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COMPARATIVE EFFECT OF ALLIUM CEPA (AC) PEEL EXTRACT WITH ITS NANO-EMULSION ON *PSEUDOMONAS* SPP. ISOLATED FROM SOME SOFT CHEESE

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ABSTRACT

Pseudomonas species are prominent opportunist pathogens. The present study was undertaken to detect the prevalence of *Pseudomonas spp.* in cheese and focused on the antibacterial effect of onion peel extract (Allium Cepa) and its nano-emulsion against *Pseudomonas aeroginosa.* One hundred and fifty samples of kareish, tallaga and cheddar cheese (50 samples each) were collected from dairy shops and supermarkets. The prevalence of the organism was 40%, 30% and 24% in the examined samples, respectively. The most prominent species were *P. aeruginosa, P. fluorescens, P. fragi, P. putrefaciens, P. proteolytica and P.alcaligenes.* The isolates of *P. aeruginosa* were confirmed by PCR utilizing the *16S rRNA gene.* The results of antibiotic sensitivity revealed that the highest antimicrobial resistance was recorded for Ampicillin (100%) and the highest sensitivity was reported for Cefotaxin (100%). AC and AC nano-emulsions were added at a concentration of 3.12 and 1.56 % in the laboratory-made kareish cheese. There was an evident antimicrobial effect on *P. aeruginosa* growth particularly for AC nano-emulsion, where, complete inhibition of the organism was observed on the 5th day. The prepared AC nano-emulsion had a Z-average diameter of 178.32± 65.73 nm and a polydispersity index (PDI) of 0.223. In examining the prepared nano-emulsion by Transmission Electron Microscope (TEM), the spherical shape was revealed and the size was 48.78 nm and the flow of active functional groups was clarified by Fourier-transform infrared spectroscopy (FTIR). Utilizing nano AC at a concentration of 1.56 % ensured its antibacterial activity on *P.aeroginosa* and did not affect on OAA of cheese.

Keywords: Allium Cepa, nano-emulsion, kareish cheese, cheddar cheese, *Pseudomonas aeruginosa*

INTRODUCTION

Customers in the Middle East choose cheese because of its high water and nutritional contents, which include proteins, lipids, carbs, and vitamins (Al-Waalan *et al.*, 2024). However, because of its high nutritional content, it is also vulnerable to a number of physicochemical changes and microbial contaminations. These pollutants include rod-shaped, aerobic, motile, Gram-negative bacteria called *Pseudomonas* species, and also the physical environmental condition, comprising low nutrition

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availability, fluctuating temperatures, high salt concentrations, and exposure to antiseptics that allow *Pseudomonas* species to thrive. They are among the most common bacterial species worldwide due to their ability to adapt to many environmental circumstances (Darwesh and Matter, 2024).

Because of its multi-antibiotic resistance, *P. aeruginosa* has become a major public health concern. Due to its ability to grow on food equipment surfaces and cause cross-contamination and post-processing food contamination, this pathogen is not only hazardous in medical settings, but also presents significant issues in the food sector (Rinky *et al.,* 2024). Furthermore, *P. aeruginosa* is a major contributor to hospital infections and frequently develops resistance to several medicines. Antibiotic abuse has also resulted in the emergence of multidrug-resistant strains of the bacteria, which in turn contributes to antimicrobial resistance (AMR) in bacteria. By 2050, it is predicted that these AMR bacteria would be responsible for 10 million deaths annually and 700,000 cases of incurable diseases worldwide (Strathdee *et al.,* 2020).

To address this challenge, natural compounds play a vital role in reducing contamination and controlling the growth of spoilage bacteria in milk and dairy products. In recent years, researchers have shown significant interest in new technologies and materials for developing antibacterial fabrics (Joshi *et al.,* 2022). Onion (Allium cepa), a member of the Allium genus, is well-known for its substantial pharmacological effects (Santas *et al.,* 2010, and Milea *et al.,* 2019) and is considered one of the richest sources of phenolic compounds, including flavonoids and anthocyanins, among vegetables (Manohar *et al.,* 2017). These phenolic compounds, particularly their hydroxyl (–OH) groups, are responsible for their antimicrobial and antioxidant activities (Ergezer *et al.,* 2018), making onions a common ingredient in meals.

Onion waste, which is produced in vast quantities by the food sector, can be turned into useful resources. Bioactive compounds with anti-inflammatory, antibacterial, anti-hyperlipidemic, antioxidant, and anti-diabetic characteristics are abundant in these by-products, particularly in the skin (Stoica *et al.,* 2023).

One fascinating field where nanotechnology is essential is the conversion of natural chemicals into nanoparticles. Applications with different degrees of complexity and adaptability have been made possible by advancements in a variety of systems and processes, which are influenced by the physicochemical characteristics of the bioactive ingredients (Taouzinet *et al.,* 2023).

