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Antimicrobial Effect of Citric Acid and Glucono-δ-lactone on some Selected Pathogenic and Lactic Acid Bacteria

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ABSTRACT

This research aimed to study the effect of citric acid and Glucono-δ-lactone (GDL) on some selected pathogenic bacteria (*Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* O157:H7 and *Pseudomonas aeruginosa*) and lactic acid bacteria LAB (*Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus paracasei*). The pH of LB medium was adjusted by citric or GDL at (4.4, 4.8, 5.2, 5.6, 6.2, 6.8 and 7.0) to test pathogens. However, the pH of MRS or M17 media were adjusted by citric or GDL at (4.4, 4.8, 5.2, 5.6, 6.2 and 6.8) to test LAB. Final pH, biomass concentration (C^B), Total viable count (TVC) and microbial growth rate % (MGR) of all studied bacteria were determined after 24 hrs of incubation. Citric acid and GDL had a high antimicrobial effect on pathogens. But they had no effect on LAB. GDL had the higher inhibition effect than citric acid against pathogenic strains. The inhibition effect of citric acid and GDL started to appear at low pH ≤ 5.2. While *Staphylococcus aureus* and *Salmonella enteritidis* were totally inhibited by GDL at pH 5.2. In addition, citric acid was more effective for enhancing the growth of LAB followed by GDL.

***Keywords*:** Antimicrobial effect, Citric acid, GDL, Pathogenic, LAB.

INTRODUCTION

Organic acids, whether naturally occurring in food and dairy products or intentionally added to them, have long been used to prevent microbial deterioration. The effectiveness of organic acids (lactic, acetic, citric, gluconic and ascorbic acid etc..) as antibacterial agents is well documented (Ayşe & Eliuz, 2020; Burin *et al.*, 2014; In *et al.*, 2013; Musfirah *et al.*, 2018). Organic acids are organic compounds with acidic properties and are produced naturally in plants and animals. Therefore, can be present in food as a result of various metabolic processes by LAB and can be added intentionally to food for a specific purpose (Bangar *et al.*, 2022). Organic acids have the ability to inhibit the microbial growth which makes it as good preservatives in the food preparation systems (Adamczak *et al.*, 2019). The antimicrobial activity of organic acids such as citric acid and GDL is related to the effect of lowering pH of food systems and inhibit the growth of microorganisms (Hauser *et al.*, 2016). Organic acids have the ability to penetrate the microbial cell and deactivate it by altering its internal pH or corrupting the metabolic reactions inside the microbial cell. Beside its antimicrobial action, organic acids possess technological functions such as regulating acidity of foods, stabilizing color, antioxidants, acidifiers, preservatives, vitamins, emulsifying, enhancing flavor and improving baking process (Hauser *et al.*, 2016).

In addition, organic acids were used as bio-preservatives in foods and considering a natural way to control the microbial environment and extend shelf life of foods (Bangar *et al.*, 2022). Citric acid is the organic acid produced from plant and animal metabolism as intermediates in metabolic pathways. It is (CA, 2-hydroxy2, 3-propanetricarboxylic acid, tricarboxylic acid) is widely used due to its numerous pharmaceutical application (Nangare *et al.*, 2021). In addition, citric acid in the biomedical applications and removing the toxic elements from plants (Nangare *et al.*, 2021).

In addition, Glucono-δ-lactone (GDL) is a ring-shaped molecule and one of glucose derivatives which contain six carbon atoms in its structure attached to hydroxyl group for each. Upon contacts to water, the ring shape molecule opened and converted to gluconic acid. Such formed acid affect the pH of foods while 1 g of Glucono-δ-lactone can reduce the pH by 0.07 to 0.09 pH unit (Herz *et al.*, 2021). Glucono-δ-lactone found its way in food industry as food ingredient. It can be used with meat and dairy products (such as yoghurt, cottage cheese and feta-like cheese) as flavoring agent and acidifier (Li *et al.*, 2023). Numerous applications in food industry are buffering, gelling, emulsifying, pickling and chelating agents. In addition, it can be used as color stabilizer, binding water, increasing viscosity and applied in edible coating (Al-Hatim *et al.*, 2021).

Citric acid and GDL can be used in cheese making as in direct acidification process, therefore, the objective of this research was to study the effect of citric acid and GDL on some pathogenic bacteria (*Salmonella enteritidis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*) and some lactic acid bacteria LAB (*Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Lactobacillus paracasei*) that are used as starter cultures in cheese making.

MATERIALS AND METHODS

Materials

Citric acid and GDL were obtained from Al-Nasr Company, Alexandria, Egypt. While, some pathogenic

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strains (*S. enteritidis, Staph. aureus, B. cereus, E. coli O157:H7 and P. aeruginosa*) and some lactic acid bacteria (LAB) (*Lb. acidophilus, Str. thermophilus, Lb. bulgaricus and Lb. paracasei*) were obtained from National Research Center, Giza, Egypt. Microbial media MRS and Luria-Bertani (LB) and Chemicals, which were used in this work, were obtained from Sigma and Merck companies. All the chemicals used for this study were analytical grade (A.R.).

