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Efficacy of pumpkin and onion essential oils against isolated *Pseudomonas aeruginosa* in cheese: A comparative study

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ABSTRACT

Greater resistance to environmental aggressors, including resistance to antibiotics and other disinfectants is a characteristic of the *Pseudomonas* spp. Consequently, one of the current study's goals was to isolate *Pseudomonas* spp. from 165 cheese samples (55 each of Tallaga, Bramily, and Ras cheese) were collected from various dairy markets and shops in Assiut city, Egypt. The isolates were identified and tested for 16S rRNA, showing positive results. According to the collected data, *Pseudomonas* spp. was found in 42.42% of the samples that were investigated, while *Pseudomonas aeruginosa* was found in 10.9% of the samples. Then, the effects of essential oils (EOs) of pumpkin (P) and onion (EO) on the viability of *Pseudomonas aeruginosa* were investigated, the minimum inhibitory concentration (MIC) of the prepared onion EO (OEO) and pumpkin EO (PEO) was detected. Tallaga cheese samples were produced utilizing the MIC of the two prepared Eos separately. The agar well diffusion method was used to assess the influence of EOs, and the results showed that the MIC for PEO and OEO was 0.39%. In conclusion, after Tallaga cheese manufacturing, the OEO proved to be the most effective against *Pseudomonas aeruginosa*. with a count of 5 log10 at 0 h and <1 after the first week.

Introduction

Cheese is one of the oldest dairy preserved foods. It was known in early civilizations. The world's oldest cheese was recently discovered in an Egyptian tomb, in 2018 researchers published proteomic analyses of the solid white mass as the ancient cheese Abdou (2024). Cheese components have demonstrated significant nutritional significance in addition to their health benefits, studies conducted by Du *et al.* (2024) have demonstrated the anticarcinogenic and antiatherogenic qualities of some fatty acids found in cheese, including conjugated linoleic acid. According to Mehanna and Rashed (1990), several varieties of soft cheese are produced in Egypt, the soft cheese known as Tallaga cheese, which is kept in cold storage. Tallaga cheese is a soft cheese from Egypt that isn't ripened. It is often created from warm milk and a small amount of salt, then refrigerated until it's ready to be consumed, which is generally two weeks away. Domiati cheese, which is salted and coagulated by enzymes; differs from one another in terms of the salt percent added to the milk used in cheese making as well as the length of time and conditions under which it is stored (El-Sharoud, 2009).

Abou-Donia (1986) mentioned that Domiati cheese, also known as Bramily cheese, is the most well-liked soft white pickled cheese in Egypt. It is manufactured from the milk of buffaloes or cows, or a combination of both, and can be eaten either fresh or after a three to six month ripening period in fermenting solution. Certain elements, like the type of milk, the time it takes for it to ripen, and the season, influence the salt concentrations utilized in the production of Domiati cheese (Ismail, 2004). Ras cheese is an Egyptian hard cheese also known by its dialectic name, Romy. It is produced in large quantities using artisan methods (Hattem *et al.*, 2012).

Despite good manufacturing practices and rigorous cleaning and sanitizing procedures established in dairy processing plants, microbiological contamination remains the main cause of products being non-compliant and/or atypical and hence not fit for human consumption. The presence of pathogenic and spoilage microorganisms can lead to non-compliant and/or atypical dairy products, which are processed dairy products that do not meet the required standards in Canada and/or have a reduced shelf life and quality defects caused by the growth of microorganisms (Sanschagrin *et al.*, 2024).

The multiplication and growth of microbes in cheese can be determined by many issues including the pH, water activity, storage temperature and availability of nutrients. Cheeses are favorable medium to the bacterial putrefaction as a result of low concentration of salt and neutral pH and high moisture content (Adam *et al.*, 2024).

Cheese spoilage caused by *Pseudomonas* spp. has increased in recent years (Carrascosa *et al.*, 2021). Their contamination routes have been largely studied, especially for milk or milk-based beverages (Martin *et al.*, 2021). In contrast, how these bacteria manage to infect cheeses even made from pasteurized milk is still much discussed. Since these are environmental microorganisms generally present in water and soil, the main sources of contamination are surfaces, and exposure to air.