So, this research was done to determine the prevalence of *Pseudomonas* spp. in some soft cheese, draw attention to the significant advances made in the identification of onion by-products as valuable sources of bioactive compounds, and notify the role of nanotechnology in changing the character of Allium Cepa extract into nano-emulsion and study the survival of this organism in cheese fortified by these agents.

MATERIALS AND METHOD

1- Collection of samples

For bacteriological analysis, 150 samples of kareish, tallaga, and cheddar cheese (50 samples each) were gathered from supermarkets and dairy stores in Assiut, Egypt, placed in sterile tubes, promptly labelled, and kept in an ice box.

2- Isolation and identification of *Pseudomonas* spp. (Patel *et al.,* 2019)

From each sample, 1 gm was inoculated into tryptone soya broth and incubated at 37° C for 24 h. After incubation, a loopful was streaked onto pseudomonas CN agar medium and incubated at 30° C for 24 h. The suspected colonies were picked up and streaked into tryptose soya agar (TSA) slants, incubated for 24 h at 30°C for biochemical identification

(Cheesbrough, 2000). Any growth on CN medium indicates the *Pseudomonas* spp. existence and the presence of blue-green or brown pigmentation, or fluorescence, were taken as presumptive evidence of *P. aeruginosa.*

2- Molecular genotyping study (Lagacé *et al.,* 2004)

Molecular confirmation of the *P. aeruginosa* isolates was done in the Reference Laboratory for Veterinary Quality Control on Poultry Production at Animal Health Research Institute, Dokki, Giza, Egypt.

The total bacterial 16S rRNA gene from the DNA-extracted samples was amplified using bacterial universal primers, AGAGTTTGATCMTGGCTCAG / TACGGYTACCTTGTTACGACTT by using a 25 µl total reaction volume containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan). The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. For gel analysis, 20 µl of the products was loaded in each gel slot. A Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra), and the data was analyzed through computer software.

3. Antimicrobial susceptibility test (CLSI, 2020)

It was carried out by the Kirby-Bauer disc diffusion method using Muller Hinton agar, where each strain was tested against 10 antimicrobial discs: vancomycin (VA), marbofloxacin (MAR), tetracycline (TET), neomycin (N), streptomycin (S), oxacillin (OXA), amoxicillin/clavulanate (AMC), penicillin (PEN), ampicillin (AMP), and cefotaxime (CTX) (Oxoid). The width of the inhibitory zone was measured with a caliper and the results were recorded using CLSI criteria.

4. Effect of allium cepa peel extract and its nano-emulsion on *P. aeruginosa* growth

4.1. Preparation of AC peel extract and AC nano-emulsion

Dry peels separated from red onions were cleaned up by washing with chlorine water (0.5%). Then, 5 g of AC was dried, ground, and mixed in 100 mL ethanol: water (50% v/v) overnight at room temperature. The mixture was centrifuged for 20 min at 2500 x g. Following this, the mixture was filtered through a Whatman No. 1 paper and kept in the fridge at 4°C (Sabire *et al.,* 2022). The AC peel extract (10 ml) was combined with Tween 80 emulsifier (40 ml) and 50 ml deionized water to prepare a stable emulsion. The nano-emulsion was created by mixing the solution in an Ultra-Truax homogenizer (M/s Ika, Germany) at 12000 rpm for 5 min. To further reduce the particle size, a probe-type ultrasound device was used for 5 min (Sarvinehbaghi *et al.,* 2021).

4.2. Characterization of AC nano-emulsion

At the Nanotechnology Unit, Al-Azhar University, Assiut Branch, Egypt, a dynamic light scattering device (Zetasizer Nano ZS, Malvern, UK) equipped with a submicron particle size analyzer was used to measure the mean diameters and polydispersity index of the nano-emulsion. Just prior to measurement, the sample was diluted 100 times with distilled water. Three measurements of the mean droplet size and polydispersity index (PDI) were made at a fixed scattered angle of 173° at 25°C. To gather and examine the data, Zeta-sizer® software (version 7.03) was employed. Fourier-transform infrared spectroscopy (FTIR, NICOLET, iS10, Thermo Scientific) was used at the Chemistry Department of the Faculty of Science, Assiut University, to determine the functional groups' attachment sites and the molecule's fingerprint.

4.3. Bacterial suspension preparation

The isolated strains of *P. aeruginosa* were cultured into selective broth and incubated at 37 ºC. The growth density was adjusted to match 0.05 MacFarland (10^8 cfu/ml) according to McFarland (1907) and diluted to reach 10^6 cfu/ml.