Methods

1. Effect of citric acid or GDL on some pathogenic and lactic acid bacteria

This experiment tested several pathogenic strains (*S. enteritidis, Staph. aureus, B. cereus, E. coli O157:H7* and *P aeruginosa*) and LAB (*Lb. acidophilus, Str. thermophilus, Lb. bulgaricus* and *Lb. paracasei*). 25 ml of LB broth was inoculated separately with each of the five pathogenic strains. Additionally, 25 ml of M17 broth and MRS broth were used to cultivate *Str. thermophilus* and other LAB strains respectively. The initial pH of the LB broth was adjusted with citric acid or GDL to (4.4, 4.8, 5.2, 5.6, 6.2, 6.8, and 7.0) for each of the inoculated pathogenic strains. After determining the initial strain count, all treatments were incubated at the appropriate temperature for each strain for 24 hr. Finally, the final pH value and total count of strains were determined after 24 hr. However, the initial pH of the M17 and MRS broths was also adjusted with citric acid or GDL to (4.4, 4.8, 5.2, 5.6, 6.2 and 6.8), followed by LAB strains were inoculated separately. All treatments were then incubated for 24 hr. at the appropriate temperature for each strain after the total viable count TVC of initial strains was determined. In addition, the final pH value and the TVC of strains were measured after 24 hr.

2. pH values

All pH values were determined using a digital pH meter (JENWAY 3510).

3. Pathogenic strains count

Initial and final pathogenic strains count in were examined using LB agar medium (Bertani, 1951).

4. Lactic acid bacteria strains count

Initial and final LAB strains count were examined using M17 (Lyttle & Petersen, 1984) and MRS ("De Man, Rogosa and Sharpe (MRS) agar," media for *Str. thermophilus* and other *Lactobacilli* LAB strains, respectively.

5. Determination of biomass concentration (C_B)

Biomass concentration is obtained by means of the measurement of the optical density at 540 nm of the inoculated broth after incubation. Biomass concentration was determined according to (Elbanna *et al.*, 2015) by this formula:

$$C_B = 0.2845 \times OD^{540\ nm}$$

Where: C_B **is biomass concentration (g/l) and** $OD^{540\ nm}$ **is optical density at 540 nm.**

6. Percentage of microbial growth rate

Percentage of microbial growth rates of pathogenic strains and LAB were calculated by this equation:

$$Microbial\ growth\ rate\% = \frac{Final\ strain\ total\ count - Intial\ strain\ total\ count}{Final\ strain\ total\ count}\ x\ 100$$

7. Multiple regression

Multiple regression analysis for microbial growth rate of all pathogenic and LAB strains was performed by Sigma plot 15 program.

8. Statistical analysis

All obtained data were subjected to the statistical analysis that performed by SPSS version 26.0 [SPSS. 2021. Statistical Package for Social Sciences. SPSS Inc., 444, North Michigan Avenue, Chicago, IL 606 11, USA.], and Sigma plot 15.0 software programs.

RESULTS AND DISCUSSION

1. Effect of citric acid and GDL on pathogenic bacteria

Effect of citric acid on pathogenic bacteria

Fig 1 shows the effect of citric acid on final pH, biomass concentration, total viable count and microbial growth rate% of five pathogenic bacterial strains (*S. enteritidis, Staph. aureus, B. cereus, E. coli* and *P. aeruginosa*). It was found that at pH 4.4, there were no significant differences between all pathogens in final pH values except *E. coli*. While, at pH 5.2, there were significant differences between all pathogens in final pH, and there was an effect of the pH change. It was obvious that citric acid affected the final pH for all pathogens and the activity was more pronounced at lower pH 4.40, 4.80 and 5.20, while its activity diminished at higher pH 5.6 to 7.00. It was reported that the inhibition of microorganisms is depended on pH as week acids loss their activity as the pH increased (Beier, 2021).

This was also apparent with biomass concentration which was lower for all five pathogens at lower pH 4.40 to 5.20. At pH 4.4, there were no significant differences in biomass concentration between *Staph. aureus, P. aeruginosa, B. cereus,* and *S. enteritidis* and *E. coli*. There was not a significant difference in biomass concentration between *Staph. aureus* and *B. cereus* at pH 5.2, while the significant was different among other three pathogens. The highest biomass concentration (0.21 gl^{-1}) was detected with *S. enteritidis* followed by *E. coli* (0.19 gl^{-1}) at pH 4.40. While, at pH 5.20, higher biomass concentration (0.43 gl^{-1}) was found with *P. aeruginosa* followed by *E. coli and S. enteritidis*. The biomass concentration increased with all pathogens with increasing pH which confirms the effectiveness of citric acid at lower pH 4.40-5.20. The effectiveness of citric acid was due to its ability to lower the pH of the internal bacterial cell. In addition, due to the dissociation of acids, the pH inside the microbial cell will change, pH become higher than the dissociation constant (pKa) of acids and a large amount of hydrogen ions (H+) is released inside the cell. In addition, the cell tries to pump out the excess of hydrogen ions (H+) consuming large amount of energy leading to cell death (Coban, 2020).

The TVC was not significant differences in all pathogens at pH 4.4 and 5.2. The higher total viable count (TVC) 4.83 log cfu ml^{-1} was observed with *E. coli* followed by *P. aeruginosa* (4.75 log cfu ml^{-1}), *B. cereus* (4.71 log cfu ml^{-1}), *S. enteritidis* (4.50 log cfu ml^{-1}) and *Staph. aureus* (4.49) at pH 4.40. While, at pH 5.2, the highest TVC was detected with *E. coli* 6.74 log cfu ml^{-1}, and the lowest number (5.18 log cfu ml^{-1}) was detected with *Staph. aureus*. This shows that citric was more effective against pathogens (*S. enteritidis* and *Staph. aureus*) at pH 5.20.

