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ROLE OF LYSOZYME AS A NATURAL ANTIBACTERIAL AGENT AGAINST *AEROCOCCUS VIRIDANS* ISOLATED FROM MILK AND SOME CHEESE TYPES

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ABSTRACT

This study aimed to identify the presence of *Aerococcus* spp. in 200 samples of raw milk and various cheese types, specifically Kariesh, Damietta, and Mozzarella (50 each sample). *Aerococcus* spp. were found in 45% of the examined samples and *A. viridans* was found in 13 samples only. PCR was utilized for the positive samples, the investigation yielded significant findings out of 90 positive samples analyzed, 13 were confirmed to be *A. viridans*, with the distribution of 7, 4, 1 and 1 from raw milk, Kariesh, Damietta, and Mozzarella cheese, respectively. The antimicrobial susceptibility profile indicated that all samples demonstrated complete susceptibility to Oxytetracycline, Gentamycin and Tetracycline. In order to increase food safety and validity, it is crucial to examine the effectiveness of the powerful antimicrobials that have recently been found against unwanted microorganisms present in food components. So, the current research focused on using Lysozyme against *A. viridans* and its potential role to extend the shelf life of Kariesh cheese. During application in Kareish cheese, 6.25% lysozyme exhibited a powerful bactericidal effect within 24 hours.

Key words: *A. viridans*, Kareish cheese, Mozzarella cheese, lysozyme, Damietta cheese.

INTRODUCTION

Milk and dairy products are critical components of the diet across all age groups, due to their diverse and nutrient-packed composition. This combination plays a significant role in ensuring a balanced nutritional state for populations. The importance of these foods in improving health highlights their value and positions them as vital elements within nutritional guidelines and practices (Michaelidou, 2008 and Pereira, 2014).

However, advancements in food safety measures, the World Health Organization (WHO) reports that food borne diseases (FBDs) claim the lives of approximately 420,000 people annually, affecting 600 million individuals worldwide (Leedom, 2020). Dairy products contribute to about 14% of these cases (WHO, 2021), as they can harbor harmful pathogens (Keba *et al.,* 2020).

As a result, both food technologists and consumers continue to prioritize food safety and quality. While there have been technological improvements in preservation, issues such as food spoilage

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and waste persist, and bacterial foodborne illnesses have not seen significant declines (Hussain, 2013).

Environmental pathogens are often introduced from the surrounding environment, especially during milking, which serves as a key source for microorganisms such as *Aerococcus* spp. (Forsman *et al.,* 1997).

Aerococcus spp. are Gram-positive, microaerophilic cocci that thrive in various environments, including hospitals, seawater, and animal farms. These bacteria grow well in low-oxygen conditions and are recognized for their presence across different settings (Vos *et al.,* 2009).

As saprophytic bacteria belonging to the *Streptococcaceae* family, *Aerococcus* spp. are commonly found in diverse environments. Their physical and biochemical properties can be similar to those of *Streptococci,* often leading to misdiagnosis (Rasmussen, 2012).

Because it has been connected to diseases like endocarditis, meningitis, and arthritis, this microbe poses serious health concerns to people (Gopalachar *et al.,* 2004 and Popescu *et al.,* 2005). With isolates discovered in both clinical and subclinical instances, *A. viridans* has been linked to bovine mastitis in various studies (Špaková *et al.,* 2012; Liu *et al.,* 2015; Saishu *et al.,* 2015 and Sun *et al.,* 2017). Additionally, it is known to contribute to arthritis, pneumonia, and meningitis in cattle, affecting livestock health (Liu *et al.,* 2015).

The rise in antibiotic resistance impacts both human health and the environment. Furthermore, consumers have become more cautious about chemical preservatives (Furkan *et al.*, 2024). Natural antimicrobial agents, sourced from plants, animals, and microorganisms, are increasingly being adopted as alternatives to chemical preservatives. These substances help to extend the shelf life of food by preventing or eliminating microbial growth (Juneja, 2012).

Lysozyme is an essential part of the innate immune response, demonstrates strong antimicrobial properties against a wide range of bacterial, fungal, and viral infections. In addition to its natural antibiotic function, it also enhances the immune system's defense and boosts the effectiveness of other antimicrobial agents (Ragland and Criss, 2017). In dairy production, lysozyme is used during cheese-making to suppress the growth of spoilage bacteria, which helps prevent issues like texture changes, gas formation, and undesirable flavors in cheese (Sinigaglia *et al.,* 2008 and Stocco *et al.,* 2015).

