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EVALUATION OF THE EFFECT OF THYME OIL NANOPARTICLES ON THE SHELF LIFE OF KARISH CHEESE

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ABSTRACT

The most widely consumed soft cheese in Egypt, particularly in the rural, is karish cheese. When cheese is produced, handled, distributed, or stored in unsanitary conditions, it could become contaminated by many types of microorganisms. The spoilage of cheese and/or foodborne diseases are caused by contamination with various microorganisms. A total of 120 Karish cheese samples were collected from El Sharkia City supermarket, Egypt. The microbiological tests performed were: aerobic plate count (APC), *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*Staph. aureus*) counts. The recorded results showed that the mean of APC, *E. coli* and *Staph. aureus* counts were 6.9, 3.45 and 4.3 $\log_{10}$ cfu/g, respectively. Researchers have investigated edible packaging materials for essential oils encapsulated in biopolymers to offer an effective and secure method of food preservation. In this study, to increase the shelf-life of Karish cheese, chitosan-based emulsions consisting of liposomes infused with thyme essential oil (TEO) were explored. Antimicrobial activities were assessed via *Staph. aureus* and *E. coli* counts over 4 weeks. High counts of *Staph. aureus* and *E. coli* recorded by the fourth week, for all cheese samples either coated or not coated with chitosan solutions. However, samples coated with TEO 1% and liposomal chitosan emulsions encapsulated TEO 0.5 and 1% v/v decreased in microbial counts up to 4 weeks, while samples coated with liposomal chitosan emulsions encapsulated TEO 2% occurred complete absence of *Staph. aureus* and *E. coli* at the end of the fourth week of storage. According to the findings, TEO 1% and liposomal chitosan emulsion encapsulated TEO 2% v/v may be promising natural solutions, with a satisfactory appearance to extend the shelf life, in addition to preserving the flavour of Karish cheese.

Key words: *E. coli- Staph. aureus-* thyme essential oil- chitosan.

INTRODUCTION

Due to its great nutritional value and low price, Karish cheese is a very popular soft cheese in Egypt, besides its low-fat content because it is made of raw skim milk. The methods of manufacturing such type of cheese by native ways are still unhygienic, the finished products are exposed to contamination from a variety of microorganisms (Deeb *et al.,* 2004). The primary causes of Karish cheese's short shelf life are its high water level and minimal salt content. (Delacroix-Buchet *et al.*, 2005).

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Karish cheese is mostly contaminated with food-borne pathogens incriminated in poisoning from food and the finished product with poor quality (Todaro *et al.,* 2013). *E coli* is one of the most serious bacteria, especially pathogenic types found in milk and its products. *E. coli* often lives in the intestinal tract of humans and animals. Some species possess virulent genes that pose a risk to both humans and animals. (Malik and Memona 2010). *Staph. aureus* is a different sort of bacteria that typically lives in both humans' and animals' skin, nose and throat. Enterotoxins produced by *Staph. aureus* results in food poisoning illnesses (Orwin *et al.,* 2003).

Currently, chemical food preservatives are not preferred by consumers worldwide. As a result, consumers are demanding the use of natural antibacterial agents for preservation (Weiss *et al.,* 2015). Numerous essential oils have been investigated for their potent antibacterial properties against a wide range of pathogens linked to food illness or spoilage. At the same time, they should not have any adverse effect on the sensory characters of the product. Numerous studies have found that using essential oils as organic preservatives in the food industry is beneficial (Hyldgaard *et al.,* 2012). However, due to their limited water solubility, potential interactions with other food matrix ingredients, and even evaporation into the packaging headspace, the direct mixing of essential oils with the product may make them less effective (Gill *et al.,* 2002; Devlieghere *et al.,* 2004; Gutierrez *et al.,* 2008; Fernandez-Pan *et al.,* 2014). Additionally, when essential oils are used as food additives, the primary taste of the product is changed. (Hayouni *et al.,* 2008).