In addition, this observation was confirmed by the higher microbial growth rate (29.03%) with *E. coli* at pH 5.20. The microbial growth rate was not significant differences in

all pathogens at pH 4.4 and 5.2. It could be noticed that at lower pH 4.40-4.80, *S. enteritidis, Staph. aureus and P. aeruginosa* were totally inhibited by citric acid, while *B. cereus, P. aeruginosa* and *E. coli* grown in lower rates. The strains; *S. enteritidis* and *Staph. aureus* had a negative growth rate (-7.45 and -2.45%) respectively at pH 4.4, while at pH 4.8, the negative growth rate (-5.23%) was found in *S. enteritidis.* The lower effect of citric acid on *B. cereus, P. aeruginosa* and *E. coli* might be due to the rate of un-dissociated, dissociated and the lower concentration of citric acid as reported by (Beier, 2021). In addition, this was due to the fact that *B. cereus, P. aeruginosa* and *E. coli* can utilize citrate under certain growth conditions which requires higher concentration of citric acid to be used for total inhibition. It was reported that citric acid was more effective against pathogenic bacteria when used in combinations with other organic acids or preservation techniques (Seok & Ha, 2021). These results were in line with (Burel *et al.*, 2021).

As shown in Fig 1, citric acid was more effective at all bacterial strains used in this study at the lowest pH values in agreement with (In *et al.*, 2013) who told that citric acid showed high activity against *S. dysenteriae*, and the number of damaged cells was highest with this organic acid. *S. dysenteriae* count decreased by 4 logs after 10 hours of treatment compared to initial bacterial count. *S. Sonnei* decreased by about 3 logs after 1 hr, and *S. boydii* and *S. flexneri* decreased by about 2 and 1 log, respectively. Citric acid showed a different reduction pattern. Although the number of damaged cells observed was relatively low.

Fig 1 A illustrated that, when adjusted or initial pH of the LB medium was reduced, the final pH, which determined after 24 hr. of incubation, was significantly constant by all pathogenic strains used. Whereas, when the initial pH of LB medium inoculated with *S. typhimurium*, *Staph. aureus*, *B. cereus*, *E. coli* O157:H7 or *P. aeruginosa* was 4.40, the final pHs had no significant differences and the strains *S. typhimurium*, *Staph. aureus*, *B. cereus*, *E. coli* O157:H7 and *P. aeruginosa* recorded 4.40, 4.80, 4.20, 4.81 and 4.50 respectively. This result was emphasized by rest of parameters in figure 1, B, C and D. Since, the TVCs of all strains, as appeared in figure 1 C, were significantly lower than or equal the initial TVC of all strains. And this result also illustrated in figure 1 D, which showed that the biomass concentration C_B of all strains had no significant differences at the lowest initial pH of LB medium 4.40. Moreover, figure 1 B, proved that citric acid had high effect on pathogenic bacteria used in this study, when initial pH was less than or equal 5.20. This result showed by the multiple regression curve of calculated microbial growth rate in figure 1 B, which started dramatically decrease at initial pH 5.50 approximately. It is worth mentioned that the microbial growth rate of *S. typhimurium* and *Staph. aureus* recorded -7.45 and -2.45% respectively at initial pH 4.40. However, other strains *B. cereus*, *E. coli* O157:H7 and *P. aeruginosa* recorded 6.27, 1.04 and 6.32% respectively at initial pH 4.40.

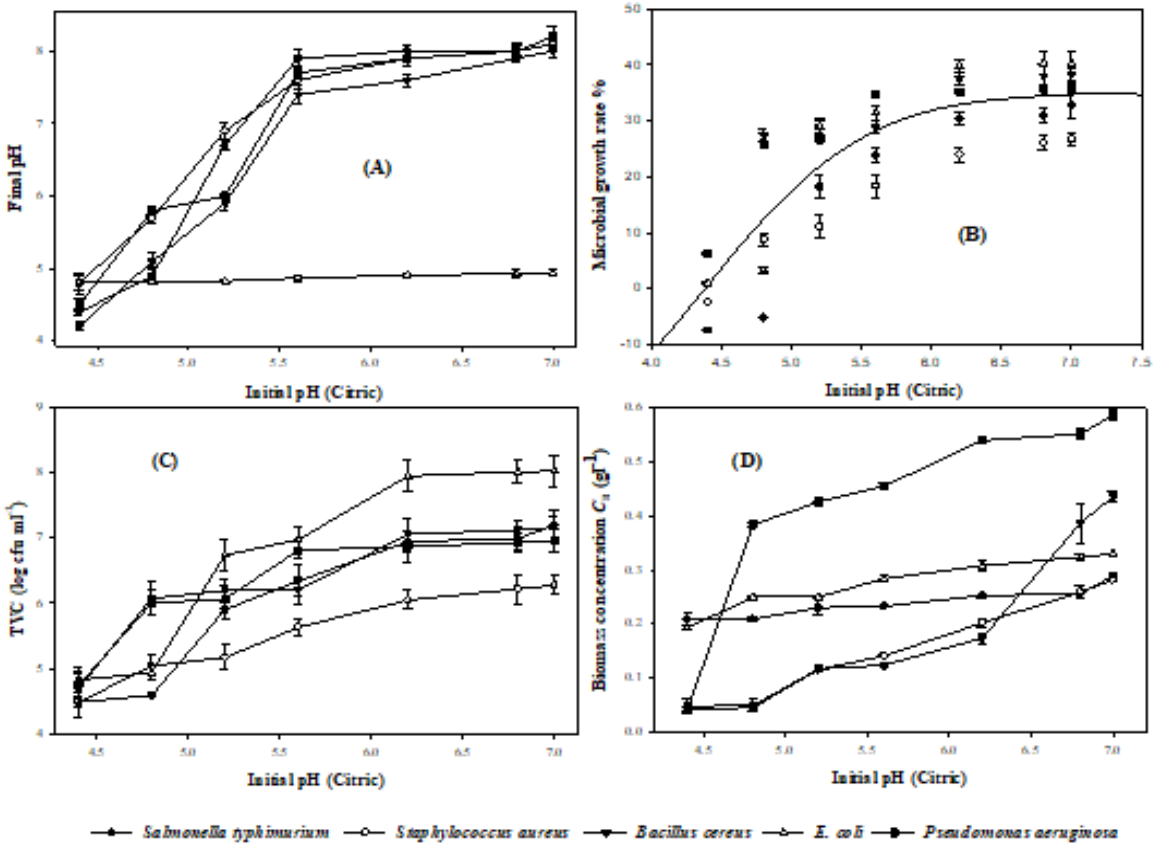

Fig 1. Effect of citric acid on final pH (A), microbial growth rate (B), TVC (C) and biomass concentration C_B (D) of pathogenic bacteria after incubation for 24 hr.

