

# Isolation and survival of *Pseudomonas aeruginosa* in yogurt fortified with pomegranate dibs

Nagah M. Saad<sup>1</sup>, Walaa S. Hassan<sup>2\*</sup>

<sup>1</sup>Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Assiut University, Egypt.

<sup>2</sup>Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt.

## ARTICLE INFO

Received: 01 October 2025

Accepted: 30 October 2025

\*Correspondence:

Corresponding author: Walaa Shaban Hassan  
E-mail address: walaa-shaban@aun.edu.eg

Keywords:

*Pseudomonas aeruginosa*, Yogurt, Pomegranate dibs

## ABSTRACT

This study aimed to investigate the prevalence of *Pseudomonas aeruginosa* in 60 random yogurt samples (20 each of plain small-scale, plain large-scale, and fruit yogurts) collected from Assiut City, Egypt from December 2023 to March 2024. Identification of *Ps. aeruginosa* was performed using biochemical and molecular methods. Pomegranate dibs were applied to evaluate its inhibitory effect on *Ps. aeruginosa* growth during the manufacture and storage of yogurt in at refrigerator (4°C). Results revealed that *Ps. aeruginosa* could be isolated from 6 (10 %) of the 60 examined yogurt samples, which were identified by chemical tests. Using PCR, the results indicated that 2 (3.33%) of the examined yogurt samples were contaminated with this bacterium. Unexpectedly, *Ps. aeruginosa* was isolated from large-scale plain yogurt samples with an incidence of 10%. The minimum inhibitory concentration (MIC) of Pomegranate dibs against *Ps. aeruginosa* growth was 4%. The results of the Pomegranate dibs 6% yogurt samples that inoculated with *Ps. aeruginosa* was the most effective one in which 6% pomegranate dibs sharply reduced the count of *Ps. aeruginosa*, until completely inhibited at the 7<sup>th</sup> day of yogurt storage, compared to the control group, in which the organism still alive until the end of storage period (15<sup>th</sup> day). The sensory evaluation of yogurt prepared by adding different concentrations of pomegranate dibs revealed that the manufactured yogurt acquired a higher score for flavor, body, and texture than the control, using the natural affordable pomegranate dibs as antibacterial against *Ps. aeruginosa* growth has been found.

## Introduction

One of the keys to the global market trend is the acquisition of unique food ingredients and flavors with enhanced health benefits (Netzel *et al.*, 2007). Yogurt is one of these food ingredients, which is classified as the most specific popular fermented milk product enjoyed all over the world for its refreshing taste and nutritional and therapeutic health benefits. On the other side, it provides an ideal condition for the growth of some pathogenic bacteria including *Pseudomonas aeruginosa* (*Ps. aeruginosa*) (Mcphee and Griffiths, 2011). This bacterium is a Gram-negative, ubiquitous rod-shaped psychrotroph (Diggle and Whiteley, 2020). It is a common pathogen of public health concern associated with nosocomial infection (Tummler *et al.*, 2014). In humans, it is a potential foodborne pathogen that can cause food poisoning (Streeter and Katouli, 2016). In Egypt, several cases of food poisoning linked to *Ps. aeruginosa* have been reported due to the consumption of dairy products (Ahmed *et al.*, 1989), furthermore, it can cause serious infections including pneumonia, endocarditis, septicemia, otitis, and keratitis (Bush and Vazquez-Pertejo, 2024). Moreover, *Ps. aeruginosa* is a hidden cause of subclinical mastitis in dairy animals, resulting in considerable economic losses (Fatima *et al.*, 2012).

This pathogen poses a challenge for antibiotic therapy owing to its antimicrobial resistance, in addition to its ability to form a biofilm (Carminati *et al.*, 2019), which exhibits its enhanced survival in adverse environmental conditions (Melchior *et al.*, 2006). Many studies have been conducted to control its survival, owing to the problems caused by *Ps. aeruginosa*. The use of Pomegranate is one of these ways, as it has been allocated the topic of some studies it is one of the important medicinal fruit and is known to have health-promoting properties such as anti-inflammatory, antimutagenic, antioxidant, and antimicrobial effects (Mastrogiovanni *et al.*, 2019). In addition to its well documented traditional use for the treatment of diarrhea, dysentery and respiratory diseases (Dey *et al.*, 2015), Pomegranate dibs is a highly nutritive product since it is