Additionally, there is now more research being done on the use of lysozyme in packaging. According to (Awad *et al.,* 2023), hydrolyzed lysozyme seems to encourage the production of peptides with bioactive qualities that improve the functionality of dairy products and have extra antimicrobial benefits.

In the present investigation, the objective was to evaluate the prevalence of *Aerococcus* spp. in milk and specific cheese types, also emphasizing the significant function of Lysozyme in improving the sensory attributes of Kariesh cheese, as well as extending its shelf life.

MATERIAL AND METHODS:

Collection of samples:

From various dairy stores, supermarkets, and street sellers in Assuit city, 200 random samples of raw milk, Kariesh, Damietta, and Mozzarella cheese (50 samples of each) with varying manufacturing dates were gathered from the period of January 2023 to March 2024. The samples were sent to the lab as soon as possible, to be inspected right away after being transported in an icebox.

Isolation of *Aerococcus* spp. (Liu *et al.,* 2015)

Loopfuls of incubated TSB were streaked onto Trypticase Soya Agar (TSA) mixed with 5% defibrinated sheep blood and incubated for 24 hours at 37ºC after being incubated on Trypticase Soya broth (TBS) for 24 hours. The small, spherical, white or grey, semi-transparent colonies of *Aerococcus* spp., which are encircled by a green zone of alpha haemolysis, were identified. For additional biochemical and PCR identification, a loopful from the probable colonies, streaked onto Brain Heart Infusion Agar (BHI), and incubated for 24 hours at 37ºC.

Identification of *Aerococcus* spp. (Vos *et al.,* 2009)

Polymerase Chain Reaction (PCR) identification for *Aerococcus* spp. (Martin *et al.,* 2007) was done in The Animal Health Research Institute in Giza, Egypt.

DNA extraction

Suspected colonies and standard strain (*A. viridans* strain were subcultured onto nutrient broth and incubated overnight at 37°C. Qiagen DNA Blood Mini kit (Cat. No. 51304, Hilden, Germany) was used to extract DNA, which was then kept at 20°C until further investigation.

DNA amplification

The Emerald Amp GT PCR master mix (Takara, Japan) Code No. RR310A kit was utilized for PCR performed in a PCR thermocycler (Biometra, Germany), with the following used reagents: a final volume of 25 µl containing 12.5 µl Emerald Amp GT PCR master mix (2x premix), 5.5 µl PCR grade water, 1 µl for each forward and reverse primer (20 pmol) and 5 µl template DNA. The primers cycling conditions during PCR started with initial denaturation at 95˚C for 5 min, followed by 35 cycles including the denaturation phase at 94˚C for 30 sec, annealing phase for 40 sec at 59˚C, extension phase at 72˚C for 45 sec. At the end, the final extension was carried at 72˚C for 10 min.

In PCR, oligonucleotide primers were utilized. The sequence of (*A. viridans 16S rRNA*) primers, Metabion (Germany) were GTG CTT GCA CTT CTG ACG TTA GC for forward primer and TGA GCC GTG GGC TTT CAC AT for reverse primer.

Agarose gel electrophoreses (Sambrook *et al*., 1989) with modification Electrophoresis grade agarose (Applichem, Germany, GmbH) (1.5 g) was prepared in 100 ml TBE buffer in a sterile flask, heated in microwave to dissolve all granules with agitation, and allowed to cool at 70˚C, then 0.5µg/ml ethidium bromide was added and mixed thoroughly. The warm agarose was poured directly into gel casting apparatus with the desired comb in apposition and left at room temperature for polymerization. The comb was then removed, and the electrophoresis tank was filled with TBE buffer. Fifteen µl of each PCR product, negative and positive control were loaded to the gel. A Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to UV cabinet, photographed by a gel documentation system (Alpha Innotech, Biometra), and the data was analyzed through computer software.

Antimicrobial susceptibility test (CLSI, M100 2020):

Each strain was tested against five antimicrobial discs: ampicillin (25µ), erythromycin (15µ), oxytetracycline (30µ), gentamycin (30µ), and tetracycline (30µ) using the Kirby-Bauer disc diffusion method with Muller Hinton agar. A caliper was used to measure the inhibitory zone's width, and the results were recorded and interpreted using CLSI criteria.