Effect of GDL on pathogenic bacteria

Fig 2 showed the effect of GDL on final pH, biomass concentration, total viable count and microbial growth rate% of five pathogenic bacterial strains (*S. enteritidis, Staph. aureus, B. cereus, E. coli and P. aeruginosa*). It was found that at pH 4.4, there were no significant differences between

all pathogens in final pH values. While, at pH 5.2, there were significant differences in final pH between all pathogens except *E.* coli, and there was an effect of the pH change. It was obvious that GDL affected the final pH for all pathogens and the activity was more pronounced at lower pH 4.40, 4.80 and 5.20, while its activity diminished at higher pH 5.6 to 7.00. This was due to the fact that when GDL contacts to water, the ring shape molecule opened and converted to gluconic acid which lower the pH of milk (Herz *et al.*, 2021).

This was obvious with biomass concentration which was lower for all five pathogens at lower pH 4.40 to 5.20. At pH 4.4, there were no significant differences in biomass concentration between *Staph. aureus, P. aeruginosa, B. cereus,* and *S. enteritidis* and *E. coli.* There was not a significant difference in biomass concentration between *Staph. aureus* and *B. cereus* at pH 5.2, while the significant was different among other three pathogens. The highest biomass concentration (0.26 gl^{-1}) was detected with *P. aeruginosa* followed by *S. enteritidis* (0.17 gl^{-1}) at pH 4.40. While, at pH 5.20, the highest biomass concentration (0.42 gl^{-1}) was found with *P. aeruginosa* followed by *E. coli and S. enteritidis.* The biomass concentration increased with all pathogens with increasing pH which confirms the effectiveness of GDL at lower pH 4.40 - 5.20. The effectiveness of GDL was due to its solubility in water and formation of gluconic acid lowering the pH and inhibiting the growth of pathogenic bacteria (Lim & Dolzhenko, 2021).

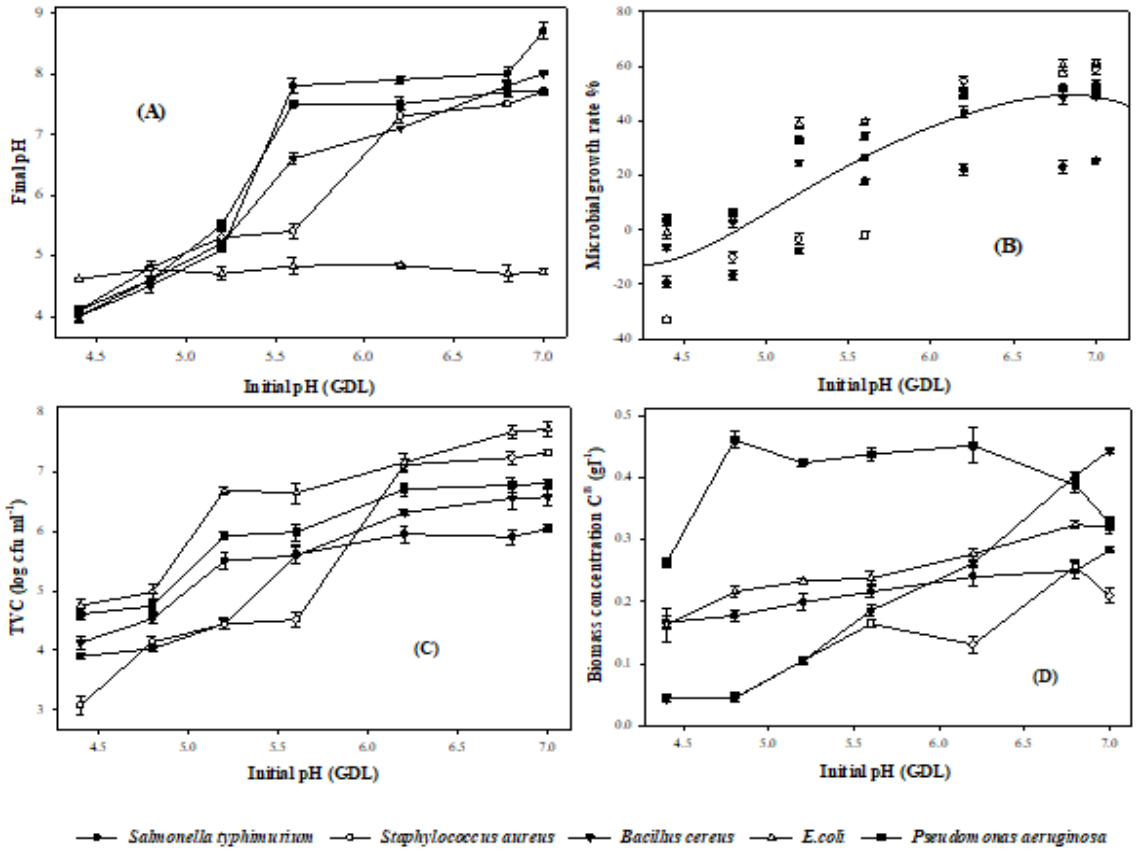

Fig 2. Effect of GDL on final pH (A), microbial growth rate (B), TVC (C) and biomass concentration C_B (D) of pathogenic bacteria after incubation for 24 hr.

