

Antibacterial activity of Chitosan-Monoterpens Nanoparticles with Application in Minced Beef Meat Preservation

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Abstract

A method was developed for synthesis of chitosan-monoterpenes nanoparticles (Ch-M-NPs). Four monoterpenes; limonen (M1), linalool (M2), minthol (M3) and thymol (M4) have been used for the synthesis of four different types of nanoparticles. Scanning electron microscopy showed that, the shape of Ch-M-NPs was spherical and the mean of particle size ranged between 33.67 to 54.19 nm. The zeta potential values ranged between -.03 to -0.169 mV. The antimicrobial activity of prepared nanoparticles was evaluated. The estimated MIC values against *E. coli* and *Salmonella typhimunium* showed that *Escherichia coli* was more susceptible to chitosan, monoterpenes and/or chitosan-monoterpenes nanoparticles than *S. typhimunium*. Among the monoterpenes, M1 was the most active (MIC = 350 and 450 mg/L against *E. coli* and *S. typhimunium*, respectively) and M3 was the lowest active (MIC =1600 and 1800 mg/L). Among Ch-M-NPs, Ch-M1-NP was the most active (MIC=180 and 250 mg/L against *E. coli* and *S. typhimunium*, respectively) and Ch-M3- NP was the lowest active nanoparticles (MIC =450 and 500) against *E. coli* and *S. typhimunium*, respectively). The antibacterial activity against *E.coil* in minced beef meat samples treated with chitosan (Ch), monoterpenes (M), chitosan- monoterpenes nanoparticles (Ch-M-NPs) after storage for ten days at 4°C expressed as 10⁴cfu/g showed that M1 was the most active through the different time of experiment (79.33 and 40.00 cfu/g) at concentrations of 1000 and 2500 ug/g. However, M2 was the lowest active (125.83 and 80.00 cfu/g) compared with control+ and control- (>300 and 243.67 cfu/g, respectively). Moreover, the antibacterial effect of Ch-M-NPs was greater compared with crude monoterpenes. The Ch-M1-NP was the most active (18.17 and 8.33cfu/gm) and Ch-M3-NP was the lowest active (35.83, 18.00 cfu/g). The results suggest that Ch-M-NPs could be used in minced meat preservation as antimicrobial agents and for shelf-life extension.

Keywords: Chitosan; Monoterpenes; Nanoparticles; Minced meat preservative.

1. Introduction

Chitosan (Ch) is a commercially biopolymer easily obtained from shellfish-processing waste and is non-toxic, biodegradable and biocompatible (Hamed et al. 2016; Kanatt et al. 2008). Ch has received a lot of attentions in food preservation process due to its antibacterial and antifungal properties.(Dutta et al. 2009; Kanatt et al. 2008). There are various methods have been reported for improvement of chitosan properties through chemical and enzymatic functionalizations. (Prashanth and Tharanathan 2007). On the other hand, monoterpenes are considered as 'natural' alternatives of chemical preservatives and their use in foods technology meet the safety and quality required by consumers. The effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres (MAP) was studied. (Doulgeraki et al. 2012). The incorporation of oregano essential oil has positive effect on microbial association of minced meat stored under MAP. Thyme has been used medicinally due to its antimicrobial, and antioxidant activity. However, thyme is used in in foods; hence, thyme was coded as "generally recognized as safe status" (GRAS) in the United States. The main chemical constituents of thyme are phenols thymol and carvacrol, glycosides, flavonoids, p-cymene, borneol, linalool, alcohols, rosmarinic acid, saponins, tannins, and terpenoids (Hanafy 2013).Anti-microbial activities of thyme and thymol have been reported *in vitro* previously (Solomakos et al. 2008). Nanoparticles (NPs) can be assimilated to envelop or reservoirs where bioactive particles are trapped and released from the large surface area (Feyzioglu and Tornuk 2016). In NP production, bioactive compounds are loaded into different polymeric nanocarriers. Protein or polysaccharide based nanocarriers may be prepared from pectin, cellulose, gum, chitosan, starch, gelatin etc. (Sonia and Sharma 2011). Various NPs have been prepared based on Ch for food due to its bioactive ingredients (Kumari et al. 2010; Nagpal et al. 2010). Terpenes compounds, such as limonene, cause the

loss of membrane integrity and dissipation of the proton-motive force (Heipieper and Martínez 2010). In this study four different chitosan-monoterpenes nanoparticles were developed using facile method (Linalool, Limonene, Menthol and Thymol). Then, their antimicrobial activity on minced meat as preservatives were investigated.

