Original Research Article

EFFET OF LACTIC ACID ON INACTIVATION OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) ISOLATED FROM TUNA LOINS PRODUCED IN CÔTE D'IVOIRE

ABSTRACT

Aims: The aim of this work was to study the effect of lactic acid on the growth of pathogenic strains of *Escherichia coli* (ETEC) isolated from tuna loins
Study design: Bacteriological study
Place and Duration of Study: Laboratory of Microbiology of the Central Laboratory of Food hygiene and Agrobusiness (LCHAI), Abidjan, Côte d'Ivoire between September 2014 and December 2014.
Methodology: Enterotoxigenic *Escherichia coli* (ETEC) strains were isolated from tuna loins. Lactic acid 1%, 2% and 3% were tested in pathogenic strains in liquid medium (brain heart broth, BCC) and in tuna loins.
Results: At lactic acid 1%, the bacterial loads decreased during the first two days and then stabilized. *E. coli* strains in tuna loins were higher (1.25 to 0.9 log CFU/g) than *E. coli* in liquid medium (0.69 to 0.3 log CFU/g). No bacterial growth was observed in the tuna loins and in BCC for concentrations of 2% and 3% of lactic acid.
Conclusion: Lactic acid has an inhibitory effect at 1% and bactericidal effect at 2% and 3% on the growth of *E. coli*. The use of lactic acid as a preservative could be a solution for the preservation of these products

Keywords: Enterotoxigenic Escherichia coli (ETEC), Lactic acid, Tuna loins, Brain heart broth

1. INTRODUCTION

Microorganisms of varying types and numbers can be found on food of animal and plant origin. The types and number of microorganisms on food can be changed due to food processing, inappropriate purchasing, storing, preparing, cooking or serving [1]. Increase in the number of these microorganisms due to the abovementioned changes may lead to spoiling of the food, causing a pathogenic effect on humans. The most important of foodborne pathogenic bacteria is *Escherichia coli*.

Comment [M1]: Reference?

The Enterotoxigenic *Escherichia coli* (ETEC) strains are mainly associated with two important clinical syndromes, choleriform watery diarrhea in children called infant diarrhea and traveler's diarrhea (or "turista") in developing countries [2]. The pathogenic power of ETEC is mainly explained by the secretion of thermostable (ST) and / or thermolabile (LT) toxins (Levine, 1987). People living in developing countries have often been reported to have this pathotype in their feces and shown to have developed immunity against this microorganism. Being a cause of mortality in children under 5, the most frequently observed

microorganism in childhood diarrhea is ETEC and it is also responsible for 30–60% of travelers' diarrhea. Infection is characterized by watery diarrhea and, depending on the person, its course may range from a normal course to cholera-like defecation with the addition of symptoms such as vomiting and high fever [3, 4, 5]. Diarrhea is the most common causes of mortality in society and among young children, especially those living in Asia and sub-Saharan Africa with inadequate healthcare systems and limited access to clean drinking water.

Comment [M2]: Here is another paragraph that may need reference.

Côte d'Ivoire through the processors and exporters of fish products, has become one of the largest exporters of tuna products to the global level [6]. There are 2 types of tuna products exported: Tuna finished products (canned) and tuna semi-finished products (tuna loins, tuna flakes, tuna skin and tuna pulp). The tuna loins are portions of the tuna flesh usually skinless and boneless and ready to use. However, industries have difficulties to export tuna loins because they don't satisfy the criteria for hygienic quality and existing standards always. ETEC has been found in these products [7], which poses a major health and public health problem and causes economic losses for companies producing tuna products.

The aim of this work was to study the effect of lactic acid on the growth of pathogenic strains of *Escherichia coli* (ETEC) isolated from tuna loins.

2. MATERIAL AND METHODS

2.1 Sample preparation

Each sample of tuna loins was crushed and aseptically distributed in Pyrex bottles then sterilized at 121°C for 15 min. Each sample was approximately 100 g in each bottle.

Brain heart broth (BCC) (Biorad, France) was prepared in accordance with the manufacturer's instructions and distributed in Pyrex bottles then sterilized at 121°C for 15 min. The volume of each broth was also 100 mL in each bottle.