Nanotechnologies have been developed to achieve this goal by enhancing bioactivities while simultaneously reducing unfavorable organoleptic effects (Guo *et al.,* 2018). According to previous studies, nanoemulsions have stronger bioactivity and antibacterial effectiveness than conventional carrier agents due to their nanometer-sized particles and improved stability (Donsì and Ferrari 2016; Chuacharoen and Sabliov 2019). Encapsulating essential oils has many benefits, including good antimicrobial packaging that eliminates their problem with strong odors (Al-Moghazy *et al.,* 2021). Some kinds of hard cheese and minced beef have been prepared effectively using Liposome encapsulated essential oils (Khosravi-Darani *et al.,* 2016; Lin *et al.,* 2016; Cui *et al.,* 2017). Thyme essential oil (TEO) and its primary component, thymol, are utilized as antioxidants, anti-inflammatory agents, topical anesthetics and scar-curing agents. They are also antibacterial agents against infections and food spoilage, in addition to their advantages for the circulatory system (Marchese *et al.,* 2016).

In this work, to provide a secure and efficient coating for Karish cheese, TEO was encapsulated into a coating emulsion consisting of chitosan and liposomes, and to distinguish the effectiveness of TEO and its antimicrobial activities against different microorganisms.

MATERIALS AND METHODS

Samples collection:

Researchers collected 120 samples of Karish cheese from EL Sharkia city supermarkets, Egypt. The obtained samples were immediately brought into the lab under strictly aseptic circumstances, where they underwent the subsequent analysis.

Microbiological examination

Serial Dilutions Preparation (ISO 6887-5:2010):

A stomacher bag containing 25 g of cheese samples and 225 ml of sodium citrate solution was stomached for one minute to achieve a dilution of 10^{-1}. The sample homogenate was processed into tenfold dilutions using diluent buffer to produce 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions.

Aerobic plate count (APC) (FDA, 2013):
Pipetting two identical sterile Petri dishes with one milliliter of each dilution was done. The Petri plates were then filled with 20 ml of melted sterile plate count agar, and the contents were properly mixed by spinning the plate several times. The plates were inverted and incubated at 35°C for 48 hours after the media had solidified. Using a Quebec counter, plates with 25–250 colonies were counted. The average number per plate was then multiplied by the dilution factor to determine the total colony forming units cfu/g.

***E. coli* count (ISO, 16649/2-2001):**
Aseptically, 1 ml of each dilution from the previously made homogenate was added to a sterile Petri dish, and 10 ml of Tryptone Bile X-Glucuronide (TBX) agar was then added. The dish was then incubated at 44 o C for 24 hours. Blue to blue-green colonies with a bluish halo zone were *E. coli's* distinctive colonies. To identify the isolated strains, biochemical tests such as Triple Sugar Iron (TSI), Indol, Methyl Red, Voges-Proskaur, and Citrate Utilization were utilized. (A.P.H.A., 2004)

***Staph. aureus* count (US-FDA, 2016):**
One ml sample suspension was aseptically transferred to 3 plates of Baird-Parker agar from the previously made homogenate, dividing the 1 ml inoculum evenly among the 3 plates (i.e., 0.4, 0.3, and 0.3 ml). A sterilized bent glass streaking rod was used to evenly distribute the inoculum over the surface of the agar plates. 48 hours were spent incubating plates at 35–37°C. *Staph. aureus* colonies are circular, smooth, convex, moist, ranging in color from gray to jet black, usually with a light-colored (off-white) edge, surrounded by an opaque zone, and frequently with an outer transparent zone. They measure 2-3 mm in diameter on plates. For counting, choose plates with 20–200 colonies. *Staph. aureus* was confirmed by tube coagulase (coagulase positive). In brief, suspicious colonies were introduced into rabbit plasma, and the tube was then incubated for three hours at 37 °C. Re-incubate for 18 hours at room temperature if the results are negative. *Staph. aureus* was thought to be present in coagulase-positive cultures.

Thyme essential oil preparation (Ozcan *et al.*, 2003)
Thyme essential oil was obtained by steam distillation from the oil extraction unit at the National Research Center, Cairo, Egypt. The essential oils diluted to 1% by mixing with ethylene glycol in the sterilized distilled water as an inert substance act as a dissolving agent for oil without any harmful effects on the food.

Chitosan-TEO coating preparation (Al-Moghazy *et al.*, 2020)
The Chitosan layer was created by mixing 2% acetic acid solution with medium molecular weight Chitosan (2 g; Sigma-Aldrich, Germany). The constant stirring persisted until a clear solution was obtained. The previously produced solution was then supplemented with TEO in three different concentrations (0.5, 1 and 2% v/v). Final solutions were homogenized by a CAT Unidrive homogenizer (12000 rpm for 2 min).

