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ORIGINAL ARTICLE

Efficacy of Octanoic Acid and Lauric Arginate, Individually and in Combination, against *Listeria monocytogenes* in Domiati Cheese

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Abstract

Listeria monocytogenes is an important foodborne pathogen implicated in outbreaks related to soft cheese, indicating the importance of its control. In the current study, the antimicrobial efficacy of octanoic acid (OA) and lauric arginate ester (LAE), individually or in combination, against *L. monocytogenes* in Domiati cheese was evaluated during storage at 4°C for 35 days. Data revealed that the population of *L. monocytogenes* in untreated Domiati cheese gradually increased during refrigerated storage. The application of 400ppm OA, 200ppm LAE, and 200ppm LAE+200ppm OA reduced the inoculated *L. monocytogenes* in Domiati cheese with 1.72±0.11, 2.14±0.01, and 2.63±0.08 log CFU/g reduction respectively, after 35 days of refrigerated storage. Moreover, the combination of 200ppm LAE+400ppm OA was the most effective treatment, leading to a 3.31±0.03 log CFU/g reduction of *L. monocytogenes* by the end of the refrigerated storage period. This study is a guide for the practical application of octanoic acid and lauric arginate during the manufacturing of Domiati cheese to control *L. monocytogenes* during the refrigerated storage.

Keywords
Cheese, Lauric arginate, *L. monocytogenes*, Octanoic acid

1. Introduction

Listeria monocytogenes is a significant foodborne pathogen that causes foodborne related deaths globally with a death rate of ~30% **(Nyarko and Donnelly, 2015).** Recently, it was reported that *L. monocytogenes* caused approximately 44% of deaths resulting from foodborne outbreaks in European Union countries in 2022 **(EFSA and ECDC, 2023).** This pathogen mostly affects immune compromised individuals, including pregnant women, neonates, and the elderly causing septicemia, stillbirth, and meningitis. Less severe symptoms associated with healthy individuals exhibiting symptoms of mild influenza and gastroenteritis **(Thakur et al., 2018).**

Soft cheese is frequently implicated in listeriosis outbreaks **(CDC, 2021; Heiman et al., 2016; Palacios et al., 2022).** During 2000-2014, recorded listeriosis outbreaks (51), a total of 17(34%) were linked to soft cheese, leading to ~180 illnesses, 17 deaths, and 14 fetal losses in the United States. Interestingly, 13(77%) of these outbreaks were linked to the consumption of pasteurized cheese **(Jackson et al., 2018)** indicating the insufficiency of pasteurization to control *L. monocytogenes* in soft cheese. Therefore, a greater effort is needed to control *L. monocytogenes* throughout the manufacturing and storage of soft cheese. Domiati cheese, as one of the most popular white soft cheeses in the Middle Eastern countries including Egypt could be a major cause of foodborne illnesses such as *L. monocytogenes* **(El-Kholy et al., 2014)**. It may be referred to the capability of *L. monocytogenes* to survive and grow at a wide range of temperatures (-.4 to 45°C), a high salt concentration, and a relatively low pH **(Bucur et al., 2018).**

Traditionally, chemical preservatives have been used to control foodborne pathogens in soft cheese. Nowadays, the incorporation of natural, effective, and approved-safe antimicrobial components into soft cheese has the potential to improve safety and consumer trust in this dairy product. Octanoic acid is commercially produced from natural sources, including milk and coconut oil **(Marina et al., 2009; Park et al., 2007).** It is generally recognized as a safe compound (GRAS) to be applied within a limit not exceeding 400ppm to cheese products **(FDA, 2023).** Furthermore, LAE, an amino acid-based cationic surfactant derived from natural dietary products including arginine and lauric acid, is applied in the food industry as a novel food additive due to its antimicrobial properties **(Ma et al., 2023).** In the human body, it is rapidly metabolized into its natural components **(Hawkins et al., 2009)**. Thus, it was approved for application in food products as a generally recognized as safe (GRAS) antimicrobial agent within a limit not exceeding 200ppm **(FDA, 2005).** Although the application of OA or LAE individually may be effective in controlling *L. monocytogenes*, it can be restricted by allowed concentrations and the effect of high concentrations on sensory attributes. Thus, combinational approaches allow a reduction of the individual antimicrobial concentration while increasing the control level.