Bacterial suspension preparation:

Prior to being inoculated onto selected agar, *Aerococcus* bacteria were cultivated on selective broth (TSB) and incubated for 24 hours. Following a vortex, the bacterial suspension was compared to a concentration of 0.5 McFarland Standard

as per (McFarland, 1907) then diluted to 10^5.

Minimum inhibitory concentration (MIC) (Valgas *et al.,* 2007)
The agar well diffusion method was conducted to find the lowest concentrations of Lysozyme that demonstrated antibacterial activity against *Aerococcus* bacteria. Lysozyme was acquired from Sigma (EC 3.2.1.17) from chicken egg white, Cat. No. L.6876, the agar well diffusion experiment was widely used to evaluate natural compounds' antibacterial properties. This assay measured the size of the growth inhibition zone surrounding the sample, which can be put into an agar well or onto a paper disc. In this experiment, 0.1 mL of previously made bacterial suspension was applied to the whole surface of the agar plate to inoculate it. Next, a full circle (6–8 mm) was made aseptically into the agar using a sterile cork borer, and a suitable volume (20–100 µL) of lysozyme solution of different concentrations of Lysozyme (pure, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.6%, and 0.8%, respectively) was added. The agar plate was then incubated at 37ºC for 24 hours to allow the antibacterial agent to diffuse into the agar medium and inhibit the growth of the tested microbial strain, resulting in a measurable growth inhibition zone.

Evaluation of the antibacterial activity of Lysozymes against *A. viridans* in Kariesh cheese manufactured in laboratory:
Ten liters of cow`s skim milk was heated to 85˚c for 15 sec and cooled to 38 - 40˚c. Subsequently, 3g/100kg rennet was added to the warm milk. Some milk was transferred and stored in a sterile jar as the negative control group, prior to introducing the prepared *Aerococcus* bacterial inoculum to the milk at a count of 10^5 cfu/ml. Following that, this inoculated milk was split into six portions so that lysozyme concentrations could be added based on MIC. Cheese lots were divided into 1- negative control (without any treatment), 2- positive control (with 10^5 *A.viridans)*, 3- cheese (with 0.8% Lysozyme and 10^5*A.viridans*) and 4- cheese (with 1.56% Lysozyme and 10^5*A. viridans*) 5-cheese (with 3.13% Lysozyme and 10^5 *A. viridans*) 6- cheese (with 6.25% Lysozyme and 10^5*A. viridans*) and examined at zero time , 1st day, 3rd day, 1st week and 10th day, the samples were kept in refrigerator at 4°C under complete aseptic conditions until *Aerococcus* spp. could no longer be found, or the samples of cheese that had been preserved began to deteriorate. 25 g of cheese were aseptically added to 225 mL of 0.1% peptone water for a tenfold serial dilution. One milliliter was plated on TSA enriched with 5% defibrinated sheep blood at 37°C for a full day to count the bacteria.

Organoleptic analysis (Badmos and Abdulsalam, 2012):
A panel of thirty judges evaluated negative control samples that contained 6.25%, 3.13%, 1.56%, and 0.8% Lysozyme, correspondingly. The judges used a nine-point hedonic scale to evaluate the cheese's sensory qualities based on five factors: taste, color, texture, and overall acceptability. Nine points were awarded for the best cheese, while one point was awarded for the worst.

Statistical analysis:
Three duplicates of each experiment were conducted. The SPSS program (SPSS Inc., Chicago, IL, USA) was used to do a one-way analysis of variance in order to determine the statistical significance of differences within the samples. The probability value (P-value) of $P<0.05$ was deemed statistically significant. The data from the microbiological tests were prepared using Excel software version 2017.