Chitosan-liposomal coating solutions preparation
By mixing 2 g of lecithin (Lio, Turkey) with 0.25, 0.5, and 1 g of steam-distilled TEO (provided by the oil extraction unit, National Research Centre, Egypt) and 50 mL of chloroform (Acros Organics, Belgium), three concentrations were created: 0.5%, 1%, and 2%. After 30 minutes of stirring at 22°C, 50 mL of chitosan/acetic acid was added. At 60°C, stirring was then carried out for an additional 30 minutes. Using compact pressure at 50°C for 2 hours, the suspension became milky and concentrated (Al-Moghazy *et al.*, 2020).

Bacterial culture, nanoemulsion and TEO application onto sliced karish cheese
Karish cheese was prepared, according to Effat *et al.* (2012), as milk heated at 85^{o}C/5 min, cooled to 42 ^{o}C and then inoculated by

a 2% active mixture starter of *Streptococcus thermophilus* & *Lactobacillus bulgaricus*. The inoculated milk was incubated at 42°C until curding was completed. The formed cheese block was cut into similar cubes with approx. 10 g each.

Karish cheese cubes were infected by immersing them in a culture of *Staph. aureus* and other *E.coli* containing 10^3 cfu/ml for 10 seconds. The extra liquid was then squeezed with a sterile cotton pad. Each cheese cube was placed in thyme essential oil at 1% (T1), CL-TEO .5% v/v (T2), CL-TEO 1% v/v (T3) and CL-TEO 2% v/v (T4) separately. 2 mL of a special coating solution was used to coat cheese cubes. Without whey, treated Karish cheeses were kept at 4°C for four weeks. At 0, 1, 2, 3, and 4 weeks of storage, microbiological studies were conducted (Al-Moghazy *et al.*, 2021).

Evaluation of the bacteriological quality of karish cheese

During their four-week cold storage phase, qualitatively in a stomacher, 10 g of each sample were aseptically homogenized for one minute in 90 ml of a sterile 2% sodium citrate solution. The samples were exposed for counting of *Staph. aureus* and *E. coli* weekly, as mentioned before.

Sensory evaluation of treated Karish cheese (Effat *et al.*, 2012)

Karish cheese samples were divided into pieces measuring approximately 5x5 cm and arranged on white plates. At room temperature (20.0°C), samples were tempered before being presented to the panelists in random order. Nine laboratory staff members, who were familiar with Karish cheese, evaluated the cheeses organoleptically at 0, 1, 2, 3, and 4 weeks of ripening at the dairy control department of the Animal Health Research Institute. Cheese was graded by the panelists on three criteria: flavor (50 points), body and texture (40 points), and appearance (10 points). The three sensory features were given scores.

RESULTS

Table 1: Statistical examination of APC, *Staph. aureus* and *E. coli* count (log_{10} cfu/g) of the examined samples.

Count/g	Min	Max	Mean±Std
APC	4.6	7.2	6.9±1.56
Staph. aureus	Not detected	5.1	3.45±0.91
E. coli	Not detected	6.00	4.3±1.1

Not detected (couldn't be detected on the plates)

Table 2: Classification of the examined 120 Karish cheese samples.

% of satisfactory samples according to the permissible limit of microbes (E.S. 1008-4, 2005)	Satisfactory (no.)	%	Unsatisfactory (no.)	%
Staph.aureus	93	77.5	27	22.5
E.coli	72	60	48	40

Table 3: Sensory evaluation of Karish cheese samples during four weeks of cold storage.