The TVC was not significant differences in all pathogens at pH 4.4 and 5.2. The higher total viable count (TVC) 4.75 log cfu ml-1 was observed with *E. coli* followed by *P. aeruginosa* (4.61 log cfu ml-1), *B. cereus* (4.13 log cfu ml-1), *S. enteritidis* (3.90 log cfu/ml) and *Staph. aureus* (3.08) at pH 4.40. While, at pH 5.2, the highest TVC was detected with *E. coli* 6.68 log cfu/ml, and the lowest number (4.44 log cfu/ml) was detected with *Staph. aureus.* This shows that GDL was more effective against pathogens (*S. enteritidis, Staph. aureus, B. cereus and P. aeruginosa*) at pH 5.2.

In addition, this observation was confirmed by the lower microbial growth rates with such organisms at pH 5.20. The microbial growth rate was not significant differences in all pathogens at pH 4.4 and 5.2. In addition, it was noticed that at lower pH 4.40, *S. enteritidis, Staph. aureus, B. cereus* and *E. coli* were totally inhibited by GDL. While at pH 5.2 *S. enteritidis* and *Staph. aureus* were totally inhibited by GDL, while *B. cereus, P. aeruginosa* and *E. coli* grown in lower rates. The strains; *S. enteritidis, Staph. aureus, B. cereus* and *E. coli* had a negative growth rate (-19.25, -33.04, -6.35 and -0.84%) respectively at pH 4.4, while at pH 5.2, the negative growth rate (-7.87 and -3.48%) was found in *S. enteritidis* and *Staph. aureus* respectively. At pH 5.6, *Staph. aureus* had -1.96% negative growth rates among all pathogens. The lower effect of GDL on *B. cereus, P. aeruginosa* and *E. coli* might be due to the lower concentration of GDL (Beier, 2021). These results were in line with Zhou *et al.* (2020), who used glucono- δ-lactone as acidifier in food processing application and it was active for reducing the microbial populations including *S.* and *E. coli* O157:H7 to lower than 1 log cfu depending on storage time. In addition, GDL is characterized by the slower release of acid into food matrix reducing water and inhibiting the growth of bacteria. It was found that glucono-δ-lactone is active against the pathogenic bacteria

Listeria monocytogenes and enhances the oxidative stability of food preparation systems (Ju *et al.*, 2022).

At initial pH higher than 5.50, all strains were not affected by citric acid and GDL. Moreover, some pathogenic strains raised the final pH more than the initial pH and this resulted from the pH homeostasis, most bacteria have mechanisms to maintain the pH inside their cytoplasm within a narrower range than the pH outside the cell. In general, three distinct strategies are used to prevent such severe drop of pH (Krulwich *et al.*, 2011; P. Lund *et al.*, 2014). First, cells often use enzyme-catalyzed reactions that consume protons: decarboxylation reactions often serve this purpose because protons are irreversibly incorporated into the reaction product after CO2 is removed. Second, cells can deploy reactions that produce basic compounds to help Neutralizes low pH (Krulwich *et al.*, 2011; Pennacchietti *et al.*, 2018). Third, many types of microbial cells remove protons from the cell at the cost of consuming ATP. Protons can be released from some bacteria using F1Fo-ATPase (Krulwich *et al.*, 2011; P. A. Lund *et al.*, 2020).

2. Effect of citric acid and GDL on lactic acid bacteria

Effect of citric acid on lactic acid bacteria

Fig 3 shows the effect of citric acid on final pH, biomass concentration, total viable count and microbial growth rate% of four lactic bacterial strains (*Lb. acidophilus, Str. thermophilus, Lb. bulgaricus, Lb. paracasei*). Citric acid affected the pH, biomass concentration, TVC and microbial growth rate of *Str.* thermophilus. It is clear that citric acid promoted the growth of LAB *Lb.* acidophilus, *Lb.* bulgaricus, *Lb.* paracasei and affected the growth of *Str.* thermophilus. It was found that at pH 4.4, there were significant differences between all LAB in final pH values. At pH 5.2, there were no significant differences between *Lb.* paracasei and *Lb.* acidophilus in final pH values and a significant difference was observed between other two LAB, and there were an effect of the pH change. The activity of LAB was more pronounced at higher pH values than lower pH values. This might be due to the fact that organic acids have an impact on the metabolism occurred by LAB due to the intermediates secondary carbon sources formed through the metabolic pathways (Adamczak *et al.*, 2019).

This was apparent with biomass concentration which was the lowest (0.02 gl^{-1}) with *Str. thermophilus* at lower pH 4.40. At the same pH level (4.40), *Lb. bulgaricus* had the highest biomass concentration (0.72 gl^{-1}) followed by *Lb. paracasei* (0.66 gl^{-1}) and *Lb. acidophilus* (0.61 gl^{-1}). At pH 4.4, there were no significant differences in biomass concentration between *Lb. acidophilus, Lb. bulgaricus, Lb. paracasei.* The same trend was observed at pH 5.2. The biomass concentration with *Str. thermophilus* was significantly different from other LAB at pH 5.2. As pH increase the biomass concentration increase with all LAB. The highest biomass concentration (0.73 gl^{-1}) was detected with *Lb. bulgaricus* followed by *Lb. paracasei* (0.70 gl^{-1}), *Lb. acidophilus* (0.67 gl^{-1}) at pH 5.20. The lowest biomass concentration (0.14 gl^{-1}) was detected with *Str. thermophilus* at the same pH level 5.20. This might be due to the effect of citric acid on the growth of *Str. thermophilus* at lower pH 4.40 to 5.20, while at higher pH values 5.60-6.80 *Str. thermophilus* was activated and higher biomass concentration was observed. It was reported that citric acid has an impact on the growth of *Str. thermophilus.* In addition, *Str. thermophilus* is characterized by higher sensitivity to antibiotics and sanitizers with lower proteolytic activity, it is unique among the streptococci in having no group-specific antigen (J. Harnett, 2011).