processed as a concentrate due to the presence of high mineral content, making it with an exceptional nutritional property, it is used as a garnish for desserts and many salad dishes. Pomegranate dibs is rich in polyphenols, such as anthocyanin and ellagitannin, which are the major class of its phytochemicals with antioxidant activity (Pirzadeh *et al.*, 2020), in addition to citric acid, lactoferrin, and lactoperoxidase, which act as antimicrobials (Dahham *et al.*, 2010). The advantages of pomegranate dibs incorporation in foods of animal origin include its antimicrobial activity, support for the activity of lactic acid bacteria, shelf-life extension, and improvement of sensory attributes. Hence, this study aimed to isolate and identify *Ps. aeruginosa* in yogurt and to study the effect of Pomegranate dibs on its viability during the preparation and storage of laboratory-manufactured yogurt.

## Materials and methods

### Isolation and identification of *Ps. aeruginosa* in yogurt

#### Collection of samples

Sixty yogurt samples divided into 3 groups (20 each of small-scale, large scale and fruit yogurt) were collected from different supermarkets in Assiut City, Egypt, during the period from December 2023 to March 2024 and transferred directly to the laboratory of the Food Hygiene, Safety, and Technology Department at the Faculty of Veterinary Medicine, Assiut University, Egypt, under complete a septic condition without delay and examined as quickly as possible.

#### Isolation of *Ps. aeruginosa* from the collected yogurt samples

10 g of each yogurt sample was inoculated into 90 ml of Tryptone soya broth (Himedia Pvt.Ltd, India) and incubated at 42°C for 24 hrs

(Oberhofer, 1979), then a loopful of culture was taken and streaked on the *Pseudomonas* agar F. base, incubated at 42°C for 48 hrs (King *et al.*, 1954). The presence of *Ps. aeruginosa* was identified by the production of blue-green pigment and a distinctive fruity smell on the media due to 2 amino acetophenone productions.

Morphological and biochemical characterization: The suspected colonies on *Pseudomonas* agar F. base were collected and screened for Gram staining and biochemical identification, which included the Indole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Triple Sugar Iron test, and Catalase test, according to the Protocol mentioned in Medical Microbiology (Cruikshank *et al.*, 1975).

#### Molecular identification of the isolated *Ps. aeruginosa* (Lavenir *et al.*, 2007)

This part has been done in the Molecular Biology Unit at Assiut University, Egypt. DNA from *Ps. aeruginosa* isolates was extracted by the boiling method and used as a template for species specific PCR amplification.

DNA extraction: Isolated *Ps. aeruginosa* strains were grown overnight at 37°C in Luria Bertani broth. DNA was extracted from the bacteria and confirmed biochemically by the boiling method, where 1 ml of the broth was taken and the sample was centrifuged until a pellet was obtained, 100 µl of PBS (Phosphate Buffer Saline) was added to the pellet, then placed on a thermal block (Biometra, Germany) at 95°C for 10 minutes. After incubation, it was centrifuged, and the supernatant was taken, which is the DNA, and stored at -20°C until use.

#### PCR Amplification

Conventional PCR was run in a thermal cycler (Veriti™ Thermal Cycler, Applied Biosystems, USA) using a bacterial primer specific for *Ps. aeruginosa*; forward (5' ATGGATGAGCGCTTCCGTG 3'), reverse (5' TCATCCTTCG-CCTCCCTG 3') to determine the presence or absence of *ecfX* gene which is restricted to *Ps. aeruginosa* and might play a role in virulence. PCR reactions were carried in a final volume of 25 µl including 12.5 µl of 2× PCR master mix (Red Master, Cosmo, England), 90 ng of DNA template (Spectrophotometer, Gene Quant 1300, England), 1 µl of each primer, and the volume was completed by nuclease-free water. The PCR conditions were as follows: 95°C for 5 min of initial denaturation; 40 cycles of 95°C for 45 sec of denaturation, annealing 48°C, 1 min for 72°C of extension; and final extension 72°C for 5min.

#### Analysis of PCR products

PCR products were run in a 1% agarose gel in TBE (Tris Borate EDTa) (pH = 8.3) and amplified using a 110-volt power supply for 1 h. A 120 Amp (VWR-300) was stained using ethidium bromide, and the image was visualized under UV illumination (UV Band-Elutor 91, Biometra) and a documentation system (Viber Loumat Kaiser-Viberloument).