The antimicrobial efficacy of OA or LAE was evaluated at concentrations that exceeded the recommended levels by the FDA **(Brown et al., 2018; Kozak et al., 2018a; Lourenço et al., 2017)** making it impractical to be applied in the dairy industry. Moreover, previous reports approved that sodium caprylate (SC), a sodium salt of octanoic acid, enhances the antimicrobial efficacy of LAE against *L. monocytogenes* in soft cheese throughout the storage period **(Brown et al., 2018; Kozak et al., 2018a).** However, SC was not approved by the FDA as a GRAS compound to be added to cheese. Therefore, the current study aimed to determine the efficacy of OA and LAE individually at the recommended levels against *L. monocytogenes* in Domiati cheese throughout storage at 4°C. Moreover, the effect of OA on the efficacy of LAE at different concentrations against *L. monocytogenes* in Domiati cheese throughout the storage period was evaluated.

2. Materials and Methods

2.1. Preparation of *L. monocytogenes* Inoculum

L. monocytogenes ATCC7644, ATCC 35152 and ATCC 19115 strains were stored in trypticase soy broth (Becton, Dickinson and Company, Sparks, MD) supplemented with 0.6% (w/v) yeast extract (Fisher Scientific, Pittsburgh, PA) (TSB-YE) and glycerol (20%, v/v) at -20°C. *L. monocytogenes* strains were twice activated in TSB-YE consecutively at 35±2°C for 24h statically. *L. monocytogenes* enumeration was carried out by spread plating of 100pl of the serially diluted culture in Phosphate Buffered Saline (PBS, 7.4pH) on Modified Oxford agar **(MOX; Biolife It.)** and incubated at 37°C for 48h. The overnight bacterial culture was serially diluted in PBS and pelleted, then re-suspended in pasteurized milk.

2.2. Antimicrobial Ingredients

Working stock solutions of generally recognized as safe (GRAS) antimicrobial agents were freshly prepared. Octanoic acid (OA) was purchased from **Sigma-Aldrich Comp., Saint Louis, USA.** Lauric arginate ester (LAE) was provided by A and B Ingredients **(CytoGuard®, Fairfield, NJ, USA),** containing 10% active LAE

2.3. Cheese Manufacturing and Inoculation

Fresh cow's milk, purchased from a local market in Beni-Suef city, Egypt, was heated at 80°C in a water bath for 10min, then left to cool to 40°C. Calcium chloride ($CaCl_2$ 0.02%, w/w) and sodium chloride (NaCl 7%, w/w) **(Youssef et al., 2016)** were added, followed by the addition of rennet as 1.5g/100kg milk with thorough mixing. The mixture was divided into different batches. Except for the negative control, all batches were inoculated with the prepared inoculum to achieve ~5.0 $\log_{10}$ CFU/g of *L. monocytogenes* culture, followed by the addition of different antimicrobial treatments before cheese curdling **(Table, 1)**. Cheese samples without bacterial inoculation and antimicrobial addition were served as a negative control. After cheese curdling, all samples were stored under the same conditions in the refrigerator at 4°C for 35 days.

2.4. Counting of *L. monocytogenes*

At zero, 1, 3, 7 days, and every week of storage, twenty-five grams of cheese samples were collected and homogenized (Lab Blender; Seward Medical Ltd., London, UK) with 225ml of sterile sodium citrate for 2min. The samples were ten-fold serially diluted and the appropriate dilutions were streak-plated into duplicate Listeria selective agar **(Oxoid, UK)** plates. The plates were aerobically incubated at 35±2°C for 48h for the enumeration of *L. monocytogenes*. The values of the counted colonies were converted into log CFU/g cheese. In addition, negative control samples were included in every examination and no colonies were observed in any of them.

2.5. Determination of pH

At each sampling time, the pH value was determined using a digital pH meter **(Adwa Instruments).**

2.6. Statistical Analysis

The results were presented as means ± standard error of the mean (SEM). Significant differences between treatments at the same time point were determined by the analysis of variance **(One-way ANOVA)** at P<0.05. All values were analyzed using SPSS 26.0 for windows **(SPSS Inc, Chicago, IL, USA).**

Table 1. Antimicrobial treatments applied to control *L. monocytogenes* in Domiati cheese.