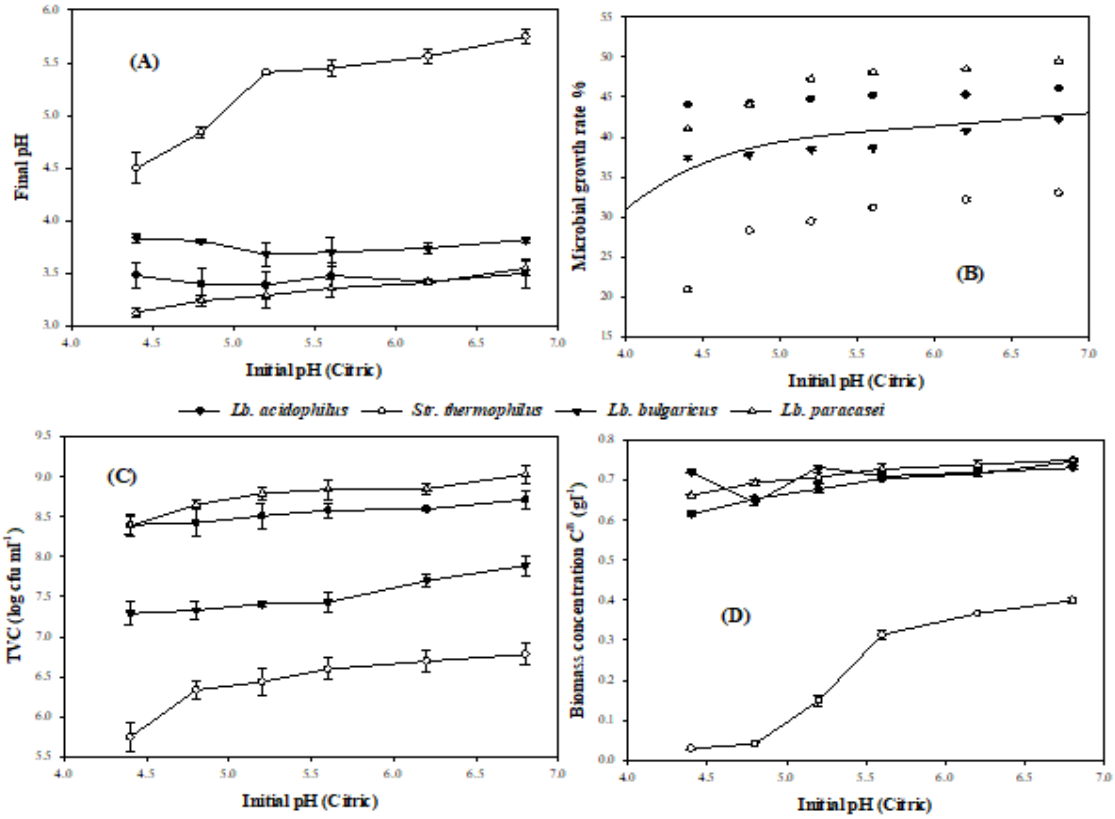

Fig. 3. Effect of citric acid on final pH (A), microbial growth rate (B), TVC (C) and biomass concentration C_B (D) of lactic acid bacteria after incubation for 24 hr.

Moreover, the higher TVC (8.40 log cfu ml-1) was observed with *Lb. acidophilus* followed by *Lb. paracasei* (8.39 log cfu ml-1), *Lb. bulgaricus* (7.29 log cfu ml-1) and the lowest TVC (5.74 log cfu ml-1) was detected with *Str. thermophilus* at pH 4.40. At pH 4.4, there were no significant differences between *Lb. acidophilus* and *Lb. paracasei* in TVC. While, at pH 5.2, there were significant differences between all LAB in TVC. The TVC increased with increasing pH level with all LAB. At pH 5.20, the highest TVC (8.79 log cfu ml-1) was observed with *Lb. paracasei* followed by *Lb. acidophilus* (8.51 log cfu ml-1), *Lb. bulgaricus* (7.41 log cfu ml-1) and the lowest TVC (6.43 log cfu ml-1) was detected with *Str. thermophilus*. This shows that citric acid was more effective for enhancing the growth of *Lb. acidophilus*, *Lb. bulgaricus*, *Lb. paracasei*.

In addition, this observation was confirmed by the higher microbial growth rate (44.05, 41.00 and 37.45%) with *Lb. acidophilus*, *Lb. paracasei* and *Lb. bulgaricus* respectively at pH 4.40. The lowest microbial growth rate (20.97%) was detected with *Str. thermophilus* at the same pH level (4.40). At pH 4.4, there were no significant differences between *Lb. acidophilus* and *Lb. paracasei* in microbial growth rate. While, at pH 5.2, there were significant differences between all LAB in microbial growth rate. It was noticed that as the pH increased, the microbial growth rate increase in all LAB. At pH 5.20, the highest microbial growth rate (47.28%) was observed with *Lb. paracasei* followed by *Lb. acidophilus* (44.77%), *Lb. bulgaricus* (38.46%) and the lowest microbial growth rate (29.45%) was detected with *Str. thermophilus*. It was reported that citric acid was used as intermediate metabolite for stimulating the growth of LAB for enhancing the production of flavoring compounds in some food preparation systems (Renouf, 2020).