#### Effect of the addition of pomegranate dabs on *Ps. aeruginosa* viability in laboratory- prepared yogurt during refrigerated storage

Preparation of the bacterial strain (International Standard, 2019): *Ps. aeruginosa* pure strain was obtained from the Reference Laboratory for Food Safety, Animal Health Research Institute (AHRI), Dokki, Giza, Egypt. The strain was maintained on tryptic soya agar slants containing 3% NaCl at 4°C. Directly, before the experiment, fresh microbial culture was adjusted to 0.5 (10<sup>8</sup> CFU/ml) McFarland and diluted to be equivalent to about 10<sup>6</sup> CFU/ml with tube dilution methods and considered as the dose to be inoculated into yogurt samples.

Pomegranate dabs: A bottle of pomegranate dabs (Lebanon, El-Fars Arabian for limited trade was obtained from the local market in Assiut City, Egypt.

#### Minimum Inhibitory Concentration (MIC) of Pomegranate dabs (CLSI, 2011).

Different concentrations of pomegranate dabs were prepared using sterile water. The study in vitro was evaluated to study the antibacterial activity of the dabs against *Ps. aeruginosa* using the agar diffusion method. Where 0.1 ml of the previously prepared bacterial strain was streaked into Muller Hinton agar plates then, 80 µl of different pomegranate dabs concentration was added in each well. The plates including the control were incubated for 24 h at 42°C. MIC was determined by observing the lowest concentration of pomegranate dabs that could inhibit the visible bacterial growth, which was (4%).

#### Yogurt preparation

Yoghurt was prepared according to Kusuma *et al.* (2010). 500 ml milk was heat treated at 90°C for 10 min., cooled to 43°C and aseptically inoculated with the starter culture (mixed culture of *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii bulgaricus* 1:1) at a rate of 3% of the milk volume. After gentle stirring, distribute the starter evenly. Before the addition of the prepared inoculum of *Ps. aeruginosa* to milk in a count of 10<sup>6</sup> CFU/ml, 100 ml milk was kept in a sterile plastic cup to be the negative control, after that the inoculated milk was divided into 4 parts and different treatments as follows:

Part 1 is the positive control (inoculated milk without any treatment) and parts 2, 3, and 4 were fortified with 2, 4, and 6 % pomegranate dabs, respectively, mixed well and incubated at 43°C till curdling, then kept in refrigerator (4°C) with the control samples and were examined for *Ps. aeruginosa* count in the 1<sup>st</sup>, 2<sup>nd</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 13<sup>th</sup>, and 15<sup>th</sup> days, 3 trials were made.

#### Sensory evaluation of pomegranate yogurt

The sensory evaluation of the fortified yoghurt samples was carried out as described by Nelson and Trout (1981), who used a quality rating scorecard for the evaluation of flavor (60 points) and body and texture (30 points) and appearance (10 points) and overall acceptability 100 points. Yoghurt samples were evaluated by 10 experienced food panelists from the Food Hygiene, Safety and Technology Department, Faculty of Vet. Medicine, Assiut University, Egypt.

#### Statistical analysis

Statistical analysis was conducted using the Statistical Program for Social Science (SPSS Inc., Chicago, IL, USA).

## Results and Discussion

Milk and milk products can be referred to as carriers of pathogenic and spoilage-causing microbes due to their high nutritional content. Raw milk is cooled as soon as it is collected to preserve its quality. Cooling retards microbial spoilage but on the other hand, it creates a favorable growth environment for potential psychrotrophs in the milk system. These microbial populations can secrete several proteases and lipases, which are highly heat stable and cannot be degraded using pasteurization. Several psychrotrophs, including *Pseudomonas* sp., have the potential to secrete exopolysaccharide, which has a further role in creating a biofilm across the milk contact surfaces (Saha *et al.*, 2024). Yogurt is a cultured milk product traditionally prepared using *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. It is rich in bioactive proteins, hydrolyzed proteins, vitamins, and minerals, which contribute to its popularity among consumers globally (Shishir *et al.*, 2024). The quality of the yogurt depends on the good quality of the raw material and efficient control at all processing stages and the manipulation of the curd after fermentation. Yogurt may be subjected to microbial contamination at various points

along the supply chain, causing a great risk to human health (Brian, 2024). *Ps. aeruginosa* is one of these pathogens that can be found in different environments and food sources.