Treatments	OA (ppm)	LAE (ppm)
T1 (Positive control)	0	0
T2	100	0
T3	200	0
T4	400	0
T5	0	50
T6	0	100
T7	0	200
T8	200	100
T9	400	100
T10	200	200
T11	400	200

3. Results and Discussion

3.1. Efficacy of Octanoic Acid against *L. monocytogenes* in Cheese

The changes in *L. monocytogenes* count in treated/untreated Domiati cheese with octanoic acid during storage at 4°C (**Fig. 1 and Table 2)**. At zero-time, initial *L. monocytogenes* counts in treated/untreated Domiati cheese with OA were significantly similar (*P*>0.05) and ranged between 4.59±0.22 and 4.71±0.18 log CFU/g. In untreated cheese, the population of *L. monocytogenes* increased during storage at 4°C, achieving 7.24±0.13 log CFU/g after 35 days of storage. Consistently, it was concluded that *L. monocytogenes* was capable of growth in cheese during refrigerated storage **(Korany et al., 2024; Tiwari et al., 2014)**. Moreover, it was reported that the population of *L. monocytogenes* gradually increased when inoculated in soft cheese, from ~3.5 to 7.7 log CFU/cm^2 during 28 days of storage at 4°C **(Soni et al., 2012)**. It might be attributed to the psychrotrophic properties of *L. monocytogenes*, which enable it to grow at refrigeration temperatures. Furthermore, it is clearly noticed that the growth rate of *L. monocytogenes* in untreated cheese samples was high during the first 3 weeks of storage, accounting for 7.14±0.26 log CFU/g. On the contrary, during the last 2 weeks of storage, the growth rate of *L. monocytogenes* in cheese was reduced. A previous study reported that the population of *L. monocytogenes* increased from ~4.0 log CFU/g at zero time to ~8.3 log CFU/g after 3 weeks of storage at 4°C, but no further growth was noticed during the 4th week of refrigerated storage **(Soni et al., 2010)**. Also, it was noticed that *L. monocytogenes* strains were able to grow exponentially in control soft cheese inoculated with nearly 3.5 log CFU/g, recording ~7.0 log CFU/g during the first 11 days, followed by slow growth to ~8.0 log CFU/g during the following 10 days of refrigerated storage **(Lourenço et al., 2017)**.

Table 2. Changes in counts of *Listeria monocytogenes* experimentally inoculated in Domiati cheese during refrigerated storage.

Day	Control	100ppm OA	200ppm OA	400ppm OA	50ppm LAE	100ppm LAE	200 ppm LAE	100 ppm LAE + 200 ppm OA	100 ppm LAE + 400 ppm OA	200 ppm LAE + 200 ppm OA	200 ppm LAE + 400 ppm OA
Zero	4.71 ± 0.18^{A}	4.65 ± 0.23^{A}	4.59 ± 0.22^{A}	4.64 ± 0.19^{A}	4.٥٩ ± 0.19^{A}	4.64 ± 0.21^{A}	4.6١ ± 0.19^{A}	4.63 ± 0.21^{A}	4.٥٨ ± 0.17^{A}	4.6٠ ± 0.21^{A}	4.٥٦ ± 0.21^{A}
1st	4.93 ± 0.24^{A}	4.76 ± 0.22^{AB}	4.69 ± 0.17^{ABC}	4.49 ± 0.21^{ABCD}	4.72 ± 0.23^{ABC}	4.59 ± 0.24^{ABCD}	4.31 ± 0.2٠^{ABCD}	4.46 ± 0.21^{ABCD}	4.22 ± 0.16^{BCD}	4.11 ± 0.26^{CD}	3.99 ± 0.14^{D}
3rd	5.77 ± 0.21^{A}	5.57 ± 0.18^{AB}	5.51 ± 0.27^{ABC}	4.98 ± 0.١٥^{CD}	5.٣٨ ± 0.26^{ABC}	5.٣١ ± 0.16^{ABCE}	4.٧٩ ± 0.١٠^{DE}	5.13 ± 0.14^{BDE}	4.83 ± 0.23^{DE}	4.60 ± 0.14^{DF}	4.22 ± 0.1١^{F}
7th	6.47 ± 0.16^{A}	6.11 ± 0.11^{AB}	5.79 ± 0.10^{BC}	5.٤٦ ± 0.١٣^{CD}	6.07 ± 0.10^{AB}	5.69 ± 0.25^{BC}	5.٠٣ ± 0.١٧^{DE}	5.51 ± 0.25^{CD}	5.19 ± 0.21^{DE}	4.74 ± 0.12^{EF}	4.3٠ ± 0.14^{F}
14th	6.95 ± 0.22^{A}	6.40 ± 0.13^{AB}	6.12 ± 0.16^{BC}	5.٥٦ ± 0.2٤^{CD}	6.٢٢ ± 0.27^{BE}	5.٩8 ± 0.18^{BC}	5.0٩ ± 0.23^{DF}	5.70 ± 0.18^{CDE}	5.1٢ ± 0.12^{DF}	4.8١ ± 0.18^{F}	4.١7 ± 0.27^{G}
21st	7.14 ± 0.26^{A}	6.56 ± 0.17^{B}	6.07 ± 0.14^{BC}	5.65 ± 0.18^{C}	6.44 ± 0.19^{B}	6.1١ ± 0.16^{BC}	5.1٢ ± 0.21^{D}	5.74 ± 0.15^{C}	5.١٠ ± 0.15^{D}	4.77 ± 0.16^{D}	4.٠٨ ± 0.15^{F}
28th	7.17 ± 0.18^{A}	6.58 ± 0.19^{B}	6.21 ± 0.11^{BC}	5.59 ± 0.09^{DE}	6.54 ± 0.22^{B}	6.13 ± 0.١٩^{BC}	5.1٠ ± 0.19^{DF}	5.72 ± 0.25^{CE}	5.2٠ ± 0.١٦^{EF}	4.67 ± 0.28^{F}	3.9٣ ± 0.21^{G}
35th	7.24 ± 0.13^{A}	6.63 ± 0.18^{B}	6.20 ± 0.15^{B}	5.52 ± 0.24^{CD}	6.57 ± 0.20^{B}	6.21 ± 0.23^{B}	5.10 ± 0.14^{CE}	5.66 ± 0.17^{D}	5.1٤ ± 0.14^{CDE}	4.62 ± 0.21^{E}	3.٩3 ± 0.16^{F}