RESULTS

Table 1: Incidence of *Aerococcus* spp. in the samples examined:

Samples	No. of examined samples	Positive samples	
		No.	%
Raw Milk	50	36	72
Kariesh cheese	50	21	42
Damietta cheese	50	5	10
Mozzarella cheese	50	28	56
Total	200	90	45

Table 2: Incidence of different *Aerococcus* spp. in the positive examined samples by using biochemical tests:

Aerococcus spp.	No. of isolates							
	Raw Milk		Kariesh cheese		Damietta cheese		Mozzarella cheese	
	No./36	%	No./21	%	No./5	%	No./28	%
A. viridans	7	19.4	4	19.04	1	20	1	3.57
A. urinae	0	0	0	0	0	0	0	0
A. sanguinicola	0	0	0	0	0	0	0	0

Table 3: Antimicrobial susceptibility profile of *A. viridans* isolated from the examined samples: (n= 13)

Antibiotic	Resistant		Intermediate		Sensitive		Mean±SD
	No.	%	No.	%	N0.	%	
Tetracycline (TE 30 mcg)	0	0	5	38.46	8	61.53	17.2±8.3
Ampicillin (AMP 10 mcg)	2	15.3	5	38.46	6	46.15	18.6±7.1
Erythromycin (E 15mcg)	3	23	7	7.69	3	7.69	12.2±7.01
Oxytetracycline (OX 30mcg)	0	0	6	46.15	7	53.84	15.9±7.3
Gentamycin (G 30 mcg)	0	0	4	30.76	9	69.23	23.8±4.5

Table 4: MIC of Lysozyme on *A. viridans* viability:

Mean ±SD	lysozyme Concentration
20±1	100%
24±1	50%
30±1	25%
24±1	12.50%
22.3±1.527	6.25%
17.1±1.75	3.13%
12.3±1.04	1.56%
0±0	0.80%

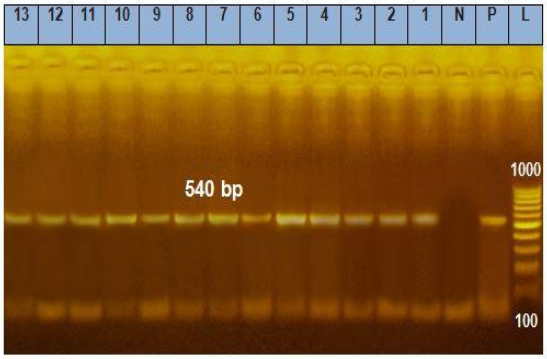

Photo 1: PCR identification of *Aerococcus viridans*.
Lane L: DNA ladder 100 bp.
Lane P: control positive of *A. viridans*.
Lane N: control negative
Lane 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and **13:** *A. viridans* positive.

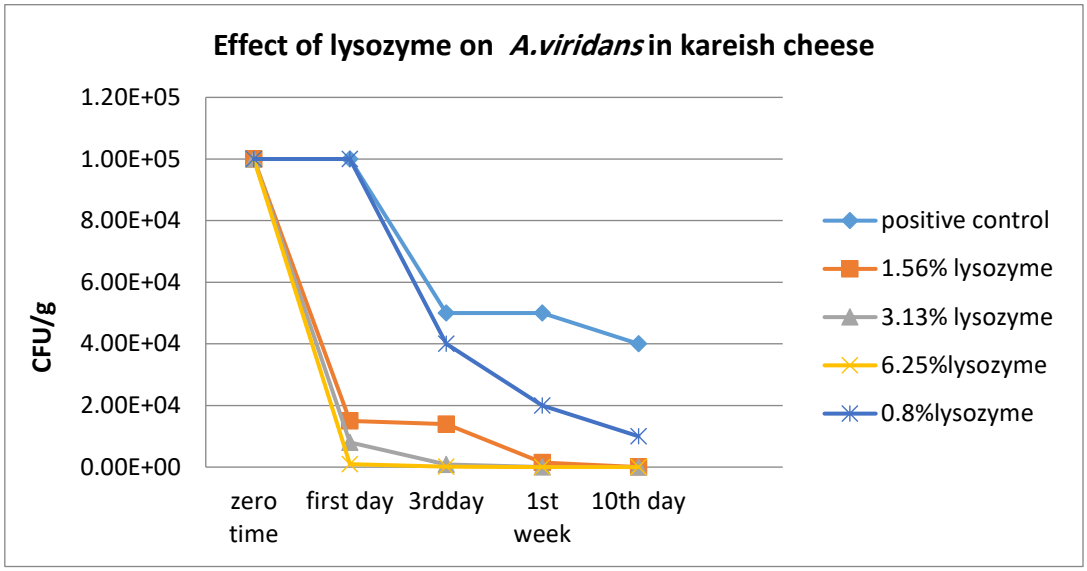

Figure 1: Antimicrobial activity of Lysozyme on *A. viridans* viability in Kariesh cheese:

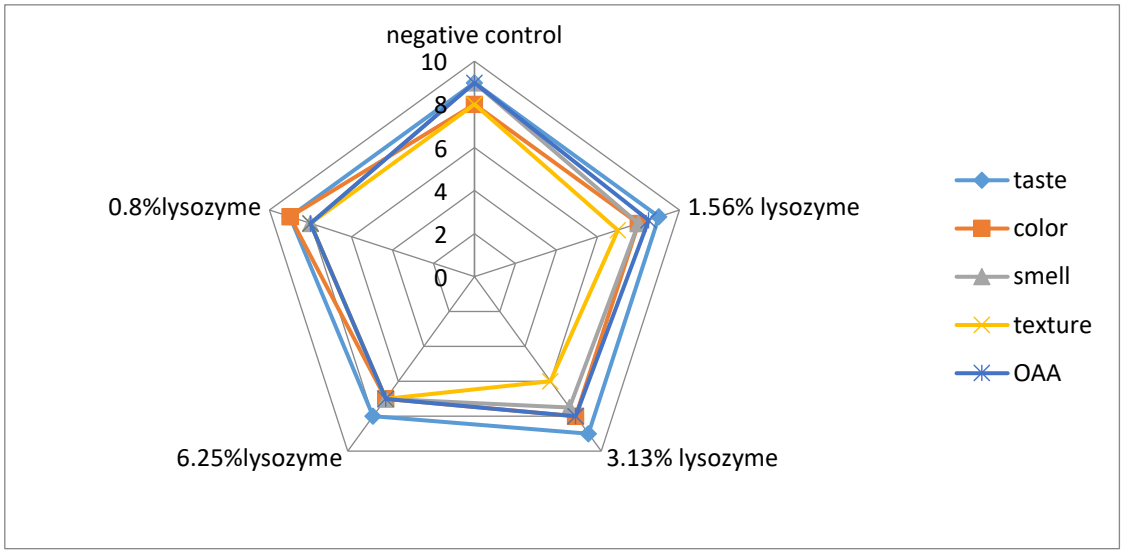

Figure 2: Sensory tests for Lysozyme application in Kariesh cheese:

DISCUSSION

Gram-positive cocci known as *Aerococcus* spp. are frequently found in a variety of dairy conditions and can aid in fermentation as well as spoiling. They flourish in milk and cheese because of their adaptability to a variety of environments, where they could contribute to the development of flavor and texture. *Aerococcus* spp. can cause unwanted consequences like spoiling or odd flavors; so, it must be closely watched and managed in dairy production. To maximize product quality and guarantee customer safety, it is essential to comprehend the ecological dynamics of *Aerococcus* in dairy systems (Facklam and Elliott, 1995).

As illustrated in Table (1), 90 isolates were identified as potentially harboring *Aerococcus* spp., accounting for 45% of the total samples assessed. The findings highlighted a significant variation in prevalence rates across different dairy types. Specifically, 36 of the tested raw milk samples were positive, yielding a prevalence of 72%. Kariesh cheese, it was 42%, with 21 positive samples, while Damietta cheese exhibited 10%, with only

5 samples containing *Aerococcus* spp., Mozzarella cheese samples showed a prevalence of 56%, with 28 testing positive.

Poor hygiene standards, unsuitable housing conditions, and a lack of bedding for dairy cows are some of the causes of the high concentrations of *Aerococcus* spp. found in raw milk (Liu *et al.,* 2015).
For many years *Aerococcus* spp. were only recognized by laboratories with a special interest in these bacteria. Now there is a greater awareness of aerococcal infections and better instruments for identifying those bacteria (Magnus, 2013).