Pseudomonas is one of the most significant bacterial totals in the dairy sector because it has been discovered as the primary Psychrotrophic bacteria associated with dairy products (Kováčová *et al.* 2024). *Pseudomonas* spp. have high genetic diversity and metabolic ability, allowing them to survive in different environments and grow at an ambient temperature of around 4°C (Navrhus *et al.* 2021). Furthermore, it causes a great hazard to animals and human health with great economic losses. The infection occurred also through cheeses because it's nature already for eating (Vrdoljak *et al.*, 2016). *Pseudomonas* one of microorganisms which are predominant in cold temperatures with a wide range of biotechnological applications (Chauhan *et al.* 2023).

Pseudomonads contamination occurs at each step of the manufacturing process of dairy products, and it becomes more persistent and resistant to sanitation procedures when bacterial cells grow as biofilm. Also, increasing amounts of enzymes as proteases and lipases with the activation of metabolic pathways related to spoilage behaviors (e.g., pig-

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ments biosynthesis) have been found in biofilm and in the fluctuating state (Quintieri *et al*., 2019). The mechanism responsible for the production and activation of spoilage enzymes, metabolites, and pathways are still poorly explored (Machado *et al*. 2020).

Pseudomonas species cause significant financial losses and pose a serious risk to both human and animal health. Due to improper handling and manufacture, *Pseudomonas* species can spread through dairy products (Quintieri *et al*., 2019). Since cheeses are prepared for consumption, the virus also spreads through them (Vrdoljak *et al*., 2016). It was previously believed that *Pseudomonas aeruginosa* biofilm contributed to the development of many chronic infectious diseases and complicated the treatment of bacterial infections. Due to the inner surface of biofilms preventing drug molecules from accessing it, antibiotics are unable to stop bacterial growth (Yuan *et al*., 2019).

Plant oils mainly composed of fatty acids esterified into a glycerol molecule including subgroups such as saturated, polyunsaturated, and monounsaturated acids. They differ in length of chain, stereoisomerisation of double bonds (cis/trans), and location of the first double bond (e.g., n-3, n-6, n-7, and n-9). They are divided into essential and non-essential fatty acids. The non-essential fatty acids group is not required in diet since the human organism is able to synthesize them, while n-6 and n-3 fatty acids are categorized as essential fatty acids (Kapoor *et al*. 2021).

Pumpkin essential oil (PEO), a rich source of nutrients, is extracted from the seeds of different pumpkin varieties for food and medicines (Hu *et al*., 2024). It is a rich source of β-carotene, dietary fiber, pectin, mineral salts, vitamins, and other health promoting elements (Kundu *et al*., 2014). Pumpkin has given the researchers access to a variety of broad spectrum anti-microbial components. *Enterococcus faecalis, Salmonella enterica, Acinetobacter baumanii, Aeromonas veronii, Escherichia coli*, *Pseudomonas aeruginosa*, bio group Sorbia, *Candida albicans, Klebsiella pneumoniae*, and *Serratia marcescens* are all inhibited by pumpkin oil (Hu *et al*., 2024). According to Tadee *et al*. (2020), the components that were isolated from pumpkin shown antibacterial action against both Gram-positive and Gram-negative bacteria. Biologically active substances found in extracts from various pumpkin sections have been shown to have antidiabetic, antibacterial, hypocholesterolemic, antioxidant, anticancer, antimutagenic, immunomodulatory, and other diverse properties (Krimer-Malešević, 2020).

The onion, or *Allium cepa*, is one of the earliest plants to be grown and has several uses. Onions have been shown to have antibacterial and antioxidant properties in addition to their nutritional benefits, and research into these properties is still ongoing. Apart from their nutritional benefits, the antimicrobial and antifungal properties against a range of Gram-positive and negative bacteria have been thoroughly studied and are still being done so (Whitemore and Naidu, 2000). The antibiotic activity of 1 mg of allicin, a (+)-S-methyl-l-cysteine Han *et al*. (1995). Aqueous extracts have also been shown in recent studies to have an inhibitory impact on a variety of bacterial and fungal species (Ward *et al*., 2002).

The current study set out to isolate *Pseudomonas* spp. from different type of cheese samples, identify the isolates using biochemical assays, and use pumpkin and onion oil in cheese manufacturing because of their potent antibacterial properties against *Pseudomonas aeruginosa*.