4.4. Minimum inhibitory concentration (MIC)

The antibacterial effect of AC and its nano-emulsion was detected by the agar well diffusion method (Valgas et al., 2007) 0.1 ml of the previously prepared bacterial strains was streaked into Muller Hinton agar plates, and 80 µl of different concentrations of AC and Nano-AC (100, 50, 25, 12.5, 6.25, 3.125, 1.6, and 0.8%, respectively) were added in each well. Then, the various sizes of the inhibitory zones were measured after 24 h.

4.5. Manufacturing of kareish cheese

Skim fresh buffalo milk was obtained from the Dairy Department, Faculty of Agriculture, Assiut Univ., Egypt. Pure cultures of *Str. salavarius* sup sp. *thermophilus* and *Lactobacillus delbruckii* sup sp. *bulgaricus* were obtained from Hansen Laboratories (Denmark). Animal powder rennet was obtained from Hansen Laboratories A/S, Copenhagen, Denmark. Sodium chloride was obtained from the local market.

Buffalo's skim milk was heated to 85°C for 15 sec and cooled to 38°-40°C. Active starters of S. thermophilus and L. bulgaricus (2% w/w) and 5% sodium chloride (salt) were added and mixed well. Rennet powder was added at a rate of 3 g/1 kg of milk, and P. aeruginosa was added at a count of 10^6 cfu/ml. Then, the cheese was divided into 3 equal portions as follows.

The first portion served as a positive control, and the second one was supplemented with AC in a concentration of 3.1% (MIC), and the AC nano-emulsion was added to the third portion in a concentration of 1.56% (MIC). The kareish cheese was manufactured as described by Effat *et al.* (2001) with slight modification. The cheese portions were packed in plastic bags and stored at 4°C for one week and were analyzed for *P. aeruginosa* growth at zero time and after curdling on the 1st, 3rd, and 5th days of storage.

4.6. Organoleptic analysis

For sensory evaluation, a group of 10 staff members from the Animal Health Research Institute, Assiut Branch, were asked to evaluate the texture, odor, appearance, and overall acceptability (OAA) of the packaged cheese samples with 3.1% AC and 1.56% AC nano-emulsion. The scale points were excellent, 5; very good, 4; good, 3; acceptable, 2; and poor, 1.

5. Statistical analysis:

Every experiment was conducted in triplicate. To ascertain the statistical significance of differences within the samples, a one-way analysis of variance was conducted using the SPSS software (SPSS Inc., Chicago, IL, USA). Excel version 2017 was used to prepare the microbiological data. Origin Lab 2021 was used to graph and analyze the FTIR results.

RESULTS

Table 1: Incidence of *Pseudomonas* spp. in different types of cheese samples.

Samples	No. of the examined samples	Positive samples	
		No.	%
Kareish cheese	50	20	40
Tallaga cheese	50	15	30
Cheddar cheese	50	12	24
Total	150	47	31.3

Table 2: Frequency distribution of the isolated *Pseudomonas* spp. in different types of cheese samples.

Pseudomonas spp.	Kareish cheese		Tallaga cheese		Cheddar cheese	
	No./20	%	No./15	%	No./12	%
P. aeruginosa	2	10	1	6.7	0	0
P. fluorescens	10	50	8	50.3	5	41.7
P. fragi	4	20	2	13.3	4	33.3
P. putrefaciens	3	15	2	13.3	1	8.3
P. proteolytica	-	-	1	6.7	1	8.3
P. alcaligenes	1	5	1	6.7	1	8.3

Table 3: Drug resistance of *Pseudomonas* spp. strains isolated from soft cheese (n = 47).

Antibiotic	**Resistant**		**Sensitive**	
	No./47	**%**	**N0./47**	**%**
Vancomycin VAN 30 mcg	2	4.3	45	95.7
Marbofloxacin MAR	17	36.2	30	63.8
Tetracycline 30 mcg	33	70.2	14	29.8
Neomycin N 30 mcg	37	78.7	10	21.3
Streptomycin S 10 mcg	40	85.1	7	14.9
Oxacillin OXA 1mcg	35	74.4	12	25.6
Amoxicillin/clavulanate AMC 20/10 mcg	40	85.1	7	14.9
Penicillin P 10 mcg	45	95.7	2	4.3
Ampicillin AMP 10 mcg	47	100	0	0
Cefotaxime CTX	0	0	47	100