In Table (2), *A. viridans* were the only species detected in all dairy products, with varying prevalence across different samples. In raw milk, *A. viridans* was identified in 19.4% of the samples (7 out of 36), and a similar prevalence of 19.04% was found in Kariesh cheese (4 out of 21 samples). In Damietta cheese, the prevalence was 20% (1 out of 5), while it dropped significantly to 3.57% in Mozzarella cheese (1 out of 28 samples). These findings align with previous studies that have identified *A. viridans* as a common microorganism in dairy products. For instance, *A. viridans* has been frequently detected in milk and cheese products, with its presence varying based on environmental and processing conditions. According to Forsman *et al.* (1997), environmental factors such as the milking process and post-processing conditions significantly influence the microbial composition of dairy products.

Compared to the present study, other research has reported different prevalence rates for *A. viridans*. In a study by Keba *et al.* (2020), *A. viridans* was found in 10-15% of dairy products, slightly lower than the obtained findings. This discrepancy could be due to regional differences in dairy processing practices, the hygiene conditions of the farms, and variations in environmental contamination during milking. Additionally, the higher prevalence of *A. viridans* in cheeses like Kariesh and Damietta in this study could reflect the influence of specific dairy product characteristics, such as the pH, moisture content, and fermentation processes, which may favor the growth of this bacterium.

Interestingly, *A. urinae* and *A. sanguinicola* were not detected in any of the samples. This was consistent with some studies that revealed these species as less common in dairy products, compared to *A. viridans*. For example, a study by Liu *et al.* (2015) noted that *A. urinae* was more commonly associated with urinary tract infections in humans, with limited occurrence in food products, including dairy. The absence of *A. sanguinicola* aligns with its classification as a rarer species in the genus *Aerococcus*, with limited research of its presence in dairy matrices.

The 13 isolates were identified using PCR and sequencing of their 16S rRNA gene, as shown in Photo1. A 540-bp 16S rRNA gene fragment was amplified by PCR using the specific primers for *A. viridans,* and all isolates produced the expected amplification product of 540 bp, which is specific for *A. viridans.* The 16S rRNA sequences were approximately 1,400 nucleotides in length, and were compared to the sequences of other Gram-positive, catalase-negative species found in the Gene Bank and EMBL databases using the FASTA program. The sequencing results showed 99.4 to 100% 16S rRNA gene sequence similarity with *A. viridans* ATCC 11536T (accession no. M58797).

According to Table (3), the susceptibility of 13 *A. viridans* isolates to various antibiotics was assessed, revealing that all 13 isolates (100%) were sensitive to Tetracycline, Gentamycin, and Oxytetra-cycline. Furthermore, 11 strains (84.61%) demonstrated a high level of susceptibility to Ampicillin, while 10 strains (76.9%)

were similarly responsive to Erythromycin. On the contrary, 2 strains were detected to be highly resistant to Ampicillin (15.38%) and 3 strains were resistant to Erythromycin (23.07%).

These findings align with Martin *et al.* (2007), who demonstrated that all *A. viridans* isolates were notably susceptible to beta-lactam antibiotics. In contrast, Špaková *et al.* (2012) and Shaker *et al.* (2019) revealed divergent resistance patterns, with their isolates exhibiting moderate susceptibility to Erythromycin (45%) and significant resistance to Penicillin (81.82%) and Tetracycline (63.63%).

The observed variability in the susceptibility patterns of *A. viridans* to various antibiotics can be attributed to several factors, including the differential exhibited responses by distinct strains within the same species. Furthermore, the emergence of bacterial resistance to certain antibiotics commonly employed in both veterinary and human medical treatments may also play a significant role, ultimately diminishing the effectiveness of these antibiotics in combating infections. This situation poses potential risks to human health, particularly when these resistant strains are transmitted through the consumption of dairy products such as milk and cheese (Scholtz *et al.,* 2022). Utilizing natural preservation techniques as treating with lysozyme may reduce the losses incurred from insufficient refrigeration and unstable cooling systems. Also, the observed reduction in fatty acids in the lysozyme-treated cheese during ripening is believed to be associated with the microbial changes driven by the inhibitory properties of lysozyme (Karaman *et al.,* 2024).

Antibacterial effect of Lysozyme on *A. viridans* viability varies according to the used concentration (Table 4), based on measuring the diameter of zone of inhibition (mm) in agar well diffusion assays, where at (0.80%, 1.56%, 3.13% and 6.25%) revealed the mean diameter of inhibitory zone (0, 12.33±1.04, 17.166± 1.75, and 22.3±1.52 mm ±STD), respectively. Whereas, Lysozyme was applied at concentrations (100%, 50% and 25%), the mean diameter of inhibitory zone was (20±1, 24±1 and 30±1 mm ±STD), respectively. The same results obtained by Yuny *et al. (*2016); Awad *et al.* (2020) and Cathrine *et al.* (2022) and also the other modification methods for Lysozyme or combine it with other antimicrobials to increase its efficiency.