Materials and methods

Sample collection and preparation

A total of 165 samples from Tallaga, Bramily and Ras cheese (55 sample each) were randomly collected from Assiut governorate, Egypt. The collected samples transferred in ice tank and rapidly transferred to the laboratory. Ten g cheese were taken in a representative manner and homogenized with 90 ml 0.1% peptone solution using a stomacher according to Meier *et al*. (2018).

Isolation and identification of Pseudomonas species (ISO 11059:2009)

Isolation of *Pseudomonas* species

A test tube containing one gram of the manufactured product was filled with nine milliliters of enrichment tryptone soya broth 10%, then was incubated at 37°C for a full day. Onto *Pseudomonas* CN agar medium, a loopful was streaked following incubation. After 24 hours of incubation at 30°C, the infected plates were removed, and any suspected colonies were streaked onto tryptose soya agar (TSA) slants. Another 24 hours of incubation at 30°C were then completed.

Morphological examination of colonies

Pseudomonas spp. was indicated by any growth on CN medium. Presumptive evidence for *Pseudomonas aeruginosa* was considered by the presence of blue-green or brown pigmentation, or fluorescence was taken as presumptive evidence of *P. aeruginosa*. Following purification, the suspicious colonies were subcultured onto nutrient agar slopes and incubated for 24 hours at 37°C. The isolated colonies underwent for further identification.

Identification of Pseudomonas species (Cheesbrough, 2000)

Morphological identification

Microscopical examination and motility test were done according to ISO (2003)) and Baron *et al*. (1994), respectively. This step was completed at the Food Analysis Center of the Banha University of Egypt's Faculty of Veterinary Medicine.

Biochemical identification

The purified *Pseudomonas* colonies were identified biochemically by pigment formation on nutrient agar (King *et al*., 1954), Oxidase test (Harrigan and McCance, 1976 (, H2S production test (Oxoid, 1998), Arginine hydrolysis (Harrigan and McCance, 1976), Gelatin liquefaction (Quinn *et al*., 1994), 10% Lactose agar test (Mac Faddin, 2000), Starch hydrolysis test) Mac Faddin, 2000) and Sugar Fermentation test (APHA., 1992).

Pseudomonas species molecular identification

Isolates that were preliminary identified as *Pseudomonas aeruginosa* were subjected to molecular analysis to confirm their identification and genetic profiles. Polymerase chain reaction was conducted to confirm the isolates as belonging to the genus *Pseudomonas aeruginosa*. This part was carried out in the Molecular Biology Research Unit (MBRU), Animal Health Institute, Giza, Egypt.

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 20 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany).

Primers sequences, target genes, amplicon sizes and cycling conditions

According to Spilker *et al*. (2004), *Pseudomonas* spp. was verified by utilizing the primers (GGGGGATCTTCGGACCTCA) and (TCCTTAGAGT-

GCCCACCCG) with Amplified segment 956 pb and by examining a genus-specific section of the 16S DNA region. Thermocycling PCR software: The Perkin-Elmer/Gene Amp® PCR System 2720 (PE Applied Biosystems) was used for the PCR amplification. It was set up to complete 35 cycles following a 5 minute denaturation cycle at 94ºC. A 30 second denaturation step at 94°C, a 30-second annealing phase at 52°C, and a 1 minute extension step at 72°C comprised each cycle. In the last cycle, the last extension section was prolonged to 10 minutes at 72ºC.

PCR technique

PCR amplification

Primers were utilized in a 25 µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. This part was carried out in the Molecular Biology Research Unit (MBRU), Animal Health Institute, Giza, Egypt. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. A Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) and gene ruler 100 bp ladder (Fermentas, thermofisher, Germany) were 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.

Agarose gel electrophoresis

Amplified products were analyzed by 1.5% agarose gel stained with ethidium bromide and visualized ultraviolet transilluminator. A 100 bp DNA ladder was used as a marker for PCR products. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Detection of OEO and PEO effect on Pseudomonas aeruginosa vitality

Preparation of the EO dilutions

The Pumpkin and Onion essential oils were purchased from the National Research Centre in Cairo, Egypt, then were stored in sealed brown vials between 2 and 8°C until they were utilized. According to Wiegand *et al*. (2008) with slight modifications: We employed the two-fold serial dilutions approach. Dimethyl sulfoxide (DMSO) was added as a fat solvent (2:2:4) to the first dilution that was produced in BHI as (oil: DMSO: BHI). The remaining dilutions were made from this stock dilution in a two-step process, using BHI just as 4 ml from it to 4 ml BHI, and so on.