OA, octanoic acid; LAE, lauric arginate ester. Mean ± SEM. $^{A-G}$ Means within a row with no common letter differ significantly ($P \leq 0.05$).

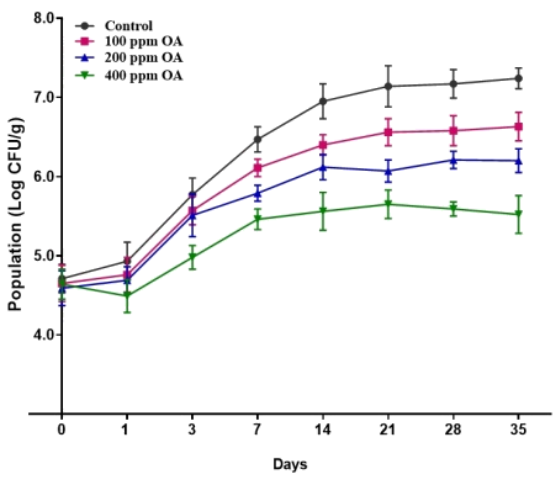

Fig. 1. Efficacy of octanoic acid (OA) against *L. monocytogenes* in experimentally inoculated Domiati cheese during refrigerated storage.

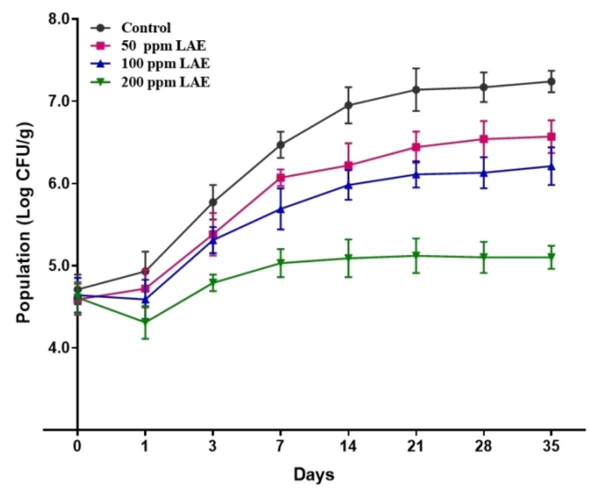

Fig. 2. Efficacy of lauric arginate (LAE) against *L. monocytogenes* in experimentally inoculated Domiati cheese during refrigerated storage.