As a preliminary survey, the results in Table 1 and Figure 1 indicated that *Ps. aeruginosa* could be detected in 3.33% of the examined yogurt samples. Unexpectedly, it is notable that this bacterium is detected only in 10% of the examined plain large-scale yogurt samples as the size of the PCR product obtained from *Ps. aeruginosa* isolates was 528 bp targeting *ecfX* gene which encodes an ECF (extracytoplasmic function) sigma factor which confirmed the identity of *Ps. aeruginosa* and might play a role in haem-uptake (Lavenir *et al.*, 2007) and could not be isolated from small-scale and fruit yogurt samples. Comparing the obtained data with the others, Ibrahim *et al.* (2022) could isolate *Ps. aeruginosa* from 16% of the examined plain yogurt samples. Meanwhile, Atia *et al.* (2023) revealed that *Ps. aeruginosa* could be detected in 25% of the examined yogurt samples. The current study proved that unhygienic handling of yogurt may lead to its contamination by *Ps. aeruginosa*. Infection with this

pathogen is difficult to treat because of resistance to antibiotics (Pang *et al.*, 2019). The consumption of *Ps. aeruginosa*-contaminated food may be the main cause of human illness causing severe enteritis.

Yogurt is easily susceptible to bacterial growth during the processing steps. So, an attempt was made to improve its safety by employing pomegranate dibs to control *Ps. aeruginosa* growth during its preparation and storage at refrigerator temperature (4°C). First, the in vitro preliminary screening of the antibacterial activity of pomegranate dibs was tested against *Ps. aeruginosa*. The minimum inhibitory concentration (MIC) was 4%. In agreement with the present results, Abu El-Wafa *et al.* (2020) found that the MIC of pomegranate dibs against *Ps. aeruginosa* reached 6.25 mg/ml, while Nozohour *et al.* (2018) showed that the MIC of pomegranate peels and seeds were 12.5 and 25 mg/ml, respectively. Moreover, Edham *et al.* (2020) found that the MIC of pomegranate dibs was 12 mm.

Results presented in Table 2 illustrate that the control yogurt samples (without any treatment) did not show a noticeable change of *Ps.*

Table 1. Prevalence of *Ps. aeruginosa* in the examined yogurt samples.

Examined samples	No. of examined samples	Positive samples by biochemical		Positive samples by PCR	
		No.	%	No.	%
Plain small-scale yogurt	20	-	-	-	-
Plain large-scale yogurt	20	6	30	2	10
Fruit yogurt	20	-	-	-	-
Total	60	6	10	2	3.33

Table 2. Effect of pomegranate dibs on the growth of *Ps. aeruginosa* during preparation and storage of yogurt.

Storage time/day	Treatments (log <sub>10</sub> CFU/g)						
	Positive control yogurt	Pomegranate dibs (2%)	Reduction (%)	Pomegranate dibs (4%)	Reduction (%)	Pomegranate dibs (6%)	Reduction (%)
1 <sup>st</sup> day	6.8	6.5	4.41	5.8	14.71	5.3	22.00
2 <sup>nd</sup> day	6.6	5.6	15.15	4.8	27.27	4	39.40
4 <sup>th</sup> day	5.9	4.8	18.64	3.8	35.60	2.7	54.24
7 <sup>th</sup> day	5	3.8	24.00	2.6	48.00	-	100.00
10 <sup>th</sup> day	4.7	2.7	42.60	-	100.00	-	100.00
13 <sup>th</sup> day	3.8	-	100.00	-	100.00	-	100.00
15 <sup>th</sup> day	3.6	-	100.00	-	100.00	-	100.00

Table 3. Sensory evaluation of control and fortified yogurt samples with pomegranate dibs.