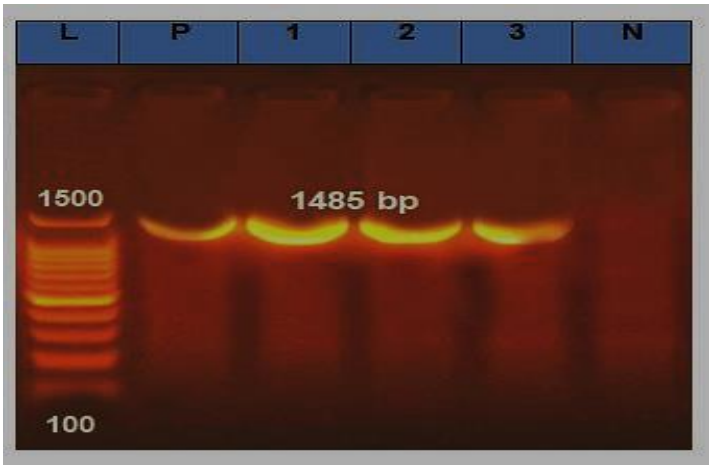

Photo 1: Agarose gel electrophoresis of the PCR product of 16S rDNA gene for the isolated *P. aeruginosa.* Lane L: ladder**,** Lane P: positive control**,** Lane N: negative control**,** Lane 1,2 ,3: positive *P. aeruginosa* (1485 bp)**.**

Photo 2: Difference between AC and Nano AC emulsions.

Table 4: Physical properties of the formulated nano-emulsion of AC by zeta-sizer.

Type of nano-AC	PDI	Size ± SD	Intensity %
Freshly prepared	0.223	178.32±65.73 nm	100%

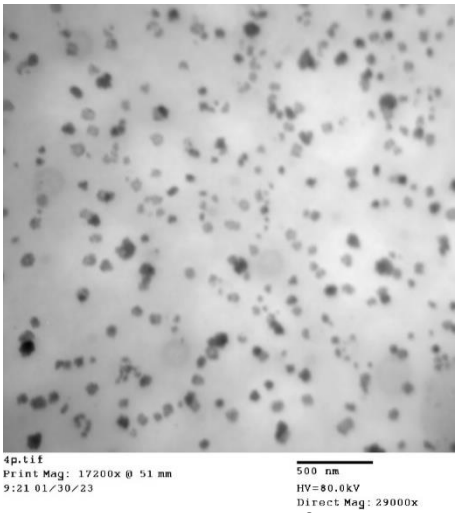

Photo 3. Transmission electron microscopy (TEM) for AC nano-emulsion with average size.

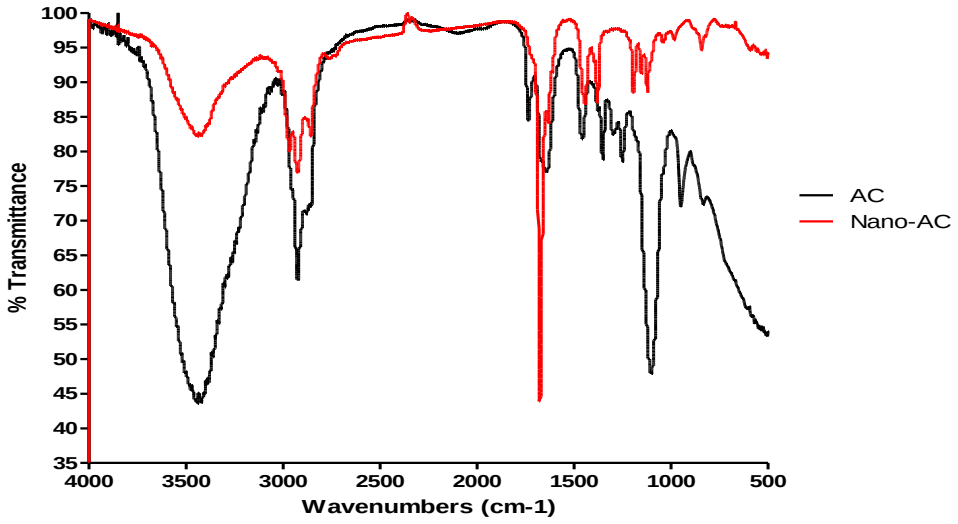

Figure 1. Fourier-transform infrared spectroscopy (FTIR) of AC and its nano-emulsion.

Table 5: The Minimum Inhibitory Concentration (MIC) of AC and its nano-emulsion effect on inoculated *Pseudomonas aeruginosa*.

Concentrations %	AC (mm)	Nano emulsion (mm)
	Mean ±SE	Mean ±SE
Pure	23±0.26	25±0.12
50	21±0.23	24±0.15
25	20±0.01	23±0.12
12.5	19±0.12	20±0.13
6.25	16±0.20	17±0.14
3.13	12±0.02	15±0.15
1.56	Zero	14±0.22
0.8	Zero	Zero

Data expressed as (mean ± SE) of three replicates.