2. Materials and Methods

2.1. Chemicals

Low molecular weight Chitosan (made from coarse ground crab, 89% degree of deacetylation), Tween 80, dimethyl sulphoxide (DMSO) and sodium tripolyphosphate (STPP), Monoterpenes include Menthol (98%), Thymol (98%), limonene (98%), and Linalool (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nutrient Broth (NB), and Nutrient Agar (NA) media were purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK),

2.2. Bacterial strains

Two food pathogenic bacteria *Escherichia coli* (ATCC 8739), and *Salmonella typhimurium* (ATCC 1402), were obtained from Microbiology Laboratory, Department of Dairy Science, Faculty of Agriculture, Alexandria University, Egypt. The selected bacterial cultures were maintained on NA medium at 37°C and stored in refrigerator at 4°C.

2.3. Minced meat

Fresh meat from Thigh was purchased from local meat packer. Samples were obtained within 72 hours of slaughtering. Samples were sterile by immersion in water solution of NaClO (10 mg/L) for one hour, then rinsed twice with sterilized distilled water, then minced using ground meat.

2.5.2. Zeta potential and size loaded chitosan-monoterpenes nanoparticles

The surface charge of the prepared chitosan monoterpenes nanoparticles was investigated by using Malvern Zeta- nano-sizer instrument (Eniga Business Park, Grove wood Road, and Malvern WR14 1XZ, UK). The fixed weight (0.1g) of the prepared particles was suspended in glycerol (50%) in isopropanol (v/v) then were sonicated for 30 min. The suspension was transferred to zeta-potential cell (Honary and Zahir 2013).

2.6. Microbiological studies

2.6.1. The *in vitro* antibacterial activity of the prepared chitosan-monoterpenes

The *in vitro* antibacterial activity of chitosan, monoterpenes, and chitosan-monoterpenes nanoparticles was assayed using NA dilution method according to the European committee for antimicrobial susceptibility testing (EUCAST) against *E. coli* (ATCC 8739), and *S. typhimurium* (ATCC1402). Preliminary screening tests were performed at concentrations ranging from 100 to 2000 mg/L of all chitosan products. For determination of MIC, different concentrations of chitosan formulations were added to NA medium immediately before it was poured into the Petri dishes at a temperature of 40-45°C. Parallel controls were maintained with distilled water mixed with NA medium. One loopful of microorganism in NB medium ($\approx 5 \mu\text{L}$) was on the surface of NA medium (ten per plate) then incubated at 37°C for 24h. Each concentration was tested in triplicate. The MIC was recorded in each case as the minimum concentration of compound which inhibited the growth of tested microorganism after incubation for 48hr at 37°C. From the MIC observed, the intermediate concentrations between MIC values were prepared by suitable dilution of stock solution and the accurate MIC values were determined (Rabea et al. 2009).

2.6.2. The *in vivo* antimicrobial activity of the prepared chitosan formulations

The minced meat samples each (100 g) were used in three replicates the following treatments; chitosan, essential oil, monoterpenes, chitosan-essential oil nanoemulsions, chitosan– essential oil, monoterpenes and silver nanoparticles at two different concentrations 1000 and 2500 $\mu\text{g/g}$ that showed good inhibition in the *in vitro* experiments, two controls, non-inoculated and inoculated control with *E.coli*. The samples of the sterile minced beef meat were placed in the polyethylene bags and contaminated with 25 μL of *E. coli* ($ca10^4$ cfu/g). In order to ensure proper distribution of the pathogen, the inoculated samples were homogenized for 2 min at room temperature. Following homogenization, different concentrations of treatments were added and to ensure uniform distribution of added compounds, treated meat samples were further homogenized as previously described. The polyethylene bags with samples from all treatments were wrapped and stored under aerobic conditions at 4°C for 10 days intervals (3, 7 and 10 days) until analysis.