On the other hand, the addition of OA to Domiati cheese during manufacturing effectively controlled the growth of *L. monocytogenes* as compared to the untreated cheese during storage at 4°C. The efficacy of OA against *L. monocytogenes* in cheese samples during storage was concentration-dependent. On the 3rd day of refrigerated storage, 400ppm OA treatment significantly ($P \leq 0.05$) diminished *L. monocytogenes* population in Domiati cheese by 0.80±0.07 log CFU/g when compared with the untreated cheese **(Fig. 1 and Table 2)**. Along the storage period, there were no significant differences between the efficacy of OA at 100 and 200ppm against *L. monocytogenes*, while significant differences were recorded between the efficacy of 100 and 400ppm OA. On the 14th day of storage, the inoculated *L. monocytogenes* in Domiati cheese were significantly reduced ($P \leq 0.05$) by 0.55±0.09, 0.83±0.06, and 1.39±0.02 log CFU/g after treatment with 100, 200, and 400ppm OA respectively, when compared to the untreated cheese. By the end of the storage period, the populations of *L. monocytogenes* in Domiati cheese treated with 100, 200, and 400ppm OA were reduced by 0.61±0.05, 1.04±0.02, and 1.72±0.11 log CFU/g respectively, when compared with the untreated cheese during storage at 4°C **(Fig. 1 and Table 2)**. In this regard, a previous report approved that the treatment of queso fresco soft cheese with 1500 and 2910 OA achieved nearly 2.0 and 5.0 log CFU/g reduction, respectively, when compared with the untreated cheese after 21 days of storage at 4°C **(Lourenço et al., 2017)**. The antimicrobial efficacy of octanoic acid is most likely due to the generation of pores throughout the cell membrane, leading to disruption of cell membrane permeability **(Choi et al., 2013)**.

3.2. Efficacy of Lauric Arginate against *L. monocytogenes in* Cheese

LAE, an immediate effective antimicrobial agent, is odorless, colorless, heat-stable, and effective in a wide range of pH, so it is ideal for application as an antimicrobial in food products **(Becerril et al., 2013; Hawkins et al., 2009)**. LAE is characterized by fast, strong, and inoculum size independent antimicrobial activity against *L. innocua*, a non-pathogenic surrogate of *L. monocytogenes* and LAE has ten times the antimicrobial activity of cinnamon and oregano essential oils **(Becerril et al., 2013)**.

Changes of the *L. monocytogenes* counts in Domiati cheese manufactured with/without the addition of LAE are illustrated in **Fig. (2)** and **Table (2)**. The initial counts of *L. monocytogenes* in all the inoculated Domiati cheese samples were significantly similar. As illustrated in **Fig. (2),** the addition of 200ppm LAE to Domiati cheese resulted in an initial reduction of *L. monocytogenes* population, followed by regrowth during subsequent storage **(Soni et al., 2012)**. Similar to OA, the efficacy of LAE against *L. monocytogenes* in cheese samples during storage was concentration-dependent. Accordingly, a previous report revealed that the efficacy of LAE against *L. monocytogenes* in both milk and soft cheese was dependent on its concentration **(Soni et al., 2010)**. Moreover, **Ma et al. (2013)** reported that LAE showed a concentration-dependent activity as it reduced *L. monocytogenes* in 2.0% reduced fat milk by 1.02±0.06 and 6.20±0.10 log CFU/ml when applied at 375 and 750ppm, respectively, after 24h of storage at 32°C. In the current study, the addition of 200ppm LAE significantly reduced *L. monocytogenes* count by 0.98±0.11 log CFU/g when compared with the untreated cheese on the 3rd day of storage **(Fig. 2 and Table 2)**. There were no significant differences (*P*>0.05) between the efficacy of 50 and 100ppm LAE against *L. monocytogenes* during the storage period. Conversely, the efficacy of 200ppm LAE against *L. monocytogenes* in Domiati cheese was significantly higher than that of 50 and 100ppm LAE from the 7th day of storage until the end of the storage period **(Table, 2)**. On the 7th day of storage, the treatment of Domiati with 100 and 200ppm LAE resulted in a 0.79±0.08 and 1.45±0.01 log CFU/g reduction of *L. monocytogenes* respectively, when compared with the untreated Domiati cheese **(Fig. 2 and Table 2)**. After 3 weeks of storage at 4°C, the application of 50, 100, and 200ppm LAE diminished *L. monocytogenes* population in cheese by 0.70±0.07, 1.03±0.10, and 2.02±0.04 log CFU/g, respectively, when compared with the untreated cheese **(Fig. 2 and Table, 2)**. In accordance, it was concluded that the application of 200ppm LAE on the surface of soft cheese reduced *L. monocytogenes* by ~2.0 log CFU/g when compared with the untreated cheese over 21 days of storage at 4°C **(Soni et al., 2010)**. Moreover, it was reported that the application of LAE at 5.0% through a coating solution reduced *L. monocytogenes* population on soft cheese by 1.7-1.8 log CFU/g after 24h of refrigerated storage **(Brown et al., 2018)**. The differences in the obtained results could be attributed to the differences in the tested bacterial strains. The antibacterial efficiency of LAE could be referred to its capability to react with the lipid portion of the bacterial cell membrane, leading to its disruption, cellular component leakage, and cellular death **(Becerril et al., 2013; Ma et al., 2013; Ma et al., 2023)**.