Parameters	Treatments	Storage period/day						
		1 <sup>st</sup> day	2 <sup>nd</sup> day	4 <sup>th</sup> day	7 <sup>th</sup> day	10 <sup>th</sup> day	13 <sup>th</sup> day	15 <sup>th</sup> day
Flavor (60 points)	Control	54	55	56	56	53	48	45
	2%	54	56	56	56	55	50	47
	4%	56	57	57	57	56	52	51
	6%	56	57	58	58	56	53	53
Body & texture (30 points)	Control	25	28	28	28	28	27	25
	2%	26	27	28	28	28	28	26
	4%	27	28	29	29	28	28	26
	6%	28	28	29	29	29	28	26
Color and Appearance (10 points)	Control	7	8	8	8	7	7	7
	2%	8	8	8	8	8	7	7
	4%	8	8	8	8	8	7	7
	6%	8	9	9	9	8	8	7
Total score (100 points)	Control	86	91	92	92	88	82	77
	2%	88	91	92	92	91	85	80
	4%	91	93	94	94	92	87	84
	6%	92	94	96	96	93	89	86

*aeruginosa* counts after milk inoculation with  $6.8 (\log_{10}/g)$ , until the 4<sup>th</sup> day of storage at the refrigerator, there was a gradual decrease in the count, reaching  $3.6 \log/g$  in the 15<sup>th</sup> day of storage. *Ps. aeruginosa* behaved differently in yogurt fortified with Pomegranate dibs, which varied from inhibition to survival depending on its concentration. The obtained results of the yogurt groups treated with pomegranate dibs indicated that all concentrations exhibited antibacterial activity against the tested organism. 6% was the best concentration that inhibited the growth of *Ps. aeruginosa* at the 7<sup>th</sup> day of storage, followed by 4% and 2% concentrations, which completely inhibited the growth of *Ps. aeruginosa* at the end of the 10<sup>th</sup> and 13<sup>th</sup> days, respectively.

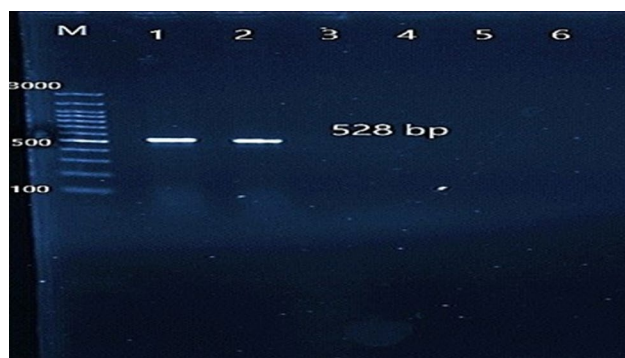


Figure 1. Agarose gel electrophoresis of the PCR product of the *ecfX* gene for the isolated *Ps. aeruginosa*.

Lane M: Ladder Marker; Lane 1 and 2: Positive samples for *Ps. aeruginosa* (528bp); Lane 3-6: Negative samples for *Ps. aeruginosa*.

The current study results are consistent with Silva *et al.* (2016) who found the antagonistic activity of pomegranate dibs against *Ps. aeruginosa*. The antibacterial effect of pomegranate dibs may be related to its contents of phytochemicals such as tannins, polyphenols, and alkaloids (Dahham *et al.*, 2010). These results indicated that pomegranate dibs can be used to aid in the treatment of *Ps. aeruginosa* infection, which was in harmony with Howell and Souza (2013). There is a growing market demand for dairy products with functional health benefits (Ziarno *et al.*, 2023) tailored to adverse demographic groups, including children, athletes, the elderly, and those seeking immune support (Gupta *et al.*, 2023).

Sensory acceptance plays an important role in consumer acceptance, Table 3 shows that the sensory evaluation of treated yogurt with pomegranate dibs was accepted compared to plain (control) yogurt during the storage period at the refrigerator. This was in accordance with Joung *et al.* (2016). El-Nagga and Abdel-Tawab (2012) revealed that yogurt with 2% and 3% pomegranate dibs were highly accepted as they had the highest score for flavor, appearance, body, and texture compared with the control. In consequence, the yogurts supplemented with pomegranate dibs were judged to have better sensory properties than plain yogurt.

## Conclusion

Yogurt may become a good carrier for pomegranate dibs. Therefore, the addition of pomegranate dibs is recommended and developed for consumers as a functional yogurt with desirable properties and long shelf life.

## Conflict of interest

The authors have no conflict of interest to declare.