Culture preparation

Using the McFarland apparatus, prepare the bacterial strains' concentration in accordance with Saad *et al*. (2017). Adjust the bacterial suspension to the point 0.5 of the McFarland standard turbidity growth.

Agar well diffusion method (El-Shenawy *et al*., 2015)

The antibacterial activity of employed EOs (Pumpkin oil and Onion oil) was tested using the agar well diffusion method. The freshly prepared bacterial inoculum (1×10^8 CFU/mL) for each strain was swabbed onto a Mueller Hinton agar plate (MHA) using a sterile cotton swab. Plates were allowed to dry and wells of 4mm were punched in the solidified agar using a sterile corkborer. Two-fold serial dilutions were performed from concentration 100% till reach to concentration0.098% from tested oil. An amount of 30-100 µl of the tested EO was instilled in the wells. The MHA plates were incubated at 37°C for 24 hours. A triplicate set of plates was made. Then measure the diameter of the zone of inhibition (DIZ) against the bacteria under investigation.

Broth dilution method (Jayana *et al*., 2010)

Two-fold serial dilutions of Pumpkin and Onion oil at different concentrations (100%, 50%, 25%, 12.5%, 6.25%, 3.125%,1.56%, 0.78%, 0.39%, 0.195% and 0.098%). The dilutions were prepared using sterile Muller Hinton broth. Each tube was inoculated by 200 µl from the 24 h age culture of the microorganism to obtain an inoculum of $1x10^7$ CFU/ ml broth. The tubes were incubated aerobically at 37ºC for 24 h. The results were interpreted based on the fact that growth occurs in the positive control and any other tube in which the concentration of the extract is not sufficient to inhibit growth.

Evaluation of EOs effect on P. aeruginosa in Tallaga cheese

Analysis of the antibacterial activity in cheese model was applied by manufacturing of Tallaga cheese according to Abdel-Salam *et al*. (2010). The prepared PEO and OEO were added during the cheese manufacture according to the obtained results that based on measurement of inhibition zone diameter formed around the well.

Laboratory manufacturing of Tallaga cheese according to Abdel-Salam (2010)

Tallaga cheese was manufactured as Fresh buffalo's milk was standardized to have 5% fat followed by a pasteurization at 72°C for 15 sec. and cooling to 38-40°C. Calcium chloride, sodium chloride and commercial rennet were added at the ratios of 0.02, 4 and 0.05% (w/v), respectively. The obtained milk was divided into four equal portions for further use as follows: (1) plain sample contained EOs for sensory evaluation (used as a negative controls), (2) samples inoculated with *Pseudomonas aeruginosa* without EOs (considered as a positive control cheese), (3) *P. aeruginosa* + 0.39% Pumpkin EO, (4) *P. aeruginosa*+ 0.39% Onion EOs, in all treatments apart from the negative control group. All cheese milk was kept coagulating at room temperature. The curds were whey out and the obtained cheeses were stored in refrigeration at 4.0±2.0°C. Samples were collected and *P. aeruginosa* was enumerated immediately at zero hour and after curdling, 1st day, 3rd day, 5th day and 7th day of storage and Isolation Onto *Pseudomonas* CN agar medium Agar.

Organoleptic assay of Tallaga cheese (negative control with EO only)

Tallga cheeses were prepared with the addition of Eos (PEO and OEO) but without pathogen inoculation. The cheeses were then used for sensory evaluation both immediately following processing and throughout storage (after 1, 3, 5, 7 days). Teams including individuals varying in age, gender, and educational background were formed to choose the thirty-five panelists. The prepared cheese samples were evaluated based on their sensory qualities, including color, flavor, body texture, and overall acceptability. (The percentage of EOs was chosen according to MIC).

Microbiological analysis of Tallaga cheese during storage period was done according to ISO 11059 (2009).