Interestingly, during the storage period, the efficacy of 200ppm LAE against *L. monocytogenes* was nearly similar to that of 400ppm OA at the same storage time **(Table, 2)**. The treatment of Domiati cheese with 400ppm OA and 200ppm LAE, individually, resulted in 1.72±0.11 and 2.14±0.01 log CFU/g reductions of *L. monocytogenes*, respectively, when compared to the untreated cheese on the 35th day of storage **(Table, 2)**.

3.3. Effect of Octanoic Acid in Enhancing the Efficacy of Lauric Arginate against *L. monocytogenes* in Cheese

Despite that the efficacy of LAE against *L. monocytogenes* in soft cheese would be enhanced at higher concentrations, its maximum concentration as a GRAS compound in cheese is 200ppm **(FDA, 2005)**. Therefore, an alternative method to potentially enhance the antimicrobial efficacy without increasing the individual allowed concentrations is the use of antimicrobial combinations to inhibit *L. monocytogenes* **(Brown et al., 2018)**. Many studies have been carried out to investigate the antimicrobial efficacy of LAE when applied with other antimicrobial compounds, aiming to reduce the added concentration of LAE in food matrices to meet the allowed requirements while increasing the antimicrobial activity in the food products. For instance, enhanced effects were recorded through the application of LAE accompanied with organic acid salts, such as sodium citrate, sodium diacetate, and sodium lactate, against different microorganisms **(Suksathit and Tangwatcharin, 2013; Terjung et al., 2014)**.

The effect of OA on the antimicrobial efficacy of LAE at low and high concentrations against *L. monocytogenes* in Domiati cheese during storage at 4°C is illustrated in **Fig. (3)** and **Table (2)**. The effect of 200ppm and 400ppm OA on the efficacy of the LAE at a low concentration (100ppm) and a high concentration (200ppm) was evaluated. At zero time, no significant differences were recorded between the populations of *L. monocytogenes* in treated and untreated cheese. On the 3rd day of refrigerated storage, the addition of 100ppm LAE + 200ppm OA and 100ppm LAE + 400ppm OA significantly reduced the population of *L. monocytogenes* in Domiati cheese by 0.65±0.08 and 0.94±0.02 log CFU/g, respectively, when compared with the untreated samples **(Fig. 3A and Table 2)**. The addition of 400ppm OA significantly enhanced the efficacy of LAE when applied at a low concentration (100ppm) on the 7th day of storage and continued till the end of the refrigerated storage **(Fig. 3A and Table, 2)**. In this regard, previous studies concluded that the application of sodium caprylate (SC) significantly (*P*≤0.05) enhanced the efficacy of LAE against *L. monocytogenes* on cheese surface **(Brown et al., 2018; Kozak et al., 2018a)**. By the end of the storage period, the population of *L. monocytogenes* in cheese was significantly reduced by 1.03±0.10, 1.59±0.04, and 2.11±0.01 log CFU/g due to the application of 100ppm LAE, 100ppm LAE + 200ppm OA, and 100ppm LAE + 400ppm OA respectively, as compared to untreated cheese samples.