## References

- Abu El-Wafa, W.M., Ahmed, R.H., Ramadan, M.A., 2020. Synergistic effects of pomegranate and rosemary extracts in combination with antibiotics against antibiotic resistance and biofilm formation of *Pseudomonas aeruginosa*. *Braz. J. Microbiol.* 51, 1079–1092.
- Ahmed, S.H., Ahmed, A.-H., Mostafa, K.M., El-Tahtawy, M.Y., 1989. Microbiological studies on *Pseudomonas aeruginosa* food poisoning associated with consumption of milk and milk products with reference to pyocine typing. *Assiut Med. J.* 13, 71–81.

- Atia, R., Mohamed, H., Abo-Elroos, N., Awad, D., 2023. Growth patterns of *Pseudomonas aeruginosa* in milk fortified with chitosan and selenium nanoparticles during refrigerated storage. *World J. Microbiol. Biotechnol.* 39, 312.
- Brian, A., 2024. Determination of microbial contamination in raw milk, processed milk and yogurt consumed in Mbarara City, western part of Uganda. *Inostr Appl. Sci.* 12, 104–11.
- Brush, L.M., Vazquez-Pertejo, M.T., 2024. *Pseudomonas* and related infection. *MSD Manual*
- Carminati, D., Bonvini, B., Rossetti, L., Zago, M., Tidona, F., Giraffa, G., 2019. Investigation on the presence of blue pigment-producing *Pseudomonas* strains along a production line of fresh mozzarella cheese. *Food Control* 100, 321–328.
- Clinical and Laboratory Standards Institute (CLSI), 2011. Performance standards for antimicrobial susceptibility testing. 21<sup>st</sup> informational supplement, M100-S21. CLSI, Wayne, PA, USA.
- Cruikshank, R., Duguid, J.P., Marmion, B.P., Swain, R.H.A., 1975. *Medical Microbiology*. Churchill Livingstone, Edinburgh, London, and New York.
- Dahham, S.S., Ali, M.N., Tabassum, H., Khan, M., 2010. Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.). *Am.-Eurasian J. Agric. Environ. Sci.* 9, 273–281.
- Dey, D., Ray, R., Hazra, B., 2015. Antimicrobial activity of pomegranate fruit constituents against drug-resistant *Mycobacterium tuberculosis* and  $\beta$ -lactamase-producing *Klebsiella pneumoniae*. *Pharmaceutical Biology* 53, 1474–1480.
- Diggle, S.P., Whiteley, M., 2020. Microbe profile: *Pseudomonas aeruginosa*—opportunistic pathogen and lab rat. *Microbiology (Reading)* 166, 30–33.
- Edham, M.H., Jafar, N.B., Fadhil, Z.H., 2020. Studying the inhibition effect of some food additives against pathogenic bacteria. *Ann. Trop. Med. Public Health* 23, S455.
- El-Nagga, E.A., Abd El-Tawab, Y.A., 2012. Compositional characteristics of date syrup extracted by different methods in some fermented dairy products. *Ann. Agric. Sci.* 57, 29–36.
- Fatima, A., Naqvi, S.B., AbdulKhalik, S., Perveen, S., Jabeen, S., 2012. Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeruginosa* isolated from patients with lower respiratory tract infections. *SpringerPlus* 1, 1–4.
- Gupta, A., San-wal, N., Bareen, M., Barua, S., Sharma, N., Olatunji, O., Nirmal, N., Sahu, J., 2023. Trends in functional beverages, functional ingredients, processing technologies, stability, health benefits, and consumer perspective. *Food Res. Int.* 170, 113046.
- Howell, A., Souza, D., 2013. The pomegranate: Effects on bacteria and viruses that influence human health. *Evid.-Based Complement. Altern. Med.* 2013, Article ID 606212, 11 pages.
- Ibrahim, M.M., Elsaied, E.I., Abd El Aal, S.F., Bayoumi, M.A., 2022. Prevalence of *Pseudomonas aeruginosa* in milk and some dairy products with reduction trials by some natural preservatives. *J. Adv. Vet. Res.* 12, 343–438.
- International Standard, 2019. ISO 20776-1: Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices—Part 1: Broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. 2<sup>nd</sup> ed. International Organization for Standardization, Geneva, Switzerland.