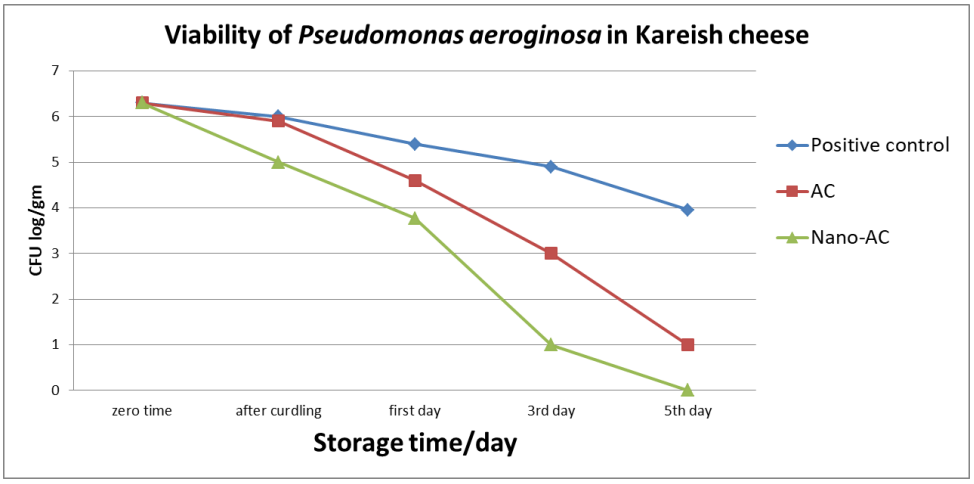

Figure 2. The viability of *Pseudomonas aeruginosa* inoculated in Kareish cheese supplemented with Ac (3.1%) and AC nano-emulsion (1.56%) during refrigerated storage.

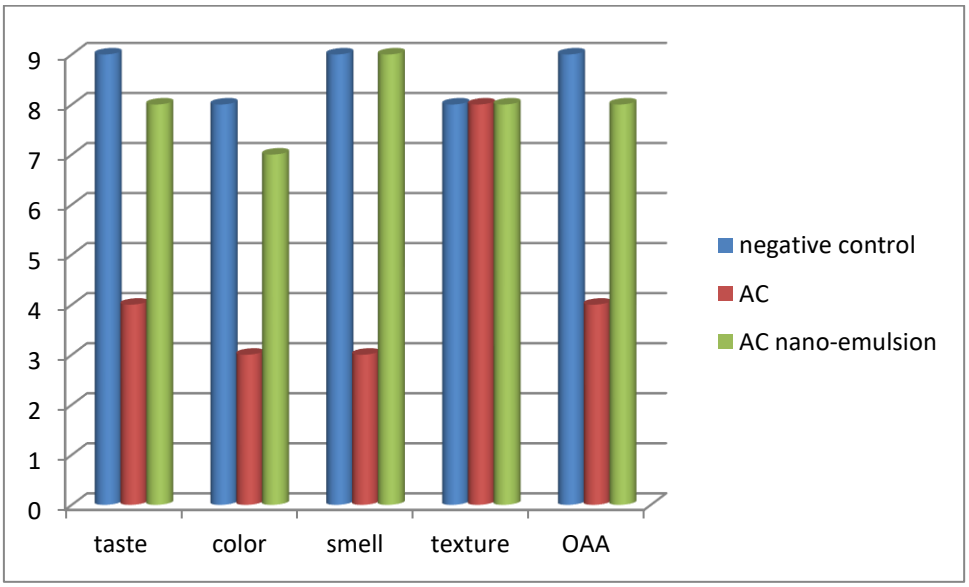

Figure 3. Sensory attributes of control and manufactured cheese with AC and AC nano emulsion during refrigeration storage.
OAA: Over All Acceptability

DISCUSSION

In addition to their capacity to produce heat-resistant spoilage enzymes, pseudomonas bacteria are psychotropic bacteria that can survive in raw, cold milk and dairy products. As such, they pose a risk to public health because they are opportunistic pathogens that can infect any part of the body (Bhargava, 2020). Because Pseudomonas species can spread from food, especially cheese and ready-to-eat food products, to consumers (Vrdoljak *et al.,* 2016).

The total prevalence of *Pseudomonas* spp. was 31.3% in the examined samples. Kareish cheese recorded the highest prevalence of 40%, while Tallaga and Cheddar cheese recorded 30% and 24%, respectively (Table 1). Kareish cheese and Tallaga cheese were more contaminated than Cheddar cheese which may be attributed to their production mainly from raw milk.

Higher results in cheese samples were recorded by Gamal *et al.* (2022), who could isolate *Pseudomonas* species from 48.5% of Tallaga cheese samples. On the other hand, a lower result was reported by Arslan *et al.* (2011), who examined 140 homemade white cheese samples, out of which 22.9% were contaminated with *Pseudomonas* spp.