2.2 Inoculum preparation

Three strains of *E. coli* were selected for the various analyzes:

- an enterotoxigenic strain of *E. coli* (ETEC), possessing both the "elt" and "est" genes resistant to amoxicillin, isolated from tuna loins;

- an *E. coli* reference strain (ATCC 25992);

- a strain of *E. coli* (KO 13) from water with the virulence gene "elt".

A colony of each strain was inoculated into 10 mL of Tryptone Soya Broth (TSB) (Mast Diagnostic, France) broth and incubated at 37°C for 24 hours. The optical density of the inoculated broth was determined using a UV 2700 spectrophotometer (Schimadzu, Germany) at a wavelength of 600 nm. Knowing that the absorbance between 0.5 and 1 corresponds to approximately 10^8 CFU/ml, the different concentrations of *E coli* have been determined and the cultures diluted to obtain a final concentration of 10^5 CFU/ml.

2.3 Kinetics of destruction of *Escherichia coli* strains

The study of the kinetics of destruction of the *Escherichia coli* strains isolated from tuna loins was carried out according to the methods described by [8]. 1 mL of each bacterial culture with a concentration of 10^5 CFU/mL was inoculated into the 100 g of tuna loins and into the 100 mL of brain hearth broth (BCC). Then, 1 ml of lactic acid (Riedel-De Haën AG Seelze-Hannover, Germany) at 1%, 2% and 3% was added. Lactic acid is used as a preservative in the food industry. They were incubated at 30°C for 5 days. At each time interval, 1 mL was

taken to determine the pH using pH meter (Milwaukee, USA) and 1 mL was taken to determine *Escherichia coli* on Tryptone Soya Agar (TSA) (Plasmatec, England). The measurement interval was (days): 0; 1; 2; 3; 4; 5

Comment [M3]: In order to give greater relevance to the results obtained, it is necessary to design a statistical analysis.

3. RESULTS AND DISCUSSION

3.1 Effect of lactic acid on the growth of *Escherichia coli* strains in non-renewed liquid medium

Figs. 1 to 3 show effect of different concentrations of lactic acid (1%, 2% and 3%) in BCC on the growth of *E. coli* ATCC 25922, *E. coli* KO strains 13 and virulent *E. coli* isolated from tuna loins respectively. The pH for lactic acid 1% concentrations was highest and the E. coli loads decreased considerably from 0.95 to 0.3 log CFU/mL (Fig. 1); from 0.77 to 0.25 log CFU/mL (Fig. 2) and from 0.69 to 0.3 log CFU/mL (Fig. 3). No bacterial growth was observed in liquid medium for concentrations of 2% and 3% of lactic acid. Whatever the curve, the bacterial loads decreased the first two days and then stabilized.

Comment [M4]: Although the UFC reduction is noticeable, it is necessary to perform a statistical analysis to verify the relevance of the results obtained, as stated before.

Fig. 4 compares the effect of lactic acid (1%) on the growth of the three strains studied. The three curves have the same appearance: the bacterial loads decreased during the first two days before stabilizing. The *E. coli* KO 13 strains were the most sensitive to the effect of lactic acid, while the *E. coli* ATCC 25922 strains were the least sensitive.

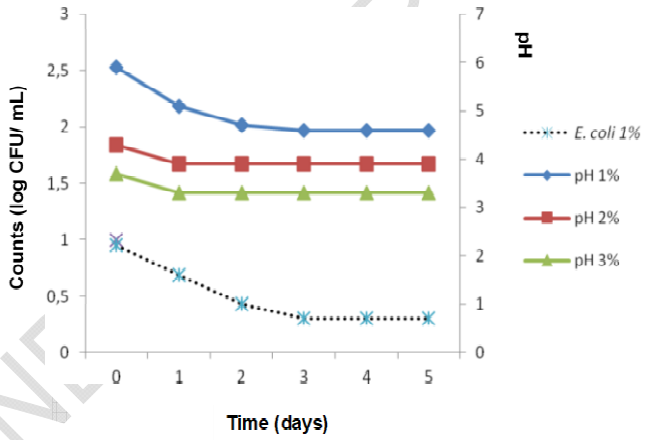

Fig. 1. Effect of lactic acid on the growth of strains of *Escherichia coli* ATCC 25922 in BCC