- Joung, J., Lee, J., Ha, Y., Shin, Y., Kim, Y., Kim, S., Oh, N., 2016. Enhanced microbial, functional and sensory properties of herbal yogurt fermented with Korean traditional plant extracts. *Korean J. Food Sci. Anim. Resour.* 36, 90–99.
- King, E.O., Ward, M.K., Raney, D.E., 1954. Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin. Med.* 44, 301.
- Kusuma, I., Arung, E., Rosamah, E., Purwatiningsih, S., Kuspradini, H., 2010. Antidermatophyte and antimelanogenesis compound from *Eleutheria americana* grown in Indonesia. *J. Nat. Med.* 64, 223–226.
- Lavenir, R., Jocktane, D., Laurent, F., Nazaret, S., Cournoyer, B., 2007. Improved reliability of *Pseudomonas aeruginosa* PCR detection by the use of the species-specific *ecfX* gene target. *J. Microbiol. Methods* 70, 20–29.
- Mastrogiovanni, F., Mukhopadhyay, A., Lacetera, N., 2019. Anti-inflammatory effects of pomegranate peel extracts on in vitro human intestinal Caco-2 cells and ex vivo porcine colonic tissue explants. *Nutrients* 11.
- McPhee, J.D., Griffiths, M.W., 2011. Psychrotrophic bacteria (*Pseudomonas* spp.). In: *Encyclopedia of Dairy Sciences*, 2<sup>nd</sup> ed. Academic Press, London, pp. 379–383.
- Melchior, M.B., Vaarkamp, H., Fink-Gremmels, J., 2006. Biofilms: A role in recurrent mastitis infections. *Vet. J.* 171, 398–407.
- Nelson, J.A., Trout, G.M., 1981. *Judging of Dairy Products*, 4<sup>th</sup> ed. INC Westport, Academic Press, pp. 345–567.
- Netzel, M., Netzel, G., Tian, Q., Schwartz, S., Konczak, I., 2007. Native Australian fruits: A novel source of antioxidants for food. *Innov. Food Sci. Emerg. Technol.* 8, 339–346.
- Nozohour, Y., Golmohammadi, R., Mirnejad, R., Fartashvand, M., 2018. Antibacterial activity of pomegranate (*Punica granatum* L.) seed and peel alcoholic extracts on *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from health centers. *J. Appl. Biotechnol. Rep.* 5, 32–36.
- Oberhofer, T.R., 1979. Growth of nonfermentative bacteria at 42°C. *J. Clin. Microbiol.* 10, 800–804.
- Pang, Z., Raudonis, R., Glick, B.R., Lin, T., Cheng, Z., 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: Mechanisms and alternative therapeutic strategies. *Biotechnol. Adv.* 37, 177–192.
- Pirzadeh, M., Caporaso, N., Rauf, A., Shariati, M.A., Yessimbekov, Z., Khan, M.U., Imran, M., Mubarak, M.S., 2020. Pomegranate as a source of bioactive constituents: A review on their characterization, properties and applications. *Crit. Rev. Food Sci. Nutr.* 1–18.
- Saha, P., Majumder, R., Rout, P., Hossain, S., 2024. Unveiling the significance of psychrotrophic bacteria in milk and milk product spoilage – A review. *Microbe* 2, 100034.
- Shishir, M.R.I., Saifullah, M., Hashim, S.B.H., Aalim, H., Bilal, M., Khan, S., Marappan, G., Tahir, H.E., Zhihua, L., Zhai, X., Arslan, M., Taip, F.S., Cheng, K.W., Zou, X., 2024. Micro- and nano-encapsulated natural products in yogurt: An emerging trend to achieve multifunctional benefits in product quality and human health. *Food Hydrocoll.* 154, 110124.
- Silva, P., Napoleão, T., Silva, L., Fortes, D., Lima, T., Zingali, R., Pontual, E., Araújo, J., Medeiros, P., Rodrigues, C., Gomes, F., Paiva, P., 2016. The juicy sarcotesta of *Punica granatum* contains a lectin that affects growth, survival as well as adherence and invasive capacities of human pathogenic bacteria. *J. Funct. Foods* 27, 695–702.
- Streeter, K., Katouli, M., 2016. *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. *Infect. Epidemiol. Med.* 2, 25–32.
- Tümmeler, B., Wiehlman, L., Klockgether, J., Cramer, N., 2014. Advances in understanding *Pseudomonas*. *F1000Prime Rep.* 6, 9.
- Ziarno, M., Kozłowska, M., Ratusz, K., Hasalliu, R., 2023. Effect of the addition of selected herbal extracts on the quality characteristics of flavoured cream and butter. *Foods* 12, 471.