The frequency distribution of isolated strains based on biochemical identification revealed that forty-seven isolates were identified as Pseudomonas species, and the frequency distribution of P. aeruginosa, *P. fluorescens, P. fragi, P. putrefaciens, P. proteolytica, and P. alcaligenes* was 10%, 50%, 20%, 15%, 0%, and 5% in Kareish cheese. While the frequency distribution was 6.7%, 50.3%, 13.3%, 13.3%, 6.7%, and 6.7% in Talaga cheese and 0%, 41.7%, 33.3%, 8.3%, 8.3%, and 8.3% in Cheddar cheese, respectively, as recorded in Table 2. The obtained results were more or less in line with those reported by Atia *et al*. (2022) and Ibrahim et al. (2022), who could detect *P.* aeruginosa in soft cheese and kareish cheese in a percentage of 18 and 8% respectively. Also, Mulet *et al.* (2010), Hammad, 2015, and Condé *et al.* (2022) reported that the predominant bacteria of the genus *Pseudomonas* was *P. fluorescens.*

In addition to its heat-resistant enzymes, *P. fluorescens* is significant in the dairy industry because it can form biofilms on the surface of milk tanks and other dairy processing equipment and can stay inside the plant for extended periods (Anntar *et al.,* 2016 and Stoeckel *et al.,* 2016). One of the most prevalent foodborne pathogens, *Pseudomonas aeruginosa*, poses serious challenges to food safety due to its ability to cause contamination, lead to antibiotic resistance, and form biofilm (Azelmad *et al.,* 2018 and Xu *et al.,* 2019). This microbe is one of the priority pathogens that the WHO should investigate for the creation and study of novel antibiotics because of its current antibiotic resistance (Botelho *et al.,* 2019).

Over 250 distinct illnesses were caused by the consumption of tainted milk products (Abrar *et al.,* 2020). It has been determined that *Pseudomonas* species, specifically *Pseudomonas aeruginosa*, are responsible for these diseases. According to Ebrahimpour *et al.* (2018), this microbe is highly significant in veterinary and medical settings and is opportunistic.

The recorded results in (Table 3) illustrated that the higher antimicrobial resistance was against Ampicillin (100%) followed by Penicillin (95.7%), Streptomycin and Amoxicillin/clavulanate (85.1%), Neomycin (78.7%), Oxacillin (74.4%), Tetracycline (70.2%), Marbofloxacillin (36.2%) and Vancomycin (4.3%). While all the tested strains were sensitive to Cefotaxime. Regarding the antimicrobial sensitivity test, the isolates lacked the susceptibility to many of the tested antimicrobials. Various *Pseudomonas* spp. isolates obtained from dairy products are resistant to penicillin, cephalosporins, carbapenems, and monobactams (Quintieri *et al.,* 2019).

In the Arab region, previous studies between 2010 and 2018 in most countries revealed that P. aeruginosa, which produces β-lactamase

enzyme, was resistant to 35 different antibiotics (Nasser et al., 2020). In Egypt, El-Shouny *et al.* (2018) examined fifty isolates of P. aeruginosa and recorded a high incidence of multiple drug resistance. The resistance of many antibiotics was due to the existence of virulence factors responsible for the pathogenicity of bacteria. Multidrug-resistant Pseudomonas aeruginosa was isolated from milk and dairy products due to a lack of hygiene (Ibrahim *et al*., 2022).

Many polymerase chain reaction (PCR)-based techniques, such as traditional PCR and real-time PCR, have been developed and used extensively in the past few decades for the identification of pathogens, such as *P. aeruginosa* (Williams *et al.*, 2010 and Lim *et al.*, 2021). Martín *et al.* (2022) confirmed that PCR is a rapid technology that has high sensitivity and specificity for particular deoxyribonucleic acid (DNA) sequences, enabling direct infection detection. Antimicrobial resistance of various bacteria is known to pose a serious threat to human health and has been detected in both industrialized and developing nations in both humans and animals.
Three isolates of antimicrobial-resistant P. aeruginosa were examined by PCR for genotypic assessments of the 16S rRNA gene, which can be detected in 100% of strains (Fig. 1). Antimicrobials made from natural sources, such as the natural extract, can aid in limiting microbial growth as an alternative to such an issue.

By-products of agricultural waste are now utilized in the food industry, rather than being thrown away. Onions produce a lot of waste due to their widespread production and worldwide, which has inspired new studies with potential approaches for reusing these by-products. So, the Allium Cepa was extracted from the external peel of red onion and transformed into nano-emulsion as cleared in (Fig. 2), which declared the difference between the nano-emulsion and the extract in physic-chemical properties.

The data recorded in (Table 4) illustrated that the created Nano-AC had a droplet size of 178.32±65.73 nm, a small droplet size, and the PDI was 0.223. While (Photo 3) revealed the image of TEM, which was almost of nanodroplets spherical in shape, had approximately uniform shape and size (48.78 nm) that provides more antibacterial activity. Moreover, (Figure 1) declared the use of FTIR to detect the functional groups, their attachment method, and molecular fingerprinting. The nano-emulsion was examined and the results suggested that the produced nanoparticles were stable and homogeneous because particle size and zeta potential of nano-emulsions are critical properties in encapsulating due to their effects on stability and application of nano systems since the zeta potential affects nano-emulsion stability (Razavi *et al.*, 2020 and Sarvinehbaghi *et al.*, 2021).