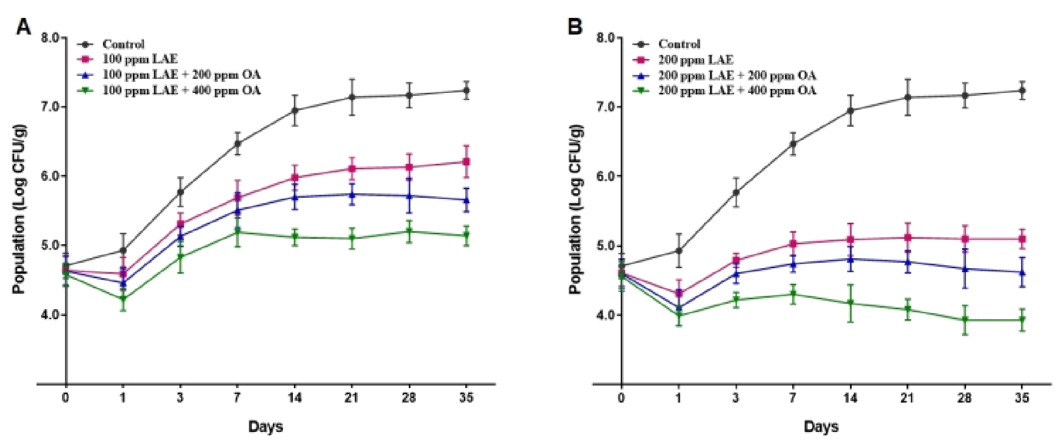

Fig. 3. Effect of octanoic acid (OA) in enhancing the efficacy of lauric arginate ester (LAE) at a low concentration (100ppm LAE) (A) and a high concentration (200ppm LAE) (B) against *L. monocytogenes* in experimentally inoculated Domiati cheese during refrigerated storage.

Furthermore, the addition of OA at 400ppm significantly enhanced the efficacy of LAE at a high concentration (200ppm) against *L. monocytogenes* on the 3rd day of storage and continued till the end of the storage period **(Fig. 3B and Table 2)**. In support, **Kozak et al. (2018b)** reported that the application of 400ppm LAE+1600 ppm sodium caprylate (SC) in whole milk reduced *L. monocytogenes* population by ~2.29 log CFU/ml as compared to the control sample after 21 days of storage at 7°C. Also, the authors concluded that the efficacy of the 400ppm LAE + 1600ppm SC combination was significantly (*P*≤0.05) more effective than the efficacy of 400 ppm LAE alone and nearly similar to the efficacy of 1600 ppm SC alone at 21 days of storage at 7°C. On the 7th day of storage, the application of

200ppm LAE + 200ppm OA and 200ppm LAE + 400ppm OA significantly diminished *L. monocytogenes* by 1.73±0.04 and 2.17±0.02 log CFU/g, respectively, when compared with the untreated cheese **(Fig. 3B and Table 2)**. On the 21st day of storage, *L. monocytogenes* population was diminished by 2.37±0.10 and 3.06 ± 0.11 log CFU/g after the application of 200ppm LAE + 200 ppm OA and 200ppm LAE + 400ppm OA, respectively, when compared with the untreated cheese. The combination of 200ppm LAE + 400ppm OA showed the highest efficacy against *L. monocytogenes* in Domiati cheese, achieving a 3.31±0.03 log CFU/g reduction at the end of the storage period as compared to the untreated cheese **(Fig. 3B and Table 2)**.

Collectively, the addition of 200ppm OA couldn't significantly enhance the efficacy of 100 or 200ppm LAE against *L. monocytogenes* in Domiati cheese. On the other hand, the addition of 400ppm OA could significantly enhance the antimicrobial efficacy of 100 or 200ppm LAE against *L. monocytogenes*. The efficacy of the 200ppm LAE + 400ppm OA combination against *L. monocytogenes* in cheese was higher than that of 200ppm LAE or 400ppm OA individually. Furthermore, 200ppm LAE + 400 ppm OA was the most effective treatment against *L. monocytogenes* in Domiati cheese along the storage period.

3.4. PH of the Domiati Cheese

The changes in pH values of untreated and treated cheese samples with different treatments during refrigerated storage are presented in **Table (3)**. The initial pH values for untreated and treated cheese samples ranged between 6.19±0.07 and 6.33±0.09. No significant (P>0.05) differences were noticed between the pH values of treated and untreated cheese samples at the same time point during the storage period. It was clearly noticed that pH values for untreated and treated cheese samples gradually decreased during the storage period, consistent with prior studies **(El-Kholy et al., 2014; Hassan et al., 2022; Korany et al., 2024)**. On the 35th day of refrigerated storage, pH values ranged between 5.19±0.12 and 5.41±0.07 **(Table, 3)**. The reduction in pH levels throughout refrigerated storage could be referred to the bacterial action, leading to the hydrolysis of lactose in cheese into some acids **(Hassan et al., 2022; Korany et al., 2024)**.