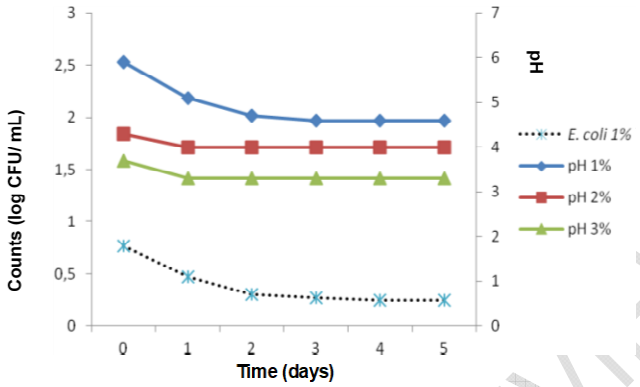

Fig. 2. Effect of lactic acid on the growth of strains of *Escherichia coli* KO 13 in BCC

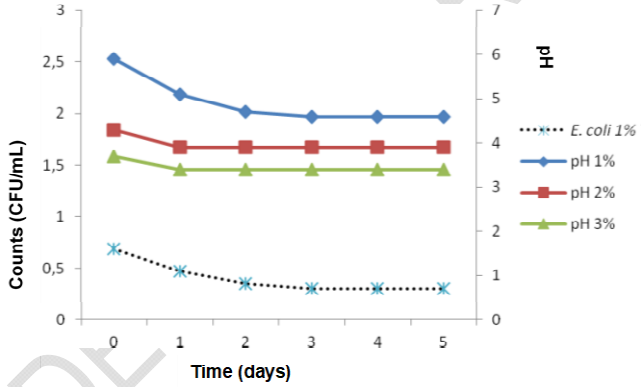

Fig. 3. Effect of lactic acid on the growth of pathogenic *Escherichia coli* from tuna loins in BCC

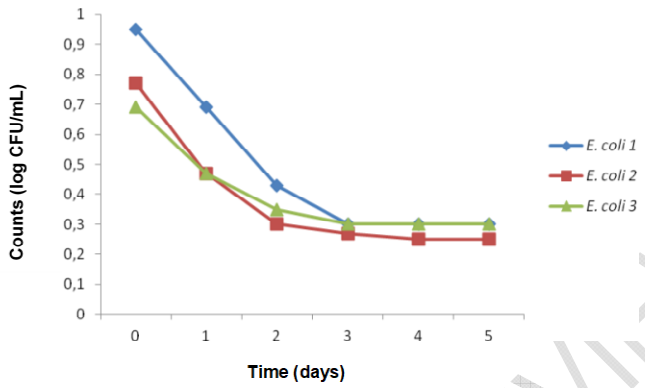

Fig. 4. Comparative evolution of the effect of lactic acid (1%) on the growth of three *Escherichia coli* strains in BCC
E. coli 1 = E. coli ATCC 25922; E. coli 2 = E. coli KO 13; E. coli 3 = pathogenic E. coli from tuna loins

3.2 Effect of lactic acid on the growth of *Escherichia coli* strains in tuna loins

Figs. 5 to 7 show the effect of different concentrations of lactic acid (1%, 2% and 3%) in tuna loins on the growth of *E. coli* ATCC 25922, *E. coli* KO 13 strains and virulent *E. coli* from tuna loins respectively. The pH for lactic acid 1% concentrations was highest and the *E. coli* loads decreased from 1.11 to 0.9 log CFU/g (Fig. 5); from 1.27 to 1.07 log CFU/g (Fig. 6) and from 1.25 to 0.9 log CFU/g (Fig. 7). No bacterial growth was observed in the tuna loins for concentrations of 2% and 3% of lactic acid. Whatever the curve, the bacterial loads decreased the first two days and then stabilized.

Fig. 8 illustrates the effect of lactic acid (1%) on the growth of the three strains studied. The three curves have the same appearance: the bacterial loads decreased during the first two days before stabilizing. The *E. coli* KO 13 strains were the most sensitive to the effect of lactic acid and the *E. coli* ATCC 25922 strains were the least sensitive.