Lysozyme may serve as a viable alternative material, either independently or integrated into hurdle technology, to enhance food safety, particularly in cheese production. Future research should explore the synergistic effects of lysozyme when it will be used alongside other antimicrobial enzymes and innovative technologies, including high-intensity pulsed electric fields, cold plasma, and irradiation, to further improve its antimicrobial efficacy in cheese manufacturing (Khorshidian *et al.,* 2021). N-acetylglucosamine and N-acetylmuramic acid 1, 4-linkages present in peptidoglycan are hydrolyzed as part of the lysozyme mechanism. Gram-negative bacteria are resistant to the effects of lysozyme, whereas Gram-positive bacteria are especially susceptible because of the peptidoglycan found in their cell walls (Conner, 1993 and Losso *et al.,* 2000). Variations in the amino acid sequences, structures, physicochemical properties, and immunological traits of lysozymes can be used to classify them. C-type (chicken-type or conventional), g-type (goose-type), and i-type (invertebrate-type) lysozymes are the three primary categories into which they are traditionally divided (Zhang and Rhim, 2022).

As declared in Figure (1), the manufactured Kareish cheese was examined at zero-time, counting of *A. viridans* was constant (10^5cfu/g) for all examined cheese samples then the count

started to decrease after 24 hours to reach 1.5 x10^4cfu/g, 8 x10^3cfu/g and 5 x 10^3cfu/g for (1.56%, 3.13% and 6.25% Lysozyme) respectively, while the count of the positive control and 0.8% Lysozyme remain constant (10^5cfu/g).

On the third day isolation, the count dropped to (1. 4 x0^4cfu/g, 8.5x10^2cfu/g and 1.3 x 10^3cfu/g), for (1.56% , 3.13% and 6.25% Lysozyme) correspondingly, positive control was (5 x 10^4cfu/g) while 0.8% Lysozyme showed significant increase of bacterial count to be 7.5 x 10^4cfu/g which highlights the significant role of Lysozyme in inhibition of *A. viridans* bacterial growth and persistence at certain concentrations at 1.56%, 3.13% and 6.25% , while the least concentration (0.8% Lysozyme) didn't have any effect.

Isolation of the first week showed complete inhibition of *A. viridans* at 6.25% Lysozyme, and the count of *A. viridans* was declined to be (1.5 x 10^3cfu/g), (1 x 10cfu/g) for (1.56% and 3.13% Lysozyme) respectively. On the other hand, positive control and 0.8% Lysozyme showed count at (5 x 10^4cfu/g). On the tenth day of isolation, there was a complete inhibition of *A. viridans* count on the plates, with a decrease in the positive control and 0.8% lysozyme count to be (4 x 10^4cfu/g).

D'Incecco *et al.* (2016) also investigated the inhibitory action of lysozyme in 16 hard cheeses produced from raw milk. According to the study, lysozyme-containing cheeses had more DNA from lysed bacterial cells and a lower count of cultivable microorganisms. Additionally, at the end of nine and sixteen months, lysozyme inhibited *Lactobacillus delbrueckii* and *Lactobacillus fermentum.*
From the obtained results, it was noticed that Lysozyme was able to inhibit the growth and multiplication of *A. viridans* in Kariesh cheese at a concentration of 6.25% Lysozyme within the 1st week and at 1.56% and 3.13% of Lysozyme within 10 days from the manufacturing date, and kept in the refrigerator at 4°C, thus prolonging the shelf life of cheese by inhibition and decreasing the number of Gram-positive spoilage bacteria present in cheese.

Animal sources contain lysozyme, a significant antibacterial that readily binds to casein micelles in dairy products like cheese (Doosh and Abdul-Rahman, 2014). It works particularly well against bacteria that are Gram-positive. Furthermore, by lowering the production of reactive oxygen species (ROS), it has been shown to possess antioxidant qualities (Benedé and Molina, 2020).