2.7. Statistical analysis

Statistical analysis was performed using the Cohort software (Costat 1986). Means and standard error (SE) were obtained from three independent replicates performed for each treatment. One-way analysis of variance (ANOVA) (Kirkpatrick LA and BC. 2013; Kotz S et al. 2006) was conducted and means property values were separated with Duncan's multiple range test to determine significant differences among the mean values at probability level of 0.05.

3. Results and discussion

3.1. Characterization of chitosan- monoterpenes nanoparticles

Morphological properties of the chitosan-monoterpenes nanoparticles were analyzed by SEM. The digital SEM images of the lyophilized samples of chitosan-monoterpenes nanoparticles are shown in **Figure 1**. The shape of particles was found to be in a spherical form Ch-M1-NP to Ch-M4-NP for chitosan-monoterpenes. The nanoparticles of chitosan-monoterpenes were almost in uniform shape and size. The average nanoparticle size of Ch-M-NPs was ranged between 34 to 54 nm Table S1. This is in agreement with previous results of NPs (Feyzioglu and Tornuk 2016; Rabea and Badawy 2014).

On the other hand, zeta potential of chitosan-monoterpenes nanoparticles was also measured understanding and predicting the interactions between particles. Therefore, the zeta potential of Ch-M-NPs is shown in Table S1 and **Figure 2**. The zeta potential values for the three combinations were ranged between -0.0346 to -0.1690 mV for chitosan -monoterpenes NPs, indicating good dispersion of nanoparticles in suspension at pH 7 and 25°C. The negative charge of zeta potential values obtained refers to the surface charge of the particles.

3.2. Microbiological studies

3.2.1. *In vitro* antibacterial activity chitosan- monoterpenes nanoparticles

The antibacterial activity was valuated as **Table 2** and Figure 3, shows the estimated MIC values of chitosan (Ch), monoterpenes (M), and chitosan- monoterpenes nanoparticles (CH-M- NPs) against *E. coli* and *Salmonella*. According to MIC values, *E. coli* was more susceptible to Ch, M and Ch-M-NPs than *S. typhimunium*. Among the monoterpenes, M1 was the most active (MIC = 350 and 450 mg/L against *E. coli* and *S. typhimunium*, respectively) followed by M4 (MIC = 450 and 500 mg/L) and M3 was the lowest active (MIC =1600 and 1800 mg/L). Among Ch-M-NPs, Ch-M1-NP was the most active (MIC=180 and 250

mg/L against *E. coli* and *S. typhimunium*, respectively) followed by Ch-M2-NP and Ch-M4-NP (MIC =200 and 350 mg/L against *E. coli* and *S. typhimunium*, respectively. While, Ch-M3-NPs were the lowest active nanoparticles (MIC =450 and 500) against *E. coli* and *S. typhimunium*, respectively. Hence, it is noted that Ch-M-NPs were more activity against the two tested bacteria than the unmodified monoterpens where the MIC values of NPs were significantly reduced which reflect higher antibacterial activity compared to the MIC values of the monoterpens alone.

The results in in consistent with previous study in which terpenes compounds, such as limonene, caused the loss of membrane integrity and dissipation of the proton-motive force (Sikkema et al. 1994),while the mechanism of action of aldehyde is based on the dissipation of the proton motive force due to the leakage of small ions (Gill and Holley 2004). NPs can be envelop bioactive particles and released from the large surface area (Feyzioglu and Tornuk 2016). Hence, nanoparticle played significant role in the positive effect of antibacterial properties of the developed nanoparticles (Bilia et al. 2014) (Kumari et al. 2010; Woranuch and Yoksan 2013).

3.2.2. *In vivo* antibacterial activity of prepared products on minced meat

Meat and meat based products are highly susceptible to microbial contamination since they are rich in essential nutrients. This is also can be accelerated by some intrinsic factors such as pH and water activity of fresh meat. *E. coli* bacteria are extensively associated with the meat and meat based products. The antibacterial activity against *E.coil* in minced beef meat samples treated with chitosan (Ch), monoterpens (M), chitosan-monoterpens nanoparticles (CH-M-NPs) individually after storage for ten days at 4°C expressed as 10⁴cfu/g are shown in **Table 3 and Figure 4**. It can be seen that M1 was the most active through the different time of experiment (79.33 and 40.00 cfu/g) followed with M4 (95.33 and 59.17 cfu/g at concentrations of 1000 and