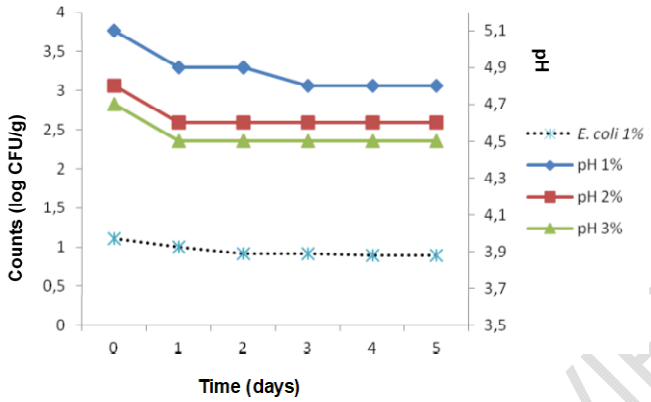

Fig. 5. Effect of lactic acid on the growth of strains of *Escherichia coli* ATCC 25922 in tuna loins

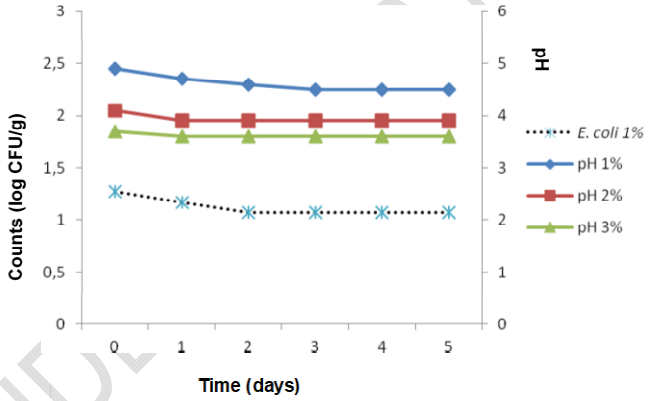

Fig. 6. Effect of lactic acid on the growth of strains of *Escherichia coli* KO 13 in tuna loins

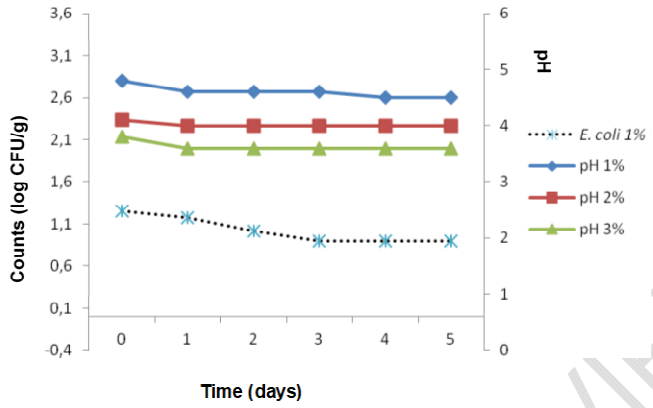

Fig. 7. Effect of lactic acid on the growth of pathogenic *Escherichia coli* from tuna loins in tuna loins

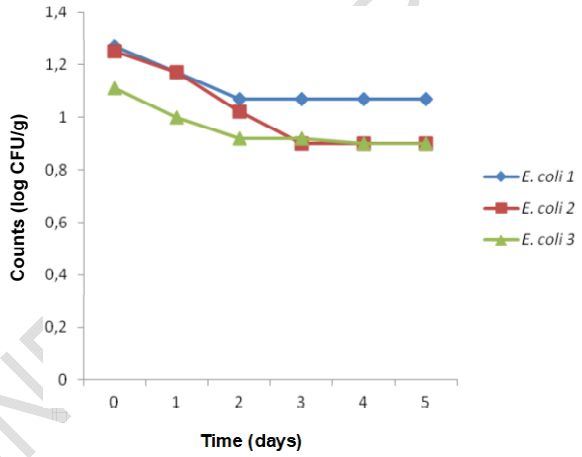

Fig. 8. Comparative evolution of the effect of lactic acid (1%) on the growth of three *Escherichia coli* strains in tuna loins

E. coli 1 = E. coli ATCC 25922; E. coli 2 = E. coli KO 13; E. coli 3 = pathogenic E. coli from tuna loins

3.3 Comparative effect of lactic acid on the growth of pathogenic strains of *Escherichia coli* from tuna loins in liquid medium and in tuna loins

Fig. 9 shows effect of lactic acid on the growth of pathogenic strains of *Escherichia coli* from tuna loins in liquid medium and in tuna loins. The bacterial loads decreased during the first two days and then stabilized. *E. coli* strains in tuna loins were higher (1.25 to 0.9 log CFU/g)

than *E. coli* in liquid medium (0.69 to 0.3 log CFU/g). The strains of *E. coli* in liquid medium were more sensitive to the effect of lactic acid than those in tuna loins.