Results

Prevalence of Pseudomonas spp. in the examined cheese samples

Results in table 1 declared the total prevalence of *Pseudomonas* spp. was 70 (42.42%). it was isolated from 34(61.8%) Tallaga cheese samples, 5(9.09%) Bramily cheese and 31(56.4%) Ras cheese.

Table 1. Prevalence of *Pseudomonas* spp. in the examined cheese samples.

Samples	No. of the examined samples	Positive samples	
		No.	%
Tallaga cheese	55	34*	61.8
Bramily cheese	55	5*	9.09
Ras cheese	55	31*	56.4
Total	165	70	42.42

Frequency distribution of different Pseudomonas spp. in the examined cheese samples

As shown in Table 2, the isolated of *Pseudomonas* spp, in Tallaga cheese could be differentiated into *P. aeruginosa* 11.76%, *P. fluorescens* 26.47%, *P. fragi* 20,58%, *P. putrefaciens* 14.70%, *P. proteolytica* 11.76, *P. alcaligenes* 8.82%, *P. acidovorans* 0% and *P. vesicularis* 5.88% while in Bramily cheese the prevalence of *Pseudomonas* spp. was .0, 40, 20, 0, 20, 20, 0 and 0 % respectively and in Ras cheese were 6.45, 64.51, 22.58, 0, 6.45, 0,0 and 0% respectively.

Table 2. Frequency distribution of different *Pseudomonas* spp. in the examined cheese samples.

Pseudomonas spp.	Tallaga cheese		Bramily cheese		Ras cheese	
	No./34	%	No./5	%	No./31	%
P. aeruginosa	4	11.76	-	-	2	6.45
P. fluorescens	9	26.47	2	40	20	64.51
P. fragi	7	20.58	1	20	7	22.58
P. putrefaciens	5	14.7	-	-	-	-
P. proteolytica	4	11.76	1	20	2	6.45
P. alcaligenes	3	8.82	1	20	-	-
P. acidovorans	-	-	-	-	-	-
P. vesicularis	2	5.88	-	-	-	-

Prevalence of P. aeruginosa in the examined cheese samples

Resuls In Table 3 showed that the total prevalence of *Pseudomonas aeruginosa* in the samples was 6(10.9%); in Tallaga cheese isolated 4(7.27%) but not detected in Bramily cheese and two isolates (3.63%) presented in Ras cheese.

Table 3. Prevalence of *P. aeruginosa* in the examined cheese samples.

Samples	No. of the examined samples	Positive samples	
		No.	%
Tallaga cheese	55	4*	7.27
Bramily cheese	55	0*	0
Ras cheese	55	2*	3.63
Total	165	6	10.9

*Significant difference ($p < 0.05$) between incidence of *P. aeruginosa* in the examined cheese samples.

MIC of PEO and OEO on P. aeruginosa by agar well diffusion method (zone of inhibition) and tube dilution method.

The antibacterial effect of PEO on *Pseudomonas aeruginosa* was shown in Table 4 with average zone of inhibition 29, 27, 25, 22, 20, 18, 16, 16, 10, 7 mm and no zone and for OEO were 31, 28, 27, 24, 22, 20, 19, 15, 13, 9 mm and no zone for concentration 100, 50, 25, 12.5, 6.25, 3.125 ,1.56, 0.78, 0.39 ,0.195 and 0.098%. the MIC for PEO and OEO was at concentration 0.195. In tube dilution method the MIC of PEO and OEO was +ve at concentration of 0.098%.

Table 4. MIC of PEO and OEO on *P. aeruginosa* by agar well diffusion method (zone of inhibition) and tube dilution method.

Conc./ well	Zone of inhibition (mm)		Tube dilution method	
	Pumpkin	Onion	pumpkin	Onion
100%	29	31	-ve	-ve
50%	27	28	-ve	-ve
25%	25	27	-ve	-ve
12.50%	22	24	-ve	-ve
6.25%	20	22	-ve	-ve
3.13%	18	20	-ve	-ve
1.56%	16	19	-ve	-ve
0.78%	16	15	-ve	-ve
0.39%	10	13	-ve	-ve
0.20	7	9	-ve	-ve
0.10	No zone	No zone	+ve	+ve

Effect of pumpkin and onion EO on P. aeruginosa in vivo (Tallaga cheese)

According to Table 5, Tallaga cheese was manufactured and injected using the determined MIC, which was 0.39% for PEO and OEO. These concentrations showed that *Pseudomonas aeruginosa* counts were successfully reduced during the first week. Due to the compounds present in pumpkin and onion essential oils contain antibacterial and antioxidant qualities, and they act against both gram-positive and gram-negative bacteria. This is why they have a greater effect.