2500 ug/g, respectively). However, M2 was the lowest active (125.83 and 80.00 cfu/g) compared with control+ and control- (>300 and 243.67 cfu/g, respectively). Moreover, the antibacterial effect of Ch-M-NPs was greater compared to crude monoterpenes. The Ch-M1-NP was the most active (18.17 and 8.33cfu/gm) followed with NPs-thymol (23.50 and 9.50 cfu/gm). However, NPs-menthol was the lowest active (35.83, 18.00 cfu/g). Furthermore, significant differences in antibacterial activities were observed between the two tested concentrations in which the higher concentrations (2500 ug/g) of the tested treatments had more antibacterial activity compared with the lower one (1000 ug/g). Meanwhile, the antibacterial activity of most treatments reduced with the time of storage.

The antibacterial properties of chitosan were reported by (Zimoch-Korzycka and Jarmoluk 2015), as Ch, L and AgNP exhibits a strong inhibiting effect of the growth of *E. coli*, *Pseudomonas fluorescens*, *Bacillus cereus*, and *Staphylococcus aureus* bacteria in minced meat stored in refrigerator. Limonene is one of the most abundant terpenes in cannabi, limonene is a monoterpene and has numerous medicinal benefits demonstrated in human and animal studies. Limonene is among a number of plant monoterpenes that have been identified as having antioxidant and anticancer properties. Linalool is considered GRAS for commercial purposes (Bickers et al. 2003). The acute oral (rat) LD₅₀ is quite high, i.e., 2.7 g/kg, suggesting substantial safety issues for mammals (Opdyke et al. 1979). Menthol, also called peppermint camphor, terpene alcohol with a strong minty, cooling odour and taste. Menthol is also used as flavouring in foods. Carvacrol and thymol, the two main phenols that constitute about 78–85% of oregano EO, are principally responsible for the antimicrobial activity of the oil (Sivropoulou et al. 1997). Application of oregano EO in meats was found to be effective in inhibiting spoilage microflora (Emiroğlu et al. 2010). Anti-microbial activities of thyme and thymol have been reported *in vitro* (Manou et al. 1998). Therefore,

antibacterial efficacy has been noted against several bacterial species, including *Salmonella typhimurium*, *Staphylococcus aureus*, and *Helicobacter pylori*. (Juven et al. 1994). A biphenyl compound and a flavonoid isolated from thyme have been reported to have antioxidant effects, in which it inhibits superoxide anion production and to protect red blood cells against oxidative damage. *In vitro* studies have noted antioxidant properties of thyme oil and thymol (Sian et al. 1999).

5. Conclusion

Four different chitosan-monoterpenes nanoparticles (Ch-M-NPs) were prepared and characterized. Monoterpenes types, limonen (M1), linalool (M2), minthol (M3) and thymol (M4) were selected and used. The prepared nanoparticles were found to be in spherical shape and an average size range of 34-54 nm. The antibacterial activity of Ch-M1-NPs were found to be superior its monoterpenes alone (MIC values 180 and 250 mg/L) against *E. coli* and *S. typhimurium*, respectively. Therefore, Ch-M-NPs are considered as an excellent cost-effective and safe antimicrobial agents for minced meat preservation.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

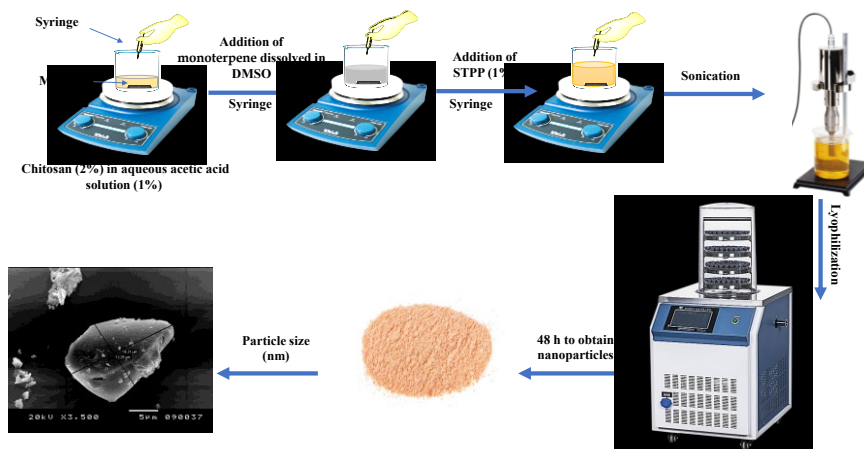
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Scheme1. Preparation of chitosan-monoterpenes nanoparticles