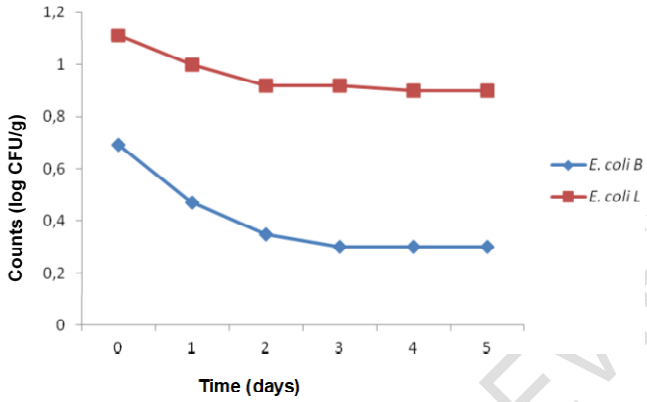

Fig. 9. Effect of lactic acid 1% on the growth of pathogenic strains of *Escherichia coli* from tuna loins in BCC and in tuna loins
E. coli L= E. coli inoculated from tuna loins; E. coli B= E. coli inoculated in liquid medium

4. DISCUSSION

The results of this work showed that bacterial loads decreased when the concentration of lactic acid at 1% was added to BCC and tuna loins. These results could be explained by the inhibitory effect of lactic acid in the various liquid and solid media. pH values after adding 1% lactic acid in this work varied from 7 to 5. *Escherichia coli* is neutrophilic but able to withstand low pH. Several authors such as [9, 10, 11, 12]; have shown that microbial growth is influenced by physicochemical conditions such as pH. The results of this study corroborate those of [13] who showed that at pH values between 4 and 5, the population of *Escherichia coli* was remarkably reduced but not completely inhibited in fermented olives in Spain. Indeed, according to [14], *E. coli* strains develop at pH between 4 and 7. [8] have shown that adding 1% lactic acid reduces the growth of *E. coli* in sausages in Sweden.

This work has shown that there is no bacterial growth when 2% and 3% lactic acid is added to the BCC and tuna loins. Indeed, the addition of lactic acid lowers the pH below 4. This very acidic pH prevents the growth of *E. coli* which is not an acidophilic bacterium. The results of this work corroborate those of [15]. Indeed, this author did not observe microbial growth below a pH 4 in "Kimchi" in Korea. Furthermore, [16] did not observe microbial growth by adding lactic acid 3% in ready-to-eat meals in Japan.

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Organic acids (lactic acid, acetic acid) and mineral acids (sulfuric acid, nitric acid) are widely used in the food industry as antimicrobial agents in order to inhibit microbial load and microbial contaminants. According to [17], acidity is the most important characteristic for determining the growth and survival of pathogenic bacteria. However, [9] have shown that organic acids are more effective than mineral acids in inhibiting the growth of *E. coli*. Lactic acid and acetic acid have been described as the most effective molecules for inhibiting the growth of *E. coli.* [18] found that lactic acid is able to reduce microbial growth in food. Other authors such as [19] showed that low pH and high acidity were associated with the reduction of the *E. coli* population.

Comment [M5]: New paragraph

Comment [M6]: There is a lack of discussion based on other articles that have compared the effectiveness of the proposed procedure when decontaminating fish.

5. CONCLUSION

The aim of this work was to study the effect of lactic acid on the growth of pathogenic strains of *Escherichia coli* (ETEC) isolated from tuna loins. Lactic acid had bacteriostatic effect at low concentrations (1%), and bactericidal effect at higher concentrations (2 and 3%) on pathogenic strains of *E. coli*. Pathovars of *E. coli* isolated from tuna loins are a hazard to be considered in the microbiological risk assessment of the consumption of these tuna products. However, the use of more than 1% lactic acid as a preservative could be a solution for the preservation of tuna loins produced in Côte d'Ivoire.

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