The inclusion of other functional groups and the differences in the peaks of nano-emulsions are considered the main reasons for their nano properties, stability, and antibacterial activity. Spectra were taken in the range of 500-3500 cm^{-1}; peaks were observed at 3500 to 1000 for AC, while for nano AC, peaks were 1700 and 3000, which indicated the different effects according to the active functional groups formed through nano-emulsion preparation.

Aslam *et al.* (2023) reported that the regions between wave numbers 1500 and 800 cm^{-1} (fingerprint region) reflected the biochemical molecules like carbohydrates, proteins, lipids, and polyphenols in (*Allium cepa L.)* extract. Regions from 4,000–2,000 cm-l were shown functional groups. Red onions (*Allium cepa L.*) extract shown in (Figure 1) peaks at 3240, 1612, and 1018 cm^{-1}. Spectra showed a strong and wide band at 3240 cm^{-1} allocated to C-H group stretching. The band at 1612 cm^{-1} showed C=C medium extending. Peak 1018 cm-1 showed C-N stretching vibration aliphatic amines. In another research by Baran *et al*. (2023), it was revealed that silver nanoparticles can be synthesized from AC peels, which is a low-cost and simple method,

where the nanoparticles allow for targeted drug administration, higher bioavailability and prolonged drug release in target tissues, and improved drug stability. However, more research is required to explore the mechanism of implication in biological applications, which is currently unknown.

Natural compounds have recently been employed in a variety of food industries through the use of nanoencapsulation, including in food matrices, packaging, dietary supplements and nutraceuticals, and functional foods. To fully comprehend the safety and toxicity of nanomaterials, their fate within the body, their capacity to form crystals that may react with living cells, and their suitability for use with various food matrices, more research is necessary. Creating international regulations that will support the safe marketing of novel nanotechnology products, which could lead to better health outcomes, is also crucial (Taouzinet *et al*., 2023). The disposal of such massive waste could be resolved by processing and using onion waste as a natural antioxidant and antimicrobial-rich food material (Sagar *et al*., 2020).

Table (5) showed the antibacterial effect of AC and AC nano-emulsion by the agar well diffusion method, which declared the antibacterial activity of AC and AC nano-emulsion against *Pseudomonas aeruginosa,* and the MIC for AC was 3.1% with a zone of inhibition of 12±0.02 mm and 15±0.15 for nano AC, while 1.56% was the recorded MIC for nano AC, and the zone of inhibition was 14±0.22 mm. (Oskay *et al.,* 2009) recorded a 16 mm inhibition zone diameter, and (Aslam *et al.,* 2023) found that the inhibition zone was 14.0, 14.2, and 14.8 mm at 5 ml of nano-emulsion against *P. aeruginosa*. The obtained results in the current study were better than the results of Oyawoye *et al.* (2022), who found the zone

of inhibition of *Pseudomonas aeruginosa* ranged from 3.5 to 4 mm, while Mardani *et al.,* (2023) reported that the zone was about 5.9 ± 0.11 mm.

The antibacterial activity of onion AC extract can be attributed to the presence of flavonoids and polyphenols, which have been reported to have a broad spectrum of antibacterial activity (Hendrich, 2006, and Grover *et al.,* 2011). Antibacterial substances dissolve the outer layer of Gm-ve bacteria and create pores in the cell membrane, and the bacterial cell leaks out, and the cell dies (Hussain *et al.,* 2021).

The data recorded in (Figure 2) clarified that Kareish cheese was manufactured in the lab and inoculated with the MIC detected *in vitro* as follows: 1.56% for nano AC and 3.12% for AC extract. These concentrations revealed good results in the reduction count of *P. aeruginosa* during the storage of cheese in the refrigerator. The initial count of *Pseudomonas aeruginosa* in samples not supplemented with either AC or Nano-AC was around 6.3 log cfu/g in all cheese samples, then the count started to decrease gradually but at a low rate, reaching 3.9 log cfu/g on the 5th day of storage, while in batches supplemented with 3.1% AC and 1.56% Nano-AC, the count decreased to 3 log cfu/g and 1 log cfu/g, respectively, on the 3rd day, while complete reduction (100%) occurred on the 5th day in the Nano-AC batch (< 1 log cfu/g), while AC recorded 1 log cfu/g at the same period.