treatments	Storage periods	C&A (10)	B &T (40)	F (50)	Total (100)
control	Zero Time	7.19±0.21^{a}	36.67±1.3^{a}	44.99±1.4^{a}	88.85±2.91^{a}
	1st week	7.10±o.1^{a}	36.51±0.69^{a}	44.79±0.35^{a}	88.40±1.14^{a}
	2nd week	7.0±0.31^{a}	36.45±0.69^{a}	44.35±0.91^{a}	87.8±1.91^{a}
	3rd week	6.80±0.31^{a}	35.00±0.59^{a}	41.57±0.78^{b}	84.37±1.68^{ab}
	4th week	6.55±0.33^{a}	34.66±0.4^{a}	39.22±0.58^{b}	80.43±1.31^{ab}
T1	Zero Time	7.1±0.21^{a}	36.7±1.3^{a}	44.88±1.4^{a}	88.68±2.91^{a}
	1st week	7.00±0.1^{a}	36.40±0.69^{a}	44.6±0.35^{a}	88.00±1.14^{a}
	2nd week	7.00±0.31^{a}	36.30±0.69^{a}	44.44±0.91^{a}	87.74±1.01^{a}
	3rd week	6.70±0.31^{a}	36.00±0.59^{a}	43.35±0.78^{b}	86.05±0.33^{ab}
	4th week	6.55±0.33^{a}	36.00±0.4^{a}	43.22±0.58^{b}	85.75±1.31^{ab}
T4	Zero Time	7.2±0.21	36.12±1.3^{a}	44.77±1.4^{a}	88.09±2.91^{a}
	1st week	7.01±0.14^{a}	36.20±1.3^{a}	44.7±0.35^{a}	87.91±1.79^{a}
	2nd week	7.00±0.31^{a}	36.15±0.69^{a}	43.35±0.91^{b}	86.35±2^{a}
	3rd week	6.80±0.40^{a}	36.00±0.59^{a}	43.35±0.78^{b}	86.15±1.77^{ab}
	4th week	6.70±0.12^{e}	36.00±1.1^{a}	43.00±0.58^{b}	85.7±1.8^{ab}

F: Flavor; B&T: Body and Texture C&A: Color and Appearance
C= without coating; T1=TEO 1%; T4= 2% v/v TEO-loaded chitosan-coated liposomes

Table 4: Total *Staph. aureus* counts (log_{10} cfu/g) of different groups of Karish cheese samples over 4 weeks.

Storage period	C	T1	T2	T3	T4
Week 0	3	3	3	3	3
Week1	3	3	2.47	2.3	2.3
Week 2	3.07	2.84	2.3	2	1.84
Week 3	3.17	2.2	2	1.69	1.2
Week 4	3.50	1.5	0.9	0	Not detected

C= without coating; T1=TEO 1%; T2= 0.5% v/v TEO-loaded chitosan-coated liposomes; T3= 1% v/v TEO-loaded chitosan-coated liposomes; T4= 2% v/v TEO-loaded chitosan-coated liposomes

Table 5: Total E. coli counts (log10 cfu/g) of different groups of Karish cheese samples over 4 weeks

Storage period	C	T1	T2	T3	T4
Week 0	3.2	3.2	3.2	3.2	3.2
Week 1	3.4	3.2	3	2.8	2.6
Week 2	3.6	2.9	3	2.8	2.4
Week 3	3.6	2.7	2.6	2.2	2
week 4	3.8	1.7	1.2	1	Not detected

C= without coating; T1=TEO 1%; T2= 0.5% v/v TEO-loaded chitosan-coated liposomes; T3= 1% v/v TEO-loaded chitosan-coated liposomes; T4= 2% v/v TEO-loaded chitosan-coated liposomes

DISCUSSION

APC is a helpful measure of cheese's microbiological status. A high viable count frequently implies raw material contamination, inadequate sanitation, or improper time and temperature during storage and/or manufacture (Kaldes, 1997). The acceptable level of APC is not specified in the Egyptian standards for Karish cheese (E.S. 1008-4, 2005), but they require that the milk used to make the cheese be pasteurized or given a comparable amount of heat together with a potent starting culture. (Kirkpcatrikand Feeney 2013).

As indicated in Table (1) the APC of Karish cheese ranged between 4.6 and 7.2 $\log_{10}$ cfu/g with an average of 6.9 $\log_{10}$ cfu/g. The present data nearly agreed with those from Hussien *et al.* (2013), who examined Karish cheese and reported that the average APC was 7.1 $\log_{10}$ cfu/g, Hassan and Gomaa (2016) who found that the APC of Karish cheese collected from Cairo and Giza were 6.2 and 7.2 log cfu/g respectively. Higher results were mentioned by Salem *et al.* (2016) Baraheem *et al.* (2007) who reported that the average APC of Karish cheese was 8.3 and 9.02 $\log_{10}$ cfu/g respectively.