Effect of GDL on lactic acid bacteria

Fig 4 shows the effect of GDL on final pH, biomass concentration, total viable count and microbial growth rate% of four lactic bacterial strains (*Lb. acidophilus*, *Str. thermophilus*, *Lb. bulgaricus*, *Lb. paracasei*). GDL affected the pH, biomass concentration, TVC and microbial growth rate of *Str. thermophilus*. It is clear that GDL promoted the growth of LAB, while *Lb. acidophilus* and *Lb. paracasei*, *Lb. bulgaricus* were activated more than *Str. thermophilus*. It was found that at pH 4.4, there were significant differences between *Lb. acidophilus* and *Lb. bulgaricus* in final pH values. At pH 5.2, there were significant differences between all LAB in final pH values, and there were an effect of the pH change. The activity of LAB was more pronounced at higher pH values than lower pH values. This was in line with Tsukahara et al. (2020) who reported that gluconic acid can stimulate the growth of LAB via the stimulation of butyrate production.

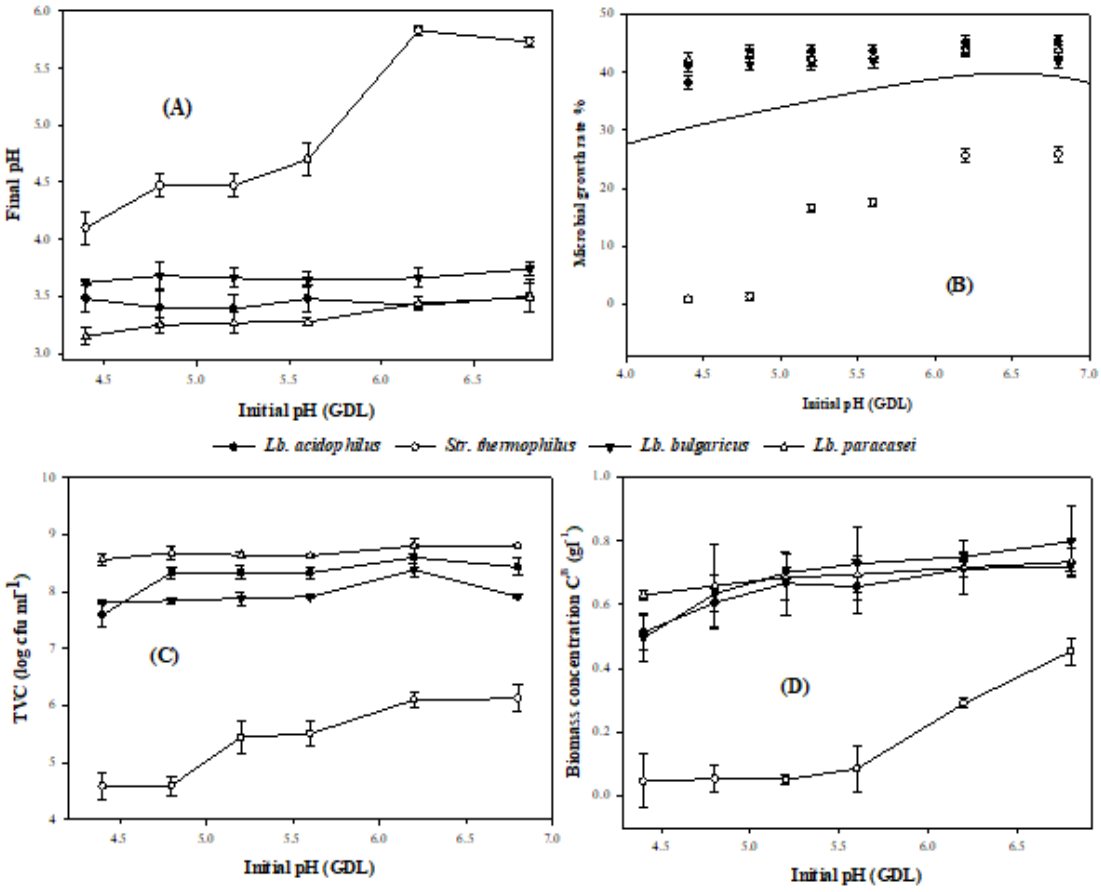

Fig. 4. Effect of GDL on final pH (A), microbial growth rate (B), TVC (C) and biomass concentration C_B (D) of lactic acid bacteria after incubation for 24 hr.

This was apparent with biomass concentration which was lower (0.04 gl^{-1}) with *Str. thermophilus* at lower pH 4.40. As pH increase the biomass concentration increase with all LAB. At pH 4.4, there were significant differences in biomass concentration between all LAB. The same trend was observed at pH 5.2. The highest biomass concentration (0.63 gl^{-1}) was

detected with *Lb. paracasei* followed by *Lb. acidophilus* (0.51 gl^{-1}), *Lb. bulgaricus* (0.49 gl^{-1}) at pH 4.40. The biomass concentration increased with *Lb. acidophilus, Lb. bulgaricus* and *Lb. paracasei* with increasing pH level. At pH 5.2, the higher biomass concentration (0.70 and 0.68 gl^{-1}) was obtained with *Lb. bulgaricus* and *Lb. paracasei* respectively followed by *Lb. acidophilus* (0.66 gl^{-1}). The lowest biomass concentration (0.05 gl^{-1}) was detected with *Str. thermophilus.* This might be due to the effect of GDL on the growth of *Str. thermophilus* at lower pH 4.40 to 5.60, while at higher pH values 6.20-6.80 *Str. thermophilus* was activated and higher biomass concentration was observed. It was reported that gluconic acid promotes the growth of lactic-acid-producing and acid-utilizing bacteria (Michiels *et al.*, 2023).