Table 5. Effect of pumpkin and onion EO on *P. aeruginosa* in vivo (Tallaga cheese).

Storage period	Negative control	Positive control	Pumpkin	Onion
		(with *P. aeruginosa*)	0.39%	0.39%
Zero hour	-ve	5 $\log_{10}$		
After curdling	-ve	4.1	3.6*	3.5*
1 day	-ve	4.0	3.1*	3.0*
3rd day	-ve	4.0	2.4*	2.6*
5th day	-ve	3.8	2.0*	2.0*
7th day	-ve	3.7	<1.0*	<1.0*

*Significant difference ($p < 0.05$) control and treatments.

Sensory evaluation of Tallaga cheese (negative control with EO only)

Figure 1 shows that the produced cheese's body, texture, and color were all good sensory acceptability. The tested concentrations of the determined MIC 0.39% of PEO and OEO exhibited a pleasant flavor with acceptable OAA for the two EOs. When comparing the treated cheese sample to the control cheese, there were minor variations in each of the scores for the various sensory characteristics.

Discussion

Low temperatures usage has permitted delay of the spoilage and retailing of several newly milky yields. On the other hand, obviously happening microbes, for example pseudomonads, have progressively turn into an actual anxiety toward refrigerated -kept fresh dairy foods since its capability to adapt themselves also grow at low temperatures. They are accountable for noticeable decay characters (rheology changes, discolor-

ations, structure loss) and non-observable faults (off-odours, off-flavours and protein breakdown) which considerably diminish the excellence and shelf-life of dairy yields (Mahmood, 2023).

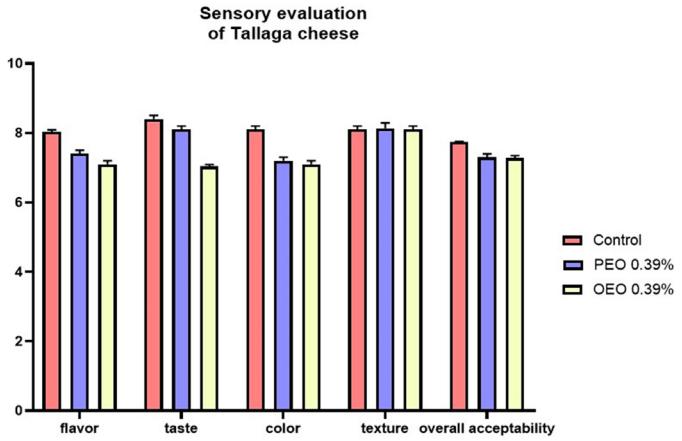

Figure 1. Sensory evaluation of Tallaga cheese (negative control with EO only).

Realizing the data outlined in Table 1, it was evident that 70% of the examined cheese samples were contaminated with *Pseudomonas* spp. The obtained result agreed with Mahmood (2023) and Al-Sabaawi *et al*. (2024). The frequency of distribution of the *Pseudomonas* spp. was presented in Table 2. While the prevalence of *P. aeruginosa* in the examined cheese samples was demonstrated in Table 3, in which, 6 out of the 165 cheese samples were positive for *P. aeruginosa* with a percentage of 10.9 %.

It was clear in the current study that the prevalence of *P. aeruginosa* was more in the examined Tallaga cheese followed by the Ras; this may be attributed to the fact that Tallaga cheese is known to be made from just warmed milk, in addition bad storage play an important role in the growth and multiplication of *Pseudomonas* and unaffected to hygienic measures in production of Tallaga and Ras cheese as mentioned by Mahmood (2023) while low prevalence of *Pseudomonas* spp. in Bramily cheese may be attributed to the effect of salt concentration present on this cheese on *Pseudomonas* count as revealed by Keskin and Ekmekçi (2008) Who reported that 7% salt concentration had bactericidal effect on *P. aeruginosa* and 5% salt concentration was bacteriostatic in all doses.