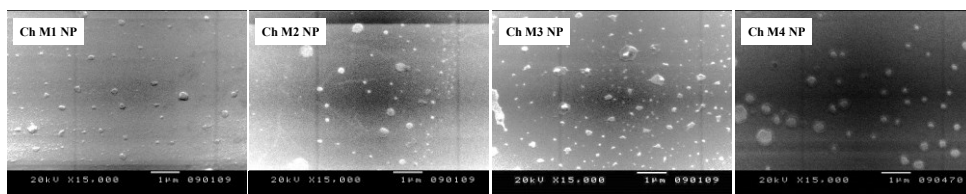


Figure 1. SEM images of the chitosan-monoterpenes nanoparticles.

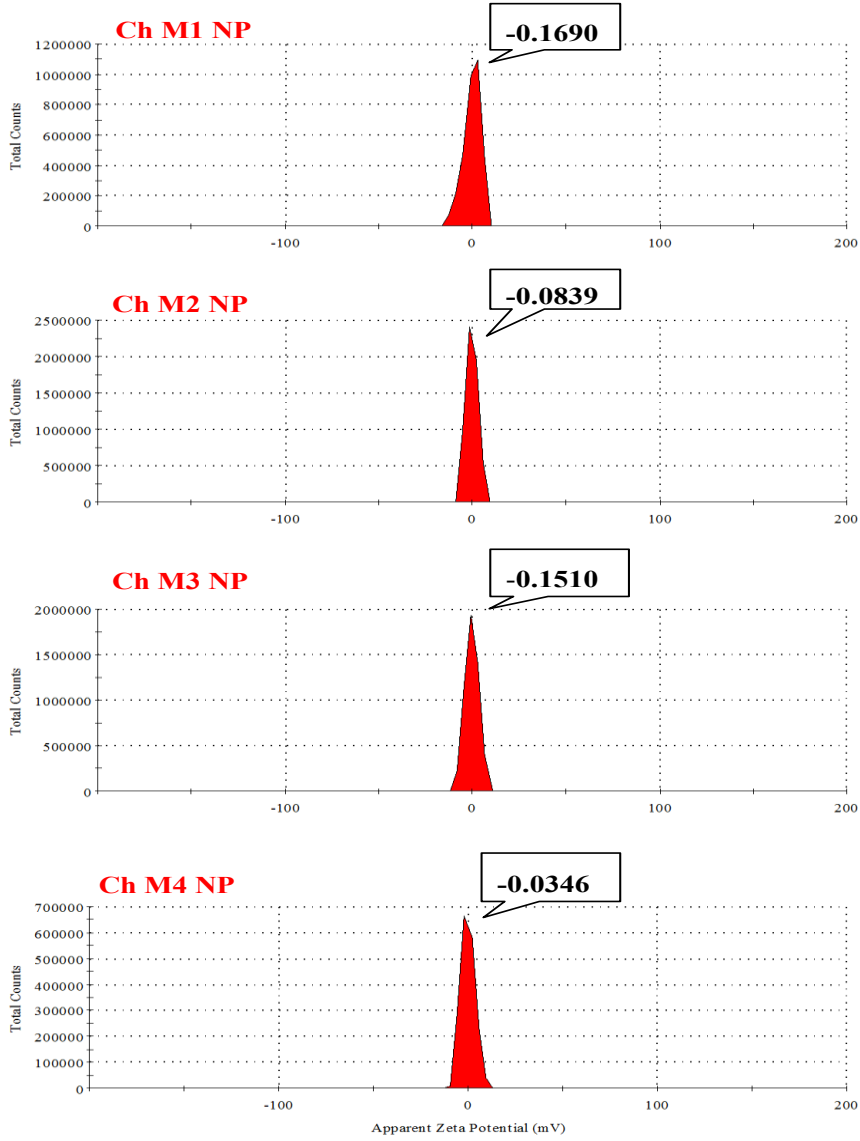


Figure 2. Zeta potential distribution of chitosan-monoterpens nanoparticles

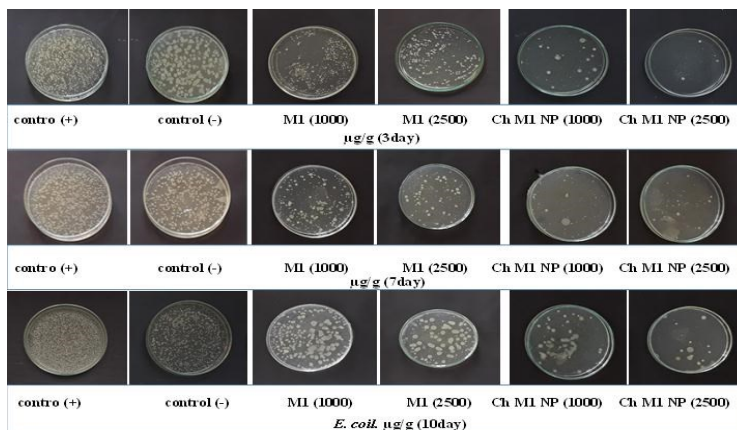


Figure 3. MIC of Ch, monoterpens, Ch M1(Limonen) nanoparticles after 48 hr *E. coli* and *S. Typhimunium*

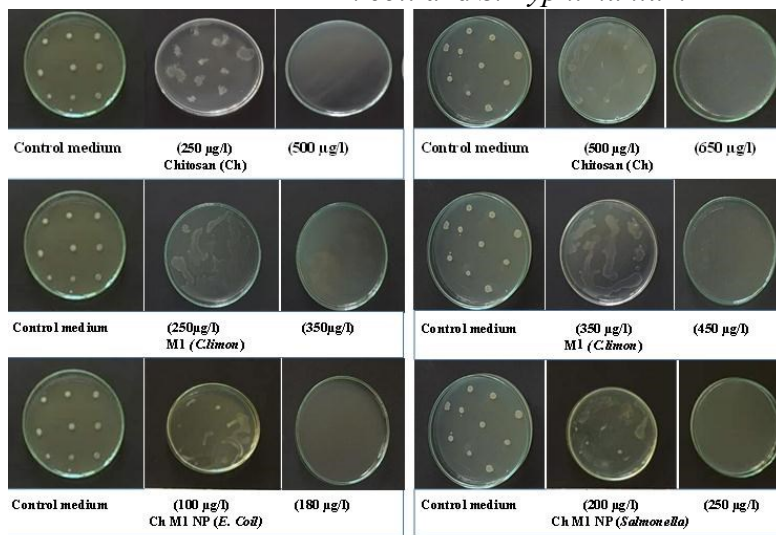


Figure 4. Representative photographs of the antibacterial activity against E. coil (104 cfu/g) of M1, Ch M1 C NE and Ch M1 C NP in minced beef meat samples after storage for ten days at 4°C

Table 1. Preparation conditions of chitosan monoterpenes nanoparticles

Formulation code	Chitosan 2% (mL)	Monoterpene (g)	Tween 80 (mL)	STPP 1% (mL)
Ch M1 NP	24.5	0.5	0.50	10
Ch M2 NP	24.5	0.5	0.50	10
Ch M3 NP	24.5	0.5	0.50	10
Ch M4 NP	24.5	0.5	0.50	10

STPP: sodium tripoly-phosphate, **EO:** Essential oil, **Ch:** Chitosan, **M:** monoterpenes **M1:** Limonen, **M2:** Linalool, **M3:** Menthol, **M4:** Thymol, **NP:** nanopart

Table 2. Zeta- potential of chitosan - monoterpenes nanoparticles

Product Code	Zeta-potential (mV)	Mobility (µmcm/Vs)	Conductivity (mS/cm)	Charge	Particle size (nm)
Ch M1 NP	-0.1690	-0.01322	0.0296	Negative	39.61±4.71
Ch M2 NP	-0.0839	-0.006575	0.0830	Negative	54.19±5.70
Ch M3 NP	-0.1510	-0.01182	0.0728	Negative	33.76±2.75
Ch M4NP	-0.0346	-0.002709	0.0544	Negative	54.12±9.07

EO: Essential oil, **Ch:** Chitosan, **NP:** nanoparticle

M: monoterpenes **M1:** Limonen, **M2:** Linalool, **M3:** Menthol, **M4:** Thymol.