Table ٣. Changes in pH of treated and untreated Domiati cheese with antimicrobials during refrigerated storage.

Day	Control	100ppm OA	200ppm OA	400ppm OA	50ppm LAE	100ppm LAE	200ppm LAE	100 ppm LAE + 200 ppm OA	100 ppm LAE + 400 ppm OA	200 ppm LAE + 200 ppm OA	200 ppm LAE + 400 ppm OA
Zero	6.٣2 ± 0.07	6.٢9 ± 0.09	6.٣3 ± 0.09	6.٢6 ± 0.07	6.٢4 ± 0.14	6.٢8 ± 0.07	6.١9 ± 0.07	6.٣0 ± 0.12	6.٣1 ± 0.13	6.٢1 ± 0.14	6.٢2 ± 0.08
1st	6.٣0 ± 0.06	6.٢4 ± 0.06	6.٢5 ± 0.14	6.٢7 ± 0.11	6.٢3 ± 0.11	6.٢0 ± 0.09	6.١4 ± 0.06	6.٢4 ± 0.07	6.٢6 ± 0.09	6.١8 ± 0.10	6.١8 ± 0.14
3rd	6.١7 ± 0.05	6.١8 ± 0.13	6.٢1 ± 0.09	6.١7 ± 0.09	6.١9 ± 0.09	6.٠9 ± 0.08	6.١1 ± 0.11	6.١6 ± 0.10	6.١4 ± 0.10	6.١1 ± 0.11	6.١3 ± 0.12
7th	6.٠1 ± 0.09	6.٠4 ± 0.09	6.٠3 ± 0.10	6.٠0 ± 0.12	6.٠5 ± 0.04	5.98 ± 0.13	٥.٩6 ± 0.10	6.٠3 ± 0.11	٥.٩9 ± 0.16	٥.٩6 ± 0.09	6.٠4 ± 0.09
14th	5.٨3 ± 0.09	5.٨7 ± 0.11	5.٨1 ± 0.11	5.٨6 ± 0.08	5.٨8 ± 0.09	٥.٩٠ ± 0.12	5.٨7 ± 0.14	٥.٩٢ ± 0.14	5.٨6 ± 0.10	5.٨8 ± 0.09	5.٨5 ± 0.15
21st	5.٦4 ± 0.11	5.٦4 ± 0.13	5.٦7 ± 0.09	5.٧0 ± 0.11	5.٧0 ± 0.11	5.٦8 ± 0.10	5.٦8 ± 0.15	5.٧6 ± 0.16	5.٧1 ± 0.08	5.٧4 ± 0.14	5.٧3 ± 0.10
28th	5.٤4 ± 0.13	5.٥2 ± 0.13	5.٤6 ± 0.10	5.٥5 ± 0.09	5.٤9 ± 0.08	5.٥1 ± 0.12	5.٥0 ± 0.10	5.٥1 ± 0.11	5.٥2 ± 0.13	5.٥6 ± 0.12	5.٤9 ± 0.13
35th	5.١9 ± 0.12	5.٣0 ± 0.05	5.٤1 ± 0.07	5.٣6 ± 0.11	5.٣3 ± 0.06	5.٣5 ± 0.08	5.٣7 ± 0.09	5.٣9 ± 0.08	5.٣0 ± 0.08	5.٤1 ± 0.07	5.٣8 ± 0.08

OA, octanoic acid; LAE, lauric arginate ester. Mean ± SEM. No significant differences (P > 0.05) between different treatments at the same time point.

4. Conclusion

The application of OA or LAE significantly reduced the population of *L. monocytogenes* in Domiati cheese during storage at 4°C. Additionally, the antimicrobial efficacy of LAE could be enhanced by the incorporation of 400ppm OA. Among all evaluated treatments, the combination of 200ppm LAE + 400ppm OA was the most effective, leading to a 3.31±0.03 log CFU/g reduction of *L. monocytogenes* in Domiati cheese after 35 days of refrigerated storage. Data obtained from this study indicated that the combination of LAE and OA can be effectively applied to Domiati cheese during the manufacturing process to control *L. monocytogenes* during refrigerated storage.

5. Conflict of Interest

The authors declare no conflict of interest.

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