Lower APC was reported by Ibrahim *et al.* (2015) and Abo El Makarem *et al.* (2017) who examined Karish cheese and found that the average APC was 6.3 and 5.07 $\log_{10}$ cfu/ g respectively.

The Egyptian Standards for Kariesh Cheese of the Egyptian government (E.S,1008-4,2005) categorize a sample as unfit for human consumption if it yielded any of the following **(**Any presence of *E. coli* and *Staph. aureus* in 1 g of food sample).

Results in the Table (1) indicated that *Staph. aureus* count of Karish cheese ranged between no colonies detected and 5.1 with an average of 3.45 $\log_{10}$ cfu/g. The present data is nearly in agreement with those obtained by Hassan and Gomaa (2016) and Abo El Makarem *et al.* (2017) and Lower findings were mentioned by Alper and Nesrin (2013), Mohamed *et al.* (2019) and El Bassiony *et al.* (2021), while higher results were mentioned by Hussien *et al.* (2013); Ibrahim *et al.* (2015) and Salem *et al.* (2016) where *Staph. aureus* counts were 7.04,5.3, and 8.62 $\log_{10}$ cfu/g, respectively.

The positive samples for *Staph. aureus* (22.5%) was incompatible with the Egyptian Standards (Table 2). Higher results were obtained by Metwalli (2011) who found that *Staph. aureus* was present in every Karish cheese sample.

The existence of *Staph. aureus* in cheese typically denotes milk infection from affected udders or outside surfaces of dairy animals, as well as infection from infected, dirty hands of dairy staff members or from their coughing and sneezing. Because *Staph. aureus* produces heat-stable enterotoxins, which can induce food poisoning at levels as low as 0.5 nanogram (ICMSF 1996), it may be the primary cause of various food poisoning outbreaks.

As shown in Table (1) *E. coli* count ranged between no colonies detected and 6 $\log_{10}$ cfu/g with an average of 4.3 $\log_{10}$ cfu/g. lesser values were reported by Salem *et al.* (2016); Hassan and Gomaa (2016); Mohammed *et al.* (2019) and El Bassiony *et al.* (2021). Forty percent of the examined samples for *E. coli* were incompatible with the Egyptian Standards (Table 2). El-Sayed *et al.* (2011) and Salem *et al.* (2016) found values that were quite similar. Hassan and Gomaa (2016) reported that *E. coli* was not detected in all cheese samples.

E. coli contamination in Karish cheese may be caused by insufficient heat processing of the raw milk used to produce the cheese or due to post-pasteurization infection during transit, handling, and sale (Ewida and Hussein, 2018).

E. coli is significant for public health, since it has been linked to gastrointestinal illnesses including severe cholera-like syndrome, gastroenteritis, epidemic diarrhea, and cases of food poisoning. Hemorrhagic colitis and the deadly hemolytic uremic syndrome were caused by outbreaks of *E. coli* that produced the Shiga toxin (Kaper *et al.*, 2004). It's necessary to keep in mind that *E. coli* is a sign of fecal contamination, either directly or indirectly, when it is found in milk and milk products.

According to the scores recorded for flavor, body and texture, color and appearance, and overall acceptability, adding T1 and T4 to Karish cheese had a minor impact on the

sensory qualities (Table 3). Karish cheese treatments' flavor ratings slightly declined in T1 and T4 treatments. On average, T1 and T4 had respectable to good flavor. Actually, EOs should only be used sparingly because of their negative effects on cheese's flavor, where the flavor of thyme-fortified soft cheese scored 35 until the end of the second week, 32 in the third week, and 29 in the fourth week of storage with a faint bitter almond flavor (Samah and Ahmed, 2019). Furthermore, Han *et al.* (2015) found that thyme oil treatment significantly ($p>0.05$) altered the flavor of shredded cheese, resulting in a less favored flavor compared to the control. As a result, this study demonstrated the benefit of essential oil encapsulation in maintaining palatable cheese flavor.

Body and texture, in addition to storage time, were among the sensory qualities that changed somewhat between all treatments. Results from El-Sayed and El-Sayed (2021) were consistent with those of the current investigation, although UF Labneh's body and texture were only marginally impacted by EO treatments. Throughout the second, third, and fourth weeks of storage, there was no statistically significant variance between the mean color and appearance scores ($p>0.05$). Additionally, the treated samples had the same hue as the control samples, which had a clear, natural white appearance, without changing the significance of the difference as indicated in Table (3). On the other hand, labneh treated with TEO nanomulsions had uniformity and a velvety sensation in color and appearance scores. (El-Sayed and El-Sayed 2021).