Table 3. The *in vitro* antibacterial activity of chitosan (Ch), monoterpens (M), chitosan- monoterpens nanoparticles (Ch M NPs) against *E. coli* and *Salmonella typhimunium*

Treatments	MIC (mg/ L)	
	<i>E. coli</i>	<i>S. typhimunium</i>
Ch	500	650
M1	350	450
M2	1000	1100
M3	1600	1800
M4	450	500
Ch M1 NP	180	250
Ch M ² NP	200	350
Ch M ³ NP	450	500
Ch M ⁴ NP	200	350

MIC is the minimum inhibitory concentration.

Code: Ch: Chitosan, **NP:** nanoparticle

M: monoterpens **M1:** Limonen, **M2:** Linalool, **M3:** Menthol, **M4:** Thymol.

Table 4. Antibacterial activity against *E. coil* (10^4 cfu/mL) in minced beef meat samples treated with chitosan (Ch), monoterpens (M), and chitosan- monoterpens nanoparticles (Ch M NPs) after storage for ten days at 4°C

Treatment	Concentration (ug/g)	Antibacterial activity against <i>E.coli</i> (10^4 cfu/g) \pm SE at time (day)			Average \pm SE
		3	7	10	
Control +	0.00	295.00 \pm 2.89	> 300	> 300	>300
Control -	0.00	137.50 \pm 1.44	295.00 \pm 2.89	298.5 \pm 0.87	243.67 ^h \pm 53.09
Ch	1000	75.00 \pm 2.89	135.00 \pm 1.44	175.00 \pm 1.44	126.67 ^g \pm 28.30
	2500	62.50 \pm 1.44	85.00 \pm 2.89	105.00 \pm 2.89	84.17 ^e \pm 12.28
M1	1000	45.50 \pm 0.29	77.50 \pm 1.44	115.00 \pm 2.89	79.33 ^d \pm 20.08
	2500	27.50 \pm 1.44	42.50 \pm 1.44	50.00 \pm 2.89	40.00 ^c \pm 6.61
M2	1000	82.50 \pm 1.44	112.50 \pm 1.44	127.50 \pm 1.44	107.50 ^f \pm 13.23
	2500	47.00 \pm 1.73	65.00 \pm 2.89	85.00 \pm 2.89	65.67 ^e \pm 10.98
M3	1000	105.00 \pm 2.89	125.00 \pm 2.89	147.50 \pm 1.44	125.83 ^g \pm 12.28
	2500	72.50 \pm 1.44	75.00 \pm 2.89	92.50 \pm 1.44	80.00 ^f \pm 6.29
M4	1000	76.00 \pm 0.58	95.00 \pm 2.89	115.00 \pm 2.89	95.33 ^c \pm 11.26
	2500	45.00 \pm 0.58	57.50 \pm 1.44	75.00 \pm 2.89	59.17 ^d \pm 8.70
Ch M1 NP	1000	13.50 \pm 0.87	17.50 \pm 0.87	23.50 \pm 0.87	18.17 ^a \pm 2.91
	2500	3.50 \pm 0.29	9.00 \pm 0.58	12.50 \pm 0.87	8.33 ^a \pm 2.62
Ch M [†] NP	1000	27.50 \pm 1.44	35.00 \pm 2.89	45.00 \pm 2.89	35.83 ^c \pm 5.07
	2500	12.50 \pm 0.87	17.50 \pm 1.44	24.00 \pm 1.15	18.00 ^a \pm 3.33
Ch M [‡] NP	1000	22.00 \pm 1.15	35.00 \pm 2.89	48.00 \pm 1.15	35.00 ^c \pm 7.51
	2500	5.00 \pm 0.58	12.50 \pm 1.44	15.00 \pm 1.73	10.83 ^b \pm 3.00
Ch M [§] NP	1000	18.50 \pm 2.02	25.00 \pm 2.89	27.00 \pm 0.58	23.50 ^b \pm 2.57
	2500	5.50 \pm 0.29	10.00 \pm 0.58	13.00 \pm 1.15	9.50 ^a \pm 2.18
Mean		73.82 ^a	99.73 ^b	112.11 ^c	Between treatment for 1000 = 3.76
LSD _{0.05}		Between time = 1.28			Between treatment for 2500 = 3.23

Values are mean of three replicates \pm standard error (SE)

Before treatment: 130×10^4 cfu/g

Control (+): Minced meat treated with *E. coil*

Control (-): Minced meat without *E. coil*

Code: Ch: Chitosan, **NP:** nanoparticle

M: monoterpens

M1: Limonen, **M2:** Linalool, **M3:** Menthol, **M4:** Thymol.