In Table 2, of all the isolated isolates, *P. fluorescens* had a high prevalence of *P. fluorescens* while, higher results have been registered by Atia *et al*. (2022). Which present in different type of cheese samples is probably due to the microbe's short production time at refrigeration temperatures and presence in the dairy processing environment which are in line with another research (Arslan *et al*., 2011 and Bhunia (2008). It was clear that in this study, Ps. fluorescens is the main bacterium that spoils cheeses like Tallaga, Ras and Bramily by secreting hydrolytic enzymes including lipase and protease. Studies by Sobeih (2000) and Dogan and Boor (2003) showed findings that were quite similar. While in Bramily and Ras cheese *P. putrefaciens*, *P. acidovorans* and *P. vesicularis* not detected. Frequency distribution of *Pseudomonas* spp in this study is lower than that reported by El-Leboudy *et al*. (2015).

The isolated *P. aeruginosa* was confirmed by PCR (Figure 2), which is a rapid method with high sensitivity and specificity for specific DNA sequences and permits direct detection of the pathogens (Spilker *et al*., 2004).

The antibacterial effect of PEO and OEO on *P. aeruginosa* was shown in Table 4, with average zone of inhibitions 29, 27, 25, 22, 20, 18, 16, 16, 10 ,7 mm and no zone, for OEO were 31, 28, 27, 24, 22, 20, 19, 15, 13, 9 mm and no zone for concentration 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195 and 0.098%, respectively. It was evident that both had an antibacterial impact on *P. aeruginosa*. However, OEO had a larger zone of inhibition than PEO due to its stronger and more effective antimicrobial action. As revealed by Khadri *et al*. (2010) which found that the essential oil of Allium sativum has an inhibitory activity of growth compared to over 50 of strains tested with MICs relatively between 32 and 128 µg/ml.

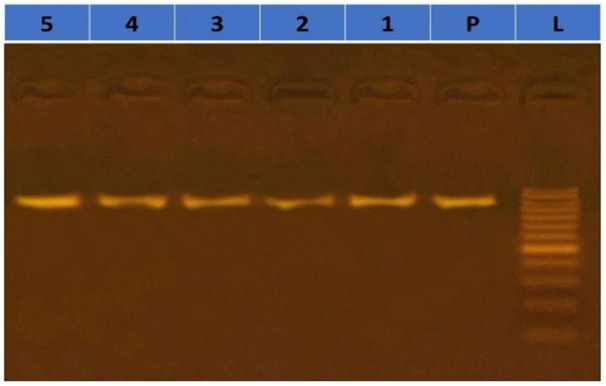

Figure 2. Agarose gel electrophoresis of the PCR product of 16SrDNA gene for the isolated *P. aeruginosa.* Lane L: ladder; Lane P: positive control; Lane 1&2&3&4&5: positive *P. aeruginosa*

The obtained results revealed that MIC for PEO and OEO was 0.39%. This result was lower than that detected by Kačániová *et al*. (2024) for PEO, but higher than the result of Octiara *et al*. (2024) for OEO. The bacteriostatic and anti-proliferative action of these EOs against these pathogenic bacteria was probably exerted through their bioactive phenolics compound. This MIC revealed good results in reduction the count of *P. aeruginosa* during the 1st week. Due to the compounds present in pumpkin and onion essential oils which contain antibacterial and antioxidant qualities, and act against both Gram-positive and Gram-negative bacteria. This is why they have a greater effect (Table 5).

Figure 1 shows that the tested cheese's color, texture and body were all of good sensory characters. The selected concentrations of the determined MIC 0.39% of PEO and OEO exhibited a pleasant flavor with good overall acceptability for the two EOs. When comparing the treated cheese sample to the control cheese, there were minor variations in each of the scores for the various sensory characteristics.

Conclusion

The research above proofed that *Pseudomonas aeruginosa* can easily spoiled soft cheese with high percentage from the Tallaga cheese due to inadequate pasteurization and cleaning and sanitizing of milking equipment and utensils of milk or due to bad storage and refrigerating facilities. That emphasizes how it is important to prevent these species from contaminating cheese within manufacturing. Furthermore, the count of *P. aeruginosa* was affected by addition of 0.39% for PEO and OEO, as they have potent antibacterial effect against *P. aeruginosa*.

Conflict of interest

The authors have no conflict of interest to declare.

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