Figure (3) shows the organoleptic evaluation of Kareish cheese made with 3.1% AC and 1.56% Nano-AC. During one week of storage at 4°C, there were no significant differences between the control and Nano-AC, while AC-supplemented cheese recorded the lowest overall acceptability, especially in smell and taste. In OAA there is a significant difference between AC and nano AC, and that may be returned to the nature of the external peel extract, which has a specific odor and color, while nano AC was white and had no effect on the nature of cheese. In addition, it nearly has no taste, so there is no effect on the taste of manufactured cheese, and that reflects the agreement in OAA of nano AC with control.

Obtained results were better than Santas *et al. (2010),* who showed that the bactericidal property of onion extracts was usually in concentrations of 15 to 20 mg/ml, and there was not much difference in the sensitivity of Gram-positive and Gram-negative bacteria. However, the results of the well diffusion assay showed that the sensitivity of Gram-positive bacteria is higher than Gram-negative bacteria, which can be attributed to the protective effect of the lipopolysaccharides coating layer.

According to Shinkafi and Dauda (2013), the thiosulfate chemical compound in onions may be the cause of the antibacterial activity. It has been shown to effectively kill a variety of common bacteria, including *Pseudomonas aeruginosa.* The volatile nature of the organosulfur compounds derived from Allium plants is one of their primary traits. This volatility is the primary cause of the distinctive scent that these plants emit, particularly when they are crushed or mashed. According to Reiter *et al.* (2017), there aren't many volatile antimicrobials available for clinical use.

CONCLUSION

Because of the organism's natural presence in the environment, which caused milk and dairy products to become contaminated, it is possible to draw the conclusion that Kareish, Tallaga, and Cheddar cheese have an impact on the spread of Pseudomonas spp. Thus, it is necessary to implement hygienic practices, manufacturing programs, hazard analysis, and critical control points in order to enhance the quality and safety of these products. However, it is evident that Allium cepa and its nano-emulsion have antibacterial properties that inhibit the growth of *P. aeruginosa.* These extracts could therefore be tested in combination with other compounds against a variety of pathogens that infect humans and animals, or they could be used as adjuvant treatments for microbial infections or for the development of new drugs.

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التأثير المقارن لمستخلص قشور البصل ومستحلبه النانوي على بكتيريا السيدوموناس ايروجينوزا المعزولة من بعض انواع الجبن الطري

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تعتبر بكتيريا السيدوموناس من مسببات الأمراض الانتهازية البارزة لذلك أجريت هذه الدراسة للكشف عن مدى انتشار بكتيريا السيدوموناس في الجبن الطري وركزت على التأثير المضاد للبكتيريا لمستخلص قشر البصل (Allium Cepa) ومستحلبه النانوي ضد السيدوموناس ايروجينوزا. تم جمع مائة وخمسين عينة من جبن القريش، جبن التلاجة، وجبن الشيدر (٥٠ عينة لكل منها) من محلات الألبان ومحلات السوبر ماركت. وكانت نسبة انتشار السيدوموناس ٤٠٪ و٣٠٪ و٢٤٪ في العينات المفحوصة على التوالي. وكانت أبرز الأنواع هي *P. aeruginosa*، *P. Fluorescens*، *P. fragi*، *P. putrefaciens*، *P. proteolytica* و*P.alcaligenes*. تم تأكيد عزلات *P. aeruginosa* بواسطة PCR باستخدام جين S rRNA١٦. أظهرت نتائج الحساسية للمضادات الحيوية أنه تم تسجيل أعلى مقاومة للمضادات الحيوية للأمبيسيلين (١٠٠٪)، كما تم تسجيل أعلى حساسية للسيفوتاكسين (١٠٠٪). وعندما تمت إضافة مستخلص قشور البصل و مستحلبه النانوي بتركيز ٣,١٢ و١,٥٦٪ في جبن القريش المصنوع في المعمل كان هناك تأثير مضاد للميكروبات واضح على نمو *P. aeruginosa* وخاصة بالنسبة للمستحلب النانوي من قشور البصل، حيث لوحظ تثبيط كامل للميكروب في اليوم الخامس. وقد بلغ متوسط قطر المستحلب النانوي AC ١٧٨,٣٢ ± ٦٥,٧٣ نانومتر وبلغ مؤشر تعدد التشتت (PDI) ٠,٢٢٣. وعند فحص المستحلب النانوي المحضر حديثًا بواسطة الميكرسكوب الإلكتروني (TEM)، تم الكشف عن الشكل الكروي وكان الحجم ٤٨,٧٨ نانومتر وتم توضيح تدفق المجموعات الوظيفية النشطة بواسطة التحليل الطيفي للأشعة تحت الحمراء (FTIR). لذلك ينصح باستعمال المستحلب النانوي لقشور البصل بتركيز ١,٥٦٪ لفاعليته ضد ميكروب السيدوموناس ايروجينوزا وعدم تأثيره على طعم الجبن.