Even though the Karish cheese was treated with T1, or T4, its general acceptance in this study was only slightly impacted during the storage period. Accordingly, fresh soft cheese produced in a lab that had been supplemented with 0.01% ginger and thyme oils received a grade A for general sensory acceptance after being stored at 4°C for a month, with the highest scores coming in the first two weeks (Ahmed *et al.*, 2021). On the other hand, according to Ayah and Saad (2016), cheese fortified with thyme oil received grades C on the third and fourth weeks in terms of general acceptability after initially receiving grades B up until the end of the second week. *Staph. aureus* counts of Karish cheese samples recorded about 3 $\log_{10}$ cfu/g at zero time for all treatments, with no discernible variation, as indicated in Table (4). The samples in group C were considerably comparable over the course of the two weeks of analysis, and by the fourth week, the *Staph. aureus* counts in group C treatments had increased by over 0.5 $\log_{10}$ cfu/g. However, the *Staph. aureus* levels were not significantly elevated in the other treatments (T1, T2, T3, and T4). Additionally, after 4 weeks of storage at 4° C, *Staph. aureus* counts for the T1, T2, and T3 samples severely dropped to 1.5, 0.9, and 0 log10 cfu/g, respectively, and for the T4 sample, there were no colonies found. Similar outcomes were reported by Ahmed *et al.* (2021), who reported a full reduction in *Staph. aureus* after the first and second weeks of storage of cheese fortified with thyme and ginger. Other studies, such as those by de Carvalho *et al.* (2015), Ayah and Saad (2016), and Hachana *et al.* (2019), reported TEO's effect on *Staph. aureus* counts by the time it reduced them until the fourth week. Engel *et al.* (2017) also looked at the antimicrobial effects of encapsulated thymol, which was towards *Staph. aureus*.

Since p-cymene did not exhibit antimicrobial action against bacteria, thymol is primarily responsible for the antimicrobial properties of TEO (Bagamboula *et al.*, 2004). According to Kloucek *et al.* (2012), Marchese *et al.* (2016), and Wang *et al.* (2016), thymol is a volatile phenol that possesses potent antibacterial properties against a wide range of food spoilage germs in both the liquid and vapor phases. According to Xu and Zhou (2008), thymol's antimicrobial action is related to its capability to permeabilize and depolarize bacterial cytoplasmic membranes.

As shown in Table (5) *E. coli* counts of Karish cheese samples recorded about 3.2 log_{10} cfu/g at zero time for all treatments. Moreover, the *E. coli* counts in C treatments rose by almost 0.6 log_{10} cfu/g by the fourth week. Furthermore, the rest of the treatments, including T1, T2, T3, and T4 did not show any significant increase in *E. coli* counts. Furthermore, *E. coli* counts decreased significantly to 1.7, 1.2 and 1 log_{10} cfu/g for T1, T2 and T3, respectively, and a complete absence (no colonies detected) at the end of 4 weeks of storage period at 4˚C. These results were consistent with Ayah and Saad (2016) and Hachana *et al.* (2019) who reported the effect of TEO on *E. coli* counts by the time reducing it till the fourth week, Govaris *et al.* (2011), who investigated the antimicrobial activity of encapsulated thymol, against *E. coli.*

Gram-negative bacteria's cell walls are more resistant to essential oils (EOS) due to the complexity of their double membrane-containing cell envelope. Hydrophobic molecules cannot enter the *E. coli* cell wall as easily as they may enter the *Staph. aureus* cell wall; as a result, EOS is less able to influence the cellular growth of the Gram-negative bacteria (*E. coli*). According to Nazzaro *et al.* (2013), the EOS works by degrading the cellular wall, harming the cytoplasmic membrane, destroying membrane proteins, decreasing adenosine triphosphate production, increasing permeability that causes ions to seep out, and killing other cellular components.