النشاط المضاد للبكتيريا لمستحضرات الحبيبات النانوية المحضرة للتربينات الاحادية مع الكيتوزان لتطبيقاتها في حفظ اللحم البقري المفروم نسبي محمد رشاد لطفي , محمد الطاهر بدوي , سمر محمد شعوبر

تم تطوير طريقه لتحضير حبيبات نانوية من التربينات الاحادية مع الكيتوزان حيث استخدم اربع انواع من التربينات الاحادية وهي (لينالول- لليمونيين - منثول- الثايمول) في تحضير الحبيبات النانوية وقد اظهر الميكروسكوب الماسح ان شكل الحبيبات النانوية كان كروي وكانت الحبيبات النانوية للكيتوزان مع المونوتريبينات منتظمة في الشكل والحجم ومتوسط حجم الحبيبات يتراوح 33.67 to 54.19 نانو متر وتراوحت قيم جهد الزيتا من -٠.٠٣ إلى -٠.١٦٩ ملي فولت . وتم تقييم النشاط المضاد للميكروبات للكيتوزان وأربعة من التربينات (لينالول – ليمونيين – مينثول- الثايمول) وأربعة من مستحضرات الحبيبات النانوية مع التربينات الاحادية والكيتوزان بتركيزات مختلفه ضد نوعين من البكتيريا المسببه للامراض الغذائيه *E-coli, Salmonella typhimunium*. وظهرت النتائج بناء على قيم اقل تركيز مثبت أن سلالة *E-coli* كانت اكثر حساسيه من السالمونيلا للمعاملات المختبره. وأظهر الليمونيين من التربينات الاحادية اكثر فاعليه كمضاد بكتيريا عن التربينات الاحادية الاخرى حيث بلغت قيم اقل تركيز مثبت ٣٥٠ - ٤٥٠ مجم/لتر ضد الإيشريشيا والسلمونيلا على التوالي. وكانت المنثول اقل التربينات الاحادية حيث بلغت قيم اقل تركيز مثبت 1600-1800 مجم/لتر ضد الإيشريشيا والسلمونيلا على التوالي وكانت مستحضرات الحبيبات النانوية لليمونيين مع الكيتوزان اكثر نشاط ضد البكتيريا عن التربينات بمفردها ضد نوعي البكتريا. وكان اقلها في التأثير الحبيبات النانوية للمنثول مع الكيتوزان ضد نوعي البكتريا. وبدراسة النشاط المضاد للميكروبات للمركبات السابقة ومشتقاتها النانوية ضد الاشرشيا كولاي في اللحم البقري المفروم بعد تخزين لمدة ١٠ ايام على درجة ٤ مئوية ان الليمونيين كان اعلى في التأثير عند تركيزي ١٠٠٠ - ٢٠٠٠ ميكروجرام / جرام بينما كان اللينالول اقلها في النشاط مقارنة بالكنترول الموجب والسالب. وعموماً فإن تأثير المونوتريبينات الاحادية النانوية المحضرة مع الكيتوزان كان اعلى من التربينات الاحادية الخام بمفردها حيث كانت الحبيبات النانوية لليمونيين مع الكيتوزان اكثر نشاطا عند التركيزين المستخدمين بينما كانت الحبيبات النانوية للمنثول مع الكيتوزان اقلها نشاطا عند التركيزين . وتقترح النتائج امكانية استخدام الحبيبات النانوية من التربينات الاحادية مع الكيتوزان في حفظ اللحم كمضادات بكتيرية وإطاله فتره الحفظ.

الكلمات الافتتاحية: الكيتوزان , التربينات الاحادية , الحبيبات النانوية , حفظ اللحم المفروم