Moreover, the higher TVC 8.56 log cfu ml^{-1} was observed with *Lb. paracasei* followed by *Lb. bulgaricus* (7.81 log cfu ml^{-1}), *Lb. acidophilus* (6.59 log cfu ml^{-1}) and the lowest TVC (4.58 log cfu ml^{-1}) was detected with *Str. thermophilus* at pH 4.40. At pH 4.4, there were no significant differences between *Lb. acidophilus* and *Lb. bulgaricus* in TVC, and the same tend was detected at pH 5.2. The TVC increased with increasing pH level with all LAB. At pH 5.20, the highest TVC (8.46 log cfu ml^{-1}) was observed with *Lb. paracasei* followed by *Lb. acidophilus* (8.33 log cfu ml^{-1}), *Lb. bulgaricus* (7.88 log cfu ml^{-1}) and the lowest TVC (5.44 log cfu ml^{-1}) was detected with *Str. thermophilus.* This shows that GDL was more effective for enhancing the growth of *Lb. acidophilus, Lb. bulgaricus, Lb. paracasei.* This might be due to the fact that *Str. thermophilus* produce large quantities of acids and it is one of LAB that can produce the higher amount of acids and contribute for fast acidification and fermentation of various food systems (Iyer *et al.*, 2010). In addition, *Str. thermophilus* has the ability to produce acetic acid with other volatile compounds (diacetyl, acetaldehyde, acetoin) during fermentation (Dan Tong, 2017).

In addition, this observation was confirmed by the higher microbial growth rate (42.17, 41.10 and 38.12%) with *Lb. paracasei, Lb. bulgaricus* and *Lb. acidophilus* respectively at pH 4.40. At pH 4.4, there were no significant differences between *Lb. acidophilus* and *Lb. bulgaricus* in microbial growth rate, and the same tend was detected at pH 5.2. The lowest microbial growth rate (0.87%) was detected with *Str. thermophilus* at the same pH level (4.40). It was noticed that as the pH increased, the microbial growth rate increase in all LAB. At pH 5.20, the highest microbial growth rate (43.16%) was observed with *Lb. acidophilus* followed by *Lb. paracasei* (42.25%), *Lb. bulgaricus* (41.62%) and the lowest microbial growth rate (16.62%) was detected with *Str. thermophilus.*

CONCLUSION

Citric acid and GDL had antimicrobial effect against the selected pathogenic bacteria. GDL had the higher inhibition effect than citric acid. The inhibition of GDL continued at pH 5.2, while *Staph. aureus* and *S. enteritidis* were totally inhibited by GDL at pH 5.2. The effect of citric acid for total inhibition of pathogens was pronounced only till pH 4.8. In addition, Citric acid and GDL enhanced the growth of LAB. Citric acid was more effective for enhancing the growth of LAB followed GDL Citric acid promoted the growth of all LAB when compared with citric acid.

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التأثير المضاد لحامض الستريك والجلوكونو دلتا لاكتون على البكتيريا المرضية وبكتيريا حامض اللاكتيك

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الملخص

يهدف هذا البحث لدراسة تأثير حامض الستريك والجلوكونو دلتا لاكتون على بعض البكتيريا المرضية (*Salmonella enteritidis, Staphylococcus aureus,* *Bacillus cereus, Escherichia coli* O157:H7 and *Pseudomonas aeruginosa*) وبعض بكتيريا حامض اللاكتيك (*Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus* and *Lactobacillus paracasei*). تم ضبط درجة pH بيئة الـ LB بحامض الستريك أو الـ GDL على درجات pH مختلفة (4.4، 4.8، 5.2، 5.6، 6.2، 6.8، 7.0) لإختبار البكتيريا المرضية. بينما تم ضبط بيئات MRS أو M17 بحامض الستريك أو الـ GDL على درجات pH مختلفة (4.4، 4.8، 5.2، 5.6، 6.2، 6.8) لإختبار بكتيريا حامض اللاكتيك. ولقد تم تقدير كل من pH البيئة النهائي و تركيز الكتلة الحيوية والعد الكلي الحيوي و معدل النمو الميكروبي المحسوب كنسبة مئوية بعد التحضين لمدة 24 ساعة. ولقد دلت النتائج على أن كلاً من حامض الستريك والـ GDL له تأثير عالٍ في تثبيط الميكروبات المرضية. ولكن وجد أن هذه الأحماض ليس لها تأثير على بكتيريا حامض اللاكتيك. وأن الـ GDL كان له أعلى تأثير مثبط للبكتيريا المرضية عن تأثير حامض الستريك عليها. ولقد بدأ ظهور التأثير المثبط لحامض الستريك والـ GDL على البكتيريا المرضية عند درجات الـ pH ≥ 5.2. بينما تم تثبيط كلاً من بكتيريا *Staphylococcus aureus*، *Salmonella enteritidis* بالكامل باستخدام الـ GDL على درجة pH 5.2. إضافة إلى ذلك، فقد حسن حامض الستريك نمو بكتيريا حامض اللاكتيك بدرجة أكبر من الـ GDL.

الكلمات الدالة: التأثير المضاد للميكروبات – حامض الستريك – جلوكونو دلتا لاكتون - البكتيريا المرضية - بكتيريا حامض اللاكتيك