Numerous researchers looked at the antibacterial benefits of adding TEO directly to the cheese matrix to prevent spoiling and/or the growth of pathogens. According to Gammariello *et al.* (2008), utilizing TEO resulted in a significant decrease in the proliferation of the bacteria that cause Italian cheese to spoil. According to Kavas *et al.* (2015), whey protein isolate edible film with 1.5% TEO applied to semi-hard Turkish cheese called Kashar significantly decreased the number of inoculated pathogenic bacteria, including *E. coli* O157:H7, *Listeria monocytogenes*, and *Staph. aureus*. Additionally, Hachana *et al.* (2019) examined the impact of 1% v/w TEO on soft cheese, which causes a significant decrease in the total APC counts. A few other research (V'azquez *et al.*, 2001) looked into the use of pure thymol to improve cheese's microbiological quality.

CONCLUSION

In this study, the microbiological quality of Karish cheese showed high APC, *Staph. aureus* and *E. coli* counts that suggest that this kind of cheese was not handled or manufactured with enough hygienic practices.

Also, the results of every microbiological examination conducted on Karish cheese demonstrate the effectiveness of chitosan, liposome, and TEO coatings in extending the cheese's shelf life and replicating it when compared to uncoated control samples.

The overall acceptability was marginally impacted during the storage period, regardless of whether the Karish cheese was treated with 1% TEO or 2% v/v TEO-containing chitosan.

Antimicrobial activity was found when coating Karish cheese with chitosan containing TEO at 0.5 and 1% v/v reduced the amounts of *E. coli* and *Staph. aureus*, and this impact outperformed that of TEO by 1%. Whereas a 2% v/v concentration of TEO in chitosan results in a complete reduction of *E. coli* and *Staph. aureus*, Karish cheese's shelf life has increased from two to four weeks as a result.

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تقييم تأثير زيت الزعتر المعالج بالنانو تكنولوجى على صلاحية الجبن القريش

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الجبن القريش هو الجبن الطري الأكثر شعبية في مصر خاصة في الريف. يمكن أن يتلوث الجبن بأنواع مختلفة من الكائنات الدقيقة أثناء إنتاجه وتوزيعه وتخزينه في ظروف غير صحية. يتسبب التلوث بالبكتريا المختلفة في تلف الجبن وأمراض التسمم الغذائي. في هذه الدراسة تم جمع ١٢٠ عينة من الجبن القريش من أسواق محافظة الشرقية- مصر. وقد تم عمل بعض الاختبارات الميكروبيولوجية وهي: العد الكلي للبكتريا, الايشريشيا كولاي والمكور العنقودي الذهبي وقد بينت النتائج المسجلة أن المتوسط العد الكلي للبكتريا والإيشريشيا كولاي والمكور العنقودي الذهبي هو ٦,٩ و ٣,٤٥ و٤,٧٣/جم, على التوالي.

وقد تم اختبار مواد التغليف للمنتجات الغذائية المصنوعة من الزيوت الطبيعية وتم معاملتها بالنانوتكنولوجي لتوفير نهج فعال وآمن لحفظ الأغذية. في هذه الدراسة، تم اختبار مستحلبات الكيتوزان المصنوعة من مواد دهنية محملة بزيت الزعتر لاطالة فترة صلاحية الجبن القريش. وقد تم تقييم الأنشطة المضادة للميكروبات عبر المكور العنقودي الذهبي وايشريشيا كولاي خلال ٤ أسابيع وقد تم عمل اختبارات حسية للجبن القريش خلال فترة التجربة واثبتت النتائج ان الصفات الحسية للجبن القريش لم تتاثر بطريقة كبيرة وأنها مقبوله حتي الأسبوع الرابع.

وقد أثبتت الدراسة وجود عدد كبير من المكور العنقودي الذهبي وايشريشيا كولاي حتي الأسبوع الرابع، لجميع عينات الجبن غير المغلفة بزيت الزعتر أو مستحلب الكيتوزان. أما العينات التي تم معاملتها بزيت الزعتر بتركيز ١% والتي تم معاملتها بمستحلب الكيتوزان المصنوعة من مواد دهنية محملة بزيت الزعتر تركيزات ٠,٥% و١% فقد وجد أن عدد ستاف أوريوس وايشريشيا كولاي أقل بشكل كبير حتي الأسبوع الرابع بينما لم يستدل علي وجودها عند الأسبوع الرابع باستخدام تركيز ٢%