوان کوسورفکتانت مناسب‌تر انتخاب شد. اگرچه فعالیت ضد باکتریایی عطرمایه و عصاره دارچین بیشتر از آنهایی بود که در نانولیپوزوم‌ها محصور شده بودند، هم عطرمایه دارچین و هم نانولیپوزوم‌های عصاره فعالیت ضد باکتریایی بالایی در برابر باکتری‌های *اشریشیا کلی* و *لیستریا مونوسایتوژنز* نشان دادند. نتایج نشان داد که بر اساس حداقل غلظت‌های بازدارنده و باکتری‌کش نمونه‌های تهیه‌شده، *لیستریا مونوسایتوژنز* مقاومت بالاتری نسبت به نانولیپوزوم‌های دارچین تهیه شده داشت. به‌منظور مطالعه اثر دارچین در افزایش عمر ماندگاری گوشت چرخ شده، اثر تیمارهای مختلف عصاره، نانو عصاره، عطرمایه، نانو عطرمایه و عصاره به همراه عطرمایه بر ویژگی‌های مختلف گوشت چرخ شده (pH، مقادیر تیوباربیتوریک اسید و بازهای نیتروژنی فرار) و همچنین اثر این تیمارها در کنترل جمعیت میکروبی *اشرشیا کلی* و *لیستریا مونوسایتوژنز* تلقیح شده در گوشت چرخ شده بررسی شدند. نتایج نشان داد که عصاره دارچین دارای خاصیت ضد میکروبی و آنتی‌اکسیدانی بوده و نانوکپسوله کردن عصاره سبب افزایش ویژگی‌های ضد میکروبی و آنتی‌اکسیدانی آن می‌شود. بیشترین مقدار pH (٦/۵۸)، مقادیر تیوباربیتوریک اسید (MDA/kg ۰/۰۸۱) و بازهای نیتروژنی فرار (mg/100 g ۷۲/۵) در تیمار شاهد در روز ۹ مشاهده شد. در حالیکه برای نمونه حاوی عطرمایه نانوکپسوله مقدار pH (٦/۰۹)، مقادیر تیوباربیتوریک اسید (MDA/kg ۰/۰۰۲) و بازهای نیتروژنی فرار (mg/100 g ۱۱/۵) به‌دست آمد. با توجه به نتایج مطالعه حاضر می‌توان از عصاره دارچین نانولیپوزومی برای افزایش ماندگاری گوشت چرخ‌کرده بدون ایجاد اثر نامطلوب و از نظر پایداری اکسیداتیو و فساد میکروبی کم استفاده کرد.