Moreover, the activity of lysozyme against Gram-negative bacteria can be enhanced by modifying it through various physical or chemical means. These modifications may increase its effectiveness in combating pathogenic bacteria, further establishing lysozyme as a valuable tool for food safety and preservation (Khorshidian *et al.,* 2022).

The addition of antimicrobial agents is crucial in controlling the growth of harmful and spoilage microorganisms in food. Dairy products, especially cheeses, offer a favorable environment for microbial growth, which highlights the importance of effective preservation methods (Franssen *et al.,* 2004).

The sensory evaluation conducted on Kariesh cheese samples produced aseptically in vitro, following the incorporation of MIC of Lysozyme. This assessment is supported by the presented data in Figure (2), which collectively illustrated the stability of the sensory characteristics of the cheese despite the addition of the antimicrobial agent by using Lysozyme at concentrations of (0.8%, 1.56%, 3.13% and 6.25%), the results were minimal to no alterations in the attributes of taste (9, 9, 9 and 9, respectively), color (8, 8, 8, 8 and 8, respectively), smell (7, 7.5, 8 and 9,

respectively), and texture (6, 6, 7 and 8, respectively) compared to other manufactured Kariesh cheese without Lysozyme addition, reinforcing the notion that the application of Lysozyme in cheese production is deemed acceptable overall (OAA): (7.5, 8, 8.5 and 9 respectively).

Lysozyme not only lowers bacteria, but also enhances sensory qualities, including texture and storage durability (Mehyar *et al.,* 2018 and Ozturkoglu-Budak *et al.,* 2021). Its capacity to attach to casein micelles could be the cause of this (Doosh and Abdul-Rahman, 2014 and Awad *et al.,* 2023). Also, in different studies, the presence of lysozyme in different products demonstrated exceptional storage stability and resilience to pH and temperature (Wang *et al.,* 2022).

CONCLUSION

Aerococcus species were found in 45% of total examined samples, and *A. viridans* was found in 13 samples only. Analyzing *Aerococci* genomes to determine phylogenetic relationships and predict virulence methods will be quite interesting. Additionally, the study sought to determine the lowest inhibitory doses necessary for the natural antibacterial agent Lysozyme to successfully stop *A. viridans* from growing in Kariesh cheese. 6.25% lysozyme exhibited a powerful bactericidal effect within 24 hours.

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دور الليسوزيم كمضاد بكتيري طبيعي ضد بكتيريا الايروكوكس فيريدانس المعزول من اللبن وبعض أنواع الجبن

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هدفت هذه الدراسة إلى تحديد وجود أنواع من المكورات الهوائية في ٢٠٠ عينة من اللبن الخام وأنواع مختلفة من الجبن، وتحديدًا الجبن القريش والدمياطي والموزاريلا (٥٠عينة من كل نوع). وتم العثور على أنواع المكورات الهوائية في ٤٥٪ من إجمالي العينات المفحوصة وتم العثور على الايروكوكس فيريدانس في ١٣ عينة فقط. استخدم اختبار تفاعل البلمرة المتسلسل للعزلات الإيجابية، أسفر البحث عن النتائج التالية من أصل ٩٠ عينة إيجابية تم تحليلها، تم تأكيد أن ١٣ عزلة هي من المكورات الهوائية، وكان التوزيع ٧،٤ ، ١ و ١من اللبن الخام، الجبن القريش، الجبن دمياطي و الجبن الموزاريلا، على التوالى. وقد أشار اختبار الحساسية ضد المضادات الحيوية أن جميع العزلات أظهرت حساسية كاملة لأوكسيتتراسيكلين، جنتاميسين وتتراسيكلين. اكتشفت العديد من مضادات الميكروبات القوية مؤخرًا، ولكن من المهم اختبار فعاليتها ضد البكتيريا غير المرغوب فيها داخل المواد الغذائية التي تساعد على تحسين سلامة الأغذية وصلاحيتها، لذا ركز هذا البحث على استخدام الليزوزيم ضد بكتيريا الايروكوكس فيريدانس ودوره المحتمل في إطالة العمر الافتراضي للجبن القريش. أثناء الاستخدام ، أظهر الليزوزيم بنسبة ٢٥.٦% تأثيرًا قويًا كمضادا للبكتيريا في غضون ٢٤ ساعة من التصنيع.